Henryk Czosnek · Murad Ghanim
Editors

Management of Insect Pests to Agriculture

Lessons Learned from Deciphering their Genome, Transcriptome and Proteome

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Part I
Adaptation to Plants and to Environment

Plant-Herbivore Interactions in the Era of Big Data

Linda L. Walling and Isgouhi Kaloshian

Abstract With the reduced costs, enhanced sensitivities and increased accessibility, the OMICs strategies of modern science are providing new insights and opportunities to understand the evolution and dynamics of plant-pest interactions. The deployment of high-throughput methods to study variation at the genome, transcriptome, proteome, and metabolome levels has allowed in-depth investigation of previously intractable questions in the field of plant-herbivore interactions. Discovery of the regulatory mechanisms within a single organism (plant, herbivore, herbivore endosymbiont, or resident microbe) or amongst multiple organisms as they interact is now possible, allowing the complexity of herbivore adaptation to plant hosts and host plant defense strategies to be revealed and providing momentum for the development of new gene-based mechanisms for controlling herbivore damage. Here the current status and challenges of OMICs technologies as they relate to plant-feeding insects important in our ecosystems, agriculture and forestry, their natural enemies, and their microbiomes are described. The genomics of plant-feeding or –pollinating insects, perspectives on the triumvirate of functional genomics (transcriptomics, proteomics, and metabolomics), as well as the increasing importance of integrating OMICs strategies to address contemporary biological questions are highlighted.

1 Introduction

Arthropods are a major contributor to the biodiversity on our planet (Janz et al. 2006). It is estimated that over 50 % of the 900,000 insect species that inhabit our planet recover their nutrients from plants (Strong et al. 1984). The insect species or biotypes adapted to crops threaten food security worldwide as they cause pre- and

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post-harvest damage exceeding 20 % annually (Oerke 2006; Pimentel 1997). An understanding of the intricacies of herbivore adaptation to plant hosts and host plant defense innovations may provide key insights into new gene-based mechanisms for controlling herbivore damage. With the decreased costs, increased sensitivity and accessibility of high-throughput methods to assess variation at the genome, transcriptome, proteome, and metabolome levels, previously intractable questions in field of plant-herbivore interactions can now be pursued. The OMICs strategies of contemporary science in conjunction with genetics and classical methods in plant biology and entomology have shed new lights on the evolution and dynamics of plant-herbivore interactions. Investigators can now dissect the regulatory mechanisms within a single organism (plant, herbivore, herbivore endosymbiont, or resident microbe) or amongst multiple organisms as they interact.

In this chapter, we highlight the current status and challenges of OMICs technologies as they relate to plant-feeding insects important in our ecosystems, agriculture and forestry, their natural enemies, and their microbiomes. As several recent reviews provide synopses of the plant OMICs arena and the utility of using model systems as vehicles to understand crop plant biochemical and physiological responses during development and in response to stress, these advances will not be described here (Feltus 2014; Hayward 2014; Lee 2014; Liberman et al. 2012). Instead, we provide an overview of the status of the genomics for plant-feeding or –pollinating insects, perspectives on the status of the triad of functional genomics (transcriptomics, proteomics, and metabolomics), as well as the increasing importance of integrating OMICs strategies to address contemporary biological questions. We also highlight two vignettes that illustrate recent advances in plant-insect interactions that have evolved due to the unique melding of plant and insect genomics, plant genetics, classical entomological techniques, and chemical profiling.

2 The Genomics of Plant Pests, Their Enemies, and Their Microbes – More is Better

2.1 Insect Genomes

In 2000, over 129 Mbp of the euchromatic sequences of *Drosophilia melanogaster* genome was released providing the first insights into insect genomes, gene complements and organization (Adams et al. 2000). Fueled by cheaper, more effective and more accessible high-throughput technologies, and enhancements to the genome assembly strategies and pipelines, over 132 insect genome projects have now been posted at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genome). In 2011, the i5K project was launched with the goal of contributing 5000 insect genome sequences with importance to agriculture, ecosystems, health, and humankind (Robinson et al. 2011). The large influx of insect genomes is beginning to be realized. These data will drive new approaches to old fields of study

and create new fields of study that, while possible with limited transcriptome data, can only be fully realized with well-annotated genomes. The post-genomics era of entomology is propelling new integrative strategies for studying insect biology, physiology, evolution, and the dynamics of insect interactions with the environment, their hosts and their microbiomes. As the advances in *Drosophilia* spp. biology, due to its multiple reference genomes and tractable genetic systems, are well established (Hoskins et al. 2015; Stark et al. 2007), they are not recounted here, despite the importance of several *Drosophilia* spp. in post-harvest crop losses (Walsh et al. 2011).

Currently there are 73 genomes for plant-feeding or -pollinating insects and for herbivore natural enemies (Table 1). These insects span 11 arthropod orders; based on NCBI dates for sequence data initial availability, most of these genome sequences became accessible nine years after publication of the *D. melanogaster* genome sequence (Fig. 1). The species of the Hymenoptera are currently best represented with an abundance of bee, ant and wasp genomes (Fig. 2; Table 1). There is also a growing number of insect enemies with the 14 parasitoid wasps (Insecta:Hymnoptera) and three predatory mites (Arachnida). For some insect orders (Diplura, Geophilomorpha, Isoptera, Orthoptera, Thysanopstera), there are only a single species with sequenced genomes (Fig. 2; Table 1).

Some of the largest genomes are found in the Hemiptera with four species (the glassy-winged sharp shooter, brown leafhopper, large milkweed bug, and brown marmorated stink bug) having genomes in excess of 1000 Mbp (Table 1). Given the large crop losses due to phloem-feeding aphids and whiteflies, it is surprising that there is only a single aphid species genome (*Acyrthosiphon pisum*; the pea aphid) and no whitefly genomes are available to date. With new enabling sequencing technologies, many additional insect genomes, both pests and natural enemies, will emerge in the near future. Deeply sequenced, assembled and well-annotated insect genomes and their complementary transcriptomes are critical for establishing an herbivore's gene complement and its dynamic expression during development, in response to its host plants, microbial communities, and natural enemies, as well as during environmental stresses imposed by current trends in climate change.

2.2 *Improvements and Discoveries Through Better Assembly and Re-sequencing*

The critical importance of a well-annotated genome is emphasized by the advances made by the recent reannotation of the honeybee (*Apis mellifera*) genome (Elsik et al. 2014; Honeybee Genome Sequencing 2006). The *A. mellifera* genome was one of the first agriculturally important insect genomes to be sequenced. The reannotated honeybee genome was driven by the generation of additional *A. mellifera* genome sequences, which had marked impacts. The new honeybee genome sequences increased the sizes of the contig N50 from 40 to 46 kb and scaffold N50

Table 1 Genomes of plant-feeding and pollinating arthropods and their natural enemies

Order/Family	Species name (Author) Common name	Genome size (Mb)	Fold coverage[a]	References/NCBI Genome link[b]
Arachnida/Phytoseiidae	*Metaseiulus occidentalis* (Nesbitt) Western predatory mite	151.7	17.7×	GCF_000255335.1
Arachnida/Tetranychidae	*Tetranychus urticae* (Koch)	89.6	8.1×	Grbic et al. (2011)
	Two-spotted spider mite	90.8	–	GCA_000239435.1
Arachnida/Varroidea	*Varroa destructor* (Anderson and Truemann)	294	4.3×	Cornman et al. (2010)
	Varroa honeybee mite	294	5×	GCA_000181155.1
Coleoptera/Buprestidae	*Agrilus planipennis* (Fairmaire) Emerald ash borer	353.5	124.7×	GCA_000699045.1
Coleoptera Cerambycidae	*Anoplophora glabripennis* (Motschulsky) Asian longhorned beetle	707.7	121×	GCA_000390285.1
Coleoptera/Chrysomelidae	*Leptinotarsa decemlineata* (Say) Colorado potato beetle	1170.2	52.3×	GCA_000500325.1
Coleoptera/Cupedidae	*Priacma serrata* (LeConte) A reticulated beetle	12.1[c]	25×	GCA_000281835.1
Coleoptera/Curculionidae	*Dendroctonus ponderosae* (Hopkins)[d]	208	400×	Keeling et al. (2013)
	Mountain pine beetle	261.3	227×	GCA_000346045.2
Coleoptera/Tenebrionidae	*Tribolium castaneum* (Herbst)	160	7.3×	Kim et al. (2010), Tribolium Genome Sequencing (2008)
	Red flour beetle	210.3	–	GCA_000002335.2

Diplura/Japygidae	*Catajapyx aquilonaris* (Silvestri)	302.1	100×	GCA_000934665.1
	Silvestri's Northern Forcepstail			
Diptera/Cecidomyiidae	*Mayetiola destructor* (Say)	185.8	34×	GCA_000149185.1
	Hessian fly			
Diptera/Tephritidae	*Bactrocera cucurbitae* (Coquillett)	374.8	66.2×	GCA_000806345.1
	Melon fly			
Diptera/Tephritidae	*Bactrocera dorsalis* (Hendel)	415.0	70×	GCA_000789215.2
	Oriental fruit fly			
Diptera/Tephritidae	*Bactrocera tryoni* (Froggatt)	700	80×	Gilchrist et al. (2014)
	Queensland fruit fly	519.0	96×	GCA_000695345.1
Diptera/Tephritidae	*Ceratitis capitata* (Wiedemann)	484.8	152.5×	GCA_000347755.1
	Mediterranean fruit fly			
Geophilomorpha/Linotaeniidae	*Strigamia maritime* (Leach)	290	–	Chipman et al. (2014)
	(European centipede)	176.2	23×	GCA_000239455.1
Hemiptera/Aphididae	*Acyrthosiphon pisum* (Harris)	464	6.2×	The International Aphid Genomics (2010)
	Pea aphid	541.7	15×	GCA_000142985.2
Hemiptera/Cicadellidae	*Homalodisca vitripennis* (Latreille)	2246.4	68.3×	GCA_000696855.1
	Glassy-winged sharpshooter			

(continued)

Table 1 (continued)

Order/Family	Species name (Author) Common name	Genome size (Mb)	Fold coverage[a]	References/NCBI Genome link[b]
Hemiptera/Coccoidea	*Dactylopius coccus* (Costa) Scale of cacti	18.6	99.8×	GCA_000833685.1
Hemiptera/Delphacidae	*Nilaparvata lugens* (Stål) Brown planthopper	1141–1220 1140.8	131.7× 133.3×	Xue et al. (2014) GCA_000757685.1
Hemiptera/Lygaeidae	*Oncopeltus fasciatus* (Dallas) Large milkweed bug	1098.7	106.9×	GCA_000696205.1
Hemiptera/Pentatomidae	*Halyomorpha halys* (Stål) Brown marmorated stink bug	1150.1	113.7×	GCA_000696795.1
Hemiptera/Pentatomidae	*Piezodorus guildinii* (Westwood) Small green stink bug	3.18	—	GCA_000786065.1
Hemiptera/Psyllidae	*Diaphorina citri* (Kuwayama) Asian citrus psyllid	485.7	180×	GCA_000475195.1
Hemiptera/Psyllidae	*Pachypsylla venusta* (Osten-Sacken) Hackberry petiole gall psyllid	701.8	181.4×	GCA_000695645.1
Hymenoptera/Apidae	*Apis dorsata* (Fabricius) Giant honeybee	230.3	60×	GCA_000469605.1
Hymenoptera/Apidae	*Apis florea* (Fabricius) Red dwarf honeybee	230.5	20.5×	GCF_000184785.1

Hymenoptera/Apidae	*Apis mellifera* (Linnaeus)	236.0	7.5×	The Honeybee Genome Sequencing (2006), Elsik et al. (2014)
	Western honeybee	250.3	8×	GCA_000002195.1
Hymenoptera/Apidae	*Apis mellifera intermissa* (Buttel-Reepen)	243.6	68×	GCA_000819425.1
	Native honeybee subspecies of Algeria			
Hymenoptera/Apidae	*Bombus impatiens* (Cresson)	249	127×	GCF_000188095.1
	Bumble bee			
Hymenoptera/Apidae	*Bombus terrestris* (Linnaeus)	248.7	21.4×	GCF_000214255.1
	Buff-tailed bumble bee			
Hymenoptera/Agaonidae	*Ceratosolen solmsi marchali* (Mayr)	277.1	93×	GCA_000503995.1
	Fig pollinating wasp			
Hymenoptera/Braconidae	*Cotesia vestalis* (Haliday)	186.1	106×	GCA_000956155.1
	Diamondback moth parasitoid			
Hymenoptera/Braconidae	*Fopius arisanus* (Sonan)	153.6	137.6×	GCA_000806365.1
	Parasitoid of Tephritid fruitflies			
Hymenoptera/Braconidae	*Microplitis demolitor* (*Wilkinson*)	250.5	26.0×	GCA_000572035.1
	Parasitoid wasp			

(continued)

Table 1 (continued)

Order/Family	Species name (Author) Common name	Genome size (Mb)	Fold coverage[a]	References/NCBI Genome link[b]
Hymenoptera/Braconidae	*Spathius agili* Yang Parasitoid wasp of emerald ash borer	148.0	96×	Kuhn et al. (2013)
Hymenoptera/Braconidae	*Spathius floridanus* (Ashmead) Parasitoid wasp of emerald ash borer	182.6	71×	Kuhn et al. (2013)
Hymenoptera/Braconidae	*Spathius galinae* Belokobylskij & Strazanac Parasitoid wasp of emerald ash borer	213.3	58×	Kuhn et al. (2013)
Hymenoptera/Braconidae	*Spathius ssp. (USA isolate)* Parasitoid wasp of emerald ash borer	147.7	96×	Kuhn et al. (2013)
Hymenoptera/Cephidae	*Cephus cinctus* (Norton) Wheat stem fly	162.2	56×	GCA_000341935.1
Hymenoptera/Encyrtidae	*Copidosoma floridanum* (Ashmead) Parasitic wasp	555×	139×	GCA_000648655.1
Hymenoptera/Formicidea	*Acromyrmex echinatior* (Forel) Panamanian leaf-cutter ant	313 295.9	123× 107×	Nygaard et al. (2011) GCA_000204515.1
Hymenoptera/Formicidea	*Atta cephalotes* (Weber) Leaf-cutter ant	290 317.7	18–20× 18–20×	Suen et al. (2011) GCA_000143395.2

Hymenoptera/Formicidea	*Camponotus floridanus* (Buckley)	240	100×	Bonasio et al. (2010)
	Florida carpenter ant			GCA_000147175.1
Hymenoptera/Formicidea	*Cerapachys biroi* (Forel)	214	124×	Oxley et al. (2014)
	Clonal raider ant	212.8	123×	GCA_000611835.1
Hymenoptera/Formicidea	*Harpegnathos saltator* (Jerdon)	330	100×	Bonasio et al. (2010)
	Jerdon's jumping ant	294.5	100×	GCA_000147195.1
Hymenoptera/Formicidea	*Linepithema humile* (Mayr)	215.6	23×	Smith et al. (2011a)
	Argentine ant	219.5	30×	GCA_000217595.1
Hymenoptera/Formicidae	*Monomorium pharaonis* (Linnaeus)	262.3	40×	GCA_000980195.2
	Pharaoh ant			
Hymenoptera/Formicidea	*Pogonomyrmex barbatus* (F.Smith)	235	10.5–12×	Smith et al.(2011b)
	Red harvester ant			GCA_000187915.1
Hymenoptera/Formicidea	*Solenopsis invicta* (Buren)	484.2	–	Wurm et al. (2011)
	Red imported fire ant	396	30×	GCA_000188075.1
Hymenoptera/Formicidae	*Vollenhovia emeryi* (Wheeler)	287.9	47×	GCA_000949405.1
	–			
Hymenoptera/Formicidae	*Wasmannia auropunctata* (Roger)	324.1	67×	GCA_000956235.1
	Little fire ant			
Hymenoptera/Megachilidae	*Megachile rotundata* (Fabricius)	272.6	300×	GCF_000220905.1
	Alfalfa leaf cutter bee			

(continued)

Table 1 (continued)

Order/Family	Species name (Author) Common name	Genome size (Mb)	Fold coverage[a]	References/NCBI Genome link[b]
Hymenoptera/Orussidae	*Orussus abietinus* (Fabricius)	201.2	467.1	GCA_000612105.1
	Parasitic wood wasp			
Hymenoptera/Pteromalidae	*Nasonia giraulti*	283.6	10–12×	Werren et al. (2010)
	Parasitoid wasp			GCA_000004775.1
Hymenoptera/Pteromalidae	*Nasonia longicornis*	285.7	12×	Werren et al. (2010)
	Jewel wasp (parasitoid wasp)		10×	GCA_000004795.1
Hymenoptera/Pteromalidae	*Nasonia vitripennis*	239.8	6×	Werren et al. (2010)
	Parasitoid wasp	295.8	6×	GCA_000002325.2
Hymenoptera/Tenthredinidae	*Athalia rosae* (Linneaus)	163.8	467.2×	GCA_000344095.1
	Turnip sawfly			
Hymenoptera/Trichogrammatidae	*Trichogramma pretiosum* (Riley)	196.2	232.7×	GCA_000599845.1
	Parasitoid wasp			
Isoptera/Termopsidae	*Zootermopsis nevadensis* (*Hagan*)	485	98×	GCA_000696155.1
	Dampwood termite			
Geophilomorpha/Linotaeniidae	*Strigamia maritime* (Leach)	290	–	Chipman et al. (2014)
	European centipede	176	23×	GCA_000239455.1
Lepidoptera/Bombycidae	*Bombyx mori* (Linnaeus)[e]	432	8.5×	The International Silkworm Genome (2008)
	Silk worm	397	Full	GCA_000151715.1

Lepidoptera/Crambidae	*Chilo suppressalis* (Walker)	824	–	Yin et al. (2014)
	Asiatic rice borer (Striped rice stemborer)	314.2	22.8×	GCA_000636095.1
Lepidoptera/Noctuidae	*Spodoptera frugiperda* (J.E. Smith)	358.0	61.3×	Kakumani et al. (2014)
	Fall armyworm			GCA_000753635.2
Lepidoptera/Nymphalidae	*Danaus plexippus* (Linnaeus)	273	74.7×	Zhan et al. (2011)
	Monarch Butterfly			GCA_000235995.1
Lepidoptera/Nymphalidae	*Heliconius melpomene melpomene* (Linnaeus)	269	38×	Consortium (2012)
	Postman butterfly	273.8	–	GCA_000313835.2
Lepidoptera/Nymphalidae	*Melitaea cinxia* (Linnaeus)	393	95×	Ahola et al. (2014)
	Glanville fritillary	389.9	70×	GCA_000716385.1
Lepidoptera/Papilionidae	*Papilio glaucus* (Linnaeus)	376	–	Cong et al. (2015)
	Eastern tiger swallowtail butterfly	374.8	72×	GCA_000931545.1
Lepidoptera/Papilionidae	*Papilio polytes* (Linnaeus)	227	595×	Nishikawa et al. (2015)
	Common Mormon swallowtail butterfly			GCA_000836215.1
Lepidoptera/Papilionidae	*Papilio xuthus* (Linnaeus)	243.9	300×	Nishikawa et al. (2015)
	Asian swallowtail butterfly			GCA_000836235.1
Lepidoptera/Plutellidae	*Plutella xylostella* (Linnaeus)	393.5	141×	You et al. (2013)
	Diamond back moth			GCA_000330985.1
Lepidoptera/Sphingidae	*Manduca sexta* (Linnaeus)	419.4	80.7×	GCA_000262585.1
	Tobacco hornworm			

(continued)

Table 1 (continued)

Order/Family	Species name (Author) Common name	Genome size (Mb)	Fold coverage[a]	References/NCBI Genome link[b]
Orthoptera/Acrididae	*Locusta migratoria* (Linnaeus)	6500	114×	Wang et al. (2014)
	Migratory Locust	5759.8	114×	GCA_000516895.1
Thysanoptera/Thripidae	*Frankliniella occidentalis* (Pergande)	415.7	158.7×	GCA_000697945.1
	Western flower thrips			

The annotations and assemblages of the arthropod genomes are continuously being refined and genome sizes are therefore in flux. Current status is best assessed at http://www.ncbi.nlm.nih.gov/genome.

[a]Genome coverage values for *So. invicta* and *St. maritime* are not available and indicated with a dash.

[b]The genome size and coverage reported is based on data in the published manuscripts and/or the NCBI GCA or GCF assembly links at http://www.ncbi.nlm.nih.gov/assembly. When genome paper and NCBI genome sizes and coverage are the same a single entry is provided.

[c]Incomplete genome representation.

[d]There are two genome annotations for *D*. ponderosae.

[e]There are four genome sequence assemblages for *B. mori*. The most recent are displayed.

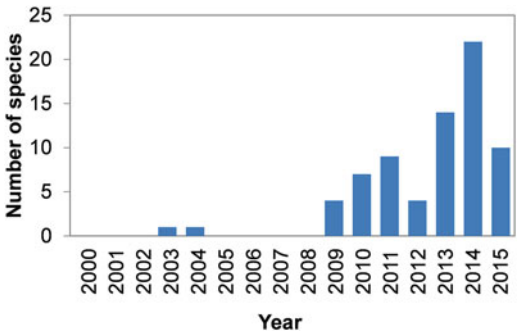

Fig. 1 Rapid increases in genome assemblies for plant-associated arachnids and insects. The *Drosophilia melanogaster* genome was released in 2000. The NCBI recorded initiation date for insect genome assemblies are indicated

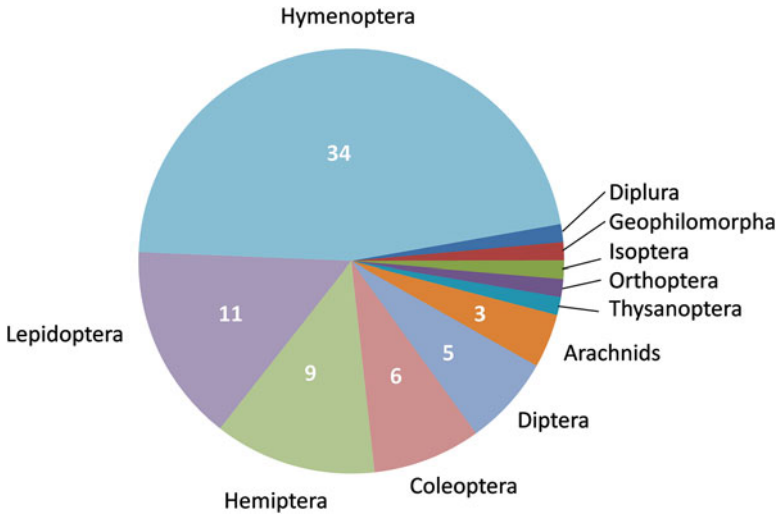

Fig. 2 Number of arthropod genome sequences with relevance to agriculture, ecosystems and forestry. Sectors without a numeral represent single genomes

from 359 to 997 kb (Elsik et al. 2014). In addition, the new *A. mellifera* genome assembly was enabled by the addition of robust transcriptome data from seven honeybee tissues and two related bee species genomes – the dwarf honey bee (*A. florea*) and the buff-tailed honeybee (*Bombus terretris*). These data increased the number of predicted protein-coding genes by nearly 34 % (10,157–15,314 genes) and increased the number of genes with orthologous sequences in other insect species. One-sixth of the new genes were identified due to the new genome assembly and the remaining new genes were revealed due to the deep-sequencing of the *A. mellifera* transcriptome.

As more complete genome sequences of agriculturally and ecologically relevant herbivores become available, transformative discoveries will flourish when re-sequencing of individuals in insect populations, species complexes, or highly related species is realized. Re-sequencing-assisted genetics of organisms is an essential

tool for understanding genotypic variation in organisms where genetic analyses are difficult to perform. A compelling example is provided by the impacts of re-sequencing of *Anopheles gambiae* and related mosquito species genomes. These initiatives have helped resolve the *An. gambiae* species complex and identified the traits that enhance vectorial capacity, as well as the process of how these traits are acquired (Fontaine et al. 2015; Neafsey et al. 2015). *An. gambiae* is a complex of morphologically indistinguishable and closely related sibling species, a subset of which transmit the malaria parasite *Plasmodium falciparum* to humans (Davidson 1964; White et al. 2011). Besides *An. gambiae*, closely related mosquitoes, also known as anopheline mosquitos, also transmit *P. falciparum* (Manguin et al. 2008). Sequencing 16 species of anophelines from different geographic locations and with varying capacities to vector the malaria parasite indicate that the ability to transmit *P. falciparum* did not arise from a common ancestor but rather from exchange of genes through interspecific mating (Neafsey et al. 2015).

Similar approaches can be used to resolve species complexes and biotype differences for plant-associated herbivores and identify critical genes underlying important pest-related traits. Of importance to world-wide agriculture is resolving the whitefly cryptic species complex of *Bemisa tabaci* (Liu et al. 2012; Tay et al. 2012). *B. tabaci* is considering one of the top 100 damaging pests of crops in the field and greenhouses worldwide (http://www.issg.org/database). In fact, *B. tabaci* is considered a regulated species by five continents including North America (the USA), Australia, Africa, China, and Europe (the European Union). Mitochondrial cytochrome oxidase I gene (mtCOI) polymorphisms and crosses between *B. tabaci* from around the world have identified over 34 distinct *B. tabaci* genetic groups that are likely to represent distinct *B. tabaci* species (Boykin 2014). Whitefly genome sequences are needed to understand the evolution and current status of this crop-damaging species complex that continues to sweep the world. Quite surprisingly, a *B. tabaci* genome has yet to be released and advances in whitefly biology and evolution have been accordingly hindered. The reported size of the *B. tabaci* haploid genome measured by flow cytometry varies from 640 to 682 Mbp (Guo et al. 2015) to 1029 Mbp (Brown et al. 2005); however, both studies indicate that whitefly genome is likely to be large, but well within the range of known Hemipteran genomes (Table 1).

Genome resequencing for most insects is in its infancy but it has already impacted our understanding of fundamental aspects of insect biology, such as insect migration and wing color. Using the reference genome of *Danaus plexippus* (the Monarch butterfly) (Zhan et al. 2011) and resequencing 101 additional *Danaus* genomes from around the world, the genetics of butterfly migration and warning coloration has been revealed (Zhan et al. 2014). Over 32 million single-nucleotide polymorphisms (SNPs) derived from *D. plexippus* populations from Central/South America, North America, Europe, and the Pacific that vary in their migratory abilities were examined. Zhan et al. (2014) showed that the North American *D. plexippus* population is the basal lineage and the Central/South American, European and Pacific populations are all distinct, independent lineages that have emanated from North America. Furthermore, they identified a genomic interval that implicated two genes with

migration; these genes, with likely roles in muscle function, encoded a component of the basement membrane (collagen type IV subunit α-1) and a flight muscle protein (kettin). Finally, using populations segregating for warning coloration, a single gene strongly associated with the bright wing coloration that warns predators of the monarch butterfly's toxicity was identified (Zhan et al. 2014).

The utility of genome re-sequencing with insects with tractable genetic systems is almost limitless. Rapid advances in understanding the function and evolution of parasitoids were made with the sequencing of three closely related *Nasonia* genomes (*N. vitripennis*, *N. giraulti*, and *N. longicornis*) (Werren et al. 2010). Furthermore, with the genetically amenable agricultural pests, the Hessian fly (*Mayetiola destructor*) and the Asian rice gall midge (*Orseolia oryzae*), advances toward identifying key traits that determine plant host-resistance responses have been made (Biradar et al. 2004; Lobo et al. 2006). For example, the first insect avirulence effector gene (*Avr13*) that mediates gene-for-gene interactions with wheat (*Triticum* spp.) expressing the *H13* resistance gene was recently discovered (Aggarwal et al. 2014). Although resistance to a number of biotypes of both *M. destructor* and *O. oryzae* have been identified in their respective plant hosts, wheat and rice, resistance-breaking biotypes of both insects exist (Bentur et al. 2008; Hao et al. 2013; Harris et al. 2003, 2006; Lakshmi et al. 2006; Rider et al. 2002). The genome sequence of *M. destructor* is currently publically available and a manuscript describing this genome is scheduled to be released soon. Availability of *M. destructor* and *O. oryzae* genomes and re-sequencing of members of these species' biotypes should begin to reveal the dynamic and rapid mechanisms used by these pests to adapt to resistant plant genotypes.

2.3 *Learning About Symbiosis Through the Genomes of Endosymbionts*

In addition to the accelerated delivery of genomes from plant-feeding/pollinating insects and their parasitoids and predators (Fig. 1, Table 1), there have been substantive advances in establishing the genomes of insect symbionts and more recently the microbiota of insect guts (Douglas 2013; Engel and Moran 2013; Jing et al. 2014). The ability to define the gene complements of the insect, its primary and secondary endosymbionts, and gut microbe genomes within each insect has provided an unprecedented research momentum that is illuminating the cross-kingdom metabolic integration of insect-bacterial mutualisms. In the hemiptera, many endosymbionts have nutritional roles by synthesizing essential amino acids and vitamins, carotenoids, or purines that cannot be made by their insect hosts and are lacking in their hosts' diets (Hansen and Moran 2014). In addition, endosymbionts impact insect fitness influencing insect host fecundity, viability, tolerance of temperature extremes, and resistance to pathogens and parasitoid wasps, as well as insecticides (Ghanim and Kontsedalov 2009; Himler et al. 2011; Montllor et al. 2002; Nachappa

et al. 2012; Oliver et al. 2003, 2005, 2006, 2014; Schmid et al. 2012; Su et al. 2013; Vorburger et al. 2010). Moreover, endosymbionts also influence insect perception by host plants (Chaudhary et al. 2014; Elzinga et al. 2014).

Obligate intracellular bacteria, such as insect endosymbionts, undergo dramatic reductions in genome size and gene content (McCutcheon and Moran 2010; Moran and Bennett 2014). Several insect endosymbiont genomes have incurred severe size contractions including the loss of genes presumed to be essential for viability of the symbiont and for meeting the nutritional needs of its insect host. For example, the genomes of the psyllid's γ-proteobacterial endosymbiont *Candidatus* Carsonella ruddii are minute (158–176 kb) and lack genes essential for DNA replication, transcription, and translation, as well as having incomplete gene complements for essential amino acid biosynthesis (Nakabachi et al. 2006; Sloan and Moran 2012). To compensate for gene loss in their primary endosymbiont genomes, several unique solutions have been deployed to assure endosymbiont and insect survival.

These mechanisms for survival have been revealed by deep sequencing of insect host and endosymbionts genomes. In some instances, hemipteran hosts have compensated for the incomplete metabolic capacity of its symbionts by expressing the "missing" metabolic genes in the insect cells (bacteriocytes) that harbor the endosymbionts (Hansen and Moran 2011). This provides an essential metabolic partnership between the insect and its symbiont. In some relationships, the insect host appears to have acquired these critical metabolic functions due to transfer of its endosymbiont gene(s) to the insect genome (Sloan et al. 2014). Alternatively, these metabolic genes have been acquired via horizontal gene transfer from other microbial sources (Husnik et al. 2013). Another strategy used in hemipteran-symbiont relationships is to have two endosymbionts committed to reconstructing a functional metabolic pathway (Wu et al. 2006).

3 Transcriptomics of Herbivores and Their Primary Endosymbionts

While the genome sequences of agricultural important insects are limited, transcriptomes of this group of insects are being sequenced in ever growing numbers. For example, the NCBI Sequence Read Archive (SRA), that stores raw sequences from high-throughput sequencing platforms, contained sequences of 369 different hempiteran insects. Not surprisingly, the largest number of entries in this database is for the pea aphid, the only hemipteran insect with a published genome sequence (Aphid Consortium 2010). As of December 2014, 166 pea aphid sequence entries were available and multiple entries for 35 additional hemipteran species. These numbers are expected to grow fast with the availability of barcoding for multiplexing samples in a single cell of a high-throughput instruments, and as scientists adapt to the use of high-throughput sequencing platforms for studies addressing differential

transcriptome profiling, transcript structure (e.g., alternative splicing), and allelic variations (e.g., single nucleotide polymorphisms, SNPs).

3.1 Sequencing is Easy, Assembly is the Challenge

Generating libraries for transcriptome profiling using high-throughput sequencing platforms has become a routine and protocols for preparing samples from single cells are easily accessible (Picelli et al. 2013). However, a challenge still remains regarding transcriptome assembly even for those organisms with fully sequenced genomes. Most popular current transcript sequencing platforms, such as RNA-Seq, typically generate short reads. The assembly of short reads and the quality of the reference genome enhances accurate transcriptome assembly. Gene annotation and transcriptome assembly is further complicated by existence of alternative splice transcript forms and due large gene families that complicate accurate transcript association with specific gene family members.

Assembly of transcriptomes of organisms with no or partial sequenced genomes is even more challenging and requires *de novo* sequence assembly and/or mapping of assembled transcripts to a divergent sister clade (Bao et al. 2013; Vijay et al. 2013; Xie et al. 2014). Therefore, in spite of accumulation of agriculture-related insect transcriptome sequences in the SRA database, future research with enhanced assembly tools and sequencing of their genomes will require reassembly of these transcriptomes to recover more accurate and full-length transcripts.

3.2 Knowledge Gained from Integration of the Genome and Transcriptome of the Endosymbiont Buchnera aphidicola

As full genome sequences of herbivores are being developed, the genome and transcriptome sequence of the aphid obligate endosymbiont *Buchnera* has already made great impact in our understanding the biology and the interaction of this endosymbiont with its host (Hansen and Moran 2014; Moran and Degnan 2006; Shigenobu et al. 2000). *Buchnera* is a γ-proteobacterium housed in aphid-derived bacteriocytes in the insect haemocoel, which is transmitted maternally. *Buchnera* provides the aphid essential amino acids, which are poorly represented in the plant sap. Similar to other endosymbionts, *Buchnera* also has a small genome (420–650 kb) and lacks genes related to regulatory functions, including transcription factors suggesting the *Buchnera* genes are not regulated at the level of transcription (McCutcheon and Moran 2012; Moran and Degnan 2006). Consistent with this is the finding that the *Buchnera* genome is transcriptionally static. Although modest fluxes in *Buchnera* RNAs have been reported during aphid development, exposing aphids to various

treatments have shown no or limited gene expression changes in this endosymbiont (Bermingham et al. 2009; Hansen and Degnan 2014). For example, near lethal heat-shock treatment do not cause increases in heat-shock gene RNAs; instead, heat-shock genes are constitutively over-expressed in *Buchnera* compared to other bacteria such as *E. coli* (Wilcox et al. 2003). Similarly, almost no changes in *Buchnera* transcriptome were detected when amino acid levels were altered in plants or aphids (Moran et al. 2005). Collectively, this information suggests that post-transcriptional, translational or post-translational controls are active in modulating *Buchnera* gene expression.

Recently, Hansen and Degnan (2014) provided a series of elegant experiments that presented evidence for post-transcriptional gene regulation by small RNAs in *Buchnera*. When *Buchnera* proteins from aphid embryos or from maternal bacteriocytes were compared, 22.5 % of the *Buchnera* proteins were differentially expressed. However, these RNAs were present at similar levels in these samples. As small RNAs are key post-transcriptional regulators, as well as transcriptional regulators, in microbes (Waters and Storz 2009), Hansen and Degnan (2014) characterized the *Buchnera* small RNA populations. Numerous small RNAs were identified and were conserved among *Buchnera* derived from four different aphid species. Importantly, small RNAs targeted untranslated regions in genes encoding differentially regulated proteins in embryos and maternal bacteriocytes. These data implicated small RNAs as key post-transcription regulators of *Buchnera* gene expression, likely acting at the level of translation since few changes were seen at the transcript level.

4 Proteomics of Herbivores

Holistic approaches to understanding changes in an organism's RNAs (via microarrays or next-generation RNA-seq) and proteins (via liquid chromatography tandem mass spectrometry [LC-MS/MS]) have gained prominence in recent years. Although proteome analysis is less sensitive than transcriptome analysis, proteomics data can be used to interpret the outcome of transcriptome. These functional genomics approaches are complementary and shed light on the orchestration of the transcriptional, post-transcriptional, translational, and post-translational processes that control plant responses to herbivory and herbivore responses to a host plant's arsenal of defenses, as well as to abiotic stresses (Hayward 2014). The field of agriculture-insect proteomics is just emerging; it has been hindered by the small number of well-annotated insect genomes and therefore the number of proteins identified are often limited. Therefore, it is not surprising, given the availability of the pea aphid genome, that the proteomics of the pea aphid is most advanced.

4.1 Proteome Analysis of Aphids

Proteome analysis of the pea aphid has resulted in the most comprehensive profile of proteins in hemipteran insects including its symbiont *Buchnera* (Hansen and Degnan 2014; Poliakov et al. 2011). The availability of a substantive number of insect genomes combined with the pea aphid genome has provided a platform for proteome analysis of additional aphid species. Homology-based proteomics analysis has been successfully used to identify aphid proteins, aphid proteins associated with virus transmission, as well as salivary secretomes of a number of different aphid species (see below) (Cilia et al. 2011a, b; Francis et al. 2006).

The literature indicates the importance of combining different approaches addressing protein-related work. For example, comparative proteome analysis of the aphid-derived bacteriocyte and the *Buchnera* endosymbiont showed no transfer of proteins between these specialized insect cells and the symbiont (Poliakov et al. 2011). This discovery indicated that in spite of the intimate biochemical relations of *Buchnera* with its insect host, this endosymbiont has more independence from its host than plant organelles such as mitochondria and plastid, where the transfer of nuclear-encoded proteins is needed for their function (Poliakov et al. 2011). However, in a recent study, Nakabachi et al. (2014) challenge this theory. Nakabachi et al. (2014) identified an aphid bacteriocyte protein RlpA4 in *Buchnera* cells. Interestingly, the aphid acquired the *RlpA4* gene via a lateral transfer from a bacterium other than *Buchnera* (Nikoh and Nakabachi 2009). These data underscore the limited sensitivity of proteomics technology and the importance of integrating different experimental approaches. With the new generation of mass spectrometers with faster scanning speeds, better mass resolution, and better mass accuracy and sensitivity, as well as a suite of new tools for qualitative and quantitative analyses (Michalski et al. 2012), new insights into the extent of protein exchange between bacteriocytes and symbionts may be revealed.

4.2 Combining Transcriptome and Proteome Analyses to Identify Aphid Effectors

One of the major research contributions of the past 5 years is the discovery of the repertoire of proteins that comprise herbivore secretomes and deciphering their roles in modulating plant defense responses (Elzinga et al. 2014; Hogenhout and Bos 2011). Transcriptome analysis of pea aphid salivary glands identified the first aphid virulence factor or effector, C002, which is secreted into the plant host tissues and is important for aphid survival and host colonization (Mutti et al. 2006, 2008; Pitino et al. 2011). Salivary gland transcriptomes of the green peach aphid (*Myzus persicae*) and potato aphid (*Macrosiphum euphorbiae*) identified additional components of the aphid secretome (Atamian et al. 2013; Bos et al. 2010; Ramsey et al. 2007); when tested for function in plant-aphid interactions, several proteins were

elicitors that stimulate plant defenses, while other are effectors that suppress host plant defenses (Atamian et al. 2013; Bos et al. 2010; Elzinga et al. 2014; Pitino and Hogenhout 2013; Rodriguez et al. 2014). Furthermore, aphid effectors, similar to phytopathogenic microbial effectors, are under positive selection to promote hemipteran virulence (Atamian et al. 2013; Carolan et al. 2011; Cui et al. 2012; Pitino and Hogenhout 2013).

Proteome analysis of the pea aphid salivary glands and saliva from a number of aphid species provide further evidence for the secretion of these proteins and underscore the complexity of the saliva composition (Carolan et al. 2009, 2011; Chaudhary et al. 2014, 2015; Cooper et al. 2010, 2011; Harmel et al. 2008; Nicholson et al. 2012; Nicholson and Puterka 2014; Rao et al. 2013; Vandermoten et al. 2014). With the exception of the pea aphid, these proteomics studies were performed with aphids that lack a genome sequence. Therefore, the identity of proteins was determined by peptide match searches to protein (or translated) databases. However, if the peptide derives from a region of high sequence divergence, a match is not found, resulting in a limited number of proteins being reliably identified. Six key findings from these saliva protein studies have been revealed. First, aphid species differ in their ability to salivate in artificial diets. Second, the composition of the saliva varies among aphids with different host ranges. Third, salivary proteins have a plethora of functions. Fourth, collecting saliva from large numbers of insects significantly increases the complexity of the salivary proteome. Fifth, many salivary proteins do not have canonical secretion signals. Finally, aphid saliva contains proteins from its primary endosymbionts. The identification of *Buchnera* proteins in the aphid saliva and the demonstration of a direct role for these microbes in plant-aphid interactions further highlights the role of endosymbiont in plant-herbivore interactions.

Recently, the power of coupling hemipteran proteome and transcriptome data was demonstrated. Enabled by a potato aphid transcriptome, a comprehensive analysis of the salivary proteome of this aphid was achieved (Chaudhary et al. 2014, 2015). Collecting saliva from over 100,000 potato aphids, 105 salivary proteins were identified. Comparison of the potato aphid secretome with the available secretomes from the pea aphid, the grain aphids, vetch aphid, and green peach aphid indicates that only a fraction of the potato aphid salivary proteins have been reported in other aphid species (Fig. 3). These studies indicate that there is still much to be learned regarding the composition and functions of proteins secreted by aphids (Chaudhary et al. 2014, 2015). Of the salivary proteins with functions inferred by homology, only a few correspond to effectors that are deployed by microbial plant pathogens (Carolan et al. 2011; Chaudhary et al. 2015). This is likely to reflect the fact that novel effectors are used in plant-aphid interactions. A large number of potato aphid salivary proteins have homologs in the pea aphid genome indicating conserved functions. Furthermore, many of these proteins are annotated as unknowns suggesting that there is still much to be learned about the mechanisms of salivary protein action *in planta*.

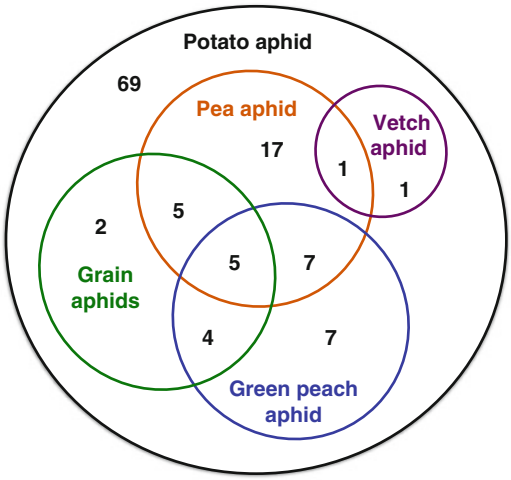

Fig. 3 Potato aphid (*Macrosiphum euphorbiae*) liquid salivary proteins and their presence in liquid saliva or in salivary gland transcriptome or proteome from pea aphid (*Acyrthosiphum pisum*), green peach aphid (*Myzus persicae*), grain feeding aphids (*Diuraphis noxia*, *Sitobion avena*, *Metapolophium dirhodum*, *Schizaphis graminum*) and the vetch aphid (*Megoura viciae*)

5 Dynamic Metabolomes

Metabolites are the end products of gene regulation. Their levels are controlled by changes in gene transcription, RNA and protein accumulation, and enzymatic activities. The literature is replete with targeted metabolite studies that have identified insect and plant natural products that mediate plant-insect and insect-insect interactions, as well as interactions of these organisms with their environment. While the value of one molecule at a time approaches remain, current MS technologies allow a new era of investigations by quantifying large numbers of diverse molecules simultaneously. The dissection of metabolite fluxes and their chemical diversity in response to injury and herbivory are now feasible, allowing investigators to assess the correlation of a multitude of plant and insect chemicals with phenotypes.

Holistic metabolomics approaches are a challenge, despite recent advances in analytical methods for resolving and identifying metabolites [eg., nuclear magnetic resonance (NMR) and MS] (Gaquerel et al. 2014; Wolfender et al. 2013). For example, today's mass spectrometers have increased mass resolution and accuracy; in addition, due to their greater ease of operation and maintenance, MS analysis of phytochemicals is now more accessible to the broader scientific community. When conjoined with the powers of multivariate data analysis approaches, fluxes in plant or insect metabolites can be successfully determined by metabolic profiling or fingerprinting (Wolfender et al. 2013). Metabolic profiling is a targeted metabolomics approach that focuses on a set of chemicals associated with a known metabolic pathway. In contrast, metabolite fingerprinting is an exploratory endeavor and novel chemical signatures associated with herbivory or other stresses can be identified. The metabolomics (aka., metabonomics) analyses can occur in the absence of a sequenced genome and used in conjunction with transcriptomics and/or proteomics, metabolomics provides an important and complementary functional genomics tool

allowing critical biochemical networks and the underlying genes to be revealed (Yonekura-Sakakibara et al. 2013).

5.1 Entomo-metabolomics

Substantive advances have been made in understanding the chemistry, function and perception of insect pheromones and fluxes in hormones that control insect behaviors and developmental transitions using targeted metabolite studies (Witzgall et al. 2010). However to date, the number metabolite profiling or fingerprinting studies in insects is relatively small. As might be anticipated, numerous reports of metabolic profiling/fingerprinting of *Drosophilia* spp. exist; this includes studies across development, as well as in response to temperature stress, hypoxia, and inbreeding (Chintapalli et al. 2013; Coquin et al. 2008; Hariharan et al. 2014; Kostal et al. 2011). In contrast, metabolomic studies for plant-consuming insects are emerging and have yet to provide the in-depth knowledge achieved in the model species *Drosophilia*. It is clear entomo-metabolomics is on a rise and studies integrating multiple OMICs technologies are beginning to appear.

Metabolomics has been used to characterize of chemical composition of haemolymph from aphids, planthoppers, the desert locust, and parasitized honeybees (Aliferis et al. 2012; Lenz et al. 2001; Moriwaki et al. 2003a, b), from abdominal secretions of grasshoppers (Buszewska-Forajta et al. 2014a, b, 2015), and defense secretions (venoms) of walking sticks (Zhang et al. 2007). In addition, metabolites correlated with larval development in the tobacco hornworm (Phalaraksh et al. 2008), diapause in an aphid parasitoid (*Praon volucre*) and the cotton bollworm (Colinet et al. 2012; Zhang et al. 2012), behavioral transitions (group vs solitary phases) in locusts (Wu et al. 2012), cold acclimation and cold tolerance in parasitoid wasps (Foray et al. 2013), and hypoxia and heat stress in stonefly nymphs (*Dinocras cephalotes*) (Verberk et al. 2013) have been determined.

To date a small number of herbivore metabolomics studies have integrated two or more OMICs strategies (Derecka et al. 2013; Wang et al. 2010; Zhang et al. 2012). For example, Zhang et al (2012) correlated the proteome and metabolome of the cotton bollworm larval brain to garner insights into the molecular events of diapause. Larvae destined for diapause (reared under short-day conditions) have two characteristic metabolic shifts relative to non-diapause destined larvae (reared under long-day conditions). In the diapause preparation phase, diapause-destined insects have a photoperiod-associated increase in many neurotransmitters and upon entry into diapause, a metabolic shift towards energy storage is observed.

A multi-OMICs strategy was taken to understand the impact of low levels of neonicotinoid pesticides that enter a honeybee hive, and therefore food chain, via contaminated nectar and pollen carried by foraging worker bees (Derecka et al. 2013). A global transcriptome and small RNA profiles, as determined by RNA-seq, showed that pesticide-exposed worker bees had changes in the levels of 15 microRNAs and over 300 genes. Up-regulated genes were enriched for proteins critical for

xenobiotic detoxification (P450s) and down-regulated genes implicated a change in lipid biosynthesis. Lipid profiling confirmed the RNA-seq data with 15 % of the lipid metabolites being different in the pesticide-exposed vs control insects (Derecka et al. 2013). These data have strong implications regarding residual insecticides and honeybee colony health.

Finally, proteome and metabolome characterization of aphids have revealed the biochemical nature of the *Buchnera*-aphid mutualisms (Chaston and Douglas 2012; Wang et al. 2010). For example, comparisons of pea aphids with their primary symbiont *Buchnera* and cured of *Buchnera* (by antibiotic treatments) were compared, as well as pea aphids with *Buchnera* fed on low or high amino acid diets. Over 238 proteins and 15 metabolites were changed relative to controls. The responses to the two treatments – limited supply of endogenous amino acids (pea aphid cured of *Buchnera*) and exogenous amino acids (low N diets) – were surprisingly distinct. These studies demonstrated the complexities of the nutritional needs of the pea aphid and provided further evidence for the critical role of *Buchnera* for aphid survival and amino acid biosynthesis.

5.2 The Role of Plant Metabolism During Herbivory

While entomo-metabolomics is in its infancy, there is a robust literature on host-plant metabolism based initially on targeted metabolite characterization, which has now transitioned to larger scale explorations into the metabolic changes *in planta*. Plants quantitatively and qualitatively alter both primary and secondary metabolism in response to injury and attack by pests and pathogens. This strategy redirects C and N resources from growth and development to the synthesis of a plant's chemical defense arsenal, resulting in protection from the attacker (Attaran et al. 2014; Jander 2014; Kliebenstein 2014). While there are short-term fitness costs associated with these metabolic transitions, these low molecular-mass molecules promote wound healing after injury and provide an induced resistance to limit further damage and attract natural enemies (Vos et al. 2013). Long term, these costly metabolic investments enhance plant survival and reproductive success (Bennett and Wallsgrove 1994; Gulati et al. 2013). It is estimated that the plant kingdom produces over 200,000 distinct metabolites, of which a small fraction is characterized (Bino et al. 2004; Wink 2003). Furthermore, a single plant species may produce 15,000 phytochemicals from primary and secondary metabolic pathways.

The primary metabolites are essential to sustain plant growth, development, reproduction, and/or viability are largely conserved across the kingdoms of life (Caspi et al. 2014). Herbivory and pathogen attack cause fluxes in primary metabolites including: amino acids for protein synthesis, non-protein amino acids, lipids, carbohydrates, and pigments (Attaran et al. 2014; Steinbrenner et al. 2011). In addition, plant hormones, which are critical for plant growth and development, play key roles during pest and pathogen attack. They are key modulators of signaling pathways that control the traits conferring basal resistance, induced resistance and

gene-for-gene resistance and have been reviewed extensively (Pieterse et al. 2012; Robert-Seilaniantz et al. 2011; Verhage et al. 2011). Targeted profiling allows quantification of many of these key regulators simultaneously (Schmelz et al. 2009).

While there is a core of natural products shared amongst most plants, some secondary metabolites are genus or species specific (Wink 2003). Not surprisingly, secondary metabolites are not essential for plant growth, development or reproduction; however, these molecules are often crucial for the survival and reproductive success of plants in stressful environments (Bennett and Wallsgrove 1994; Wink 2003). In conjunction with structural barriers, a plant's robust arsenal of secondary metabolites prevents colonization by most insects. Only the insects that have found adaptive mechanisms (i.e., chemical sequestration, detoxification or down-regulation of secondary metabolite pathways) are sufficiently protected from the biological ramifications of a host plant's secondary metabolites and are able to colonize and successfully reproduce on their host (Kaloshian and Walling 2015). The diversity of plants natural products has largely evolved due to this continual pressure by pests and pathogens. This drives the evolution and recruitment of enzymes to modify the core structures of defense metabolites in a unique manner to generate new, potent biological activities that interfere with insect and pathogen success. This evolution-based strategy for chemical diversity is clearly seen in all classes of key defense compounds: alkaloids, flavonoids, glucosinolates, phenolics, and terpenoids (Burow et al. 2010; Facchini 2001; Gershenzon and Dudareva 2007; Sumner et al. 2015).

Secondary metabolites are particularly important in plant defense to pests and pathogens by mediating plant-to-plant communication, attracting an herbivore's natural enemies, and interfering with herbivore feeding, fecundity, settling, growth, and development (Pierik et al. 2014). Over the past decade, scientists have made significant inroads into understanding a number of secondary metabolite pathways in crops and model plants. Leveraging the genetic and genomic tools of the model plant *Arabidopsis thaliana*, as well as the advances in computational analysis, a comprehensive systems biology approach to plant metabolic changes in response to pest and pathogen attack has been realized (Fukushima et al. 2014; Kliebenstein 2014; Roessner et al. 2002). In Arabidopsis, the primary focus has been on the aliphatic and indolic glucosinolates revealing: the entire glucosinolate biochemical pathway, key regulators of this pathway, the genetic variation in Arabidopsis ecotypes that drive the evolution of glucosinolate diversity, as well as fluxes in primary metabolism (Burow et al. 2010; Jensen et al. 2014; Kliebenstein 2012; Pentzold et al. 2014; Rowe et al. 2008; Wentzell et al. 2007).

Greater challenges are encountered with non-model organisms or orphan crops with limited genome sequences, transcriptomes, robust genetics, collections of mutants, or genomics tools. Correlated expression of genes and metabolites has provided significant insights into the genes associated with key secondary metabolite production in plants (Gaquerel et al. 2014; Saito et al. 2010; Wolfender et al. 2013; Yonekura-Sakakibara et al. 2013). For example, small dedicated microarrays were used to correlate the temporal changes in gene expression in response to spider mite (*Tetranychus urticae*) feeding or jasmonic acid treatments (a key regulator of

volatile production) (Kant et al. 2004; Mercke et al. 2004). In cucumber, this strategy led to the identification of cDNAs encoding (E,E)-α-farnesene and (E)-β-caryophyllene synthases (Mercke et al. 2004). Since that time, there have been numerous integrated studies (metabolite profiling/fingerprinting with transcriptomics and/or proteomics) that have revealed the dynamics and chemical complexity of secondary metabolites, as well as new the identification of new metabolites in well studied pathways, in non-model plants and crops (Dafoe et al. 2011; Gulati et al. 2013; Huffaker et al. 2011; Jansen et al. 2009; Kersten et al. 2013; Sugimoto et al. 2014).

Today, research is beginning to emerge that leverages the natural genetic diversity of plants, mutant collections, with one or more OMICs technologies. These strategic fusions have allowed previously unapproachable research questions in plant-pest interactions to be explored. In Sects. 6 and 7, we illustrate this integrative approach in two systems: maize and Arabidopsis.

6 Mining Natural Variation for Loci Controlling Resistance

In this section, we highlight advances in the genetic basis of aphid resistance and susceptibility in maize that was achieved by screening a genetically diverse collect of maize lines in conjunction with targeted metabolomics. Quantitative trait loci (QTLs) associated with resistance to chewing and stem-boring insects have been identified revealing untapped genetic resources in maize (McMullen et al. 2009a; Meihls et al. 2012; Smith and Clement 2012). However, the genes underlying these mechanisms of resistance are largely unexplored. Recently, a maize nested association mapping (NAM) population was made from a set of 25 genetically diverse maize lines that were crossed with the genome-sequenced, inbred line B73 to create over 5000 recombinant inbred lines (RILs) (Flint-Garcia et al. 2005; McMullen et al. 2009b). The NAM population was screened for susceptibility to the maize leaf aphid (*Rhopalosiphum maidis*) and has provided surprising new insights into benzoxazinoid metabolism (Meihls et al. 2013).

6.1 The Benzoxazinoids and Resistance to Herbivores

Benzoxazinoids are abundant in the Graminaceae (Niemeyer 1988, 2009). These secondary metabolites accumulate in a cultivar-, age- and organ-dependent manner (Cambier et al. 2000). In some lines, benzoxazinoids are constitutively expressed, while in others they are induced by biotic stress (Cambier et al. 2000; Meihls et al. 2013). Benzoxazinoids confer resistance to aphids, chewing insects, as well as pathogens, and are associated with allelopathy (Ahmad et al. 2011; Glauser et al. 2011; Niemeyer 2009). The many of the genes and enzymes required for the synthesis of benzoxazinoids from indole are well established, with seven enzymes

associated with the linear biosynthetic pathway for DIBOA and two additional enzymes for the conversion of DIBOA to DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) (Dutartre et al. 2012). The most abundant benzoxazinoid in maize is DIMBOA-glucoside (DIMBAO-Glc), which is converted to HDMBOA-Glc (2-hydroxy-4,7-dimethoxy-1,4- benzoxazin-3-one) by a methyltransferase (Oikawa et al. 2002) and to DIM2BOA-Glc (2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3-one glucoside) by an unknown enzyme. For chewing insects that cause tissue damage, these benzoxazinoid glucosides contact stored β-glucosidases after cell disruption to release the corresponding benzoxazinoid aglycones (DIMBOA, HDIMBOA, and DIM2BOA) (Meihls et al. 2013; Niemeyer 2009). Aglycone degradation products also accumulate and these chemicals are more potent insect deterrents than DIMBOA and HDIMBOA (Grambow et al. 1986). Additionally, since the production of HDIMBOA is insect induced and the non-enzymatic breakdown of HDIMBOA is more rapid than DIMBOA, high levels of HDIMBOA-Glc are correlated with enhanced chewing insect and pathogen resistance (Dafoe et al. 2011; Glauser et al. 2011; Maresh et al. 2006; Oikawa et al. 2004).

6.2 *New Insights into Benzoxazinoids Regulation in Aphid Resistance*

Correlated with the more limited tissue damage that is associated with phloem-feeding, aphids do not increase overall levels of benzoxazinoids (Ahmad et al. 2011; Cambier et al. 2001). However, levels of DIMBOA, DIMBOA-Glc and HDMBOA-Glc increase in the apoplast of aphid-infested plants, suggesting differences in benzoxazinoid localization (Ahmad et al. 2011). Since aphid mouthparts must transverse the apoplast while searching for a feeding site in the vasculature and aphids "taste" their surroundings, aphids likely come in contact with these deterrents. In addition, DIMBOA-Glc appears to be a component of the phloem sap, the primary site of aphid feeding; while the aglycone DIMBOA is absent (Caillaud and Niemeyer 1996; Givovich et al. 1992, 1994). It is not known if HDMBOA and HDMBOA-Glc are phloem-localized. Supplementation of benzoxazinoids in aphid-artificial diets shows that HDMBOA-Glc is more detrimental to aphid survival and reproduction than DIMBOA-Glc (Cambier et al. 2001; Meihls et al. 2013).

By assessing maize leaf aphid population growth on RILs from the NAM population, a single, dominant QTL located on chromosome 1 was correlated with aphid resistance/susceptibility and has provided new insights into benzoxazinoid regulation (Meihls et al. 2013). Analysis of RILs allowed the QTL to be rapidly mapped to a ~4-Mb region containing 31 genes, of which three genes were O-methyltransferases, which are known to be important in the conversion of DIMBOA-Glc to HDMBOA-Glc (Oikawa et al. 2002). One of these genes *Bx10c* (*Benzoxazinoneless 10c*) was disrupted by a transposon in the aphid-resistant lines

but remained functional in aphid-susceptible lines. Targeted metabolic profiling of benzoxazinoid showed high levels of DIMBOA-Glc and extremely low levels of HDMBOA-Glc in aphid-resistant lines. At first glance these data seem to conflict with the finding that *in vitro* HDMBOA-Glc is more toxic to aphids than DIMBOA-Glc (Cambier et al. 2001; Meihls et al. 2013). One of two possibilities explain this surprising finding. It is possible that aphids will have limited contact with HDMBOA-Glc, since it is not clear if HDMBOA is phloem localized. Alternatively, it appears that aphid resistance is correlated with DIMBOA's secondary role *in planta*. Similar to glucosinolates of the Brassicaeae (Clay et al. 2009), the benzoxazinoid DIMBOA activates MAMP (microbe-associated molecular pattern)-triggered immunity. Callose deposition is a hallmark of MAMP-triggered immunity (Zipfel 2014) and DIMBOA, but not HDMBOA-Glc, is required for callose deposition after aphid feeding (Ahmad et al. 2011). Consistent with this observation, there are fewer callose deposits in the aphid-susceptible plants with low DIMBOA-Glc and high HDMBOA-Glc levels (Meihls et al. 2013).

The Meihls et al. (2013) study clearly demonstrated the power of combining genetics and genomics with metabolomics. Not only did they demonstrate the critical importance of DIMBOA-induced defense responses in resistance to aphids, they identified one of the elusive genes in DIMBOA catabolism (*Bx10c*) (Meihls et al. 2013; Niemeyer 2009). Furthermore, Meihls et al. identified two additional DIMBOA-methyltransferase genes (*Bx10a* and *Bx10b*) and several other closely related methyltransferase-like homologs, which have unknown roles in benzoxazinoid catabolism. Finally, natural variation in DIM2BOA-Glc levels were also revealed in the NAM parental lines providing opportunities for identifying the genes controlling this trait.

7 Integrating Genomics, Genetics and Electrical Recordings: How Plants Perceive Electrical Signals

Long-distance signaling in plant-insect interactions is supported by a robust literature. However, in some cases the identity of the mobile signals and their hierarchical status within signaling networks have been controversial and difficult to uncover. Jasmonates are undisputed long-distance signals that are critical for plant resistance to herbivores (Browse 2009; Koo and Howe 2009), as plants that are unable to perceive or produce jasmonates are compromised in their resistance to herbivores (Howe and Jander 2008; Kombrink 2012). The biologically active jasmonic acid isoleucine conjugate (JA-Ile) and its precursor JA accumulate within minutes in an injured leaf, as well as in non-damaged leaves (Glauser et al. 2009). The importance of electrical and hydraulic signals as long-distance wound-associated signals was established over two decades ago (Davies and Schuster 1981; Stahlberg and Cosgrove 1992; Wildon et al. 1992). Until recently, the mechanism of electrical signal perception has remained elusive. By leveraging the powers of Arabidopsis

genetics, genomics and established electrical monitoring technologies, the transmission and perception of electrical signals generated by herbivory and tissue damage have been elucidated; furthermore, a new model for linking hydraulic signals to electrical signaling has emerged (Farmer et al. 2014; Maffei et al. 2007; Mousavi et al. 2013; Salvador-Recatala et al. 2014).

7.1 Unraveling Plant Perception of Electrical Signals Induced by Injury or Herbivory

When caterpillar feeding disrupts the integrity of cells, two of the earliest responses are changes in the plasma transmembrane potential (V_m) and modulation of ion fluxes across the plasma membrane (Ebel and Mithoefer 1998; Maffei et al. 2007). The plasma membrane depolarizations are rapidly propagated (1 cm min^{-1}) from the site of damage across the expanse of the wounded leaf (Maffei et al. 2004). In addition to cellular breaches, danger signals such as defense peptides and DAMPs (damage-associated molecular patterns) also cause plasma membrane depolarizations (Boller and Felix 2009; Krol et al. 2010). The herbivory-induced V_m changes are followed by calcium fluxes into the cytosol, ROS production, and subsequent changes in the levels of the defense hormones JA and salicylic acid to activate defense genes. The change in V_m also has a more immediate impact, as it is followed by a transient electrical signal (action potential) that propagates rapidly along plant cell surfaces traveling from the site of damage throughout the entire plant (Maffei et al. 2007).

Two recent papers have revealed mechanisms of the electrical signal perception after caterpillar feeding and wounding (Mousavi et al. 2013; Salvador-Recatala et al. 2014). Using non-invasive surface electrodes, Mousavi et al (2013) showed that *Spodoptera littoralis* (Egyptian cotton leafworm) feeding and mechanical wounding of Arabidopsis leaves generate electrical signals (wound-activated surface potentials, WASPs) that are rapidly propagated within the damaged leaf. The WASPs also rapidly spread into distal leaves with direct vascular interconnections (parastichies) and proximal leaves with contact parastichies (Dengler 2006; Mousavi et al. 2013). Within the wounded leaf, WASPs moved along the midrib at a rate of 9 cm min^{-1}, while the average signal speed from a wounded leaf to a distal leaf with a direct vascular connection was 5.8 cm min^{-1}. This rate of signal propagation is well correlated with the timing of JA accumulation and *JAZ10* (*JASMONATE-ZIM DOMAIN 10*) gene expression in distal leaves of Arabidopsis (Chauvin et al. 2013).

Using wild-type plants or plants that lack a functional jasmonate receptor (*coi1*; *coronatine insensitive 1*) and current injections into leaves via platinum electrodes, it was demonstrated that the mechanism of WASP generation is upstream or independent of jasmonate perception and independent of insect oral secretions (Mousavi et al. 2013). Furthermore, based on analyses of an NADPH oxidase mutant (*rbohD*, *respiratory burst oxidase homolog D*), WASP generation is independent of RbohD;

RbohD-dependent reactive oxygen species are known to influence V_m and travel at speeds similar to WASPs (Miller et al. 2009). Comparisons of transcriptomes from current-injected leaves, wounded leaves, and distal leaves after wounding showed that there is substantial overlap in the gene sets for these treatments. For example, 70 % of the Arabidopsis genes induced by current injection were also up-regulated in both local and distal leaves after wounding. Many wound-responsive genes, including nine of the 12 *JAZ* genes, which are regulators of jasmonate responses, were induced by current injection.

Ion fluxes, ion channels and pumps have been implicated in jasmonate signaling (Bonaventure et al. 2007; Bruex et al. 2008; Kang et al. 2006) and therefore these proteins are candidates for perception of the early long-distance signals. By mining the Arabidopsis genome the suite of ion channels and pumps were identified. Leveraging the Arabidopsis insertional mutant collection, homozygous mutants in these genes were identified and tested for their ability to transmit local and systemic WASPs. Genes tested included: glutamate receptor-like (GLR) proteins, putative cyclic nucleotide gated channels, chloride and potassium channels, calcium-binding proteins, and vacuolar and plasma membrane H^+-ATPases. Mutations in four *GLRs* (*glr3.1*, *glr3.2*, *glr3.3*, and *glr3.6*) altered the duration of WASPs in the wounded leaf and/or distal unwounded leaf. Furthermore, the double mutant *glr3.2 glr3.6* had reduced electrical activity and damped *JAZ10* RNA levels after current injection relative to wild-type plants.

The ability to leverage the deep genetic/genomics resources of Arabidopsis has unambiguously revealed the *GLR* genes involved in propagation of the electrical signals that promote JA biosynthesis and signaling. These GLRs encode putative ion channels. Intriguingly, these proteins are similar to the vertebrate ionotropic glutamate receptors that are involved in the fast excitatory synaptic events of neurons suggesting a conservation of function across kingdoms. It will be of interest to see if the mechanisms of WASP propagation remain conserved in other plant species.

7.2 Electrical Signaling via the Phloem and Xylem

Phloem sieve elements are also known to propagate electrical signals in response to biotic and abiotic stress (Rhodes et al. 1999; van Bel et al. 2014). To monitor electrical signaling events within the phloem after herbivory, Salvador-Recatala et al. (2014) used aphids (*Brevicoryne brassicae*; cabbage aphid) as live "electrodes". In these experiments, electrical penetration graphs (EPG) were used to demonstrate that the aphid was in phloem phase (e.g., consuming phloem sap or salivating into the phloem). At this time, *Pieris brassicae* (cabbage white butterfly) caterpillars were placed on the aphid-infested leaf or on a lower leaf with direct vascular interconnections to the distal leaf with the feeding aphid. Two types of membrane depolarization waves (fast and slow) were observed. Fast depolarization waves (2 s) were detected in both the damaged and distal leaves and were correlated with *JAZ10*

expression in distal leaves; while slow depolarization waves (14 s) were only detected in the distal leaves. The *glr3.2 glr3*.6 double mutant suppressed the depolarization waves in non-wounded distal leaves indicating that phloem propagates electrical signals throughout the plant in a GLR3.2 GLR3.6-dependent manner.

These phloem-propagated electrical signals appear to represent a portion of the total wound-activated electrical activity (Farmer et al. 2014; Rhodes et al. 1999; Salvador-Recatala et al. 2014; Wildon et al. 1992). Pressure changes within the xylem have also been implicated as generating a long-distance signal resulting in changes in membrane potential (Malone and Stankovic 1991; Stahlberg and Cosgrove 1997). A model integrating hydraulic pressure, electrical signaling, and jasmonate biosynthesis has recently been proposed (Farmer et al. 2014). Four of the Arabidopsis 13-lipoxygenase genes (*LOX2*, *LOX3*, *LOX4* and *LOX6*) contribute to JA biosynthesis in wounded leaves (Chauvin et al. 2013). However, only *LOX6* is required for the rapid systemic accumulation of JA and, interestingly, *LOX6* is expressed in cells in close association with the xylem cell wall (Chauvin et al. 2013). These cells may be "contact cells" that are hydrostatically linked to the xylem (Van Bel and Van Der Schoot 1988). Farmer's Squeeze Cell Hypothesis proposes that the changes in water column tension in xylem are transmitted upon tissue damage to contact cells and other cells adjacent to the xylem by physically squeezing these cells. The rapid pressure changes in the cells adjacent to the xylem are proposed to cause the propagated electrical signal.

8 Future Prospectives

While studies on some organisms have advanced into the post-genomics era, research on most agriculturally important plants and herbivores is just beginning to enter the OMICs phase of discovery. The current and emerging technologies have revolutionized the way we do science, accelerated discoveries, and allowed the plant and insect scientific communities to address questions that were previously not feasible. At the core of these discoveries are the rapidly growing number of insect and orphan crop genome sequences that have been advanced by multi-species genome initiatives. When complemented with robust transcriptomics, proteomics, and metabolomics approaches, the intricate multi-level relationships of arthropods with their host plants and resident microbes will emerge. Research is now moving from model organisms to less well-studied crops and their pests. These translational agriculture and ecosystem initiatives will be empowered by the foundations established in more tractable model systems, which provide roadmaps for discovery.

The OMICs strategies present myriad opportunities and, of course, present challenges. First, there is an acute need for our next generation of entomologists and plant biologists to have rigorous training in OMICs technologies, integrative scientific approaches, and, most importantly, the analysis of increasingly large data sets. Second, as the methods for analyzing big data sets are continuously emerging, it is critical that data sets be analyzed and re-analyzed with the improved computational

tools to assure that conclusions stand the test of time. Third, advances in plant-herbivore interactions will require reliable methods to develop transgenic insects and crops to quickly deploy reverse-genetic strategies to test hypotheses derived from OMICs research. Finally, pest interactions with plants are inherently more complex than pathogen interactions, as both the genotype of the pest and all of its resident microbes are likely to influence pest perception and plant susceptibility. In addition, we study animals that make active choices and display a wide variety of feeding behaviors that vary by feeding guild; the frequency and duration of these behaviors may cause significant variations in plant responses at the transcript, protein and metabolite levels. Therefore, as our community engages in integrative OMICs strategies, it may be advantageous to establish a strict set of guidelines for best practices for our field. It is likely that increased numbers of biological replications, strategies to "synchronize" insect behaviors, and defining standard protocols for controlled infestations for insects in the different feeding guilds will be essential for the community to extract meaningful data and enable comparisons. Leveraging these big data sets will be critical for rapid advances for the sustainability of agriculture and our ecosystems.

References

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A., Galle, R. F., George, R. A., Lewis, S. E., Richards, S., Ashburner, M., Henderson, S. N., Sutton, G. G., Wortman, J. R., Yandell, M. D., Zhang, Q., Chen, L. X., Brandon, R. C., Rogers, Y. H., Blazej, R. G., Champe, M., Pfeiffer, B. D., Wan, K. H., Doyle, C., Baxter, E. G., Helt, G., Nelson, C. R., Gabor, G. L., Abril, J. F., Agbayani, A., An, H. J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R. M., Basu, A., Baxendale, J., Bayraktaroglu, L., Beasley, E. M., Beeson, K. Y., Benos, P. V., Berman, B. P., Bhandari, D., Bolshakov, S., Borkova, D., Botchan, M. R., Bouck, J., Brokstein, P., Brottier, P., Burtis, K. C., Busam, D. A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J. M., Cawley, S., Dahlke, C., Davenport, L. B., Davies, P., de Pablos, B., Delcher, A., Deng, Z., Mays, A. D., Dew, I., Dietz, S. M., Dodson, K., Doup, L. E., Downes, M., Dugan-Rocha, S., Dunkov, B. C., Dunn, P., Durbin, K. J., Evangelista, C. C., Ferraz, C., Ferriera, S., Fleischmann, W., Fosler, C., Gabrielian, A. E., Garg, N. S., Gelbart, W. M., Glasser, K., Glodek, A., Gong, F., Gorrell, J. H., Gu, Z., Guan, P., Harris, M., Harris, N. L., Harvey, D., Heiman, T. J., Hernandez, J. R., Houck, J., Hostin, D., Houston, K. A., Howland, T. J., Wei, M. H., Ibegwam, C., Jalali, M., Kalush, F., Karpen, G. H., Ke, Z., Kennison, J. A., Ketchum, K. A., Kimmel, B. E., Kodira, C. D., Kraft, C., Kravitz, S., Kulp, D., Lai, Z., Lasko, P., Lei, Y., Levitsky, A. A., Li, J., Li, Z., Liang, Y., Lin, X., Liu, X., Mattei, B., McIntosh, T. C., McLeod, M. P., McPherson, D., Merkulov, G., Milshina, N. V., Mobarry, C., Morris, J., Moshrefi, A., Mount, S. M., Moy, M., Murphy, B., Murphy, L., Muzny, D. M., Nelson, D. L., Nelson, D. R., Nelson, K. A., Nixon, K., Nusskern, D. R., Pacleb, J. M., Palazzolo, M., Pittman, G. S., Pan, S., Pollard, J., Puri, V., Reese, M. G., Reinert, K., Remington, K., Saunders, R. D., Scheeler, F., Shen, H., Shue, B. C., Siden-Kiamos, I., Simpson, M., Skupski, M. P., Smith, T., Spier, E., Spradling, A. C., Stapleton, M., Strong, R., Sun, E., Svirskas, R., Tector, C., Turner, R., Venter, E., Wang, A. H., Wang, X., Wang, Z. Y., Wassarman, D. A., Weinstock, G. M., Weissenbach, J., Williams, S. M., Woodage, T., Worley, K. C., Wu, D., Yang, S., Yao, Q. A., Ye, J., Yeh, R. F., Zaveri, J. S., Zhan, M., Zhang, G., Zhao, Q., Zheng, L., Zheng, X. H., Zhong, F. N., Zhong, W., Zhou, X., Zhu, S., Zhu, X., Smith, H. O.,

Gibbs, R. A., Myers, E. W., Rubin, G. M., & Venter, J. C. (2000). The genome sequence of *Drosophila melanogaster. Science, 287*, 2185–2195.

Aggarwal, R., Subramanyam, S., Zhao, C. Y., Chen, M. S., Harris, M. O., & Stuart, J. J. (2014). Avirulence effector discovery in a plant galling and plant parasitic arthropod, the Hessian Fly (*Mayetiola destructor*). *PloS One, 9*, e100958.

Ahmad, S., Veyrat, N., Gordon-Weeks, R., Zhang, Y., Martin, J., Smart, L., Glauser, G., Erb, M., Flors, V., Frey, M., & Ton, J. (2011). Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiology, 157*, 317–327.

Ahola, V., Lehtonen, R., Somervuo, P., Salmela, L., Koskinen, P., Rastas, P., Välimäki, N., Paulin, L., Kvist, J., Wahlberg, N., Tanskanen, J., Hornett, E. A., Ferguson, L. C., Luo, S., Cao, Z., de Jong, M. A., Duplouy, A., Smolander, O.-P., Vogel, H., McCoy, R. C., Qian, K., Chong, W. S., Zhang, Q., Ahmad, F., Haukka, J. K., Joshi, A., Salojärvi, J., Wheat, C. W., Grosse-Wilde, E., Hughes, D., Katainen, R., Pitkänen, E., Ylinen, J., Waterhouse, R. M., Turunen, M., Vähärautio, A., Ojanen, S. P., Schulman, A. H., Taipale, M., Lawson, D., Ukkonen, E., Mäkinen, V., Goldsmith, M. R., Holm, L., Auvinen, P., Frilander, M. J., & Hanski, I. (2014). The Glanville fritillary genome retains an ancient karyotype and reveals selective chromosomal fusions in Lepidoptera. *Nature Communications, 5*, 4737.

Aliferis, K. A., Copley, T., & Jabaji, S. (2012). Gas chromatography-mass spectrometry metabolite profiling of worker honey bee (*Apis mellifera* L.) hemolymph for the study of *Nosema ceranae* infection. *Journal of Insect Physiology, 58*, 1349–1359.

Atamian, H. S., Chaudhary, R., Cin, V. D., Bao, E., Girke, T., & Kaloshian, I. (2013). In planta expression or delivery of potato aphid *Macrosiphum euphorbiae* effectors Me10 and Me23 enhances aphid fecundity. *Molecular Plant-Microbe Interactions, 26*, 67–74.

Attaran, E., Major, I. T., Cruz, J. A., Rosa, B. A., Koo, A. J., Chen, J., Kramer, D. M., He, S. Y., & Howe, G. A. (2014). Temporal dynamics of growth and photosynthesis suppression in response to jasmonate signaling. *Plant Physiology, 165*, 1302–1314.

Bao, E., Jiang, T., & Girke, T. (2013). BRANCH: Boosting RNA-Seq assemblies with partial or related genomic sequences. *Bioinformatics, 29*, 1250–1259.

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *The New Phytologist, 127*, 617–633.

Bentur, J. S., Cheralu, C., & Rao, P. R. M. (2008). Monitoring virulence in Asian rice gall midge populations in India. *Entomologia Experimentalis et Applicata, 129*, 96–106.

Bermingham, J., Rabatel, A., Calevro, F., Vinuelas, J., Febvay, G., Charles, H., Douglas, A., & Wilkinson, T. (2009). Impact of host developmental age on the transcriptome of the symbiotic bacterium *Buchnera aphidicola* in the pea aphid (*Acyrthosiphon pisum*). *Applied and Environmental Microbiology, 75*, 7294–7297.

Bino, R. J., Hall, R. D., Fiehn, O., Kopka, J., Saito, K., Draper, J., Nikolau, B. J., Mendes, P., Roessner-Tunali, U., Beale, M. H., Trethewey, R. N., Lange, B. M., Wurtele, E. S., & Sumner, L. W. (2004). Potential of metabolomics as a functional genomics tool. *Trends in Plant Science, 9*, 418–425.

Biradar, S. K., Sundaram, R. M., Thirumurugan, T., Bentur, J. S., Amudhan, S., Shenoy, V. V., Mishra, B., Bennett, J., & Sarma, N. P. (2004). Identification of flanking SSR markers for a major rice gall midge resistance gene *Gm1* and their validation. *Theoretical and Applied Genetics, 109*, 1468–1473.

Boller, T., & Felix, G. (2009). A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology, 60*, 379–406.

Bonasio, R., Zhang, G., Ye, C., Mutti, N. S., Fang, X., Qin, N., Donahue, G., Yang, P., Li, Q., Li, C., Zhang, P., Huang, Z., Berger, S. L., Reinberg, D., Wang, J., & Liebig, J. (2010). Genomic comparison of the ants *Camponotus floridanus* and *Harpegnathos saltator. Science, 329*, 1068–1071.

Bonaventure, G., Gfeller, A., Proebsting, W. M., Hoertensteiner, S., Chetelat, A., Martinoia, E., & Farmer, E. E. (2007). A gain-of-function allele of *TPC1* activates oxylipin biogenesis after leaf wounding in Arabidopsis. *The Plant Journal, 49*, 889–898.

Bos, J. I., Prince, D., Pitino, M., Maffei, M. E., Win, J., & Hogenhout, S. A. (2010). A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS Genetics, 6*, e1001216.

Boykin, L. M. (2014). *Bemisia tabaci* nomenclature: Lessons learned. *Pest Management Science, 70*, 1454–1459.

Brown, J. K., Lambert, G. M., HGhanim, M., Czosnek, H., & Galbraith, D. W. (2005). Nuclear DNA content of the whitefly *Bemisia tabaci* (Aleyrodidae: Hempitera) estimated by flow cytometry. *Bulletin of Entomological Research, 95*, 309–312.

Browse, J. (2009). Jasmonate passes muster: A receptor and targets for the defense hormone. *Annual Review of Plant Biology, 60*, 183–205.

Bruex, A., Liu, T.-Y., Krebs, M., Stierhof, Y.-D., Lohmann, J. U., Miersch, O., Wastermack, C., & Schumacher, K. (2008). Reduced V-ATPase activity in the trans-Golgi network causes oxylipin-dependent hypocotyl growth inhibition in Arabidopsis. *Plant Cell, 20*, 1088–1100.

Burow, M., Halkier, B. A., & Kliebenstein, D. J. (2010). Regulatory networks of glucosinolates shape *Arabidopsis thaliana* fitness. *Current Opinion in Plant Biology, 13*, 348–353.

Buszewska-Forajta, M., Siluk, D., Struck-Lewicka, W., Raczak-Gutknecht, J., Markuszewski, M. J., & Kaliszan, R. (2014a). Identification of lipid fraction constituents from grasshopper (*Chorthippus* spp.) abdominal secretion with potential activity in wound healing with the use of GC-MS/MS technique. *Journal of Pharmaceutical and Biomedical Analysis, 89*, 56–66.

Buszewska-Forajta, M., Struck-Lewicka, W., Bujak, R., Siluk, D., & Kaliszan, R. (2014b). Determination of water-soluble components of abdominal secretion of grasshopper (*Chorthippus* spp.) by GC/MS/MS in search for potential wound healing agents. *Chromatographia, 77*, 1091–1102.

Buszewska-Forajta, M., Bujak, R., Yumba-Mpanga, A., Siluk, D., & Kaliszan, R. (2015). GC/MS technique and AMDIS software application in identification of hydrophobic compounds of grasshoppers' abdominal secretion (*Chorthippus* spp.). *Journal of Pharmaceutical and Biomedical Analysis, 102*, 331–339.

Caillaud, C. M., & Niemeyer, H. M. (1996). Possible involvement of the phloem sealing system in the acceptance of a plant as host by an aphid. *Experientia, 52*, 927–931.

Cambier, V., Hance, T., & de Hoffmann, E. (2000). Variation of DIMBOA and related compounds content in relation to the age and plant organ in maize. *Phytochemistry, 53*, 223–229.

Cambier, V., Hance, T., & De Hoffmann, E. (2001). Effects of 1,4-benzoxazin-3-one derivatives from maize on survival and fecundity of *Metopolophium dirhodum* (Walker) on artificial diet. *Journal of Chemical Ecology, 27*, 359–370.

Carolan, J. C., Fitzroy, C. I., Ashton, P. D., Douglas, A. E., & Wilkinson, T. L. (2009). The secreted salivary proteome of the pea aphid *Acyrthosiphon pisum* characterised by mass spectrometry. *Proteomics, 9*, 2457–2467.

Carolan, J. C., Caragea, D., Reardon, K. T., Mutti, N. S., Dittmer, N., Pappan, K., Cui, F., Castaneto, M., Poulain, J., Dossat, C., Tagu, D., Reese, J. C., Reeck, G. R., Wilkinson, T. L., & Edwards, O. R. (2011). Predicted effector molecules in the salivary secretome of the pea aphid (*Acyrthosiphon pisum*): A dual transcriptomic/proteomic approach. *Journal of Proteome Research, 10*, 1505–1518.

Caspi, R., Altman, T., Billington, R., Dreher, K., Foerster, H., Fulcher, C. A., Holland, T. A., Keseler, I. M., Kothari, A., Kubo, A., Krummenacker, M., Latendresse, M., Mueller, L. A., Ong, Q., Paley, S., Subhraveti, P., Weaver, D. S., Weerasinghe, D., Zhang, P., & Karp, P. D. (2014). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Research, 42*, D459–D471.

Chaston, J., & Douglas, A. E. (2012). Making the most of "omics" for symbiosis research. *The Biological Bulletin, 223*, 21–29.

Chaudhary, R., Atamian, H. S., Shen, Z., Briggs, S. P., & Kaloshian, I. (2014). GroEL from the endosymbiont *Buchnera aphidicola* betrays the aphid by triggering plant defense. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 8919–8924.

Chaudhary, R., Atamian, H. S., Shen, Z., Briggs, S. P., & Kaloshian, I. (2015). Potato aphid salivary proteome: Enhanced salivation using resorcinol and identification of aphid phosphoproteins. *Journal of Proteome Research, 14*, 1762–1778.

Chauvin, A., Caldelari, D., Wolfender, J.-L., & Farmer, E. E. (2013). Four 13-lipoxygenases contribute to rapid jasmonate synthesis in wounded *Arabidopsis thaliana* leaves: A role for lipoxygenase 6 in responses to long-distance wound signals. *The New Phytologist, 197*, 566–575.

Chintapalli, V. R., Al Bratty, M., Korzekwa, D., Watson, D. G., & Dow, J. A. T. (2013). Mapping an atlas of tissue-specific *Drosophila melanogaster* metabolomes by high resolution mass spectrometry. *PloS One, 8*, e78066.

Chipman, A. D., Ferrier, D. E. K., Brena, C., Qu, J., Hughes, D. S. T., Schröder, R., Torres-Oliva, M., Znassi, N., Jiang, H., Almeida, F. C., Alonso, C. R., Apostolou, Z., Aqrawi, P., Arthur, W., Barna, J. C. J., Blankenburg, K. P., Brites, D., Capella-Gutiérrez, S., Coyle, M., Dearden, P. K., Du Pasquier, L., Duncan, E. J., Ebert, D., Eibner, C., Erikson, G., Evans, P. D., Extavour, C. G., Francisco, L., Gabaldón, T., Gillis, W. J., Goodwin-Horn, E. A., Green, J. E., Griffiths-Jones, S., Grimmelikhuijzen, C. J. P., Gubbala, S., Guigó, R., Han, Y., Hauser, F., Havlak, P., Hayden, L., Helbing, S., Holder, M., Hui, J. H. L., Hunn, J. P., Hunnekuhl, V. S., Jackson, L., Javaid, M., Jhangiani, S. N., Jiggins, F. M., Jones, T. E., Kaiser, T. S., Kalra, D., Kenny, N. J., Korchina, V., Kovar, C. L., Kraus, F. B., Lapraz, F., Lee, S. L., Lv, J., Mandapat, C., Manning, G., Mariotti, M., Mata, R., Mathew, T., Neumann, T., Newsham, I., Ngo, D. N., Ninova, M., Okwuonu, G., Ongeri, F., Palmer, W. J., Patil, S., Patraquim, P., Pham, C., Pu, L.-L., Putman, N. H., Rabouille, C., Ramos, O. M., Rhodes, A. C., Robertson, H. E., Robertson, H. M., Ronshaugen, M., Rozas, J., Saada, N., Sánchez-Gracia, A., Scherer, S. E., Schurko, A. M., Siggens, K. W., Simmons, D., Stief, A., Stolle, E., Telford, M. J., Tessmar-Raible, K., Thornton, R., van der Zee, M., von Haeseler, A., Williams, J. M., Willis, J. H., Wu, Y., Zou, X., Lawson, D., Muzny, D. M., Worley, K. C., Gibbs, R. A., Akam, M., & Richards, S. (2014). The first myriapod genome sequence reveals conservative arthropod gene content and genome organisation in the centipede *Strigamia maritima. PLoS Biology, 12*, e1002005.

Cilia, M., Tamborindeguy, C., Fish, T., Howe, K., Thannhauser, T. W., & Gray, S. (2011a). Genetics coupled to quantitative intact proteomics links heritable aphid and endosymbiont protein expression to circulative polerovirus transmission. *Journal of Virology, 85*, 2148–2166.

Cilia, M., Tamborindeguy, C., Rolland, M., Howe, K., Thannhauser, T. W., & Gray, S. (2011b). Tangible benefits of the aphid *Acyrthosiphon pisum* genome sequencing for aphid proteomics: Enhancements in protein identification and data validation for homology-based proteomics. *Journal of Insect Physiology, 57*, 179–190.

Clay, N. K., Adio, A. M., Denoux, C., Jander, G., & Ausubel, F. M. (2009). Glucosinolate metabolites required for an Arabidopsis innate immune response. *Science, 323*, 95–101.

Colinet, H., Renault, D., Charoy-Guevel, B., & Com, E. (2012). Metabolic and proteomic profiling of diapause in the aphid parasitoid *Praon volucre. PloS One, 7*, e32606.

Cong, Q., Borek, D., Otwinowski, Z., & Grishin Nick, V. (2015). Tiger swallowtail genome reveals mechanisms for speciation and caterpillar chemical defense. *Cell Reports, 10*, 910–919.

Consortium, T. H. G. (2012). Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature, 487*, 94–98.

Cooper, W. R., Dillwith, J. W., & Puterka, G. J. (2010). Salivary proteins of Russian wheat aphid (Hemiptera: Aphididae). *Environmental Entomology, 39*, 223–231.

Cooper, W. R., Dillwith, J. W., & Puterka, G. J. (2011). Comparisons of salivary proteins from five aphid (Hemiptera: Aphididae) species. *Environmental Entomology, 40*, 151–156.

Coquin, L., Feala, J. D., McCulloch, A. D., & Paternostro, G. (2008). Metabolomic and flux-balance analysis of age-related decline of hypoxia tolerance in Drosophila muscle tissue. *Molecular Systems Biology, 4*, 233. doi:http://dx.doi.org/210.1038/msb.2008.1071.

Cornman, S. R., Schatz, M. C., Johnston, S. J., Chen, Y. P., Pettis, J., Hunt, G., Bourgeois, L., Elsik, C., Anderson, D., Grozinger, C. M., & Evans, J. D. (2010). Genomic survey of the ectoparasitic mite Varroa destructor, a major pest of the honey bee Apis mellifera. *BMC Genomics, 11*, 602.

Cui, F., Smith, C. M., Reese, J., Edwards, O., & Reeck, G. (2012). Polymorphisms in salivary-gland transcripts of Russian wheat aphid biotypes 1 and 2. *Insect Science, 19*, 429–440.

Dafoe, N. J., Huffaker, A., Vaughan, M. M., Duehl, A. J., Teal, P. E., & Schmelz, E. A. (2011). Rapidly induced chemical defenses in maize stems and their effects on short-term growth of *Ostrinia nubilalis. Journal of Chemical Ecology, 37*, 984–991.

Davidson, G. (1964). *Anopheles gambiae*, a complex of species. *Bulletin of the World Health Organization, 31*, 625–634.

Davies, E., & Schuster, A. (1981). Intercellular communication in plants: Evidence for a rapidly generated, bidirectionally transmitted wound signal. *Proceedings of the National Academy of Sciences of the United States of America, 78*, 2422–2426.

Dengler, N. G. (2006). The shoot apical meristem and development of vascular architecture. *Canadian Journal of Botany, 84*, 1660–1671.

Derecka, K., Blythe, M. J., Malla, S., Genereux, D. P., Guffanti, A., Pavan, P., Moles, A., Snart, C., Ryder, T., Ortori, C. A., Barrett, D. A., Schuster, E., & Stoeger, R. (2013). Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae. *PloS One, 8*, e68191.

Douglas, A. E. (2013). Microbial brokers of insect-plant interactions revisited. *Journal of Chemical Ecology, 39*, 952–961.

Dutartre, L., Hilliou, F., & Feyereisen, R. (2012). Phylogenomics of the benzoxazinoid biosynthetic pathway of Poaceae: Gene duplications and origin of the *Bx* cluster. *BMC Evolutionary Biology, 12*, 64.

Ebel, J., & Mithoefer, A. (1998). Early events in the elicitation of plant defence. *Planta, 206*, 335–348.

Elsik, C., Worley, K., Bennett, A., Beye, M., Camara, F., Childers, C., de Graaf, D., Debyser, G., Deng, J., Devreese, B., Elhaik, E., Evans, J., Foster, L., Graur, D., Guigo, R.; HGSC production teams, Hoff, K., Holder, M., Hudson, M., Hunt, G., Jiang, H., Joshi, V., Khetani, R., Kosarev, P., Kovar, C., Ma, J., Maleszka, R., Moritz, R., Munoz-Torres, M., & Murphy, T. (2014). Finding the missing honey bee genes: lessons learned from a genome upgrade. *BMC Genomics, 15*, 86.

Elzinga, D. A., De Vos, M., & Jander, G. (2014). Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. *Molecular Plant-Microbe Interactions, 27*, 747–756.

Engel, P., & Moran, N. A. (2013). The gut microbiota of insects – Diversity in structure and function. *FEMS Microbiology Reviews, 37*, 699–735.

Facchini, P. J. (2001). Alkaloid biosynthesis in plants: Biochemistry, cell biology, molecular regulation, and metabolic engineering applications. *Annual Review of Plant Physiology and Plant Molecular Biology, 52*, 29–66.

Farmer, E. E., Gasperini, D., & Acosta, I. F. (2014). The squeeze cell hypothesis for the activation of jasmonate synthesis in response to wounding. *The New Phytologist, 204*, 282–288.

Feltus, F. A. (2014). Systems genetics: A paradigm to improve discovery of candidate genes and mechanisms underlying complex traits. *Plant Science, 223*, 45–48.

Flint-Garcia, S. A., Thuillet, A. C., Yu, J. M., Pressoir, G., Romero, S. M., Mitchell, S. E., Doebley, J., Kresovich, S., Goodman, M. M., & Buckler, E. S. (2005). Maize association population: A high-resolution platform for quantitative trait locus dissection. *The Plant Journal, 44*, 1054–1064.

Fontaine, M. C., Pease, J. B., Steele, A., Waterhouse, R. M., Neafsey, D. E., Sharakhov, I. V., Jiang, X., Hall, A. B., Catteruccia, F., Kakani, E., Mitchell, S. N., Wu, Y. C., Smith, H. A., Love, R. R., Lawniczak, M. K., Slotman, M. A., Emrich, S. J., Hahn, M. W., & Besansky, N. J. (2015). Mosquito genomics. Extensive introgression in a malaria vector species complex revealed by phylogenomics. *Science, 347*, 1258524.

Foray, V., Desouhant, E., Voituron, Y., Larvor, V., Renault, D., Colinet, H., & Gibert, P. (2013). Does cold tolerance plasticity correlate with the thermal environment and metabolic profiles of a parasitoid wasp? *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology, 164*, 77–83.

Francis, F., Gerkens, P., Harmel, N., Mazzucchelli, G., De Pauw, E., & Haubruge, E. (2006). Proteomics in *Myzus persicae*: Effect of aphid host plant switch. *Insect Biochemistry and Molecular Biology, 36*, 219–227.

Fukushima, A., Kusano, M., Mejia, R. F., Iwasa, M., Kobayashi, M., Hayashi, N., Watanabe-Takahashi, A., Narisawa, T., Tohge, T., Hur, M., Wurtele, E. S., Nikolau, B. J., & Saito, K. (2014). Metabolomic characterization of knockout mutants in Arabidopsis: Development of a metabolite profiling database for knockout mutants in Arabidopsis. *Plant Physiology, 165*, 948–961.

Gaquerel, E., Gulati, J., & Baldwin, I. T. (2014). Revealing insect herbivory-induced phenolamide metabolism: From single genes to metabolic network plasticity analysis. *The Plant Journal, 79*, 679–692.

Gershenzon, J., & Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology, 3*, 408–414.

Ghanim, M., & Kontsedalov, S. (2009). Susceptibility to insecticides in the Q biotype of *Bemisia tabaci* is correlated with bacterial symbiont densities. *Pest Management Science, 65*, 939–942.

Gilchrist, A., Shearman, D., Frommer, M., Raphael, K., Deshpande, N., Wilkins, M., Sherwin, W., & Sved, J. (2014). The draft genome of the pest tephritid fruit fly *Bactrocera tryoni*: Resources for the genomic analysis of hybridising species. *BMC Genomics, 15*, 1153.

Givovich, A., Morse, S., Cerda, H., Niemeyer, H. M., Wratten, S. D., & Edwards, P. J. (1992). Hydroxamic acid glucosides in honeydew of aphids feeding on wheat. *Journal of Chemical Ecology, 18*, 841–846.

Givovich, A., Sandstrom, J., Niemeyer, H. M., & Pettersson, J. (1994). Presence of a hydroxamic acid glucoside in wheat phloem sap, and its consequences for performance of *Rhopalosiphum padi* (L.) (Homoptera: Aphididae). *Journal of Chemical Ecology, 20*, 1923–1930.

Glauser, G., Dubugnon, L., Mousavi, S. A. R., Rudaz, S., Wolfender, J. L., & Farmer, E. E. (2009). Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded *Arabidopsis. The Journal of Biological Chemistry, 284*, 34506–34513.

Glauser, G., Marti, G., Villard, N., Doyen, G. A., Wolfender, J. L., Turlings, T. C. J., & Erb, M. (2011). Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. *The Plant Journal, 68*, 901–911.

Grambow, H. J., Luckge, J., Klausener, A., & Muller, E. (1986). Occurrence of 2-(2-hydroxy-4,7-dimethoxy-2h-1,4-benzoxazin-3-one)-beta-d-glucopyranoside in *Triticum aestivum* leaves and its conversion into 6-methoxy-benzoxazolinone. *Zeitschrift für Naturforschung Section C: A Journal of Biosciences, 41*, 684–690.

Grbic, M., Van Leeuwen, T., Clark, R. M., Rombauts, S., Rouze, P., Grbic, V., Osborne, E. J., Dermauw, W., Ngoc, P. C., Ortego, F., Hernandez-Crespo, P., Diaz, I., Martinez, M., Navajas, M., Sucena, E., Magalhaes, S., Nagy, L., Pace, R. M., Djuranovic, S., Smagghe, G., Iga, M., Christiaens, O., Veenstra, J. A., Ewer, J., Villalobos, R. M., Hutter, J. L., Hudson, S. D., Velez, M., Yi, S. V., Zeng, J., Pires-daSilva, A., Roch, F., Cazaux, M., Navarro, M., Zhurov, V., Acevedo, G., Bjelica, A., Fawcett, J. A., Bonnet, E., Martens, C., Baele, G., Wissler, L., Sanchez-Rodriguez, A., Tirry, L., Blais, C., Demeestere, K., Henz, S. R., Gregory, T. R., Mathieu, J., Verdon, L., Farinelli, L., Schmutz, J., Lindquist, E., Feyereisen, R., & Van de Peer, Y. (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature, 479*, 487–492.

Gulati, J., Kim, S. G., Baldwin, I. T., & Gaquerel, E. (2013). Deciphering herbivory-induced gene-to-metabolite dynamics in *Nicotiana attenuata* tissues using a multifactorial approach. *Plant Physiology, 162*, 1042–1059.

Guo, L., Wang, S., Wu, Q., Zhou, X., Xie, W., & Zhang, Y. (2015). Flow cytometry and K-mer analysis estimates of the genome sizes of *Bemisia tabaci* B and Q (Hemiptera: Aleyrodidae). *Frontiers in Physiology, 6.*http://dx.doi.org/2010.3389/fphys.2015.00144

Hansen, A. K., & Degnan, P. H. (2014). Widespread expression of conserved small RNAs in small symbiont genomes. *The ISME Journal, 8*, 2490–2502.

Hansen, A. K., & Moran, N. A. (2011). Aphid genome expression reveals host-symbiont cooperation in the production of amino acids. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 2849–2854.

Hansen, A. K., & Moran, N. A. (2014). The impact of microbial symbionts on host plant utilization by herbivorous insects. *Molecular Ecology, 23*, 1473–1496.

Hao, Y., Cambron, S. E., Chen, Z., Wang, Y., Bland, D. E., Buntin, G. D., & Johnson, J. W. (2013). Characterization of new loci for Hessian fly resistance in common wheat. *Theoretical and Applied Genetics, 126*, 1067–1076.

Hariharan, R., Hoffman, J. M., Thomas, A. S., Soltow, Q. A., Jones, D. P., & Promislow, D. E. L. (2014). Invariance and plasticity in the *Drosophila melanogaster* metabolomic network in response to temperature. *BMC Systems Biology, 8*, 139.

Harmel, N., Letocart, E., Cherqui, A., Giordanengo, P., Mazzucchelli, G., Guillonneau, F., De Pauw, E., Haubruge, E., & Francis, F. (2008). Identification of aphid salivary proteins: A proteomic investigation of *Myzus persicae. Insect Molecular Biology, 17*, 165–174.

Harris, M. O., Stuart, J. J., Mohan, M., Nair, S., Lamb, R. J., & Rohfritsch, O. (2003). Grasses and gall midges: Plant defense and insect adaptation. *Annual Review of Entomology, 48*, 549–577.

Harris, M. O., Freeman, T. P., Rohfritsch, O., Anderson, K. G., Payne, S. A., & Moore, J. A. (2006). Virulent Hessian fly (Diptera: Cecidomyiidae) larvae induce a nutritive tissue during compatible interactions with wheat. *Annals of the Entomological Society of America, 99*, 305–316.

Hayward, S. A. (2014). Application of functional 'Omics' in environmental stress physiology: Insights, limitations, and future challenges. *Current Opinion in Insect Science, 4*, 35–41.

Himler, A. G., Adachi-Hagimori, T., Bergen, J. E., Kozuch, A., Kelly, S. E., Tabashnik, B. E., Chiel, E., Duckworth, V. E., Dennehy, T. J., Zchori-Fein, E., & Hunter, M. S. (2011). Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science, 332*, 254–256.

Hogenhout, S. A., & Bos, J. I. B. (2011). Effector proteins that modulate plant-insect interactions. *Current Opinion in Plant Biology, 14*, 422–428.

Hoskins, R. A., Carlson, J. W., Wan, K. H., Park, S., Mendez, I., Galle, S. E., Booth, B. W., Pfeiffer, B. D., George, R. A., Svirskas, R., Krzywinski, M., Schein, J., Carmela Accardo, M., Damia, E., Messina, G., Mendez-Lago, M., de Pablos, B., Demakova, O. V., Andreyeva, E. N., Boldyreva, L. V., Marra, M., Carvalho, A. B., Dimitri, P., Villasante, A., Zhimulev, I. F., Rubin, G. M., Karpen, G. H., & Celniker, S. E. (2015). The Release 6 reference sequence of the *Drosophila melanogaster* genome. *Genome Research, 25*, 445–458.

Howe, G. A., & Jander, G. (2008). Plant immunity to insect herbivores. *Annual Review of Plant Biology, 59*, 41–66.

Huffaker, A., Kaplan, F., Vaughan, M. M., Dafoe, N. J., Ni, X., Rocca, J. R., Alborn, H. T., Teal, P. E. A., & Schmelz, E. A. (2011). Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins in maize. *Plant Physiology, 156*, 2082–2097.

Husnik, F., Nikoh, N., Koga, R., Ross, L., Duncan, R. P., Fujie, M., Tanaka, M., Satoh, N., Bachtrog, D., Wilson, A. C., von Dohlen, C. D., Fukatsu, T., & McCutcheon, J. P. (2013). Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. *Cell, 153*, 1567–1578.

Jander, G. (2014). Revisiting plant-herbivore co-evolution in the molecular biology era. *Annual Plant Reviews, 47*, 361–384.

Jansen, J. J., Allwood, J. W., Marsden-Edwards, E., van der Putten, W. H., Goodacre, R., & van Dam, N. M. (2009). Metabolomic analysis of the interaction between plants and herbivores. *Metabolomics, 5*, 150–161.

Janz, N., Nylin, S., & Wahlberg, N. (2006). Diversity begets diversity: Host expansions and the diversification of plant-feeding insects. *BMC Evolutionary Biology, 6*, 4.

Jensen, L. M., Halkier, B. A., & Burow, M. (2014). How to discover a metabolic pathway? An update on gene identification in aliphatic glucosinolate biosynthesis, regulation and transport. *Biological Chemistry, 395*, 529–543.

Jing, X., Wong, A. C., Chaston, J. M., Colvin, J., McKenzie, C. L., & Douglas, A. E. (2014). The bacterial communities in plant phloem-sap-feeding insects. *Molecular Ecology, 23*, 1433–1444.

Kakumani, P. K., Malhotra, P., Mukherjee, S. K., & Bhatnagar, R. K. (2014). A draft genome assembly of the army worm, *Spodoptera frugiperda. Genomics, 104*, 134–143.

Kaloshian, I., & Walling, L. (2015). Plant immunity: connecting the dots between microbial and hemipteran immune responses. In H. Czosnek, & M. Ghanim (Eds.), *Management of insect pests to agriculture: Lessons learned from deciphering their genome, transcriptome, and proteome.* (chap. 9, pp. XX). Cham: Springer.

Kang, S., Kim, H. B., Lee, H., Choi, J. Y., Heu, S., Oh, C. J., Kwon, S. I., & An, C. S. (2006). Overexpression in Arabidopsis of a plasma membrane-targeting glutamate receptor from small radish increases glutamate-mediated Ca^{2+} influx and delays fungal infection. *Molecular Cell, 21*, 418–427.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A., & Schuurink, R. C. (2004). Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiology, 135*, 483–495.

Keeling, C. I., Yuen, M. M., Liao, N. Y., Docking, T. R., Chan, S. K., Taylor, G. A., Palmquist, D. L., Jackman, S. D., Nguyen, A., Li, M., Henderson, H., Janes, J. K., Zhao, Y., Pandoh, P., Moore, R., Sperling, F. A., Huber, D. P., Birol, I., Jones, S. J., & Bohlmann, J. (2013). Draft genome of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major forest pest. *Genome Biology, 14*, R27.

Kersten, B., Ghirardo, A., Schnitzler, J.-P., Kanawati, B., Schmitt-Kopplin, P., Fladung, M., & Schroeder, H. (2013). Integrated transcriptomics and metabolomics decipher differences in the resistance of pedunculate oak to the herbivore *Tortrix viridana* L. *BMC Genomics, 14*, 737.

Kim, H. S., Murphy, T., Xia, J., Caragea, D., Park, Y., Beeman, R. W., Lorenzen, M. D., Butcher, S., Manak, J. R., & Brown, S. J. (2010). BeetleBase in 2010: Revisions to provide comprehensive genomic information for *Tribolium castaneum. Nucleic Acids Research, 38*, D437–D442.

Kliebenstein, D. J. (2012). Plant defense compounds: Systems approaches to metabolic analysis. *Annual Review of Phytopathology, 50*, 155–173.

Kliebenstein, D. J. (2014). Quantitative genetics and genomics of plant resistance to insects. *Insect-Plant Interactions, 47*, 235–262.

Kombrink, E. (2012). Chemical and genetic exploration of jasmonate biosynthesis and signaling paths. *Planta, 236*, 1351–1366.

Koo, A. J. K., & Howe, G. A. (2009). The wound hormone jasmonate. *Phytochemistry, 70*, 1571–1580.

Kostal, V., Korbelova, J., Rozsypal, J., Zahradnickova, H., Cimlova, J., Tomcala, A., & Simek, P. (2011). Long-term cold acclimation extends survival time at 0°C and modifies the metabolomic profiles of the larvae of the fruit fly *Drosophila melanogaster. PloS One, 6*, e25025.

Krol, E., Mentzel, T., Chinchilla, D., Boller, T., Felix, G., Kemmerling, B., Postel, S., Arents, M., Jeworutzki, E., Al-Rasheid, K. A. S., Becker, D., & Hedrich, R. (2010). Perception of the Arabidopsis danger signal peptide 1 involves the pattern recognition receptor AtPEPR1 and its close homologue AtPEPR2. *The Journal of Biological Chemistry, 285*, 13471–13479.

Kuhn, K. L., Duan, J. J., & Hopper, K. R. (2013). Next-generation genome sequencing and assembly provides tools for phylogenetics and identification of closely related species of Spathius, parasitoids of *Agrilus planipennis* (emerald ash borer). *Biological Control, 66*, 77–82.

Lakshmi, P. V., Amudhan, S., Bindu, K. H., Cheralu, C., & Bentur, J. S. (2006). A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh. *Indian International Journal of Tropical Insect Science, 26*, 207–211.

Lee, I. (2014). A showcase of future plant biology: moving towards next-generation plant genetics assisted by genome sequencing and systems biology. *Genome Biology, 15*, 305–305.

Lenz, E. M., Hagele, B. F., Wilson, I. D., & Simpson, S. J. (2001). High resolution H-1 NMR spectroscopic studies of the composition of the haemolymph of crowd- and solitary-reared nymphs of the desert locust, *Schistocerca gregaria. Molecular Biology, 32*, 51–56.

Liberman, L. M., Sozzani, R., & Benfey, P. N. (2012). Integrative systems biology: An attempt to describe a simple weed. *Current Opinion in Plant Biology, 15*, 162–167.

Liu, S.-S., Colvin, J., & De Barro, P. J. (2012). Species concepts as applied to the whitefly *Bemisia tabaci* systematics: How many species are there? *Journal of Integrative Agriculture, 11*, 176–186.

Lobo, N. F., Behura, S. K., Aggarwal, R., Chen, M. S., Collins, F. H., & Stuart, J. J. (2006). Genomic analysis of a 1 Mb region near the telomere of Hessian fly chromosome X2 and avirulence gene *vH13. BMC Genomics, 7*, 7.

Maffei, M., Bossi, S., Spiteller, D., Mithofer, A., & Boland, W. (2004). Effects of feeding *Spodoptera littoralis* on lima bean leaves. I. Membrane potentials, intracellular calcium variations, oral secretions, and regurgitate components. *Plant Physiology, 134*, 1752–1762.

Maffei, M. E., Mithofer, A., & Boland, W. (2007). Before gene expression: Early events in plant-insect interaction. *Trends in Plant Science, 12*, 310–316.

Malone, M., & Stankovic, B. (1991). Surface potentials and hydraulic signals in wheat leaves following localized wounding by heat. *Plant, Cell & Environment, 14*, 431–436.

Manguin, S., Carnevale, P., Mouchet, J., Coosemans, M., Julvez, J., Richard-Lenoble, D., & Sircoulon, J. (2008). *Biodiversity of malaria in the world* (p. 428). Montrouge: John Libbey Eurotext.

Maresh, J., Zhang, J., & Lynn, D. G. (2006). The innate immunity of maize and the dynamic chemical strategies regulating two-component signal transduction in *Agrobacterium tumefaciens. ACS Chemical Biology, 1*, 165–175.

McCutcheon, J. P., & Moran, N. A. (2010). Functional convergence in reduced genomes of bacterial symbionts spanning 200 My of evolution. *Genome Biology and Evolution, 2*, 708–718.

McCutcheon, J. P., & Moran, N. A. (2012). Extreme genome reduction in symbiotic bacteria. *Nature Reviews Microbiology, 10*, 13–26.

McMullen, M., Frey, M., & Degenhardt, J. (2009a). Genetics and biochemistry of insect resistance in maize. In J. L. Bennetzen & S. Hake (Eds.), *Handbook of maize: Its biology* (pp. 271–289). New York: Springer.

McMullen, M. D., Kresovich, S., Villeda, H. S., Bradbury, P., Li, H., Sun, Q., Flint-Garcia, S., Thornsberry, J., Acharya, C., Bottoms, C., Brown, P., Browne, C., Eller, M., Guill, K., Harjes, C., Kroon, D., Lepak, N., Mitchell, S. E., Peterson, B., Pressoir, G., Romero, S., Rosas, M. O., Salvo, S., Yates, H., Hanson, M., Jones, E., Smith, S., Glaubitz, J. C., Goodman, M., Ware, D., Holland, J. B., & Buckler, E. S. (2009b). Genetic properties of the maize nested association mapping population. *Science, 325*, 737–740.

Meihls, L. N., Kaur, H., & Jander, G. (2012). Natural variation in maize defense against insect herbivores. *Cold Spring Harbor Symposia on Quantitative Biology, 77*, 269–283.

Meihls, L. N., Handrick, V., Glauser, G., Barbier, H., Kaur, H., Haribal, M. M., Lipka, A. E., Gershenzon, J., Buckler, E. S., Erb, M., Kollner, T. G., & Jander, G. (2013). Natural variation in maize aphid resistance is associated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity. *Plant Cell, 25*, 2341–2355.

Mercke, P., Kappers, I. F., Verstappen, F. W., Vorst, O., Dicke, M., & Bouwmeester, H. J. (2004). Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. *Plant Physiology, 135*, 2012–2024.

Michalski, A., Damoc, E., Lange, O., Denisov, E., Nolting, D., Müller, M., Viner, R., Schwartz, J., Remes, P., Belford, M., Dunyach, J.-J., Cox, J., Horning, S., Mann, M., & Makarov, A. (2012). Ultra high resolution linear ion trap Orbitrap mass spectrometer (Orbitrap Elite) facilitates top down LC MS/MS and versatile peptide fragmentation modes. *Molecular & Cellular Proteomics, 11*, O111.013698.

Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M. A., Shulaev, V., Dangl, J. L., & Mittler, R. (2009). The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling, 2*, ra45.

Montllor, C. B., Maxmen, A., & Purcell, A. H. (2002). Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecological Entomology, 27*, 189–195.

Moran, N. A., & Bennett, G. M. (2014). The tiniest tiny genomes. *Annual Review of Microbiology, 68*, 195–215.

Moran, N. A., & Degnan, P. H. (2006). Functional genomics of *Buchnera* and the ecology of aphid hosts. *Molecular Ecology, 15*, 1251–1261.

Moran, N. A., Dunbar, H. E., & Wilcox, J. L. (2005). Regulation of transcription in a reduced bacterial genome: Nutrient-provisioning genes of the obligate symbiont Buchnera aphidicola. *Journal of Bacteriology, 187*, 4229–4237.

Moriwaki, N., Matsushita, K., Nishina, M., & Kono, Y. (2003a). High concentrations of trehalose in aphid hemolymph. *Applied Entomology and Zoology, 38*, 241–248.

Moriwaki, N., Matsushita, K., Nishina, M., Matsuda, K., & Kono, Y. (2003b). High myo-inositol concentration in the hemolymph of planthoppers. *Applied Entomology and Zoology, 38*, 359–364.

Mousavi, S. A. R., Chauvin, A., Pascaud, F., Kellenberger, S., & Farmer, E. E. (2013). *GLUTAMATE RECEPTOR-LIKE* genes mediate leaf-to-leaf wound signalling. *Nature, 500*, 422–426.

Mutti, N. S., Park, Y., Reese, J. C., & Reeck, G. R. (2006). RNAi knockdown of a salivary transcript leading to lethality in the pea aphid, *Acyrthosiphon pisum. Journal of Insect Science, 6*, 1–7.

Mutti, N. S., Louis, J., Pappan, L. K., Pappan, K., Begum, K., Chen, M. S., Park, Y., Dittmer, N., Marshall, J., Reese, J. C., & Reeck, G. R. (2008). A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 9965–9969.

Nachappa, P., Shapiro, A. A., & Tamborindeguy, C. (2012). Effect of '*Candidatus* Liberibacter solanacearum' on fitness of its insect vector, *Bactericera cockerelli* (Hemiptera: Triozidae), on tomato. *Phytopathology, 102*, 41–46.

Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H. E., Moran, N. A., & Hattori, M. (2006). The 160-kilobase genome of the bacterial endosymbiont Carsonella. *Science, 314*, 267.

Nakabachi, A., Ishida, K., Hongoh, Y., Ohkuma, M., & Miyagishima, S. Y. (2014). Aphid gene of bacterial origin encodes a protein transported to an obligate endosymbiont. *Current Biology, 24*, R640–R641.

Neafsey, D. E., Waterhouse, R. M., Abai, M. R., Aganezov, S. S., Alekseyev, M. A., Allen, J. E., Amon, J., Arca, B., Arensburger, P., Artemov, G., Assour, L. A., Basseri, H., Berlin, A., Birren, B. W., Blandin, S. A., Brockman, A. I., Burkot, T. R., Burt, A., Chan, C. S., Chauve, C., Chiu, J. C., Christensen, M., Costantini, C., Davidson, V. L., Deligianni, E., Dottorini, T., Dritsou, V., Gabriel, S. B., Guelbeogo, W. M., Hall, A. B., Han, M. V., Hlaing, T., Hughes, D. S., Jenkins, A. M., Jiang, X., Jungreis, I., Kakani, E. G., Kamali, M., Kemppainen, P., Kennedy, R. C., Kirmitzoglou, I. K., Koekemoer, L. L., Laban, N., Langridge, N., Lawniczak, M. K., Lirakis, M., Lobo, N. F., Lowy, E., MacCallum, R. M., Mao, C., Maslen, G., Mbogo, C., McCarthy, J., Michel, K., Mitchell, S. N., Moore, W., Murphy, K. A., Naumenko, A. N., Nolan, T., Novoa, E. M., O'Loughlin, S., Oringanje, C., Oshaghi, M. A., Pakpour, N., Papathanos, P. A., Peery, A. N., Povelones, M., Prakash, A., Price, D. P., Rajaraman, A., Reimer, L. J., Rinker, D. C., Rokas, A., Russell, T. L., Sagnon, N., Sharakhova, M. V., Shea, T., Simao, F. A., Simard, F., Slotman, M. A., Somboon, P., Stegniy, V., Struchiner, C. J., Thomas, G. W., Tojo, M., Topalis, P., Tubio, J. M., Unger, M. F., Vontas, J., Walton, C., Wilding, C. S., Willis, J. H., Wu, Y. C., Yan, G., Zdobnov, E. M., Zhou, X., Catteruccia, F., Christophides, G. K., Collins, F. H., Cornman, R. S., Crisanti, A., Donnelly, M. J., Emrich, S. J., Fontaine, M. C., Gelbart, W., Hahn, M. W., Hansen, I. A., Howell, P. I., Kafatos, F. C., Kellis, M., Lawson, D., Louis, C., Luckhart, S., Muskavitch, M. A., Ribeiro, J. M., Riehle, M. A., Sharakhov, I. V., Tu, Z., Zwiebel, L. J., & Besansky, N. J. (2015). Mosquito genomics. Highly evolvable malaria vectors: the genomes of 16 *Anopheles* mosquitoes. *Science, 347*, 1258522.

Nicholson, S. J., & Puterka, G. J. (2014). Variation in the salivary proteomes of differentially virulent greenbug (*Schizaphis graminum* Rondani) biotypes. *Journal of Proteomics, 105*, 186–203.

Nicholson, S. J., Hartson, S. D., & Puterka, G. J. (2012). Proteomic analysis of secreted saliva from Russian wheat aphid (*Diuraphis noxia* Kurd.) biotypes that differ in virulence to wheat. *Journal of Proteomics, 75*, 2252–2268.

Niemeyer, H. M. (1988). Hydroxamic acids 4 hydroxy-1 4-benzoxazin-3-ones defense chemicals in the Gramineae. *Phytochemistry, 27*, 3349–3358.

Niemeyer, H. M. (2009). Hydroxamic acids derived from 2-hydroxy-*2H*-1,4-benzoxazin-3(*4H*)-one: Key defense chemicals of cereals. *Journal of Agricultural and Food Chemistry, 57*, 1677–1696.

Nikoh, N., & Nakabachi, A. (2009). Aphids acquired symbiotic genes via lateral gene transfer. *BMC Biology, 7*, 12. doi:10.1186/1741-7007-7-12.

Nishikawa, H., Iijima, T., Kajitani, R., Yamaguchi, J., Ando, T., Suzuki, Y., Sugano, S., Fujiyama, A., Kosugi, S., Hirakawa, H., Tabata, S., Ozaki, K., Morimoto, H., Ihara, K., Obara, M., Hori, H., Itoh, T., & Fujiwara, H. (2015). A genetic mechanism for female-limited Batesian mimicry in Papilio butterfly. *Nature Genetics, 47*, 405–409.

Nygaard, S., Zhang, G., Schiøtt, M., Li, C., Wurm, Y., Hu, H., Zhou, J., Ji, L., Qiu, F., Rasmussen, M., Pan, H., Hauser, F., Krogh, A., Grimmelikhuijzen, C. J. P., Wang, J., & Boomsma, J. J. (2011). The genome of the leaf-cutting ant *Acromyrmex echinatior* suggests key adaptations to advanced social life and fungus farming. *Genome Research, 21*, 1339–1348.

Oerke, E. C. (2006). Crop losses due to pests. *The Journal of Agricultural Science, 144*, 31–43.

Oikawa, A., Ishihara, A., & Iwamura, H. (2002). Induction of HDMBOA-Glc accumulation and DIMBOA-Glc 4-O-methyltransferase by jasmonic acid in poaceous plants. *Phytochemistry, 61*, 331–337.

Oikawa, A., Ishihara, A., Tanaka, C., Mori, N., Tsuda, M., & Iwamura, H. (2004). Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves. *Phytochemistry, 65*, 2995–3001.

Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 1803–1807.

Oliver, K. M., Moran, N. A., & Hunter, M. S. (2005). Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 12795–12800.

Oliver, K. M., Moran, N. A., & Hunter, M. S. (2006). Costs and benefits of a superinfection of facultative symbionts in aphids. *Proceedings of the Royal Society B: Biological Sciences, 273*, 1273–1280.

Oliver, K. M., Smith, A. H., & Russell, J. A. (2014). Defensive symbiosis in the real world – Advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Functional Ecology, 28*, 341–355.

Oxley, P. R., Ji, L., Fetter-Pruneda, I., McKenzie, S. K., Li, C., Hu, H., Zhang, G., & Kronauer, D. J. (2014). The genome of the clonal raider ant *Cerapachys biroi. Current Biology, 24*, 451–458.

Pentzold, S., Zagrobelny, M., Rook, F., & Bak, S. (2014). How insects overcome two-component plant chemical defence: Plant β-glucosidases as the main target for herbivore adaptation. *Biological Reviews, 89*, 531–551.

Phalaraksh, C., Reynolds, S. E., Wilson, I. D., Lenz, E. M., Nicholson, J. K., & Lindon, J. C. (2008). A metabonomic analysis of insect development: H-1-NMR spectroscopic characterization of changes in the composition of the haemolymph of larvae and pupae of the tobacco hornworm, *Manduca sexta. Science Asia, 34*, 279–286.

Picelli, S., Bjorklund, A. K., Faridani, O. R., Sagasser, S., Winberg, G., & Sandberg, R. (2013). Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nature Methods, 10*, 1096–1098.

Pierik, R., Ballare, C. L., & Dicke, M. (2014). Ecology of plant volatiles: Taking a plant community perspective. *Plant, Cell & Environment, 37*, 1845–1853.

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology, 28*, 489–521.

Pimentel, D. (1997). *Pest management in agriculture: Techniques for reducing pesticide use: Environmental and economic benefits* (p. 456). Chichester: Wiley.

Pitino, M., & Hogenhout, S. A. (2013). Aphid protein effectors promote aphid colonization in a plant species-specific manner. *Molecular Plant-Microbe Interactions, 26*, 130–139.

Pitino, M., Coleman, A. D., Maffei, M. E., Ridout, C. J., & Hogenhout, S. A. (2011). Silencing of aphid genes by dsRNA feeding from plants. *PloS One, 6*, e25709.

Poliakov, A., Russell, C. W., Ponnala, L., Hoops, H. J., Sun, Q., Douglas, A. E., & van Wijk, K. J. (2011). Large-scale label-free quantitative proteomics of the pea aphid-*Buchnera* symbiosis. *Molecular and Cellular Proteomics, 10*, M110.007039.

Ramsey, J. S., Wilson, A. C., de Vos, M., Sun, Q., Tamborindeguy, C., Winfield, A., Malloch, G., Smith, D. M., Fenton, B., Gray, S. M., & Jander, G. (2007). Genomic resources for *Myzus persicae*: EST sequencing, SNP identification, and microarray design. *BMC Genomics, 8*, 423.

Rao, S. A., Carolan, J. C., & Wilkinson, T. L. (2013). Proteomic profiling of cereal aphid saliva reveals both ubiquitous and adaptive secreted proteins. *PloS One, 8*, e57413.

Rhodes, J. D., Thain, J. F., & Wildon, D. C. (1999). Evidence for physically distinct systemic signalling pathways in the wounded tomato plant. *Annals of Botany, 84*, 109–116.

Rider, S. D., Jr., Sun, W., Ratcliffe, R. H., & Stuart, J. J. (2002). Chromosome landing near avirulence gene *vH13* in the Hessian fly. *Genome, 45*, 812–822.

Robert-Seilaniantz, A., Grant, M., & Jones, J. D. G. (2011). Hormone crosstalk in plant disease and defense: More than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology, 49*, 317–343.

Robinson, G. E., Hackett, K. J., Purcell-Miramontes, M., Brown, S. J., Evans, J. D., Goldsmith, M. R., Lawson, D., Okamuro, J., Robertson, H. M., & Schneider, D. J. (2011). Creating a buzz about insect genomes. *Science, 331*, 1386.

Rodriguez, P. A., Stam, R., Warbroek, T., & Bos, J. I. B. (2014). Mp10 and Mp42 from the aphid species *Myzus persicae* trigger plant defenses in *Nicotiana benthamiana* through different activities. *Molecular Plant-Microbe Interactions, 27*, 30–39.

Roessner, U., Willmitzer, L., & Fernie, A. R. (2002). Metabolic profiling and biochemical phenotyping of plant systems. *Plant Cell Reports, 21*, 189–196.

Rowe, H. C., Hansen, B. G., Halkier, B. A., & Kliebenstein, D. J. (2008). Biochemical networks and epistasis shape the *Arabidopsis thaliana* metabolome. *Plant Cell, 20*, 1199–1216.

Saito, N., Ohashi, Y., Soga, T., & Tomita, M. (2010). Unveiling cellular biochemical reactions via metabolomics-driven approaches. *Current Opinion in Microbiology, 13*, 358–362.

Salvador-Recatala, V., Tjallingii, W. F., & Farmer, E. E. (2014). Real-time, in vivo intracellular recordings of caterpillar-induced depolarization waves in sieve elements using aphid electrodes. *The New Phytologist, 203*, 674–684.

Schmelz, E. A., Engelberth, J., Alborn, H. T., Tumlinson, J. H., & Teal, P. E. A. (2009). Phytohormone-based activity mapping of insect herbivore-produced elicitors. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 653–657.

Schmid, M., Sieber, R., Zimmermann, Y. S., & Vorburger, C. (2012). Development, specificity and sublethal effects of symbiont-conferred resistance to parasitoids in aphids. *Functional Ecology, 26*, 207–215.

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y., & Ishikawa, H. (2000). Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature, 407*, 81–86.

Sloan, D. B., & Moran, N. A. (2012). Genome reduction and co-evolution between the primary and secondary bacterial symbionts of psyllids. *Molecular Biology and Evolution, 29*, 3781–3792.

Sloan, D. B., Nakabachi, A., Richards, S., Qu, J., Murali, S. C., Gibbs, R. A., & Moran, N. A. (2014). Parallel histories of horizontal gene transfer facilitated extreme reduction of endosymbiont genomes in sap-feeding insects. *Molecular Biology and Evolution, 31*, 857–871.

Smith, C. M., & Clement, S. L. (2012). Molecular bases of plant resistance to arthropods. *Annual Review of Entomology, 57*, 309–328.

Smith, C. D., Zimin, A., Holt, C., Abouheif, E., Benton, R., Cash, E., Croset, V., Currie, C. R., Elhaik, E., Elsik, C. G., Fave, M.-J., Fernandes, V., Gadau, J., Gibson, J. D., Graur, D., Grubbs, K. J., Hagen, D. E., Helmkampf, M., Holley, J.-A., Hu, H., Viniegra, A. S. I., Johnson, B. R., Johnson, R. M., Khila, A., Kim, J. W., Laird, J., Mathis, K. A., Moeller, J. A., Muñoz-Torres, M. C., Murphy, M. C., Nakamura, R., Nigam, S., Overson, R. P., Placek, J. E., Rajakumar, R., Reese, J. T., Robertson, H. M., Smith, C. R., Suarez, A. V., Suen, G., Suhr, E. L., Tao, S., Torres, C. W., van Wilgenburg, E., Viljakainen, L., Walden, K. K. O., Wild, A. L., Yandell, M., Yorke, J. A., & Tsutsui, N. D. (2011a). Draft genome of the globally widespread and invasive Argentine ant (*Linepithema humile*). *Proceedings of the National Academy of Sciences of the United States of America, 108*, 5673–5678.

Smith, C. R., Smith, C. D., Robertson, H. M., Helmkampf, M., Zimin, A., Yandell, M., Holt, C., Hu, H., Abouheif, E., Benton, R., Cash, E., Croset, V., Currie, C. R., Elhaik, E., Elsik, C. G., Favé, M.-J., Fernandes, V., Gibson, J. D., Graur, D., Gronenberg, W., Grubbs, K. J., Hagen, D. E., Viniegra, A. S. I., Johnson, B. R., Johnson, R. M., Khila, A., Kim, J. W., Mathis, K. A., Munoz-Torres, M. C., Murphy, M. C., Mustard, J. A., Nakamura, R., Niehuis, O., Nigam, S., Overson, R. P., Placek, J. E., Rajakumar, R., Reese, J. T., Suen, G., Tao, S., Torres, C. W., Tsutsui, N. D., Viljakainen, L., Wolschin, F., & Gadau, J. (2011b). Draft genome of the red harvester ant *Pogonomyrmex barbatus. Proceedings of the National Academy of Sciences of the United States of America, 108*, 5667–5672.

Stahlberg, R., & Cosgrove, D. J. (1992). Rapid alterations in growth-rate and electrical potentials upon stem excision in pea seedlings. *Planta, 187*, 523–531.

Stahlberg, R., & Cosgrove, D. J. (1997). The propagation of slow wave potentials in pea epicotyls. *Plant Physiology, 113*, 209–217.

Stark, A., Lin, M. F., Kheradpour, P., Pedersen, J. S., Parts, L., Carlson, J. W., Crosby, M. A., Rasmussen, M. D., Roy, S., Deoras, A. N., Ruby, J. G., Brennecke, J., Hodges, E., Hinrichs, A. S., Caspi, A., Park, S.-W., Han, M. V., Maeder, M. L., Polansky, B. J., Robson, B. E., Aerts, S., van Helden, J., Hassan, B., Gilbert, D. G., Eastman, D. A., Rice, M., Weir, M., Hahn, M. W., Park, Y., Dewey, C. N., Pachter, L., Kent, W. J., Haussler, D., Lai, E. C., Bartel, D. P., Hannon, G. J., Kaufman, T. C., Eisen, M. B., Clark, A. G., Smith, D., Celniker, S. E., Gelbart, W. M., & Kellis, M. (2007). Discovery of functional elements in 12 *Drosophila* genomes using evolutionary signatures. *Nature, 450*, 219–232.

Steinbrenner, A. D., Gomez, S., Osorio, S., Fernie, A. R., & Orians, C. M. (2011). Herbivore-induced changes in tomato (*Solanum lycopersicum*) primary metabolism: A whole plant perspective. *Journal of Chemical Ecology, 37*, 1294–1303.

Strong, D. R., Lawton, J. H., & Southwood, T. R. E. (1984). *Insects on plants: Community patterns and mechanisms*. Cambridge, MA: Harvard University Press.

Su, Q., Oliver, K. M., Pan, H., Jiao, X., Liu, B., Xie, W., Wang, S., Wu, Q., Xu, B., White, J. A., Zhou, X., & Zhang, Y. (2013). Facultative symbiont Hamiltonella confers benefits to *Bemisia tabaci* (Hemiptera: Aleyrodidae), an invasive agricultural pest worldwide. *Environmental Entomology, 42*, 1265–1271.

Suen, G., Teiling, C., Li, L., Holt, C., Abouheif, E., Bornberg-Bauer, E., Bouffard, P., Caldera, E. J., Cash, E., Cavanaugh, A., Denas, O., Elhaik, E., Favé, M.-J., Gadau, J., Gibson, J. D., Graur, D., Grubbs, K. J., Hagen, D. E., Harkins, T. T., Helmkampf, M., Hu, H., Johnson, B. R., Kim, J., Marsh, S. E., Moeller, J. A., Muñoz-Torres, M. C., Murphy, M. C., Naughton, M. C., Nigam, S., Overson, R., Rajakumar, R., Reese, J. T., Scott, J. J., Smith, C. R., Tao, S., Tsutsui, N. D., Viljakainen, L., Wissler, L., Yandell, M. D., Zimmer, F., Taylor, J., Slater, S. C., Clifton, S. W., Warren, W. C., Elsik, C. G., Smith, C. D., Weinstock, G. M., Gerardo, N. M., & Currie, C. R. (2011). The genome sequence of the leaf-cutter ant *Atta cephalotes* reveals insights into its obligate symbiotic lifestyle. *PLoS Genetics, 7*, e1002007.

Sugimoto, K., Matsui, K., Iijima, Y., Akakabe, Y., Muramoto, S., Ozawa, R., Uefune, M., Sasaki, R., Alamgir, K. M., Akitake, S., Nobuke, T., Galis, I., Aoki, K., Shibata, D., & Takabayashi,

J. (2014). Intake and transformation to a glycoside of (Z)-3-hexenol from infested neighbors reveals a mode of plant odor reception and defense. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 7144–7149.

Sumner, L. W., Lei, Z., Nikolau, B. J., & Saito, K. (2015). Modern plant metabolomics: Advanced natural product gene discoveries, improved technologies, and future prospects. *Natural Product Reports, 32*, 212–229.

Tay, W. T., Evans, G. A., Boykin, L. M., & De Barro, P. J. (2012). Will the real *Bemisia tabaci* please stand up? *PloS One, 7*, e50550.

The Honeybee Genome Sequencing Consortium. (2006). Insights into social insects from the genome of the honeybee *Apis mellifera. Nature, 443*, 931–949.

The International Aphid Genomics Consortium. (2010). Genome sequence of the pea aphid *Acyrthosiphon pisum. PLoS Biology, 8*, e1000313.

The International Silkworm Genome Consortium. (2008). The genome of a lepidopteran model insect, the silkworm *Bombyx mori. Insect Biochemistry and Molecular Biology, 38*, 1036–1045.

Tribolium Genome Sequencing Consortium. (2008). The genome of the model beetle and pest *Tribolium castaneum. Nature, 452*, 949–955.

Van Bel, A. J. E., & Van Der Schoot, C. (1988). Primary function of the protective layer in contact cells buffer against oscillations in hydrostatic pressure in the vessels? *International Association of Wood Anatomists Bull, 9*, 285–288.

van Bel, A. J. E., Furch, A. C. U., Will, T., Buxa, S. V., Musetti, R., & Hafke, J. B. (2014). Spread the news: Systemic dissemination and local impact of Ca^{2+} signals along the phloem pathway. *Journal of Experimental Botany, 65*, 1761–1787.

Vandermoten, S., Harmel, N., Mazzucchelli, G., De Pauw, E., Haubruge, E., & Francis, F. (2014). Comparative analyses of salivary proteins from three aphid species. *Insect Molecular Biology, 23*, 67–77.

Verberk, W. C. E. P., Sommer, U., Davidson, R. L., & Viant, M. R. (2013). Anaerobic metabolism at thermal extremes: A metabolomic test of the oxygen limitation hypothesis in an aquatic insect. *Integrative and Comparative Biology, 53*, 609–619.

Verhage, A., Vlaardingerbroek, I., Raaymakers, C., Van Dam, N. M., Dicke, M., Van Wees, S. C. M., & Pieterse, C. M. J. (2011). Rewiring of the jasmonate signaling pathway in Arabidopsis during insect herbivory. *Frontiers in Plant Science, 2*, article 47:1–12. doi:10.3389/fpls.2011.00047.

Vijay, N., Poelstra, J. W., Kunstner, A., & Wolf, J. B. (2013). Challenges and strategies in transcriptome assembly and differential gene expression quantification. A comprehensive in silico assessment of RNA-seq experiments. *Molecular Ecology, 22*, 620–634.

Vorburger, C., Gehrer, L., & Rodriguez, P. (2010). A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biology Letters, 6*, 109–111.

Vos, I. A., Pieterse, C. M. J., & van Wees, S. C. M. (2013). Costs and benefits of hormone-regulated plant defences. *Plant Pathology, 62*, 43–55.

Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V. M., O'Neal, S. D., & Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management, 2*, G1–G7.

Wang, Y., Carolan, J. C., Hao, F., Nicholson, J. K., Wilkinson, T. L., & Douglas, A. E. (2010). Integrated metabonomic-proteomic analysis of an insect-bacterial symbiotic system. *Journal of Proteome Research, 9*, 1257–1267.

Wang, X., Fang, X., Yang, P., Jiang, X., Jiang, F., Zhao, D., Li, B., Cui, F., Wei, J., Ma, C., Wang, Y., He, J., Luo, Y., Wang, Z., Guo, X., Guo, W., Wang, X., Zhang, Y., Yang, M., Hao, S., Chen, B., Ma, Z., Yu, D., Xiong, Z., Zhu, Y., Fan, D., Han, L., Wang, B., Chen, Y., Wang, J., Yang, L., Zhao, W., Feng, Y., Chen, G., Lian, J., Li, Q., Huang, Z., Yao, X., Lv, N., Zhang, G., Li, Y., Wang, J., Wang, J., Zhu, B., & Kang, L. (2014). The locust genome provides insight into swarm formation and long-distance flight. *Nature Communications, 5*, 2957.

Waters, L. S., & Storz, G. (2009). Regulatory RNAs in bacteria. *Cell, 136*, 615–628.
Wentzell, A. M., Rowe, H. C., Hansen, B. G., Ticconi, C., Halkier, B. A., & Kliebenstein, D. J. (2007). Linking metabolic QTLs with network and cis-eQTLs controlling biosynthetic pathways. *PLoS Genetics, 3*, 1687–1701.
Werren, J. H., Richards, S., Desjardins, C. A., Niehuis, O., Gadau, J., Colbourne, J. K. & Group TNGW. (2010). Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science, 327*, 343–348.
White, B. J., Collins, F. H., & Besansky, N. J. (2011). Evolution of *Anopheles gambiae* in relation to humans and malaria. *Annual Review of Ecology, Evolution, and Systematics, 42*, 111–132.
Wilcox, J. L., Dunbar, H. E., Wolfinger, R. D., & Moran, N. A. (2003). Consequences of reductive evolution for gene expression in an obligate endosymbiont. *Molecular Microbiology, 48*, 1491–1500.
Wildon, D. C., Thain, J. F., Minchin, P. E. H., Gubb, I. R., Reilly, A. J., Skipper, Y. D., Doherty, H. M., Odonnell, P. J., & Bowles, D. J. (1992). Electrical signaling and systemic proteinase inhibitor induction in the wounded plant. *Nature, 360*, 62–65.
Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry, 64*, 3–19.
Witzgall, P., Kirsch, P., & Cork, A. (2010). Sex pheromones and their impact on pest management. *Journal of Chemical Ecology, 36*, 80–100.
Wolfender, J. L., Rudaz, S., Choi, Y. H., & Kim, H. K. (2013). Plant metabolomics: From holistic data to relevant biomarkers. *Current Medicinal Chemistry, 20*, 1056–1090.
Wu, D., Daugherty, S. C., Van Aken, S. E., Pai, G. H., Watkins, K. L., Khouri, H., Tallon, L. J., Zaborsky, J. M., Dunbar, H. E., Tran, P. L., Moran, N. A., & Eisen, J. A. (2006). Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS Biology, 4*, 1079–1092.
Wu, R., Wu, Z., Wang, X., Yang, P., Yu, D., Zhao, C., Xu, G., & Kang, L. (2012). Metabolomic analysis reveals that carnitines are key regulatory metabolites in phase transition of the locusts. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 3259–3263.
Wurm, Y., Wang, J., Riba-Grognuz, O., Corona, M., Nygaard, S., Hunt, B. G., Ingram, K. K., Falquet, L., Nipitwattanaphon, M., Gotzek, D., Dijkstra, M. B., Oettler, J., Comtesse, F., Shih, C.-J., Wu, W.-J., Yang, C.-C., Thomas, J., Beaudoing, E., Pradervand, S., Flegel, V., Cook, E. D., Fabbretti, R., Stockinger, H., Long, L., Farmerie, W. G., Oakey, J., Boomsma, J. J., Pamilo, P., Yi, S. V., Heinze, J., Goodisman, M. A. D., Farinelli, L., Harshman, K., Hulo, N., Cerutti, L., Xenarios, I., Shoemaker, D., & Keller, L. (2011). The genome of the fire ant *Solenopsis invicta. Proceedings of the National Academy of Sciences of the United States of America, 108*, 5679–5684.
Xie, Y., Wu, G., Tang, J., Luo, R., Patterson, J., Liu, S., Huang, W., He, G., Gu, S., Li, S., Zhou, X., Lam, T. W., Li, Y., Xu, X., Wong, G. K., & Wang, J. (2014). SOAPdenovo-Trans: De novo transcriptome assembly with short RNA-Seq reads. *Bioinformatics, 30*, 1660–1666.
Xue, J., Zhou, X., Zhang, C.-X., Yu, L.-L., Fan, H.-W., Wang, Z., Xu, H.-J., Xi, Y., Zhu, Z.-R., Zhou, W.-W., Pan, P.-L., Li, B.-L., Colbourne, J., Noda, H., Suetsugu, Y., Kobayashi, T., Zheng, Y., Liu, S., Zhang, R., Liu, Y., Luo, Y.-D., Fang, D.-M., Chen, Y., Zhan, D.-L., Lv, X.-D., Cai, Y., Wang, Z.-B., Huang, H.-J., Cheng, R.-L., & Zhang, X.-C. (2014). Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. *Genome Biology, 15*, 521.
Yin, C., Liu, Y., Liu, J., Xiao, H., Huang, S., Lin, Y., Han, Z., & Li, F. (2014). ChiloDB: A genomic and transcriptome database for an important rice insect pest Chilo suppressalis. *Database, 2014*: article ID bau065; doi:10.1093/database/bau065.
Yonekura-Sakakibara, K., Fukushima, A., & Saito, K. (2013). Transcriptome data modeling for targeted plant metabolic engineering. *Current Opinion in Biotechnology, 24*, 285–290.
You, M., Yue, Z., He, W., Yang, X., Yang, G., Xie, M., Zhan, D., Baxter, S. W., Vasseur, L., Gurr, G. M., Douglas, C. J., Bai, J., Wang, P., Cui, K., Huang, S., Li, X., Zhou, Q., Wu, Z., Chen, Q.,

Liu, C., Wang, B., Li, X., Xu, X., Lu, C., Hu, M., Davey, J. W., Smith, S. M., Chen, M., Xia, X., Tang, W., Ke, F., Zheng, D., Hu, Y., Song, F., You, Y., Ma, X., Peng, L., Zheng, Y., Liang, Y., Chen, Y., Yu, L., Zhang, Y., Liu, Y., Li, G., Fang, L., Li, J., Zhou, X., Luo, Y., Gou, C., Wang, J., Wang, J., Yang, H., & Wang, J. (2013). A heterozygous moth genome provides insights into herbivory and detoxification. *Nature Genetics, 45*, 220–225.

Zhan, S., Merlin, C., Boore, J. L., & Reppert, S. M. (2011). The monarch butterfly genome yields insights into long-distance migration. *Cell, 147*, 1171–1185.

Zhan, S., Zhang, W., Niitepold, K., Hsu, J., Haeger, J. F., Zalucki, M. P., Altizer, S., de Roode, J. C., Reppert, S. M., & Kronforst, M. R. (2014). The genetics of monarch butterfly migration and warning colouration. *Nature, 514*, 317–321.

Zhang, F., Dossey, A. T., Zachariah, C., Edison, A. S., & Bruschweiler, R. (2007). Strategy for automated analysis of dynamic metabolic mixtures by NMR. Application to an insect venom. *Analytical Chemistry, 79*, 7748–7752.

Zhang, Q., Lu, Y.-X., & Xu, W.-H. (2012). Integrated proteomic and metabolomic analysis of larval brain associated with diapause induction and preparation in the cotton bollworm, *Helicoverpa armigera*. *Journal of Proteome Research, 11*, 1042–1053.

Zipfel, C. (2014). Plant pattern-recognition receptors. *Trends in Immunology, 35*, 345–351.

Proteomic Insights into the Hidden World of Phloem Sap Feeding

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Abstract The physical interface between a phloem-feeding insect and its host plant is a single cell buried deep within the plant tissue. As such, the molecular interactions between these notorious agricultural pests and the crop plants upon which they feed are difficult to study. 'Omic' technologies have proved crucial in revealing some of the fascinating detail of the molecular interplay between these partners. Here we review the role of proteomics in identifying putative components of the secreted saliva of phloem-feeding insects, particularly aphids, and discuss the limited knowledge concerning the function of these proteins.

1 Overview

Phloem feeding insects represent a guild of agricultural pests that are notoriously difficult to study and even harder to control (van Emden and Harrington 2007). Much of the problem lies in the location of the feeding site since the physical interface between the insect and plant is a single sieve element cell within the phloem bundle buried deep in the leaf. As a consequence, ingestion of the diet cannot be observed directly as is possible in most chewing insects. Several approaches have been developed over the last few decades to address these issues and some notable achievements include the electrical penetration graph and the use of non-persistent plant viruses to determine the sequence of feeding behaviours between plant surface penetration and phloem sap ingestion (see Powell et al. 2006 for full review).

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However much of the mechanistic detail involved in the location, acquisition and preparation of the feeding site remain obscure.

Advances in 'omics' technologies have improved the identification of putative genes and proteins with a direct involvement in the insect-plant interaction. Here, we review advances made by proteomic technologies in understanding the molecular interplay between phloem-feeding insects and plants, focussing on the proteins that have been identified as being secreted in the saliva. Much of the evidence comes from studies involving aphids, since this group of insects has extensive genomic and, to a lesser extent, proteomic resources available in the public domain coupled with a long history of agronomic research aimed at improving control strategies.

2 Feeding Behaviour

Phloem-feeding insects such as aphids, whiteflies and leafhoppers cause direct damage by removing photoassimilates and by the transmission of a variety of plant viruses. Host plant location and acceptance involves many steps that must occur in the correct sequence before feeding can commence. Initial plant recognition is mediated by the antennae and the mouthparts. Aphid antennae bear many sensilla which are used in chemoreception and the perception of the leaf surface (Bromley and Anderson 1982) and tactile receptors on the tip of the proboscis respond to contact and surface texture and enable aphids to detect the contours of leaf veins, their preferred feeding site (Tjallingii 1978; Powell et al. 2006).

Once a plant has been accepted, the aphid will settle and initiate penetration to the sieve element. Aphids feed from a single phloem cell within the sieve element and can continuously imbibe phloem sap for prolonged periods (Tjallingii 1995). The mouthparts are modified as piercing stylets formed by the paired mandibles and maxillae (Pollard 1973) that come together to form two distinct channels: (1) a salivary canal that transports saliva into the plant and (2) a food canal, through which phloem sap is ingested. The food canal has a larger diameter (0.7 μm) than the salivary canal (0.3 μm) (Ponsen 1987). Plant penetration can be monitored using the electrical penetration graph (EPG; McLean and Kinsey 1965; Tjallingii 2006) that allows the recording of signal waveforms reflecting different insect activities and locations of the stylet tips. Perhaps unsurprisingly there is a direct relationship between the length of the stylets and the depth of the phloem tissue within the host plant, but despite differences in insect size and the internal architecture of the host plant the mechanism of penetration appears to be similar across aphid groups – the stylets move intercellularly along and within cell walls without directly passing through any cell, to form a convoluted stylet track (Fig. 1). However, the aphid appears to taste and reject the contents of many cells as the stylet tips journey towards the sieve element (Tjallingii and Esch 1993).

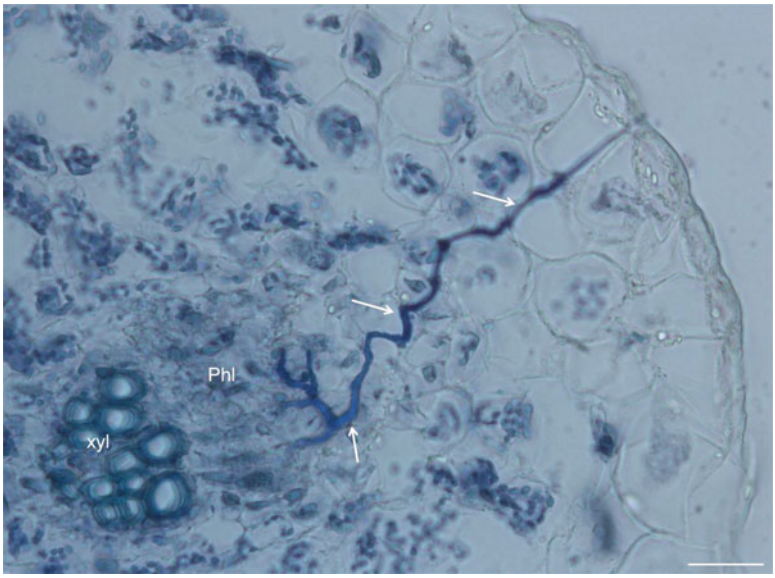

Fig. 1 Hand-cut section of broad bean *Vicia faba* cv. The Sutton showing salivary sheath remaining in the plant following feeding by the pea aphid *Acyrthosiphon pisum. Phl* phloem, *xyl* xylem, *arrows* sheath material. Scale bar = 100 μm

3 Recognition of the Sieve Tube

The ability to detect and find sieve tubes suggests that phloem feeding insects receive cues for sieve-tube recognition along the stylet route perhaps by sampling cells. Specific conditions such as pH, sugar species and concentration, viscosity, and oxygen pressure may enable sieve-tube detection (Hewer et al. 2010). In early experiments, sucrose was determined as the most suitable carbohydrate substrate for aphid growth and reproduction, leading to the suggestion that sucrose is an important cue for aphid orientation (Auclair 1963, 1969; Mittler and Dadd 1964). However, recent experiments using artificial choice-chamber systems have suggested that pH is also an important orientation cue used by aphids to locate the sieve element (Hewer et al. 2010). Different aphid species (*Megoura viciae, Myzus persicae, Rhopalosiphum padi* and *Macrosiphum euphorbiae*) showed a significant preference for sucrose at concentrations of 15 % and pH 7 (over a test range of pH 5–8) that matches the composition of the sieve-tube sap of their host plants (Hewer et al. 2010). However, further studies are warranted to determine the precise navigational cues employed by aphids and other phloem-feeders since this is an obvious potential target for disrupting feeding behaviour.

4 Aphid Salivation

The secretion of saliva prior to and during phloem feeding has attracted significant attention. Two types of saliva are secreted during feeding – gelling saliva and watery saliva (Miles 1999; Cherqui and Tjallingii 2000). Prior to stylet insertion, the aphid secretes a small amount of gelling saliva onto the plant surface. This is termed the salivary flange and hardens almost immediately, perhaps upon contact with oxygen in the air. The aphid inserts its stylet through this gel and begins to probe into the plant. An individual aphid may make several probes over an extended period of time before accepting a sieve element for sustained ingestion. As the stylets progress through the plant gelling saliva is continuously secreted which hardens to form a rigid, protective sheath around the stylet. The so-called salivary sheath remains in the plant after stylet withdrawal irrespective of whether the aphid has successfully acquired a sieve element or not (see Fig. 1). During probing activities, very small amounts of watery saliva are discharged when parenchymal cells are briefly punctured, followed by ingestion of minute amounts of cell sap, before any sieve elements are punctured (Prado and Tjallingii 1994; Martin et al. 1997). The frequency of the brief cell punctures (referred to as 'potential drops' in EPG traces) often increases immediately before the onset of sustained ingestion, again suggesting that the aphid is obtaining some kind of navigational cue from the cell contents. Once the sieve element is located the gelling saliva forms a seal around the site of insertion. The secretion of gelling saliva ceases at this point and the aphid begins to secrete watery saliva. The transition between the secretion of gelling and watery saliva appears to be instantaneous but the mechanism controlling the switch in consistency and perhaps composition is unclear. The ingestion of phloem sap into the food canal is resisted initially and watery saliva is released into the contents of the sieve tube for approximately 5 min (Miles 1999). Thereafter, during ingestion, there is a continual secretion of watery saliva but, since the end of the salivary canal is a short distance behind the tip of the stylets, the saliva is ingested with the sieve tube sap under positive turgor pressure (Tjallingii 1995). Regulation of phloem sap intake is thought to be controlled by the precibarial valve at the opening of the anterior gut (Pollard 1973), but again this aspect of aphid feeding is poorly understood.

5 Composition of Aphid Saliva

From the previous discussion of the mechanistic aspects of aphid feeding it is clear that the salivary secretions play a crucial role in the aphid-plant interaction, not least because they represent a significant investment of resources by the insect. However, before realistic hypotheses concerning the function of salivary secretions can be formulated, a thorough understanding of the composition of the different types of saliva is required, and it is here that modern analytical techniques, particularly proteomics, have had a significant impact.

Aphid saliva is an aqueous solution containing amino acids, haemolymph components (including proteins, see below) and proteins derived from the salivary glands (Miles 1999). Early studies in the literature adopted substrate-specific enzyme assays to determine bioactive components of saliva, but these are limited since they can only detect, by their very nature, those proteins that interact with the substrate. Nevertheless, these methods provided early recognition of the importance of bioactive molecules in the aphid-plant interaction. The salivary components detected by these methods can be divided into two broad categories: (i) hydrolases (pectinases, cellulases, oligosaccharases); and (ii) oxidation/reduction enzymes (phenol oxidase and peroxidases) (Miles 1999; Campbell and Dreyer 1985, 1990; Peng and Miles 1988; Madhusudhan and Miles 1998). The roles of most of these enzymes during aphid penetration and feeding are not well understood. The salivary sheath apparently contains proteins with active sulphydryl groups and it exhibits enzyme activity, including phenoloxidases and peroxidases, while, in addition, oxidases and pectinases have been reported in the watery saliva (Miles 1999). Aphids are able to access the chemical nature of the matrix polysaccharides in the cell wall and cell components of plants and would seem to do so by action of the hydrolytic enzymes that occur in the saliva (Miles 1999).

In the last decade our understanding of the complexities of aphid saliva has increased with the use of mass spectrometry and proteomics to identify salivary proteins. These techniques provide positive identities based on sequence homology with publicly available databases, and can detect novel proteins. The approach is therefore more comprehensive since it does not rely on a single substrate to detect the presence of a bioactive protein. However, in the absence of species-specific sequence information (either as genomic or transcriptomic data) only highly conserved proteins can be detected. Significant advances in the ability to identify aphid salivary proteins was achieved following the publication of the complete genome sequence of the pea aphid *Acyrthosiphon pisum* (International Aphid Genomics Consortium 2010) and further advances can be expected as more genomic resources become available for other phloem-feeding insects.

A detailed proteomic analysis of saliva secreted by the pea aphid *Acyrthosiphon pisum* showed the presence of nine proteins following GE-LC-MS/MS and LC-MS/MS, with reference to expressed sequence tags (EST) and genomic sequence data for *A. pisum* (Carolan et al. 2009). Four proteins were identified by sequence homology: an M2 metalloprotease (a homolog of angiotensin-converting enzyme); an M1 zinc-dependant metalloprotease; a glucose-methanol-choline (GMC)-oxidoreductase; and a homolog to regucalcin (a putative calcium-binding protein). The other five proteins were not homologous to any previously described sequence and included an abundant salivary protein (ACYPI009881, see below) with a putative role in the formation of the salivary sheath (based on its amino acid composition). The metalloproteases and regucalcin were predicted to be directly involved in maintenance of sustained feeding through the inactivation of plant protein defences and inhibition of calcium-mediated occlusion of phloem sieve elements, respectively, and the oxidoreductase may promote gelling of the sheath protein or mediate oxidative detoxification of plant allelochemicals (Carolan et al. 2009).

A multi-approach experiment based on both in-solution and in-gel (after 2D gel electrophoresis) protein digestion and complementary mass spectrometry techniques was used to investigate the salivary proteome of *Myzus persicae* (Harmel et al. 2008). Some proteins were identified with a known function in other insects, while others were related to aphid expressed sequence tag (EST) sequences from specific tissue locations (i.e. head and/or salivary glands).

The secreted saliva of the vetch aphid *Megoura viciae* (Will et al. 2007) comprised at least 29 proteins ranging in weight from 20 to 170 kDa when analysed using one dimensional gel electrophoresis. Although no protein identities were obtained, a conformational change in two proteins at 40 and 43 kDa when the gel was incubated in the presence of free calcium suggests that these proteins may play an important role in preventing formation of protein plugs (so-called forisomes) in Fabaceae (Will et al. 2007; see Will et al. 2012 for review). Interestingly, these proteins have a similar molecular weight to the regucalcin identified in the saliva of *A. pisum* (Carolan et al. 2009).

As our understanding of the detailed composition of aphid saliva increases, comparative analysis of saliva from different species or between 'biotypes' or clones of the same species is becoming possible. These studies have an applied implication since they reveal potential targets in pest species that might have an important role in future control strategies, but they can also reveal evolutionary links between feeding strategies, such as polyphagy and monophagy, and host plant choice including host plant alteration during the insect life cycle. As an example, a recent study (Rao et al. 2013) of secreted saliva from aphids that feed on cereals (colloquially referred to as 'cereal aphids', although the species concerned are not necessarily close phylogenetically) determined only three individual proteins that were also detected in the secreted salivary proteomes of *A. pisum* (Carolan et al. 2009, 2011) and *M. persicae* (Harmel et al. 2008) which feed on dicotyledonous plants. These common proteins (two paralogues from the GMC-oxidoreductase family referred to as glucose dehydrogenase or GLD, and the novel protein ACYPI009881) are both implicated in the formation of the salivary sheath and are discussed in more detail below.

The salivary sheath is a crucial structure common to a wide range of sap-feeding insects, including aphids, whiteflies and planthoppers (see Fig. 1). The abundance and amino-acid composition of the ACYPI009881 protein (Carolan et al. 2009) suggests that it may contribute to the sheath saliva. The conserved nature of this protein is significant given that the salivary sheath plays an important role in masking the presence of feeding aphids from plant defences, including preventing leakage of sieve element contents into the apoplast, a known trigger of plant defences (Tjallingii 2006; Will and van Bel 2006; Will et al. 2007). The hypothetical protein ACYPI009881 (referred to as sheath protein or SHP) was common to the cereal aphids *Sitobion avenae* and *Metopolophium dirhodum* and has previously been identified from the secreted saliva and salivary gland of *A. pisum* (Carolan et al. 2009, 2011) indicating that SHP may be common to a wide variety of aphid species. Immunoblotting using antibodies raised against SHP confirmed the presence of the protein in both secreted saliva and salivary gland extracts from *S. avenae* and *M. dirhodum*. In addition, SHP was localized to specific cell types within the salivary gland (Rao et al. 2013).

The other common proteins belong to the GMC-oxidoreductase family and these are the most frequently reported bioactive proteins in studies of aphid saliva detected either by substrate–specific assays (Madhusudhan and Miles 1998) or by direct identification using mass spectrometry (Harmel et al. 2008; Carolan et al. 2009; Nicholson et al. 2012; Rao et al. 2013; Vandermorten et al. 2014; Nicholson and Puterka 2014; see Table 1). GMC oxidoreductase in insect saliva in general has been implicated in the modification of plant defence mechanisms (Eichenseer et al. 1999; Musser et al. 2002; 2005), and in aphids specifically is speculated to be involved in the detoxification of noxious phytochemicals and in promoting the gelling of sheath saliva by enhancing disulphide bridge formation (Miles and Oertli 1993). GMC-oxidoreductase has been detected in the secreted saliva, but the protein does not originate in the salivary gland (Rao et al. 2013) and is most likely imported from the haemolymph. A model indicating the putative origin and routes of secretion of two common salivary proteins is suggested in Fig. 2. Interestingly, glucose dehydrogenase, another member of the GMC-oxidoreductase family, was also detected in the saliva of *Diuraphis noxia* and *Schizaphis graminum*, two pests of cereals in the USA that cause phytotoxic lesions following feeding (Nicholson et al. 2012; Nicholson and Puterka 2014). Glucose dehydrogenase was the only significant protein in the watery saliva of *S. graminum* in common with other aphid salivary proteomes (Nicholson and Puterka 2014), but analysis of the saliva from virulent and avirulent strains of this notorious pest suggested that the protein composition of the saliva might be an important factor in determining host plant responses to aphid feeding.

Evidence for the involvement of a third party in the composition of aphid saliva is slowly emerging. The presence in the saliva of the chaperonin GroEL with sequence homology matching to the primary endosymbiotic bacteria *Buchnera aphidicola* (Filichkin et al. 1997; Vandermorten et al. 2014; Chaudhary et al. 2014) suggests an intriguing role for the bacteria in supplying molecular patterns that can be recognized by plant defenses. A detailed analysis following artificial introduction of GroEL via either direct application or transfection demonstrated recognition by and activation of the plant immune response, and a negative impact on the performance of feeding aphids (Chaudhary et al. 2014). However, the direct involvement of *Buchnera*-derived GroEL in the priming of plant defenses requires further confirmation, and no study has detected a *Buchnera*-derived protein *in planta*.

All the secreted salivary proteomes reported to date have been obtained by analysis of proteins recovered from artificial liquid diets held between layers of stretched membrane. The recovery of sufficient quantities of secreted protein for analysis by mass spectrometry requires the concentration of large volumes of diet and multiple collections that are pooled into a single sample. There are obvious technical difficulties associated with this technique, including awareness of contamination and false positives, but the major drawback concerns the biological significance of the protein libraries. The diet system is by its very nature artificial and requires a phloem-feeding insect to actively rather than passively ingest (as is normally the case), and there is no consensus on the most appropriate composition of the diet from which to retrieve salivary proteins. Nevertheless, the identification of secreted proteins is a valuable first step towards more detailed functional studies, including the identification of salivary proteins *in planta*, which will provide a deeper

Table 1 Publications using mass spectrometry to identify secreted salivary proteins from aphids

Reference	Aphid species	Technique	Number of identified proteins	Selected key proteins implicated in the molecular insect-plant interaction
Harmel et al. (2008)	*Myzus persicae*	In gel LC-MS/MS	14 (plus several peptides matched to ESTs)	Glucose oxidase, glucose dehydrogenase, NADH dehydrogenase, α-glucosidase, α-amylase
		In solution LC-MS/MS		
Carolan et al. (2009)	*Acyrthosiphon pisum*	In gel LC-MS/MS	9 (5 with no known function)	M1 metalloprotease, M2 metalloprotease, GMC-oxidoreductase, regucalcin, putative sheath protein (SHP)
		In solution LC-MS/MS		
Nicholson et al. (2012)	*Diuraphis noxia*	In solution LC-MS/MS	34	Glucose dehydrogenase, lipophorin, chitinase, CiV16.8g1-like, lava lamp
Rao et al. (2013)	*Metopolophium dirhodum, Sitobion avenae*	In gel LC-MS/MS	19 (2 with no known function)	Putative sheath protein (SHP), GMC-oxidoreductase, trehalase, peroxidase, β-galactosidase
Vandermorten et al. (2014)	*Acyrthosiphon pisum*	In solution LC-MS/MS	14	Oxidoreductases, peptidases, lipid-binding proteins, ATP-binding proteins
	Megoura viciae	In gel MALDI-TOF MS	61	
	Myzus persicae			
Nicholson and Puterka (2014)	*Schizaphis graminum*	In solution LC-MS/MS	32 (9 with no known function)	Glucose dehydrogenase, lipophorin, complementary sex determiner, carbonic anhydrase
Chaudhary et al. (2014)	*Macrosiphum euphorbiae*	In solution LC-MS/MS	94 (62 with no known function)	GroEL (*Buchnera*), glucose dehydrogenase, trehalase, C002 (putative elicitor), putative sheath protein (SHP)

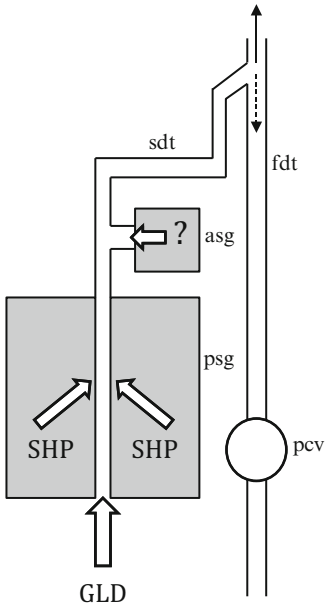

Fig. 2 Schematic representation of the origin and secretion of salivary proteins by phloem-feeding aphids. Only one half of the paired salivary gland is shown (*shaded*). *Blocked arrows* indicate origin of salivary proteins; *line arrows* indicate direction of saliva flow with *solid arrow* if pre-cibarial valve is closed (salivation into the plant) and *dashed arrow* if pre-cibarial valve is open (during ingestion). The contribution of the accessory gland to the secreted saliva remains unclear. *SHP* putative sheath protein (ACYPI009881), *GLD* glucose dehydrogenase, *asg* accessory salivary gland, *psg* principal salivary gland, *fdt* food duct, *sdt* salivary duct, *pcv* pre-cibarial valve

understanding of the role of the saliva in the insect-plant interaction. The limited numbers of publications that have adopted mass spectrometry to identify secreted salivary proteins from aphids are detailed in Table 1.

6 Importance of the Salivary Gland

The origin of the secreted saliva (i.e. the material of aphid origin that enters the plant prior to and during ingestion) is the salivary gland. In aphids, the glands are paired and consist of two principal glands and two accessory glands located between the head and pro-thorax. A large, bi-lobed principal gland joins with a smaller accessory gland to form one half of the gland and the two sides unite through the common salivary duct that leads to the mouthparts (Ponsen 1972). A similar distinction between accessory and principal glands is seen in other phloem feeding insects, although the principal gland may be structurally more complicated. As an example,

the principal glands of leafhoppers have multiple lobes each consisting of distinct groups of follicle cells (Sogawa 1965). The distinction between principal and accessory gland is largely based on size and morphology, with no direct evidence of the functional significance of each tissue. However, the accessory gland has been implicated as the conduit for plant virus accession to the saliva (Gildow and Gray 1993), perhaps in conjunction with the influx of water, and consequently further investigation is warranted. The importance of the principal gland in the production of at least some of the salivary proteins has already been highlighted, but it also appears to act as a conduit for the introduction of components from the haemolymph that could be derived from a variety of different tissues, including the fat body. As a consequence, putative libraries of salivary proteins derived from analysis of the salivary gland alone could be misleading.

A dual transcriptomic-proteomic analysis of the salivary gland of *A. pisum* used a bioinformatics approach to select a suite of identified proteins that had the potential to be included in the secreted proteome (Carolan et al. 2011). Amongst these were a group of so-called effector proteins (i.e. proteins that alter host plant cell structure or function) based on their homology or similarity to pathogenesis- or parasitism-related effector proteins secreted by other plant pathogens, particularly plant pathogenic nematodes. In particular, proteins in aphid saliva could have homologous or analogous functions to the giant cell modifying proteins of plant parasitic nematodes (Carolan et al. 2011).

7 Conclusions

In contrast to feeding by leaf chewing insects, the damage to plants caused by phloem feeding insects such as aphids is largely hidden from view and difficult to study. Consequently, modern approaches to combat phloem feeding pests have lagged behind advances in targeting leaf chewing insects despite their economic impact and predicted increasing global importance. The damage inflicted by aphids can be directly related to their ability to bypass and/or overcome host plant defences and a detailed mechanistic understanding of the process at a molecular level could lead to novel control strategies, e.g. through the use of highly selective RNA interference (RNAi; Pitino et al. 2011). Aphids can puncture and feed from plants for long periods without inducing a plant wound response and it has long been suspected that components of aphid saliva play a critical role in preventing blockage of the feeding site and/or detection of the mouthparts by the plant (Miles 1999; Will and van Bel 2006), but a detailed understanding of the composition of aphid saliva has only recently emerged. On a wider scale, the ever increasing human population requires a parallel increase in crop yields, yet increasing concerns about environmental safety have led to more stringent restrictions in the use of insecticides particularly in Europe. In addition, insects are renowned for developing resistance against insecticides, which further reduces the effectiveness of this control measure in the long term. For these reasons it is of importance to understand the molecular mechanisms that mediate the interaction between aphids and their host plants so

that new methodologies can be developed that reduce agrochemical inputs. With the availability of more genomic and proteomic resources, the field will expand rapidly and will have increasing relevance to other phloem-feeding insects such as planthoppers and whiteflies.

References

Auclair, J. L. (1963). Aphid feeding and nutrition. *Annual Review of Entomology, 8*, 439.

Auclair, J. L. (1969). Nutrition of plant-sucking insects on chemically defined diets. *Entomologia Experimentalis Et Applicata, 12*, 623.

Bromley, A. K., & Anderson, M. (1982). An electrophysiological study of olfaction in the aphid Nasonovia ribis-nigri. *Entomologia Experimentalis et Applicata, 32*, 101–110.

Campbell, D. C., & Dreyer, D. L. (1985). Host-plant resistance of sorghum – Differential hydrolysis of sorghum pectic substances by polysacharases of greenbug biotypes (*Schizaphis graminum* Homoptera, Aphididae). *Archives of Insect Biochemistry and Physiology, 2*, 203–215.

Campbell, D. C., & Dreyer, D. L. (1990). The role of plant matrix polysaccharides in aphid-plant interactions. In R. K. Campbell & R. D. Eikenbary (Eds.), *Aphid-plant genotype interactions* (pp. 149–169). Amsterdam: Elsevier.

Carolan, J. C., Fitzroy, C. I., Ashton, P. D., Douglas, A. E., & Wilkinson, T. L. (2009). The secreted salivary proteome of the pea aphid Acyrthosiphon pisum characterised by mass spectrometry. *Proteomics, 9*, 2457–2467.

Carolan, J. C., Caragea, D., Reardon, K. T., Mutti, N. S., Dittmer, N., Pappan, K., Cui, F., Castaneto, M., Poulain, J., Dossat, C., Tagu, D., Reese, J. C., Reeck, G. R., Wilkinson, T. L., & Edwards, O. R. (2011). Predicted effector molecules in the salivary secretome of the pea aphid (*Acyrthosiphon pisum*): a dual transcriptomic/proteomic approach. *Journal of Proteome Research, 10*, 1505–1518.

Chaudhary, R., Atamian, H. S., Shen, Z., Briggs, S. P., & Kaloshian, I. (2014). GroEL from the endosymbiont *Buchnera aphidicola* betrays the aphid by triggering plant defense. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 8919–8924.

Cherqui, A., & Tjallingii, W. F. (2000). Salivary proteins of aphids, a pilot study on identification, separation and immunolocalisation. *Journal of Insect Physiology, 46*, 1177–1186.

Eichenseer, H., Mathews, M. C., Bi, J. L., Murphy, J. B., & Felton, G. W. (1999). Salivary glucose oxidase: Multifunctional roles for helicoverpa zea? *Archives of Insect Biochemistry and Physiology, 42*, 99–109.

Filichkin, S. A., Brumfield, S., Filichkin, T. P., & Young, M. J. (1997). *In vitro* interactions of the aphid endosymbiont SymL chaperonin with barley yellow dwarf virus. *Journal of Virology, 71*, 569–577.

Gildow, F. E., & Gray, S. M. (1993). The aphid salivary gland basal lamina as a selective barrier associated with vector-specific transmission of barley yellow dwarf luteoviruses. *Phytopathology, 83*, 1293–1302.

Harmel, N., Letocart, E., Cherqui, A., Giordanengo, P., Mazzucchelli, G., Guillonneau, F., DE Pauw, E., Haubruge, E., & Francis, F. (2008). Identification of aphid salivary proteins: A proteomic investigation of Myzus persicae. *Insect Molecular Biology, 17*, 165–174.

Hewer, A., Will, T., & van Bel, A. J. (2010). Plant cues for aphid navigation in vascular tissues. *The Journal of Experimental Biology, 213*, 4030–4042.

International Aphid Genomics Consortium. (2010). Genome sequence of the pea aphid *Acyrthosiphon pisum. PLoS Biology, 8*(2), e1000313. doi:10.1371/journal.pbio.1000313.

Madhusudhan, V. V., & Miles, P. W. (1998). Mobility of salivary components as a possible reason for differences in the responses of alfalfa to the spotted alfalfa aphid and pea aphid. *Entomologia Experimentalis Et Applicata, 86*, 25–39.

Martin, B., Collar, J. L., Tjallingii, W. F., & Fereres, A. (1997). Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *The Journal of General Virology, 78*(Pt 10), 2701–2705.

Mclean, D. L., & Kinsey, M. G. (1965). Identification of electrically recorded curve patterns associated with aphid salivation and ingestion. *Nature, 205*, 1130–1131.

Miles, P. W. (1999). Aphid saliva. *Biological Reviews, 74*, 41–85.

Miles, P. W., & Oertli, J. J. (1993). The significance of antioxidants in the aphid-plant interaction – The redox hypothesis. *Entomologia Experimentalis Et Applicata, 67*, 275–283.

Mittler, T. E., & Dadd, R. H. (1964). Gustatory discrimination between liquids by the aphid *Myzus persicae* (Sulzer). *Entomologia Experimentalis Et Applicata, 7*, 315–328.

Musser, R. O., Hum-Musser, S. M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J. B., & Felton, G. W. (2002). Herbivory: caterpillar saliva beats plant defences. *Nature, 416*, 599–600.

Musser, R. O., Cipollini, D. F., Hum-Musser, S. M., Williams, S. A., Brown, J. K., & Felton, G. W. (2005). Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. *Archives of Insect Biochemistry and Physiology, 58*, 128–137.

Nicholson, S. J., & Puterka, G. J. (2014). Variation in the salivary proteomes of differentially virulent greenbug (*Schizaphis graminum* Rondani) biotypes. *Journal of Proteomics, 105*, 186–203.

Nicholson, S. J., Hartson, S. D., & Puterka, G. J. (2012). Proteomic analysis of secreted saliva from Russian Wheat Aphid (*Diuraphis noxia* Kurd.) biotypes that differ in virulence to wheat. *Journal of Proteomics, 75*, 2252–2268.

Peng, Z., & Miles, P. W. (1988). Studies on the salivary physiology of plant bugs – Function of the catechol oxidase of the rose aphid. *Journal of Insect Physiology, 34*, 1027–1033.

Pitino, M., Coleman, A. D., Maffei, M. E., Ridout, C. J., & Hogenhout, S. A. (2011). Silencing of aphid genes by dsRNA feeding from plants. *PLoS One, 6*(10), e25709. doi:10.1371/journal.pone.0025709.

Pollard, D. G. (1973). Plant penetration by feeding aphids (Hemiptera, Aphidoidea). *Bulletin of Entomological Research, 62*, 631–714.

Ponsen, M. B. (1972). The site of potato leaf roll virus multiplication in its vector Myzus persicae, an anatomical study. *Meded Landbouwhogesch Wageningen, 16*, 1–147.

Ponsen, M. B. (1987). Alimentary tract. In A. K. Minks & P. Harrewijn (Eds.), *Aphids, their biology, natural enemies and control* (World crop pests, Vol. 2A, pp. 79–96). Amsterdam: Elsevier.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology, 51*, 309–330.

Prado, E., & Tjallingii, W. F. (1994). Aphid activities during sieve element punctures. *Entomologia Experimentalis Et Applicata, 72*, 157–165.

Rao, S. A. K., Carolan, J. C., & Wilkinson, T. L. (2013). Proteomic profiling of cereal aphid saliva reveals both ubiquitous and adaptive secreted proteins. *PLoS One, 8*(2), e57413. doi:10.1371/journal.pone.0057413.

Sogawa, K. (1965). Studies on the salivary glands of rice plant leafhoppers. I Morphology and histology. *Japanese Journal of Applied Entomology and Zoology, 9*, 275–304.

Tjallingii, W. F. (1978). Electronic recording of penetration behavior by aphids. *Entomologia Experimentalis Et Applicata, 24*, 721–730.

Tjallingii, W. F. (1995). *Regulation of phloem sap feeding by aphids. Regulatory mechanisms in insect feeding*. New York: Chapman and Hall.

Tjallingii, W. F. (2006). Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany, 57*, 739–745.

Tjallingii, W. F., & Esch, T. H. (1993). Fine-structure of aphid stylet routes in plant-tissues in correlation with EPG signals. *Physiological Entomology, 18*, 317–328.

van Emden, H. F., & Harrington, R. (2007). *Aphids as crop pests*. London/Wallingford: CABI.

Vandermorten, S., Harmel, N., Mazzucchelli, G., De Pauw, E., Haubrage, E., & Francis, F. (2014). Comparative analysis of salivary proteins from three aphid species. *Insect Molecular Biology, 23*, 67–77.

Will, T., & van Bel, A. J. (2006). Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany, 57*, 729–737.

Will, T., Tjallingii, W. F., Thonnessen, A., & van Bel, A. J. (2007). Molecular sabotage of plant defense by aphid saliva. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 10536–10541.

Will, T., Carolan, J. C., & Wilkinson, T. L. (2012). Breaching the sieve element – The role of saliva as the molecular interface between aphids and the phloem. In G. A. Thompson & A. J. E. van Bel (Eds.), *Phloem: molecular cell biology, systemic communication, biotic interactions*. London: Wiley.

Part II
Development and Regulation

Genomics of Phenotypic Plasticity in Aphids

Jenn A. Brisson, Julie Jaquiery, Fabrice Legeai, Gaël Le Trionnaire, and Denis Tagu

Abstract This chapter aims at explaining how the understanding of a complex adaptive trait (phenotypic plasticity) of insect pests (aphids) can gain from genomics approaches. Aphids adapt their capacity of dispersal to escape natural enemies or poor plant quality, as well as they adapt capacity to produce eggs or viviparous larvae upon the seasons. Genomes of aphids are now available, and post-genomics strategies (including quantitative genetics) allow the community to identify key regulatory gene circuits that govern the phenotypic adaptation of these insects to their changing environment.

1 Introduction: Being Adapted, Being Plastic

1.1 Genomics and Post-genomics

The description, understanding and prediction of how insects adapt to their environments are three important features that must be taken into account in order to contribute to plant protection against pests. This is particularly true today, when (i)

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global change is a new player in the fight between pests and plants, and (ii) genome studies are particularly amenable due to innovations and new technologies. We are more and more equipped to decipher the link between genotype, phenotype and environment by using large-scale experiments (*e.g.*, high throughput phenotyping and genotyping) in this era of "big data". These approaches still, however, require smart analyses to extract valuable knowledge.

Genomics is based on the acquisition of genome sequences, often imperfect but still very useful, which serve as a sort of "anatomy" of the genetic material that provides the basis for post-genomics studies, the same way that body anatomy provides the basis for physiology (Tagu et al. 2010). Post-genomic approaches can be developed in four areas for addressing environmental adaptations of organisms: phylogenomics, genome variation (or genetic diversity), microbiome, and genome functioning. Phylogenomics is used for studying the history of genomes during evolution and is useful for understanding the way traits (gene and protein functions) appeared or disappeared during evolution. Genome variation is largely used to understand the variation of traits within or between populations in relation to ecological and environmental patterns (The International HapMap Consortium 2007). Microbiome studies must be included in trait investigations since most multicellular eukaryotes (including insects) live in close or loose association with microorganisms; we are all biological and genomic chimeras. Microbes largely influence the phenotype of their hosts, and aphids are good examples: the primary symbiont *Buchenera aphidicola* is essential for nutrition of aphids, and a series of facultative symbionts can influence several diverse traits such as heat tolerance (*Serratia symbiotica*, *Rickettsia*), body color change (*Rickettsiella*), protection against parasitoids (*Hamiltonella parasitica*, *Regiella insecticola*) and fungi (*R. insecticola*), male killing (*Spiroplasma*), or host plant adaptation (*R. insecticola*) (reviewed in Oliver et al. 2010). Examining gene function at a whole genome scale is still challenging and has been developed for *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Homo sapiens* (Consortium et al. 2012; Gerstein et al. 2010; Roy et al. 2010); but we think that tackling the different DNA elements that are functional in a given genome is no longer restricted to model species (see Tagu et al. 2014a, b) and could be developed on aphids.

1.2 Aphids, Genomics and Adaptation

Here we will focus on aphids as an example of insect pests that are particularly efficient in adapting to their environment, both by genetic variation among populations and by developing alternative phenotypes as a short term (one generation) phenotypically plastic response. We will first describe the different genomic resources that have been developed in the last 5 years before giving examples of phenotypic plasticities for two main traits: the mode of reproduction and wing phenotype. We will also demonstrate the power of using quantitative genetic approaches to understand the mechanisms involved in phenotypic plasticity.

1.3 Phenotypic Plasticity in Insects of Agroecosystems

Phenotypic plasticity occurs in many different insect Orders and species, and we invite readers to refer to published reviews (Le Trionnaire et al. 2008; Ogawa and Miura 2014; Simpson et al. 2011). Phenotypic plasticity in insects concerns the appearance of morphological forms or morphs that gradiently respond to changing local environments (Davidowitz et al. 2004; Moraiti et al. 2014; Nijhout 2003) or are discrete (polyphenism). Here, we will concentrate on aphid polyphenisms, but we can mention the textbook-case of polyphenism in social insects with specialized morphs such as workers, foragers, soldiers, and reproducers (queens and drones) mainly found (but not exclusively) in Hymenoptera (Cronin et al. 2013). We can also mention the case of locusts that remarkably and quickly change morphology and behavior from solitary morphs to gregarious, migrating morphs (Wang and Kang 2014). These morphologically plastic phenotypes have strong correlations with insect behavior and thus are of agronomic import. For example, aphids attack plants and transmit plant viruses. The foraging behavior of bees is required for pollination, for pollen and nectar harvest, and together with workers, for honey production. Migratory morphs of crickets are responsible for drastic and quick damage to crops.

1.4 Polyphenism in Aphids

Aphids display phenotypic plasticity for four main traits. First, some species develop soldiers that defend colonies often protected in galls, formed by host plant tissues under the effect of aphids (Tian and Zhou 2014). These gall-forming aphids belong to phylogenetically basal groups of aphids. Second, many aphid species alternate host plants between autumn/winter and spring/summer, and the nature of host plants can vary from annual to perennial plants that often belong to different plant families. This requires nutritional adaptations that occur by phenotypic plasticity. In this review we will, however, concentrate on two other plastic traits for which genomic and post-genomic approaches have been applied: the reproductive and the dispersal polyphenisms. In these two cases, during embryonic development inside the mother's abdomen, external changes of the local environment trigger developmental fates within embryos that lead to the development of alternative phenotypes more adapted to the appropriate environment (Fig. 1). We discuss each of these two polyphenisms below.

Aphids can alternate between sexual and asexual reproduction during their annual life cycle, called cyclical parthenogenesis. It has been known since the mid-eighteenth century that aphid larvae are born by viviparity from their mother, and that this reproduction is independent of males. Viviparous females are thus parthenogenetic. In their two ovaries and many ovarioles they develop chains of embryos, all at different stages of development. The result is that one adult parthenogenetic

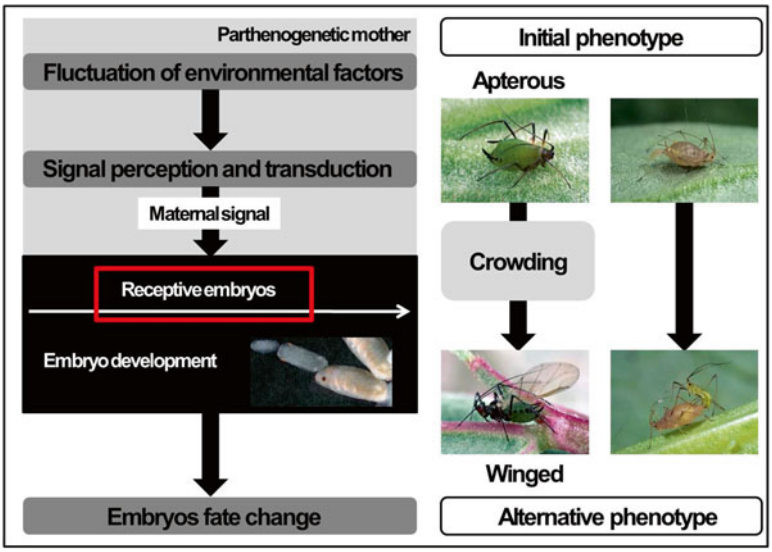

Fig. 1 Phenotypic plasticity in aphids. A critical developmental period (receptive embryos) permits a developmental response to a local change of environmental conditions; this will orientate development towards an alternative phenotype adapted to this new environmental condition. Because of viviparity and the large number of developing embryos at different stages of development within the female aphid's abdomen, the parthenogenetic female exposed to this new environmental cue has a large probability of containing embryos within the critical period of development. In aphids, the alternative phenotypes could be apterous/winged (in response to crowding, for instance) or asexual/sexual in response photoperiod shortening

female can develop and give birth to up to 80–90 embryos. During early oogenesis occurring in the ovaries of these females, pre-oocytes do not enter into meiosis and remain diploid with no recombination events. Viviparous embryos are thus genetic clones of their mother, and are clones between them, all being females. This reproductive process occurs in spring and summer, which corresponds to crop season. Consequently, crop protection against aphids is required to limit the extraordinary capacity of multiplication of aphids by asexual viviparous reproduction.

In autumn, the reproductive mode alternates: viviparous parthenogenetic females – after sensing the changes in photoperiod length and temperature occurring in fall – give birth to clonal progeny with sexual forms (sexual females and males) and behavior (Fig. 2). There are thus three different morphs in aphids concerning the reproductive mode that is formed under environmental cue changes: parthenogenetic females, sexual females and males. Sexual individuals (males and females) resume true meiosis to form haploid recombinant gametes, and these two sexes mate. Sexual females are oviparous and lay eggs after fertilization. Fecundity of sexual females is reduced compare with parthenogenetic females with the production of approximately a dozen eggs. Eggs are diapausing during autumn and winter; this allows genotypes to survive during the low temperatures of winter. Eventually, this plastic trait confers a strong adaptive value to aphids during their

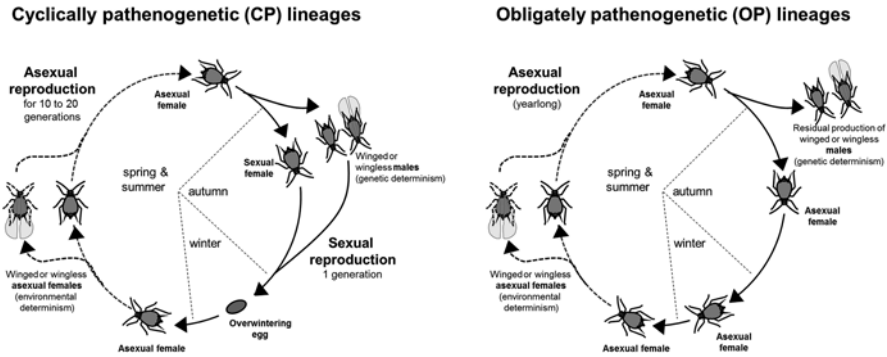

Fig. 2 Life cycle of the pea aphid. *Left*: populations that alternate reproductive mode between seasons. *Right*: populations that do not alternate their reproductive mode, remaining asexual all year, except when producing males in autumn. In both cases, phenotypic plasticity of winged/wingless females occurs among asexual females in the spring and summer. Males produced in autumn can be winged or wingless, a trait that is genetically determined (not plastic). See text for details

annual life cycle (for reviews, see Le Trionnaire et al. 2008; Ogawa and Miura 2014). However, there are variations of this trait within populations and species that make the story more complicated (and interesting!). This variation is detailed below (Sect. 3).

Aphids can also alternate between winged and wingless morphologies, referred to as the wing or dispersal polyphenism. This polyphenism occurs during the parthenogenetic portion of the aphid life cycle (Fig. 2). Under ideal, stress-free conditions, females typically are wingless and produce wingless clonal daughters. When a host plant becomes overcrowded, or if a danger is sensed (such as the presence of natural enemies), however, mothers produce winged clonal daughters that are capable of dispersing to other host plants (reviewed in Müller et al. 2001; Braendle et al. 2006). One adult female can produce both types of daughters. Those daughters are genetically identical but phenotypically quite distinct.

The winged and wingless phenotypes as well as the sexual/asexual phenotypes are examples of developmental plasticity: environmental cues affect developing embryos and fix their ultimate, adult phenotype. More specifically, because of its viviparous nature, an aphid mother can directly influence the development of her offspring. Only embryos that have not yet been born are competent to respond to inducing signals. Thus the pea aphid mother likely perceives the local environmental cue and transmits this information to her daughter embryos. Only embryos that are within a critical, sensitive embryonic period can receive this maternal signal. Once born, the nymphs' developmental trajectory is set.

After this introduction to aphid phenotypic plasticity, we invite you to a journey: first, we get the equipment in our camp base, then we go for a trip to quantitative genetics before spending most of our time traveling in the reproductive and dispersal polyphenisms of aphids, and conclude by dreaming of new tools.

2 Aphid Genomics Resources: Data and Tool-Kits

2.1 The Pea Aphid Genome

In 2010, the sequence of the pea aphid genome was published (International Aphid Genomics Consortium 2010). This was not only the first aphid genome available but also the first of a Hemipteran. But what do we mean by "the genome sequence of the pea aphid"? We have to keep in mind that this is still a draft genome sequence, meaning an imperfect one. Even though new versions of the pea aphid genome have become available online (aphidbase.com) since the 2010 publication, the number of scaffolds (23,924) far exceeds the number of chromosomes (n = 4). This means that the physical distribution of the genome sequences is far from been completed: all the pieces have been sequenced, but partially assembled and the genome partially reconstructed. This is common in genome projects and only a few eukaryotic genomes have been fully assembled (e. g., *Homo sapiens*, *D. melanogaster*, *C. elegans*, and *Arabidopsis thaliana*). This is because sequencing techniques still provide mainly short sequences, and because a large portion of genomes (up to 90 %) contains repetitive DNA (heterochromatin) that is difficult to assemble. For the pea aphid genome, as for other genomes, we expect that new technologies will improve the quality of the assembly. For example, optical mapping can now provide physical maps at several hundred kb scales and sequencing of long-fragment DNA techniques are becoming available (*e.g.*, Nanopore and Illumina Moleculo).

The partially assembled pea aphid genome provides a good starting catalog of protein-coding genes and of various functional DNA elements such as transposons, mRNAs and non-coding RNAs (Table 1). We can summarize the main characteristics of the pea aphid genome into four highlights. First, the number of predicted genes is approximately twice that of other sequenced arthropod genomes (except *Daphnia pulex*, Colbourne et al. 2011), with approximately 34,000 predicted genes compared to 13,955 for *D. melanogaster* (Flybase v5.57). It is intriguing that the *Daphnia* and *Acyrthosiphon* genomes share this particularity, since both exhibit

Table 1 The pea aphid genome by the numbers

Genome size	541,675,471
Number of scaffolds	23,924
Mean scaffold size	22,642
N50	518,546
Number of transcripts	36,939
Size of the transcripts	Mean: 8,684; median: 3,984
Mean number of exons by transcript	4.97
Exon size	Mean: 393.7; median: 204
Intron size	Mean: 1662; median: 201
Number of transcripts with a NR match	24,998
Number of transcripts with a Gene Ontology annotation	10,062

phenotypic plasticity (see comparison and discussion in Simon et al. 2011). One cannot totally exclude that some of the predicted genes might be false annotations due to scattered scaffolds or allelic variants. However, deep, precise and genome-scale phylogenetic analyses strongly indicate that this high number of predicted genes correlates with a large number of duplicated genes or even expanded gene families: miRNA and piRNA machinery (Jaubert-Possamai et al. 2010; Lu et al. 2011), cathepsins (Rispe et al. 2008), cuticular proteins (Gallot et al. 2010), and kelch proteins (actin binding proteins) (Huerta-Cepas et al. 2010). Further, 2459 pea aphid gene families underwent lineage-specific gene duplication, with transposases and retrotransposases being the most amplified (from 50 to 200 members) (International Aphid Genomics Consortium 2010; Huerta-Cepas et al. 2010). Second, despite the high number of duplications and expansions in genes encoding proteins, there are still some metabolic and developmental pathways that lack genes (International Aphid Genomics Consortium 2010). For example, several genes central to immunity are missing: this is the case with the immunodeficient pathway. This could be explained by either the presence of several bacteria within the pea aphid body (requiring a decrease of anti-microbe response to allow microbe-host interactions) and/or the fact that the phloem-sap (the unique food source of aphids) is poor in microbes and does not require strong immunoprotection (Gerardo et al. 2010). Third, by sequencing the pea aphid genome, the discovery of lateral gene transfers of bacterial genes from the ancient and obligate symbiont *Buchnera aphidicola* was expected: this symbiont has a very shortened genome compared to a free living bacterium, with only approximately 600 genes compared to 4200 in *E. coli*. However, no trace of *Buchnera* genes were found in the pea aphid genome and unexpectedly, lateral gene transfers were observed from other bacteria species (Nikoh et al. 2010). And fourth, lateral gene transfers occurred from fungal partners. This last point concerns the carotenoid pathway that confers the green or rose body color of the pea aphid (Moran and Jarvik 2010). The pea aphid genome acquired different genes involved in carotenoid biosynthesis, with the rose color of individuals due to the carotenoid torulent pigment. These genes are from fungal origin. The green individuals have deletions within the carotenoid desaturase gene, disrupting the capacity to accumulate torulent. It is important to note that bacterial secondary symbionts can also influence body color of the pea aphid (Tsuchida et al. 2010).

As mentioned earlier, a genome sequence is only the beginning of the story, and the exploitation of these data are very promising for different disciplines such as evolutionary biology, population genomics, system biology, ecology (such as behavioral ecology), etc. (Tagu et al. 2010). Further, the description of the pea aphid genome is far from complete and we need input from several communities to unravel the structure, dynamic, evolution and functioning of this genome. The four different genomic features we mentioned above (as well as others) have to be examined in terms of generalization. In other words, are they specific to *Acyrthosiphon pisum*? If not, when did they appear in the evolution of aphids? Do other aphid genomes have their own specificities? Can we identify common key functions and candidates for plant protection methods?

2.2 Other Aphid Genomes and Resources

It is important to continue our efforts on sequencing more aphid genomes, by following at least two main tracks: first, sequencing most of the aphid crop pests in order to have genomic resources required to identify candidate functions involved in the pest features of these species. And second, sequencing aphid species that cover the evolutionary tree of this group to extract evolutionary traits related to genome structure and evolution (Fig. 3). This will require many more years of work primarily dedicated to annotation, analyses, and exploitation of data.

Today, there are a series of ongoing aphid genome projects. Most of them are have just started and thus no information is available yet (*e.g.*, several cereal and corn aphids; Nicholson et al. 2015). Other projects have encountered technical difficulties mostly at the assembly step. The i5K consortium (i5K Consortium 2013) provides some recommendations to decrease the risk of assembly problems. The most important recommendation is to reduce the heterozygosity of the samples in order to avoid allelic variants that can cause misassemblies. There are several ways to reduce this heterozygosity: the use of haploid individuals (such as males for Hymenoptera), DNA extraction from a single individual (if the insect is large enough for sufficient DNA material), or the production of inbred, nearly homozygous lineage. Unfortunately, there are no haploid morphs in aphids and they do not tolerate inbreeding. However, one possibility is to screen natural populations to find newly introduced populations with a low level of heterozygosity (for example, populations that experienced a bottleneck). That is what the *Phylloxera* genome project

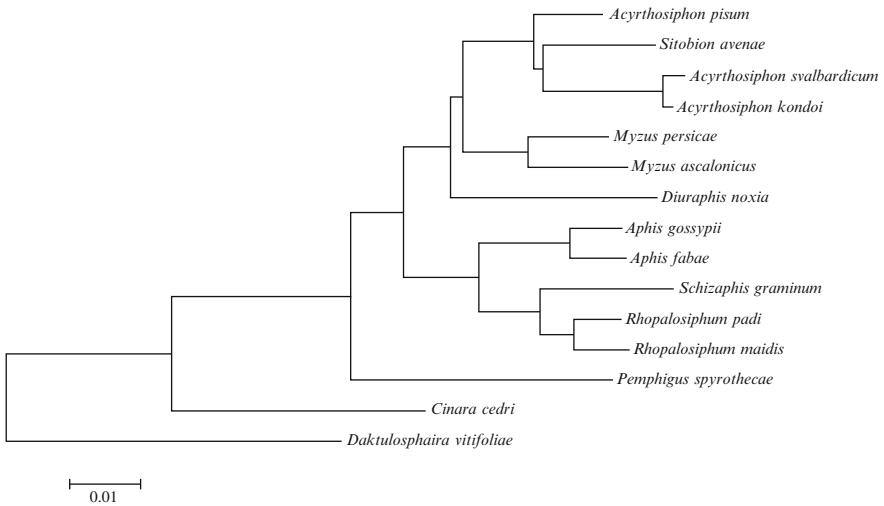

Fig. 3 A simplified phylogenetic tree of select aphid species. Tree created from DNA sequence alignment by Neighbour Joining, with distance correction by Kimura 2 parameters (Courtesy of Claude Rispe, INRA)

did to find a population with low heterozygosity (0.11 using microsatellite markers), and the ongoing assembly of this genome is promising.

It is probable that the next released aphid genome will be *Myzus persicae*, the green peach or peach-potato aphid. An international genome project within the framework of the International Aphid Genomics Consortium (IAGC) is ongoing to assemble and annotate this 350 Mb genome. This is a very important step since the green peach aphid is a polyphagous pest (while the pea aphid is restricted to Fabaceae), is probably separated by approximately 50 million years from the pea aphid (Kim et al. 2011), and alternates host plants between asexual and sexual reproduction, while the pea aphid does not. Soon, we will be able to compare for the first time two different aphid genomes and eventually identify general and specific features for these two genomes.

Apart from genome projects, transcriptomic approaches have been initiated on different aphid species. Transcriptomes consist of a list of transcribed mRNAs coding proteins. Trancriptome sequencing can be used to identify the main developmental and metabolic functions active in a given group of cells, as well as to compare expression profiles between different biotic and/or abiotic conditions. The first aphid transcriptomes were produced by Sanger sequencing of cDNAs (Expressed Sequenced Tags or ESTs) from *Rhopalosiphum padi*, *Toxoptera citricida*, and *Aphis glycines* (Hunter et al. 2003; Tagu et al. 2004; Bai et al. 2010), as well as *A. pisum* (Sabater-Muñoz et al. 2006; Nakabachi et al. 2005) and *M. persicae* (Ramsey et al. 2007). They allowed the characterization of expressed genes in different morphs, organs and tissues (parthenogenetic females, males, bacteriocytes, head, and gut). Most of these sequences were also used afterward for genome annotation. Transcriptomes can, for example, be useful for inferring the gene evolutionary rates from sequence comparisons. One of the most striking examples is the 1KITE initiative (1000 Insect Transcriptomes for Evolution) that aims at studying the transcriptomes of more than 1,000 insect species encompassing all recognized insect orders, with the objective of unraveling the evolution of insects for understanding how life in terrestrial versus limnetic environments evolved (Misof et al 2014). This is not specifically related to aphids, but it demonstrates how the comparison of a large number of conserved gene sequences can solve phylogenetic discrepancies and can help provide information about the evolution of specific traits in insects. Concerning aphids, the comparison of eight transcriptomes from eight species, half being cyclically parthenogenetic and the other half having totally lost the ability to reproduce sexually (hence referred to as obligatory parthenogens) was performed (Ollivier et al. 2012). After reconstruction of a shared protein set and comparison between groups of species, no significant increase in nonsynonymous/synonymous mutation was observed between cyclically and obligatory parthenogenetic species; but for *R. maidis* – a completely asexual species – a significant increase of synonymous mutations was recorded compared to *R. padi* (cyclically parthenogenetic species), indicating in this case a genetic signature of diversification between sexual and asexual species.

In conclusion, it is clear that in the following years, many more genomic resources for aphid species will be developed and made available. This follows the international i5K initiative that aims to help entomologists develop genomic

resources for most of the arthropod Orders (i5K Consortium 2013). Best practices are published, as well as web training for different steps such are annotation tools or help in bioinformatic centralization of data. The aphidologists have the most interest to be active in i5K, as it is already involved with several ongoing aphid genome projects. And finally, understanding aphid adaptations to their environment involves the integration of ecological studies that can exploit genomic resources. Ecological networks have to enter the genomic area since aphids can have several interactions such as with conspecific, natural enemies, plants and microbe symbionts (Fig. 4). Aphid genomics is thus not restricted to aphid species, but rather extends to other insects and organisms that interact with aphids.

2.3 *Database and Data Integration*

Setting up a centralized bioinformatic warehouse is crucial to organize, display, and distribute genomic resources among a dispersed community, and to facilitate their handling by non-specialist bioanalysts. In that regard, AphidBase (http://www.aphidbase.org), a comprehensive information system set up to safely centralize and promulgate data generated by the IAGC, has been implemented (Legeai et al. 2010). It has been built using software tools from GMOD including several Chado instances: genome browsers Gbrowse (Stein 2013) and JBrowse (Skinner et al. 2009), gene and protein reports, an ontology navigator (WebApollo), an application for manual curation (Lee et al. 2013), and various other tools such as a blast search and a full text search facilities.

Using this system, data can be visualized and extracted as features organized and linked over a genome sequence. It includes the automatic or manual prediction of

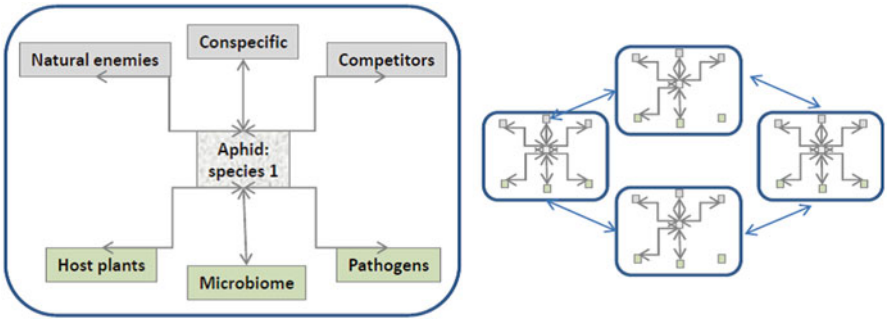

Fig. 4 A model for connecting genomics with ecological networks. *Left*: each aphid species can be seen as the center of biotic interactions with different organisms (host plants, microbes, etc.) including other insects (conspecific, competitors, natural enemies). This interaction network will help to choose species of interest for developing genomic resources (and ecological data). *Right*: each species of the left panel can also be seen as a centre of interactions, showing a (complex) network that multiplies the possibilities and choices to be made

protein-coding genes, but can be complemented by many other features such as noncoding RNA (*e.g.*, tRNAs, rRNAs, miRNAs or lncRNAs), transposable elements, SNPs, or larger genetics variants.

But, the automatic prediction of genes may be subject to errors due to the incompleteness, fragmentation, or duplications of the genome, complexity of the structure of the genes (*e.g.*, long introns and/or very short exons), or the lack of evidence of transcription. Thus, occasionally manual curation is needed to convert the raw machine-generated data into useable, robust and useful data. The overall goals of manual annotation are:

- To establish almost exhaustive lists of genes playing a key role in some crucial processes.
- To provide names for known genes, based primarily on homology to what is known in other organisms.
- To fix obvious errors in the automated gene models and improve them where additional data are available – *i.e.*, to get the intron/exon coordinates right.
- To add value by connecting information about a gene from different sources in a coherent and accessible way.

For all steps, we used WebApollo (Lee et al. 2013) because it offers many functionalities facilitating the correction of gene structures and allows users to probe, manipulate and alter the interpretation of gene models. Within WebApollo, annotations can be created, deleted, merged, split, classified and commented on. For example, one can easily locate and correct incorrect splice sites or start/stop codons, classify a gene as a pseudogene, and even create a new alternatively spliced RNA.

Furthermore, we are collecting millions of RNA-Seq reads and data from various ChIP-Seq/FAIRE-Seq, and proteomics projects. These resources are valuable to produce or confirm gene predictions. And, as they give information about the expression level of the transcripts or their regulation by epigenetics factors in different tissues or conditions, they also help to better understand the role of known or unknown genes involved in biological processes such as phenotypic plasticity. Consequently, powerful extraction, integration and mining tools dedicated to next generation sequence data will facilitate data analyses.

Centralizing the resources in one information system is the first step to achieve data integration and is critical for taking advantage of the richness of the data produced by IAGC, to increase the knowledge of the tissue-specific expression of genes, and to make them available for meta-analyses. But, the increasing amount of expression data from transcriptomic and proteomic projects requires the standardization of morphological descriptions by controlled-structured vocabulary. This structuring information system is indispensable for eventually cross-referencing the anatomical or other phenotypic data with gene expression data from various genotypes. For this purpose, we are supporting AphidAtlas, a computing resource for the management of anatomical and developmental data that includes a controlled and structured vocabulary (ontology), as a mediator to link the genes from AphidBase and the -omics data (transcriptomics, proteomics or epigenetics) produced by biologists.

Finally, in order to help a broad community of scientist carry out their own data manipulation and analyses, we set up a dedicated Galaxy server (Goecks et al. 2010) with tools for extracting data from AphidBase and for running elaborated analyses using a large set of sophisticated programs on a powerful linux cluster. Galaxy is facilitating the implementation of most commonly used Unix command line interface programs. It also helps with maintaining, manipulating and sharing data through a archive of history of actions. Finally, it allows the development of workflows and their implementation, thereby facilitating data at medium or high-speed processing. Finally, Galaxy is dedicated to an audience of biologists wishing to run applications through a web interface, who do not have access to a Unix server or who do not know how to implement Unix commands.

In conclusion, AphidBase presents an integrated environment to advance the knowledge about important aphid biological processes. It includes manual gene prediction curation and the compilation and integration of large-scale datasets with meta-data and ontologies, using standardized analysis pipelines, as well as providing sophisticated tools for analyzing, browsing and querying heterogeneous data.

3 Quantitative Genetics: Using Polymorphism to Identify Loci Involved in Polyphenic Traits

3.1 Reproductive Mode Polymorphism

In some cases, polyphenic traits have analogues that are not environmentally sensitive. An example of an aphid trait that is either environmentally or genetically controlled is their ability to reproduce sexually or asexually. The ancestral life cycle of aphids is cyclical parthenogenesis (Davis 2012). Yet, several aphid species also exhibit lineages that are characterized by an altered response to sex-inducing environmental cues, such as the inability to produce sexual females or both sexual females and males (Simon et al. 2010). Instead, they produce asexual females year-round. These "obligatory parthenogenetic" lineages are frost sensitive because they are unable to produce the only stage that resists winter coldness, eggs. So, although the switch from clonal to sexual reproduction in cyclically parthenogenetic lineages is triggered by photoperiodic changes, the lack of production of sexual forms in these obligatory parthenogenetic lineages is genetically determined since changes in environmental conditions have little or no effect on their reproductive phenotype (Simon et al. 2010; Dedryver et al. 2013). This genetically controlled variation of reproductive mode is referred to as "reproductive polymorphism".

Interestingly, geographical variations in climate favor the stable co-existence of these two reproductive strategies. Indeed, a cyclically parthenogenetic life cycle is required to survive in regions with cold winters. In contrast, obligatory parthenogenetic lineages are favoured in regions with mild winters since they continue to reproduce year-long and thus have a demographic advantage (Dedryver et al. 2001; Rispe et al. 1998). Accordingly, cyclically parthenogenetic lineages dominate in cold areas

and obligatory parthenogenetic lineages in warmer regions, and both coexist in regions with fluctuating winter temperatures (Dedryver et al. 2001; Simon et al. 2002, 2010). Intraspecific variation for the mode of reproduction (reproductive polymorphism) has been documented in most pest aphid species, including *A. pisum* (Frantz et al. 2006), *M. persicae* (Blackman 1971), the birdcherry-oat aphid *Rhopalosiphum padi* (Simon et al. 1991), the melon and cotton aphid *Aphis gossypii* (Ebert and Cartwright 1997) and the grain aphid *Sitobion avenae* (Dedryver et al. 1998).

The genetic architecture of reproductive mode polymorphism has been studied through crossing experiments in only a few aphid species. This approach is possible thanks to the production of functional males by some obligatory asexual lineages, which can mate with sexual females from cyclically parthenogenetic lineages (Fig. 2). The principle is to cross different lineages for at least two generations and to record the reproductive phenotype (under sex-inducing conditions) of a large number of progeny. From these data, one can deduce the number of loci involved and the dominance of the different alleles.

The first investigation of the inheritance of reproductive modes was performed by Blackman (1972) in *M. persicae*. By crossing genotypes with distinct reproductive phenotypes, Blackman (1972) concluded that cyclical parthenogenesis is dominant and obligatory parthenogenesis, recessive. More recently, Dedryver et al. (2013) showed that obligate parthenogenesis is also recessive in the grain aphid *S. avenae*. They further demonstrated that this key ecological trait is likely to be determined by two or three loci in *S. avenae*. The simplest genetic model they proposed includes a major gene interacting with a suppressor gene.

The genetic architecture of the polymorphism of reproduction is currently being investigated in the pea aphid, *A. pisum*. A similar two-generation crossing approach was used. The availability of the genome sequence made it possible to easily develop hundreds of genetic markers (here microsatellite markers, Jaquiéry et al. 2012). A genetic map was constructed based on the marker genotypes using the two-generation pedigree. Then, the joint analysis of the segregation of the phenotypic trait in F2 individuals and genetic markers in a QTL framework demonstrated that reproductive mode is controlled by a single genomic region located on the X chromosome (Jaquiéry et al. 2014). Alleles associated with cyclical parthenogenesis are also dominant in this aphid species. In parallel, a genome scan approach was conducted on wild populations under opposite selective pressures for reproductive mode. The principle of such a population genomic approach is to measure genetic differentiation between populations under divergent selection on the trait of interest on a large number of markers that cover the whole genome. Genome scans rely on the fact that neutral factors (including dispersal, drift, and demography) affect all loci, while selection acts only on loci involved in the control of the trait or on loci located nearby (Lewontin and Krakauer 1973). Hence, loci located within a genomic region under divergent selection are expected to show stronger genetic differentiation than loci located in genomic regions not involved in the control of the trait. The genome scan performed on populations selected for opposite reproductive regime revealed four outlier loci. Interestingly, three of the outliers were found within the genomic region pinpointed by the QTL approach, providing independent evidence

that the genomic region identified controls reproductive mode in the pea aphid (Jaquiéry et al. 2014). Next steps will include additional crosses to generate recombinant individuals in this genomic region, as well as high density genome scans based on whole genome resequencing approaches to refine the location of the portion of genome controlling reproductive strategies. The final aim will be to identify a group of candidate genome sequences that will be tested for more detailed functional analyses.

3.2 Wing Polymorphism in the Pea Aphid

Another example of a polyphenic trait with a genetic analogue is the male wing dimorphism in pea aphids, in which males can be either winged or wingless due to genetic (not environmental as in the asexual females) variation. The alternative morphs are determined by a single unidentified locus on the X chromosome called *aphicarus* (*api*) (Caillaud et al. 2002; Braendle et al. 2005a). The name "aphicarus" comes from "aphid" plus "Icarus", the Greek mythological figure that flew too close to the sun and lost his wings. Winged and wingless male siblings produced by an *api* heterozygous female are genetically identical except that they carry an X chromosome with either the *api* winged (winged males) or *api* wingless allele (wingless males). The male wing dimorphism is referred to as a wing polymorphism rather than a polyphenism because of its allelic basis.

Because of the many similarities shared by the asexual female polyphenism and male polymorphism, identifying *api* may provide significant insight into the mechanistic basis of the female polyphenism. This is supported by a previous study (Braendle et al. 2005b) that showed that in an *api* F2 mapping population, asexual females of the three different *api* genotypes differed in their propensity to produce winged offspring. This result indicated that there is genetic linkage between the two dimorphisms. In other words, genotype by environment interaction at or near the *api* locus explain genetic variation in the female wing polyphenism (Braendle et al. 2005b).

Both recombination mapping and association mapping approaches can be taken to identify *api*. Braendle et al. (2005a) previously established an *api* linkage mapping population. Using ~170 F2 individuals, they generated a coarse X chromosome map that located *api* to a 10 cm region (i.e., tens of millions of bases). Since then, additional sequencing (using the Illumina platform) of a subset of genomic DNA from 384 F2s (technique of Andolfatto et al. 2011) was used to discover and score single nucleotide polymorphisms (SNPs) as mapping markers (Brisson and colleagues, unpublished data). They identified 32 genomic scaffolds with significant linkage to *api* and ordered them using restriction fragment length polymorphism markers that were developed for each scaffold. Simultaneously, an association analysis was also used to identify the *api* region. 20 *api* heterozygous females from natural populations were used to produce winged and wingless males from each female. Winged and wingless males produced from the same female are identical at their autosomes but carry different X chromosomes. Genomic DNA from these 20 winged and wingless males was sequenced (using Illumina) at low coverage (~0.6×)

(Bickel et al. 2013) and the data were used to identify SNPs perfectly associated with the male phenotypes.

The two approaches pointed to the same genomic region as the location of *api*. In particular, one genomic scaffold contained SNPs highly associated with *api* in the natural isolates and was in perfect linkage with the male phenotypes in the mapping population. Thus, by using these next generation sequencing approaches, it was possible to move fairly rapidly from no sense of *api*'s location to a region of approximately 100 kb. Experiments are now in progress to identify a putative *api* gene using gene expression analysis. Future experiments will be able to address what functions *api* has in asexual females, providing information about the molecular mechanisms that control the female wing plasticity.

4 Post-genomics of Phenotypic Plasticity

Aphid genomic resources have been effectively utilized to examine the gene expression basis of their polyphenisms. Here we will briefly review what has been learned by applying these resources to the reproductive polyphenism and the wing polyphenism. Following this, we will discuss future genomic efforts that will primarily focus on epigenetic mechanisms.

4.1 Reproductive Polyphenism

The physiological basis and the neuroendocrine control of the reproductive polyphenism has been extensively studied since the early 70s, but the molecular basis of this phenomenon has only been investigated in the last few years due to the recent availability of genomic resources. Initial approaches used DD-RT-PCR and cDNA arrays to identify transcripts differentially expressed between aphids reared under long and short photoperiod and respectively producing asexual and sexual phenotypes in their offspring, mostly on the pea aphid (Cortés et al. 2008; Le Trionnaire et al. 2007, 2009). These studies allowed the identification of genetic programs regulated during the initial steps of photoperiodic signal detection and transduction that occur in head tissues of parthenogenetic females that can produce sexual forms. Transcripts coding proteins involved in the visual system, the nervous system and the endocrine system (especially genes involved in the insulin signaling pathway) were differentially expressed, supporting earlier physiological studies implicating the neuroendocrine system in this signal transduction step. The regulation of the insulin pathway is intriguing since insulin is known in other insects to be involved in photoperiod sensitive traits such as diapause (see Huybrechts et al. 2010 for discussion).

Photoperiod shortening also had an impact on cuticle structure: several cuticular protein transcripts were downregulated under the short-day condition. On top of that, transcripts involved in cuticle formation – namely *Drosophila* homologous genes *black* and *ebony* involved in the conjugation of dopamine and β-alanin to

form NBAD within the cuticle – were also underexpressed. Taken together, these data suggest that a possible desclerotization (loosening) of cuticle structure occurs under the short-day condition. Additional RT-PCR experiments (Gallot et al. 2010) indeed showed that two transcripts involved in dopamine biosynthesis (dopa-decarboxylase and tyrosine hydroxylase) were downregulated in the heads of short day-reared aphids, suggesting a putative role for dopamine in the regulation of the reproductive polyphenism. An interesting parallel can be drawn with phase polyphenism in locust, where the transition from gregarious to solitarious phase is controlled by the dopamine pathway (Ma et al. 2011), involving regulation by a microRNA (Yang et al. 2014a).

It has also long been hypothesized that photoperiodic time measurement in insects involves a specific photoperiodic clock and the circadian clock (Saunders 2008). The pea aphid genome contains components of the circadian clock and their expression levels were measured in aphid heads under long and short-day conditions at 4-h time intervals (Cortés et al. 2010). Expression analysis for some core genes revealed circadian rhythmicity as well as a significant effect of the photoperiod on the amplitude of oscillations, suggesting a putative involvement of the circadian clock in the photoperiodic response associated with reproductive polyphenism. Finally, the role of melatonine as a regulator of the photoperiodic signal has been hypothesized (Gao and Hardie 1997). More recently, Barberà et al. 2013 described the expression of arylalkylamine N-acetyltransferase in the pea aphid, showing that gene expression varied by photoperiod.

The involvement of Juvenile Hormones (JH) in the control of photoperiodism has also been studied. Topical application of JH or Kinoprene (a JH analogue) on the abdomen of viviparous aphids producing sexual individuals resulted in the reversion of the response to production of asexual individuals (Hardie and Lees 1985; Corbitt and Hardie 1985; Gallot et al. 2012). Ishikawa et al. (2012b) recently demonstrated that JHIII titer was lower in aphids producing sexual morphs under short-day conditions than in aphids producing parthenogenetic morphs under long-day conditions. They demonstrated that the level of expression of the JH esterase gene – coding for a protein involved in JH degradation – was significantly higher in aphids producing sexual individuals. Altogether, those studies strongly suggest that JH plays a significant role in the transduction of the photoperiodic signal.

Once the photoperiod shortening signal is perceived and transduced, the embryos – the target tissue – will display the alternative phenotypes, embryos being parthenogenetic (with a diploid germline) under long days conditions or sexual (with a true haploid germline) under short-day conditions. A transcriptomic comparison of asexual versus sexual embryogenesis (Gallot et al. 2012) revealed that specific genetic programs were involved in this transition. Some transcripts coding proteins involved in oogenesis – such as *Drosophila* homologues *orb* and *nudel* involved in oocyte axis formation and specification – were differentially expressed. Transcripts involved in post-transcriptional regulation, such as *pop2* (known to play a role in polA-tail stabilization,) cell cycle control (*cyclin J*) and epigenetic regulation (*Suv4-20H1*) were also detected. The fine localization by *in situ* hybridization of these transcripts revealed that they were mostly localized within the germline

cells, which suggests that the initial steps of the embryogenesis switch occur in a limited number of cells. A complementary study (Duncan et al. 2013) examined the effect of reproductive mode on the expression of key maternal and axis patterning genes, namely orthodenticle (*otd*), hunchback (*hb*), caudal (*cad*) and nanos (*nos*) (see also Chang et al. 2009). These genes displayed some differences in their expression levels as well as on their localization within the sexually and asexually produced oocytes and embryos. These differences reflect substantial changes in gene regulatory networks controlling early development in the pea aphid.

The development of genomic resources thus allowed the identification of key genetic programs involved in the control of reproductive polyphenism, from the initial steps of photoperiodic signal perception and transduction to the later steps of the asexual to sexual embryogenesis switch. Small RNAs, and especially microRNAs, are key post-transcriptional regulators of gene expression. In the pea aphid, only a few studies focusing on that aspect of gene regulation have been performed. Jaubert-Possamai et al. (2010) showed that the different genes coding proteins involved in the pea aphid miRNA machinery were duplicated or expanded: *dicer*, *argonaute* and *pasha*. Some of the duplicated copies (such as one copy of *argonaute 1*) showed strong signatures of positive selection (Ortiz-Rivas et al. 2012). Further, these duplicated copies have different expression profiles between the different morphs, when the original copies did not. This strongly suggests the involvement of the miRNA pathway in reproductive morph regulation. High-throughput sequencing of small RNA samples from parthenogenetic individuals established the first catalogue of pea aphid microRNAs (Legeai et al. 2010). MicroRNA arrays were also used to examine differences in microRNAs expression between different aphid morphs (sexual versus asexual females) identifying, for instance, mir34 as being regulated between morphs. In *Drosophila*, mir34 is regulated by ecdysone and JH.

Clearly, the transition of reproductive mode is a complex process that is driven by environmental, biochemical and genetic cues: there are strong modifications of genetic programs at different levels (photoperiod sensing, signal transduction, morphogenesis) and more work is required to form a global picture of the integration of these mechanisms (see Sect. 4.3).

4.2 Wing Polyphenism

Less is known about the physiological and molecular mechanisms underlying the wing polyphenism as compared to the reproductive polyphenism. As mentioned above, the wing polyphenism consists of two genetically identical morphs: a winged morph and a wingless morph. Although we refer to the two morphs as winged and wingless, this is shorthand for systemic differences between the morphs. For example, wingless morphs are more sedentary, have no wings, have fewer sense organs on their antennae, and develop faster than winged morphs (Dixon and Howard 1986; Braendle et al. 2006). These individual phenotypes are always found together

and therefore it is likely that a large number of gene expression differences are responsible for their morphological differences.

The transcriptional basis of the adult winged and wingless morphs has been profiled in several aphid species (Ghanim et al. 2006; Brisson et al. 2007; Yang et al. 2014b). A particular advantage of gene expression profiling in the aphid wing polyphenism, as in the reproductive polyphenism, is that the potentially confounding effect of genotype can be controlled by using aphids of the same clone, which are genetically identical. These studies have demonstrated that alternative gene expression states underlie these alternative morphologies. They have also provided insight into the specific genes that underlie the tradeoffs that characterize a dispersive versus reproductive life history strategy. For example, genes encoding proteins related to flight structures and energy production were found at higher levels in the winged morphs as compared to the wingless morph.

Much less is known about the genomic basis of wing morph determination. Recall that morph choice is determined embryonically. Only embryos that have not yet been born are competent to respond to wing-inducing signals (Sutherland 1969). Thus the pea aphid mother perceives the wing induction cue (crowding) and transmits this information to her daughter embryos. Only embryos that are within a critical, sensitive embryonic period can receive this maternal signal (Ishikawa and Miura 2013). Once born, the nymphs' developmental trajectory is set; they cannot be induced to produce wings. Therefore, to understand the molecular basis of wing determination rather than morph development or maintenance, genomic profiling must be done on embryos.

Only one published study has profiled gene expression (using differential display) of adult females and embryos as they were exposed or not exposed to the wing-inducing cue of crowding (Ishikawa et al. 2012a). They identified three genes (Uba1, McrNaca, and *wingless*) expressed at higher levels in high density treated females relative to low density controls. These genes are therefore candidates for being involved in wing determination. Since then, a RNA-Seq study identified an additional ~3500 genes that may be involved in early stages of the polyphenic response, such as genes involved in odorant binding, the ecdysone hormone signaling pathways, and chromatin remodeling (N. Vellichirammal and J. A. Brisson, 2015, unpublished data). Much work remains to be done on deciphering the molecular basis of wing-induction in aphids, but promising candidate pathways have been identified and work is ongoing.

4.3 Future Work: What Is the Contribution of Epigenetic Mechanisms in the Control of Polyphenism?

An especially promising area of future research is to use epigenetic analyses to interrogate the molecular basis of the two polyphenisms. Epigenetic mechanisms provide a critical link between environmental inputs and phenotypic outputs; they

can affect gene expression in a heritable manner without changing the underlying DNA sequence (Feil and Fraga 2012). They are therefore excellent candidate mechanisms for mediating plasticity. The main epigenetic mechanisms include DNA methylation, histone modifications, and noncoding RNAs. Each one of these can be analyzed at the whole genome level in either adult females as they are receiving morph-determination environmental cues or in receptive embryos during sensitive stages.

DNA methylation concerns the modification of DNA nucleotides by methyl groups, the presence of which can affect the transcriptional capacity of the DNA. DNA methylation has been largely studied in mammals where it is associated with promoter regions. Highly methylated promoters are correlated with repressed transcription. In insects, methylation occurs primarily in gene bodies and methylation is associated with gene expression, not repression. DNA methylation is required for honey bee queen versus worker development (Kucharski et al. 2008), and there are strong correlations between methylation patterns and splicing sites for genes involved in caste determination, which is another well-known case of phenotypic plasticity (Lyko et al. 2010; Lyko and Maleszka 2011). The pea aphid genome annotation confirmed that all components of the DNA methylation pathway are present (*e.g.*, components of *de novo* methylation and maintenance methylation) and LC-ESI-MS/MS showed that 0.69 % of cytosines are methylated (Walsh et al. 2010; Dombrovsky et al. 2009). A more comprehensive analysis of the methylomes of the different pea aphid morphs is now needed, as well as clear analysis by functional tools (see Sect. 5) on the role of methylation on aphid polyphenisms.

DNA accessibility to transcription and regulation mainly depends on nucleosome distribution along the DNA molecule. Nucleosomes are the core element of chromatin and are made of histone octamers wrapped around the DNA. Combinations of post-translational histone modifications (*e.g.*, acetylation, methylation, or sumoylation), also called epigenetic marks, have consequences on the level of DNA accessibility and determine distinct chromatin states. Genome-wide profiling of the combinatorial pattern of enrichment or depletion of specific histone modification or chromosomal protein marks has been established for all *Drosophila* chromosomes (Kharchenko et al. 2011). This analysis allowed the establishment of a nine-state model for *Drosophila* chromatin. Some combinations of marks are associated with transcription start sites (H3K4me2/me3 for a di- or tri-methylation of the K4 on the Histone 3 and H3K9ac for an acetylation of the K9 on the Histone 3) or pericentromeric heterochromatin (H3K9me/me3). The epigenetic state of a genome can then be precisely characterized by studying those epigenetic marks.

Whether specific epigenetic marks are associated with the genomic regions involved in the establishment of alternative phenotypes in the context of polyphenism remains an open question. A recent study demonstrated that gene proximal changes in H3K27ac could discriminate between two female worker and male ant castes and partially explained differential gene expression between castes (Simola et al. 2013). Similarly, we can hypothesize that the "plastic" genomic regions associated with the establishment of polyphenism – wing and reproductive – in aphids might be under epigenetic control. The comparison of sexual and asexual embryo

transcriptomes already showed that transcripts coding Histones H2B.3 and H1 were differentially expressed, suggesting that some regions of the embryonic genome might display changes in chromatin organization and accessibility (Gallot et al. 2012). As well, the main genes involved in chromatin modification exist in the pea aphid genome, with a tendency towards duplication and expansion of some gene families (Rider et al. 2010). A genome-scale analysis of differentially open chromatin regions – using methods such as FAIRE (Formaldehyde Assisted Isolation of regulatory Elements, Simon et al. 2012) – between alternative phenotypes would allow the identification of all the regions displaying changes in chromatin accessibility. The enrichment of specific epigenetic marks at these locations could then be tested by Chromatin Immunoprecipitation (ChIP) experiments. Such analyses should show how chromatin accessibility dynamic changes within pea aphid genomes in response to environmental cues can trigger the establishment of plastic phenotypes.

Noncoding RNAs can also act as guides for epigenetic modifications. This includes long noncoding RNAs and piwiRNAs (piRNAs). The former are not very well described in insects and represent a heterogeneous group of molecules with diverse modes of action. No characterization of long noncoding RNAs has been made in aphids so far, even if they are potential key regulators of chromatin structure and dynamics. piRNAs, which are 23–30 nt small RNAs, silence transposable elements in order to protect the integrity of the genome; they preferentially act in germline tissues at the very beginning of embryo development. A study by Wang and Elgin (2011) showed in *Drosophila* that piRNAs could guide chromatin modifications such as HP1a and H3K9me2 marks at the genomic locations associated with transposons normally silenced by these piRNAs. These small RNAs thus appear as important intermediates between transposon silencing and guidance of histone modifications. Reproductive polyphenism in aphids results in a switch of asexual to sexual embryogenesis, with a transition from a diploid germline to a true haploid germline. A correlative analysis of piRNAs cluster changes between the two types of germlines with changes in specific epigenetic marks would also help illuminate germline genome dynamics during the establishment of the reproductive polyphenism. And the fact that the piRNA machinery pathway is made of expanded gene family members with different expression profiles is intriguing (Lu et al. 2011).

5 Functional Genomics

Post-genomic approaches allowed the identification of the genetic programs involved in the regulation of polyphenism in aphids. Among those, some transcripts might be key putative regulators of this phenomenon. To address the specific role of these elements in the control of polyphenism, dedicated functional analyses (including forward and reverse genetics) are needed.

5.1 Transient Expression of Interfering RNAs

RNA interference is a tool based on the sequence-specific suppression of gene expression and offers various opportunities for insect science, from gene function analysis to pest population management. This technology aims at expressing/injecting small non-coding RNA molecules (usually short-interfering RNAs or siRNAs) in an organism/tissue that will target a transcript in a sequence-specific manner. The biological process involves the recognition and cleavage of long double-stranded RNAs by the RNAse III Dicer enzyme into 20–25 bp fragments (siRNAs). These small RNAs are then loaded onto a RNA-induced Silencing Complex (RISC). The catalytic component of RISC is the RNAseH-like domain of an Argonaute protein, which cleaves single-stranded mRNAs that share sequence similarity with the small RNA (Scott et al. 2013). Such silencing tools exploit the siRNA pathway, and especially the capacity of cells to degrade mRNAs after recognition by specific siRNAs.

In aphids, RNAi tools have been developed for functional analyses of specific transcripts. Mutti et al. (2006, 2008) injected Coo2 siRNAs and observed that Coo2-siRNAs injected aphids had reduced fitness. Coo2 is a salivary protein delivered into the plant by the aphid. Coo2-siRNas injected aphids showed an altered electropenetrography profile, suggesting that this protein is essential for aphid feeding. In this study, a clear link between Coo2 RNAi knockdown and an aphid feeding phenotype was established. Jaubert-Possamai et al. (2007) injected double-stranded RNAs (dsRNAs) directed against a calreticulin and a cathepsin L transcript. They also observed a significant reduction (around 40 %) of transcript levels but could not observe any specific phenotype. These pioneering studies in aphids first demonstrated that this gene knockdown tool was efficient to silence specific transcripts, but also that RNAi efficiency was transient, since the effect did not last for more than a few days. Since injection has been shown to be quite damaging for aphids, an alternative method based on the feeding of aphids on artificial media complemented with siRNAs or dsRNAs was subsequently developed. In one study, aphids were fed on artificial media containing dsRNAs directed against an aquaporin gene; a reduction of around 50 % of transcript expression could be observed. Treated aphids showed a reduced osmotic pressure, which suggested that the aquaporin gene was essential for water cycling within the aphid gut (Shakesby et al. 2009). In another study, aphids were fed on media containing siRNAs directed against a VTPase transcript. A 31 % reduction of transcripts level expression as well as an increase of mortality (69 %) could be observed in treated aphids (Whyard et al. 2009). Another study used this artificial media feeding strategy to target the *hunchback* transcript in the pea aphid, which is essential for axis patterning during oogenesis in *Drosophila*. Aphids fed with dsRNAs against this transcript displayed a transcript level reduction of 50 % as well as an increase lethality of 30 % (Mao and Zeng 2012). A recent study made the comparison of injection and feeding administration methods for delivery of dsRNAs targeting the cathepsin-L gene in the pea aphid (Sapountzis et al. 2014). The effect of the treatment was analyzed within single aphids in five different body parts: bacteriocytes, gut, embryos, head and remaining body carcass. Injection was more effi-

cient in the head and carcass, resulting in morphological defects suggesting a role for cathepsin-L in the molting process. In contrast, feeding was more efficient in the gut since specific gut epithelial cells defects could be observed. RNAi efficiency thus appears to depend on the administration method and the targeted tissue. Other RNAi studies used the potential of plant stable transformation to increase the ingestion of small RNAs by aphids. Pitino et al. (2011) stably transformed *Arabidopsis thaliana* plants to express dsRNAs directed against Rack1 and Coo2 transcripts from *Myzus persicae* aphids, since they are able to feed on this model plant. They observed a reduction of nearly 60 % of both transcripts and reduced fecundity of treated aphids. The same *Arabidopsis*/*M. persicae* system was used to target serine protease transcripts, using *Arabidopsis* plants transformed with vectors expressing dsRNAs against this gene. This again resulted in reduced fecundity of treated aphids (Bhatia et al. 2012). Recent studies also transformed *Triticum aestivum* and *Nicotiana benthamiana* plant species to express dsRNAs specifically directed against a *Sitobion avenae* carboxylesterase (Xu et al. 2014) and the *hunchback* gene of *M. persicae* transcripts (Mao and Zeng 2014). In both cases, gene expression of targeted transcripts was reduced by at least 30 % and treated aphids showed reduced fecundity.

These studies on RNAi in aphids are summarized in Table 2. As a general trend, RNAi efficiency is rarely above 60 % and is usually transient. The tissue where targeted genes are expressed can also limit RNAi efficiency. So far, mainly gut and salivary gland tissues have been targeted, since genes expressed in those places are more likely to be in direct contact with siRNAs. It is also rare to identify a clear phenotype other than general characteristics such as mortality or fecundity. RNAi nevertheless is a valuable tool for functional analyses as well as a possible alternative for pest management strategies.

In terms of transient expression, the use of aphid viruses such as Densoviruses (DNV), which are parvoviruses that infect different insect species, to introduce DNA or RNA into the aphid body with a strong replication and expression level could be a good alternative method. DNVs replicate in the nuclei of insect cells and cause the characteristic nuclear hypertrophy (densonucleosis). One possibility would be to create synthetic viruses inserted with specific DNA sequences and use them as a gene vector to silence or overexpress target transcripts. So far, no studies have been reported in the aphid literature, but the *M. persicae* DNV (Van Munster et al. 2003) isolate might represent a remarkable tool to be used as a gene vector. An efficient recombinant mosquito DNV-mediated RNA interference system has indeed been recently developed (Gu et al. 2011) so such a system might be worth trying in the aphid system.

5.2 Towards Stable Transgenesis?

Stable transgenesis relies on integrating DNA fragments within the genome of a given organism or generating random or targeted mutations within the sequence of genes of interest. In order to get a stable transformation composed of only

Table 2 List of RNAi experiments performed on aphids

Study	Aphid species	Administration mode	Targeted transcripts	Tissue expression	RNAi efficiency (%)	Phenotype
Mutti et al. (2006)	*A. pisum*	siRNAs injection	C002	Salivary glands	40	Lethal
Jaubert-Possamai et al. (2007)	*A. pisum*	dsRNAs injection	Cathepsin-L	Gut	40	No phenotype
			Calreticulin	Whole body	40	No phenotype
Mutti et al. (2008)	*A. pisum*	siRNAs injection	C002	Salivary glands	40	Reduced performance
Whyard et al. (2009)	*A. pisum*	Feeding on artificial media with dsRNAs	vATPase	Gut	31	Lethal
Shakesby et al. (2009)	*A. pisum*	Feeding on artificial media with dsRNAs	Aquaporin 1	Gut	50	Elevated osmotic pressure
Pitino et al. (2011)	*M. persicae*	Transgenic plant (Arabidopsis thaliana and icotiana benthamiana) expressing dsRNAs	Rack1	Gut	50	Reduced aphid fecundity
			C002	Salivary glands	60	Reduced aphid fecundity
Bhatia et al. (2012)	*M. persicae*	Transgenic plant (Arabidopsis thaliana) expressing dsRNAs	Serine protease	Gut	30–60	Reduced gut protease activity and altered fecundity
Mao and Zeng 2012)	*A. pisum*	Feeding on artificial media with dsRNAs	Hunchback	Embryo	50	Lethal
Xu et al. (2014)	*S. avenae*	Transgenic plant (Triticum aestivum) expressing dsRNAs	Carboxylesterase (CbE E4)	Whole body	30–60	Reduced fecundity

(continued)

Table 2 (continued)

Study	Aphid species	Administration mode	Targeted transcripts	Tissue expression	RNAi efficiency (%)	Phenotype
Mao and Zeng (2014)	*M. persicae*	Transgenic plant (Nicotiana benthamiana) expressing dsRNAs	Hunchback	Embryo	30	Reduced fecundity
						Reduced biomass production
Sapountzis et al. (2014)	*A. pisum*	dsRNAs Injection and feeding	Cathepsin-L	Gut, head and carcass	Depending on administration mode and body part	Molting and gut epithelial cells defect

transgenic cells within an adult, it is necessary that the transgene integrates into the germ cell nucleus. Aphid species that produce either viviparous larvae or eggs make the system particularly promising. Viviparity could be an advantage since the fecundity is very high, with about 80 oocytes developed per mother. Injecting foreign DNA into parthenogenetic females should, in theory, be favored by this high number of putatively receptive cells. However, targeting foreign molecules in ovaries is difficult since several physical barriers separate ovarioles from the hemolymph where the foreign DNA is injected. Eggs thus may be more appropriate, but the fecundity of oviparous aphid sexual females is not high (approximately a dozen eggs) and the production of sexual females requires the induction of the sexual cycle and the co-production of males for mating. Further, oviposition is not synchronous. Injection into insect eggs is usually performed at a precise stage after oviposition, just after the second meiosis and before fusion with the sperm. Aphid eggs also diapause for almost 3 months, which delays producing transgenic individuals. Integrating foreign DNA within an aphid genome thus represents a challenging task, especially because transgenesis frequencies and efficacies are usually low in insects. Some years ago, several labs belonging to the IAGC tried injection of the Piggy-BAC system largely used in other insect species (such as *Bombyx*), but trials in aphids were unsuccessful.

New methodologies that target mutations by transgenesis have proven to be a powerful tool to generate specific mutation events within the genome of various organisms and link them to a wide range of phenotypes (Joung and Sander 2013; Larson et al. 2013). These methodologies use zinc-finger nuclease, TALEN (TAL-effectors), or even more recently the CRISPR-Cas nine systems to selectively create point mutation events at selected genomic locations. Such methods rely on DNA or RNA micro-injections into eggs, which could be done in aphids. Initial trials are needed to demonstrate that injected eggs are able to hatch 3 months later and that genome editing can be efficient.

5.3 Forward Genetics?

Stable transgenesis can also be performed by genome editing, using a forward genetics approach (generating mutants and screening for the phenotype of interest). EMS mutagenesis has been successful in generating random mutations within the genome of various model organisms. Such an approach was recently applied to the pea aphid and proved its efficiency (Tagu et al. 2014a). The authors first developed a protocol where pea aphids were treated with various concentrations of EMS and they could observe a reduced proportion of males within the offspring of treated aphids. Males are haploid for the X chromosome, so they used this proportion as read-out for EMS causing random lethal recessive mutations. They then developed another protocol that allowed the maintenance of putative mutant aphid lines. Some of these were analyzed for their reproductive morph production, and among them, one line showed a reduced and reproducible males proportion as well as some males

showing a range of morphological defects. Chemical mutagenesis thus appears feasible in aphids and opens the way for the development of forward genetic screens for specific phenotypes.

6 Conclusions: Integration

Genomic and post-genomic approaches in aphids are growing up! They are not fully mature yet… As stressed in the introduction, it is important to improve our capacity to precisely understand aphid adaptation mechanisms in order to propose new methods for controlling these pests in agriculture.

We already see some limitations of our studies: first, we focused in recent years on a limited number of aphid species. The development of genomic and post-genomic strategies is required for more species that have specific adaptations (such as host plants for instance). Second, we lack strong functional analysis tools, despite efforts made by the community.

There are, however, alternative ways to enrich our knowledge of aphid biology and phenotypic plasticity. We can learn from the large natural variation of these traits *in natura*, by comparing natural populations that differ in the trait of interest. Also, we can develop and integrate large post-genomic data regarding the different functional DNA elements: this concerns all kind of RNAs and chromatin modifications, at different levels of regulation (pre- post-transcriptional or translational). These integrations require not only genomic data but also bioinformatics analyses and modeling to develop gene networks. This has been initiated with the goal of constructing a gene network encompassing mRNA and miRNA in the pea aphid (Wucher et al. 2015) but much remains to be done (see discussion in Tagu et al. 2014b). Such integrative approaches might eventually lead to a modENCODE-like initiative in a non-model species such as aphids.

References

Andolfatto, P., Davison, D., Erezyilmaz, D., Hu, T. T., Mast, J., Sunayama-Morita, T., & Stern, D. L. (2011). Multiplexed shotgun genotyping for rapid and efficient genetic mapping. *Genome Research, 21*, 610–617.

Bai, X., Zhang, W., Orantes, L., Jun, T.-H., Mittapalli, O., Mian, M. R., & Michel, A. P. (2010). Combining next-generation sequencing strategies for rapid molecular resource development from an invasive aphid species, Aphis glycines. *PLoS One, 5*, e11370.

Barberà, M., Mengual, B., Collantes-Alegre, J., Cortés, T., González, A., & Martínez-Torres, D. (2013). Identification, characterization and analysis of expression of genes encoding arylalkylamine N-acetyltransferases in the pea aphid Acyrthosiphon pisum. *Insect Molecular Biology, 22*, 623–634.

Bhatia, V., Bhattacharya, R., Uniyal, P. L., Singh, R., & Niranjan, R. S. (2012). Host generated siRNAs attenuate expression of serine protease gene in myzus persicae. *PLoS One, 7*, e46343.

Bickel, R. D., Dunham, J. P., & Brisson, J. A. (2013). Widespread selection across coding and noncoding DNA in the Pea Aphid genome. *G3: Genes|Genomes|Genetics, 3*, 993–1001.

Blackman, R. (1971). Variation in the photoperiodic response within natural populations of Myzus persicae (Sulz.). *Bulletin of Entomological Research, 60*, 533–546.

Blackman, R. (1972). The inheritance of life-cycle differences in Myzus persicae (Sulz.)(Hem., Aphididae). *Bulletin of Entomological Research, 62*, 281–294.

Braendle, C., Caillaud, M. C., & Stern, D. L. (2005a). Genetic mapping of aphicarus -- a sex-linked locus controlling a wing polymorphism in the pea aphid (Acyrthosiphon pisum). *Heredity, 94*, 435–442.

Braendle, C., Davis, G. K., Brisson, J. A., & Stern, D. L. (2006). Wing dimorphism in aphids. *Heredity, 97*, 192–199.

Braendle, C., Friebe, I., Caillaud, M. C., & Stern, D. L. (2005b). Genetic variation for an aphid wing polyphenism is genetically linked to a naturally occurring wing polymorphism. *Proceedings of the Royal Society B: Biological Sciences, 272*, 657–664.

Brisson, J. A., Davis, G. K., & Stern, D. L. (2007). Common genome-wide patterns of transcript accumulation underlying the wing polyphenism and polymorphism in the pea aphid (Acyrthosiphon pisum). *Evolution & Development, 9*, 338–346.

Caillaud, M., Boutin, M., Braendle, C., & Simon, J. (2002). A sex-linked locus controls wing polymorphism in males of the pea aphid, Acyrthosiphon pisum (Harris). *Heredity, 89*, 346–352.

Chang, C.-C., Huang, T.-Y., Cook, C. E., Lin, G.-W., Shih, C.-L., & Chen, R. P. (2009). Developmental expression of Apnanos during oogenesis and embryogenesis in the parthenogenetic pea aphid Acyrthosiphon pisum. *International Journal of Developmental Biology, 53*, 169.

Colbourne, J. K., Pfrender, M. E., Gilbert, D., Thomas, W. K., Tucker, A., Oakley, T. H., Tokishita, S., Aerts, A., Arnold, G. J., & Basu, M. K. (2011). The ecoresponsive genome of Daphnia pulex. *Science, 331*, 555–561.

Consortium, E. P., et al. (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature, 489*, 57–74.

Consortium i5K. (2013). The i5K initiative: Advancing arthropod genomics for knowledge, human health, agriculture, and the environment. *Journal of Heredity*, 104, 595–600.

Corbitt, T. S., & Hardie, J. (1985). Juvenile hormone effects on polymorphism in the pea aphid, A cyrthosiphon pisum. *Entomologia Experimentalis et Applicata, 38*, 131–135.

Cortés, T., Ortiz-Rivas, B., & Martínez-Torres, D. (2010). Identification and characterization of circadian clock genes in the pea aphid Acyrthosiphon pisum. *Insect Molecular Biology, 19*, 123–139.

Cortés, T., Tagu, D., Simon, J., Moya, A., & Martínez-Torres, D. (2008). Sex versus parthenogenesis: A transcriptomic approach of photoperiod response in the model aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae). *Gene, 408*, 146–156.

Cronin, A. L., Molet, M., Doums, C., Monnin, T., & Peeters, C. (2013). Recurrent evolution of dependent colony foundation across eusocial insects. *Annual Review of Entomology, 58*, 37–55.

Davidowitz, G., D'Amico, L. J., & Nijhout, H. F. (2004). The effects of environmental variation on a mechanism that controls insect body size. *Evolutionary Ecology Research, 6*, 49–62.

Davis, G. K. (2012). Cyclical parthenogenesis and viviparity in aphids as evolutionary novelties. *Journal of Experimental Zoology Part B-Molecular and Developmental Evolution, 318B*(6 Special Issue), 448–459.

Dedryver, C., Le Gallic, J., Gauthier, J., & Simon, J. (1998). Life cycle of the cereal aphid Sitobion avenae F.: polymorphism and comparison of life history traits associated with sexuality. *Ecological Entomology, 23*, 123–132.

Dedryver, C.-A., Hullé, M., Le Gallic, J.-F., Caillaud, M. C., & Simon, J.-C. (2001). Coexistence in space and time of sexual and asexual populations of the cereal aphid Sitobion avenae. *Oecologia, 128*, 379–388.

Dedryver, C., Le Gallic, J., Mahéo, F., Simon, J., & Dedryver, F. (2013). The genetics of obligate parthenogenesis in an aphid species and its consequences for the maintenance of alternative reproductive modes. *Heredity, 110*, 39–45.

Dixon, A., & Howard, M. (1986). Dispersal in aphids, a problem in resource allocation. In *Insect flight* (pp. 145–151). Heidelberg: Springer.

Dombrovsky, A., Arthaud, L., Ledger, T. N., Tares, S., & Robichon, A. (2009). Profiling the repertoire of phenotypes influenced by environmental cues that occur during asexual reproduction. *Genome Research, 19*, 2052–2063.

Duncan, E. J., Leask, M. P., & Dearden, P. K. (2013). The pea aphid (*Acyrthosiphon pisum*) genome encodes two divergent early developmental programs. *Developmental Biology, 377*, 262–274.

Ebert, T., & Cartwright, B. (1997). Biology and ecology of Aphis gossypii Glover (Homoptera: Aphididae). *Southwestern Entomologist, 22*, 116–153.

Feil, R., & Fraga, M. F. (2012). Epigenetics and the environment: emerging patterns and implications. *Nature Reviews Genetics, 13*, 97–109.

Frantz, A., Plantegenest, M., & Simon, J.-C. (2006). Temporal habitat variability and the maintenance of sex in host populations of the pea aphid. *Proceedings of the Royal Society B: Biological Sciences, 273*, 2887–2891.

Gallot, A., Rispe, C., Leterme, N., Gauthier, J.-P., Jaubert-Possamai, S., & Tagu, D. (2010). Cuticular proteins and seasonal photoperiodism in aphids. *Insect Biochemistry and Molecular Biology, 40*, 235–240.

Gallot, A., Shigenobu, S., Hashiyama, T., Jaubert-Possamai, S., & Tagu, D. (2012). Sexual and asexual oogenesis require the expression of unique and shared sets of genes in the insect Acyrthosiphon pisum. *BMC Genomics, 13*, 76.

Gao, N., & Hardie, J. (1997). Melatonin and the pea aphid, Acyrthosiphon pisum. *Journal of Insect Physiology, 43*, 615–620.

Gerardo, N. M., Altincicek, B., Anselme, C., Atamian, H., Barribeau, S. M., De Vos, M., Duncan, E. J., Evans, J. D., Gabaldón, T., & Ghanim, M. (2010). Immunity and other defenses in pea aphids, Acyrthosiphon pisum. *Genome Biology, 11*, R21.

Gerstein, M. B., Lu, Z. J., Van Nostrand, E. L., Cheng, C., Arshinoff, B. I., Liu, T., Yip, K. Y., Robilotto, R., Rechtsteiner, A., & Ikegami, K. (2010). Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project. *Science, 330*, 1775–1787.

Ghanim, M., Dombrovsky, A., Raccah, B., & Sherman, A. (2006). A microarray approach identifies ANT, OS-D and takeout-like genes as differentially regulated in alate and apterous morphs of the green peach aphid *Myzus persicae* (Sulzer). *Insect Biochemistry and Molecular Biology, 36*, 857–868.

Goecks, J., Nekrutenko, A., Taylor, J., & Galaxy Team. (2010). Galaxy: A comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biology, 11*, R86.

Gu, J., Liu, M., Deng, Y., Peng, H., & Chen, X. (2011). Development of an efficient recombinant mosquito densovirus-mediated RNA interference system and its preliminary application in mosquito control. *PLoS One, 6*, e21329.

Hardie, J., & Lees, A. (1985). Endocrine control of polymorphism and polyphenism. *Comprehensive Insect Physiology, Biochemistry and Pharmacology, 8*, 441–490.

Huerta-Cepas, J., Marcet-Houben, M., Pignatelli, M., Moya, A., & Gabaldón, T. (2010). The pea aphid phylome: A complete catalogue of evolutionary histories and arthropod orthology and paralogy relationships for Acyrthosiphon pisum genes. *Insect Molecular Biology, 19*, 13–21.

Hunter, W., Dang, P., Bausher, M., Chaparro, J., McKendree, W., Shatters, R., Jr., McKenzie, C., & Sinisterra, X. (2003). Aphid biology: Expressed genes from alate Toxoptera citricida, the brown citrus aphid. *Journal of Insect Science, 3*, 1–7.

Huybrechts, J., Bonhomme, J., Minoli, S., Prunier-Leterme, N., Dombrovsky, A., Abdel-Latief, M., Robichon, A., Veenstra, J. A., & Tagu, D. (2010). Neuropeptide and neurohormone precursors in the pea aphid, Acyrthosiphon pisum. *Insect Molecular Biology, 19*, 87–95.

International Aphid Genomics Consortium. (2010). Genome sequence of the Pea Aphid Acyrthosiphon pisum. *PLoS Biology, 8*(2), e1000313.

Ishikawa, A., Ishikawa, Y., Okada, Y., Miyazaki, S., Miyakawa, H., Koshikawa, S., Brisson, J. A., & Miura, T. (2012a). Screening of upregulated genes induced by high density in the vetch aphid Megoura crassicauda. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 317*, 194–203.

Ishikawa, A., & Miura, T. (2013). Transduction of high-density signals across generations in aphid wing polyphenism. *Physiological Entomology, 38*, 150–156.

Ishikawa, A., Ogawa, K., Gotoh, H., Walsh, T. K., Tagu, D., Brisson, J. A., Rispe, C., Jaubert-Possamai, S., Kanbe, T., & Tsubota, T. (2012b). Juvenile hormone titre and related gene expression during the change of reproductive modes in the pea aphid. *Insect Molecular Biology, 21*, 49–60.

Jaquiéry, J., Stoeckel, S., Nouhaud, P., Mieuzet, L., Mahéo, F., Legeai, F., Bernard, N., Bonvoisin, A., Vitalis, R., & Simon, J. C. (2012). Genome scans reveal candidate regions involved in the adaptation to host plant in the pea aphid complex. *Molecular Ecology, 21*, 5251–5264.

Jaquiéry, J., Stoeckel, S., Larose, C., Nouhaud, P., Rispe, C., Mieuzet, L., Bonhomme, J., Mahéo, F., Legeai, F., Gauthier, J.-P., Prunier-Leterme, N., Tagu, D., & Simon, J.-C. (2014). Genetic control of contagious asexuality. *PLoS Genetics, 10*(12), e1004838.

Jaubert-Possamai, S., Le Trionnaire, G., Bonhomme, J., Christophides, G. K., Rispe, C., & Tagu, D. (2007). Gene knockdown by RNAi in the pea aphid Acyrthosiphon pisum. *BMC Biotechnology, 7*, 63.

Jaubert-Possamai, S., Rispe, C., Tanguy, S., Gordon, K., Walsh, T., Edwards, O., & Tagu, D. (2010). Expansion of the miRNA pathway in the hemipteran insect Acyrthosiphon pisum. *Molecular Biology and Evolution, 27*, 979–987.

Joung, J. K., & Sander, J. D. (2013). TALENs: A widely applicable technology for targeted genome editing. *Nature Reviews Molecular Cell Biology, 14*, 49–55.

Kharchenko, P. V., Alekseyenko, A. A., Schwartz, Y. B., Minoda, A., Riddle, N. C., Ernst, J., Sabo, P. J., Larschan, E., Gorchakov, A. A., Gu, T. T., Linder-Basso, D., Plachetka, A., Shanower, G., Tolstorukov, M. Y., Luquette, L. J., Xi, R. B., Jung, Y. L., Park, R. W., Bishop, E. P., Canfield, T. K., Sandstrom, R., Thurman, R. E., MacAlpine, D. M., Stamatoyannopoulos, J. A., Kellis, M., Elgin, S. C. R., Kuroda, M. I., Pirrotta, V., Karpen, G. H., & Park, P. J. (2011). Comprehensive analysis of the chromatin landscape in Drosophila melanogaster. *Nature, 471*, 480–485.

Kim, H., Lee, S., & Jang, Y. (2011). Macroevolutionary patterns in the Aphidini aphids (Hemiptera: Aphididae): diversification, host association, and biogeographic origins. *PLoS One, 6*, e24749.

Kucharski, R., Maleszka, J., Foret, S., & Maleszka, R. (2008). Nutritional control of reproductive status in honeybees via DNA methylation. *Science, 319*, 1827–1830.

Larson, M. H., Gilbert, L. A., Wang, X., Lim, W. A., Weissman, J. S., & Qi, L. S. (2013). CRISPR interference (CRISPRi) for sequence-specific control of gene expression. *Nature Protocols, 8*, 2180–2196.

Le Trionnaire, G., Francis, F., Jaubert-Possamai, S., Bonhomme, J., De Pauw, E., Gauthier, J. P., Haubruge, E., Legeai, F., Prunier-Leterme, N., Simon, J. C., Tanguy, S., & Tagu, D. (2009). Transcriptomic and proteomic analyses of seasonal photoperiodism in the pea aphid. *BMC Genomics, 10*, 456.

Le Trionnaire, G., Hardie, J., Jaubert-Possamai, S., Simon, J. C., & Tagu, D. (2008). Shifting from clonal to sexual reproduction in aphids: physiological and developmental aspects. *Biology of the Cell, 100*, 441–451.

Le Trionnaire, G., Jaubert, S., Sabater-Munoz, B., Benedetto, A., Bonhomme, J., Prunier-Leterme, N., Martinez-Torres, D., Simon, J. C., & Tagu, D. (2007). Seasonal photoperiodism regulates the expression of cuticular and signalling protein genes in the pea aphid. *Insect Biochemistry and Molecular Biology, 37*, 1094–1102.

Lee, E., Helt, G. A., Reese, J. T., Munoz-Torres, M. C., Childers, C. P., Buels, R. M., Stein, L., Holmes, I. H., Elsik, C. G., & Lewis, S. E. (2013). Web Apollo: A web-based genomic annotation editing platform. *Genome Biology, 30*, R93.

Legeai, F., Rizk, G., Walsh, T., Edwards, O., Gordon, K., Lavenier, D., Leterme, N., Mereau, A., Nicolas, J., Tagu, D., & Jaubert-Possamai, S. (2010). Bioinformatic prediction, deep sequencing of microRNAs and expression analysis during phenotypic plasticity in the pea aphid, Acyrthosiphon pisum. *BMC Genomics, 11*, 281.

Lewontin, R., & Krakauer, J. (1973). Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics, 74*, 175–195.

Lu, H.-L., Tanguy, S., Rispe, C., Gauthier, J.-P., Walsh, T., Gordon, K., Edwards, O., Tagu, D., Chang, C.-C., & Jaubert-Possamai, S. (2011). Expansion of genes encoding piRNA-associated argonaute proteins in the pea aphid: Diversification of expression profiles in different plastic morphs. *PLoS One, 6*, e28051.

Lyko, F., Foret, S., Kucharski, R., Wolf, S., Falckenhayn, C., & Maleszka, R. (2010). The honey bee epigenomes: Differential methylation of brain DNA in queens and workers. *PLoS Biology, 8*, e1000506.

Lyko, F., & Maleszka, R. (2011). Insects as innovative models for functional studies of DNA methylation. *Trends in Genetics, 27*, 127–131.

Ma, Z., Guo, W., Guo, X., Wang, X., & Kang, L. (2011). Modulation of behavioral phase changes of the migratory locust by the catecholamine metabolic pathway. *Proceedings of the National Academy of Sciences, 108*, 3882–3887.

Mao, J., & Zeng, F. (2012). Feeding-based RNA intereference of a gap gene is lethal to the pea aphid, Acyrthosiphon pisum. *PLoS One, 7*, e48718.

Mao, J., & Zeng, F. (2014). Plant-mediated RNAi of a gap gene-enhanced tobacco tolerance against the Myzus persicae. *Transgenic Research, 23*(1), 145–152.

Misof, B., Liu, S., Meusemann, K., Peters, R. S., Donath, A., Mayer, C., Frandsen, P. B., Ware, J., Flouri, T., Beutel, R. G., Niehuis, O., Petersen, M., Izquierdo-Carrasco, F., Wappler, T., Rust, J., Aberer, A. J., Aspöck, U., Aspöck, H., Bartel, D., Blanke, A., Berger, S., Böhm, A., Buckley, T. R., Calcott, B., Chen, J., Friedrich, F., Fukui, M., Fujita, M., Greve, C., Grobe, P., Gu, S., Huang, Y., Jermiin, L. S., Kawahara, A. Y., Krogmann, L., Kubiak, M., Lanfear, R., Letsch, H., Li, Y., Li, Z., Li, J., Lu, H., Machida, R., Mashimo, Y., Kapli, P., McKenna, D. D., Meng, G., Nakagaki, Y., Navarrete-Heredia, J. L., Ott, M., Ou, Y., Pass, G., Podsiadlowski, L., Pohl, H., von Reumont, B. M., Schütte, K., Sekiya, K., Shimizu, S., Slipinski, A., Stamatakis, A., Song, W., Su, X., Szucsich, N. U., Tan, M., Tan, X., Tang, M., Tang, J., Timelthaler, G., Tomizuka, S., Trautwein, M., Tong, X., Uchifune, T., Walzl, M. G., Wiegmann, B. M., Wilbrandt, J., Wipfler, B., Wong, T. K., Wu, Q., Wu, G., Xie, Y., Yang, S., Yang, Q., Yeates, D. K., Yoshizawa, K., Zhang, Q., Zhang, R., Zhang, W., Zhang, Y., Zhao, J., Zhou, C., Zhou, L., Ziesmann, T., Zou, S., Li, Y., Xu, X., Zhang, Y., Yang, H., Wang, J., Wang, J., Kjer, K. M., & Zhou, X. (2014). Phylogenomics resolves the timing and pattern of insect evolution. *Science, 346*(6210), 763–767.

Moraiti, C., Nakas, C., & Papadopoulos, N. (2014). Diapause termination of Rhagoletis cerasi pupae is regulated by local adaptation and phenotypic plasticity: Escape in time through bet-hedging strategies. *Journal of Evolutionary Biology, 27*, 43–54.

Moran, N. A., & Jarvik, T. (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science, 328*, 624–627.

Müller, C. B., Williams, I. S., & Hardie, J. (2001). The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology, 26*, 330–340.

Mutti, N. S., Louis, J., Pappan, L. K., Pappan, K., Begum, K., Chen, M.-S., Park, Y., Dittmer, N., Marshall, J., & Reese, J. C. (2008). A protein from the salivary glands of the pea aphid, Acyrthosiphon pisum, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences, 105*, 9965–9969.

Mutti, N. S., Park, Y., Reese, J. C., & Reeck, G. R. (2006). RNAi knockdown of a salivary transcript leading to lethality in the pea aphid, Acyrthosiphon pisum. *Journal of Insect Science, 6*, 1–7.

Nakabachi, A., Shigenobu, S., Sakazume, N., Shiraki, T., Hayashizaki, Y., Carninci, P., Ishikawa, H., Kudo, T., & Fukatsu, T. (2005). Transcriptome analysis of the aphid bacteriocyte, the symbiotic host cell that harbors an endocellular mutualistic bacterium, Buchnera. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 5477–5482.

Nicholson, S. J., Nickerson, M. L., Dean, M., Song, Y., Hoyt, P. R., Rhee, H., Kim, C., & Puterka, G. J. (2015). The genome of Diuraphis noxia, a global aphid pest of small grains. *BMC Genomics, 16*(1), 429.

Nijhout, H. F. (2003). Development and evolution of adaptive polyphenisms. *Evolution & Development, 5*, 9–18.

Nikoh, N., McCutcheon, J. P., Kudo, T., Miyagishima, S.-Y., Moran, N. A., & Nakabachi, A. (2010). Bacterial genes in the aphid genome: Absence of functional gene transfer from Buchnera to its host. *PLoS Genetics, 6*, e1000827.

Ogawa, K., & Miura, T. (2014). Aphid polyphenisms: Trans-generational developmental regulation through viviparity. *Frontiers in Physiology, 5*, 1.

Oliver, K. M., Degnan, P. H., Burke, G. R., & Moran, N. A. (2010). Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annual Review of Entomology, 55*, 247–266.

Ollivier, M., Gabaldón, T., Poulain, J., Gavory, F., Leterme, N., Gauthier, J.-P., Legeai, F., Tagu, D., Simon, J., & Rispe, C. (2012). Comparison of gene repertoires and patterns of evolutionary rates in eight aphid species that differ by reproductive mode. *Genome Biology and Evolution, 4*, 155–167.

Ortiz-Rivas, B., Jaubert-Possamai, S., Tanguy, S., Gauthier, J.-P., Tagu, D., & Claude, R. (2012). Evolutionary study of duplications of the miRNA machinery in aphids associated with striking rate acceleration and changes in expression profiles. *BMC Evolutionary Biology, 12*, 216.

Pitino, M., Coleman, A. D., Maffei, M. E., Ridout, C. J., & Hogenhout, S. A. (2011). Silencing of aphid genes by dsRNA feeding from plants. *PLoS One, 6*, e25709.

Ramsey, J. S., Wilson, A. C., De Vos, M., Sun, Q., Tamborindeguy, C., Winfield, A., Malloch, G., Smith, D. M., Fenton, B., & Gray, S. M. (2007). Genomic resources for Myzus persicae: EST sequencing, SNP identification, and microarray design. *BMC Genomics, 8*, 423.

Rider, S., Jr., Srinivasan, D., & Hilgarth, R. (2010). Chromatin-remodelling proteins of the pea aphid, Acyrthosiphon pisum (Harris). *Insect Molecular Biology, 19*, 201–214.

Rispe, C., Kutsukake, M., Doublet, V., Hudaverdian, S., Legeai, F., Simon, J.-C., Tagu, D., & Fukatsu, T. (2008). Large gene family expansion and variable selective pressures for cathepsin B in aphids. *Molecular Biology and Evolution, 25*, 5–17.

Rispe, C., Pierre, J. S., Simon, J. C., & Gouyon, P. H. (1998). Models of sexual and asexual coexistence in aphids based on constraints. *Journal of Evolutionary Biology, 11*, 685–701.

Roy, S., Ernst, J., Kharchenko, P. V., Kheradpour, P., Negre, N., Eaton, M. L., Landolin, J. M., Bristow, C. A., Ma, L., & Lin, M. F. (2010). Identification of functional elements and regulatory circuits by Drosophila modENCODE. *Science, 330*, 1787–1797.

Sabater-Muñoz, B., Legeai, F., Rispe, C., Bonhomme, J., Dearden, P., Dossat, C., Duclert, A., Gauthier, J. P., Ducray, D. G., & Hunter, W. (2006). Large-scale gene discovery in the pea aphid Acyrthosiphon pisum (Hemiptera). *Genome Biology, 7*, R21.

Sapountzis, P., Duport, G., Balmand, S., Gaget, K., Jaubert-Possamai, S., Febvay, G., Charles, H., Rahbé, Y., Colella, S., & Calevro, F. (2014). New insight into the RNA interference response against *cathepsin-L* gene in the pea aphid, *Acyrthosiphon pisum*: Molting or gut phenotypes specifically induced by injection or feeding treatments. *Insect Biochemistry and Molecular Biology, 51*, 20–32.

Saunders, D. (2008). Photoperiodism in insects and other animals. In *Photobiology* (pp. 389–416). New York: Springer.

Scott, J. G., Michel, K., Bartholomay, L. C., Siegfried, B. D., Hunter, W. B., Smagghe, G., Zhu, K. Y., & Douglas, A. E. (2013). Towards the elements of successful insect RNAi. *Journal of Insect Physiology, 59*, 1212–1221.

Shakesby, A., Wallace, I., Isaacs, H., Pritchard, J., Roberts, D., & Douglas, A. (2009). A water-specific aquaporin involved in aphid osmoregulation. *Insect Biochemistry and Molecular Biology, 39*, 1–10.

Simola, D. F., Ye, C., Mutti, N. S., Dolezal, K., Bonasio, R., Liebig, J., Reinberg, D., & Berger, S. L. (2013). A chromatin link to caste identity in the carpenter ant Camponotus floridanus. *Genome Research, 23*, 486–496.

Simon, J.-C., Pfrender, M. E., Tollrian, R., Tagu, D., & Colbourne, J. K. (2011). Genomics of environmentally induced phenotypes in 2 extremely plastic arthropods. *Journal of Heredity, 102*, 512–525.

Simon, J.-C., Rispe, C., & Sunnucks, P. (2002). Ecology and evolution of sex in aphids. *Trends in Ecology & Evolution, 17*, 34–39.

Simon, J.-C., Stoeckel, S., & Tagu, D. (2010). Evolutionary and functional insights into reproductive strategies of aphids. *Comptes Rendus Biologies, 333*, 488–496.

Simon, J., Blackman, R., & Le Gallic, J. (1991). Local variability in the life cycle of the bird cherry-oat aphid, Rhopalosiphum padi (Homoptera: Aphididae) in western France. *Bulletin of Entomological Research, 81*, 315–322.

Simon, J. M., Giresi, P. G., Davis, I. J., & Lieb, J. D. (2012). Using formaldehyde-assisted isolation of regulatory elements (FAIRE) to isolate active regulatory DNA. *Nature Protocols, 7*, 256–267.

Simpson, S. J., Sword, G. A., & Lo, N. (2011). Polyphenism in insects. *Current Biology, 21*, R738–R749.

Skinner, M. E., Uzilov, A. V., Stein, L. D., Mungall, C. J., & Holmes, I. H. (2009). JBrowse: A next-generation genome browser. *Genome Research, 19*, 1630–1638.

Stein, L. D. (2013). Using GBrowse 2.0 to visualize and share next-generation sequence data. *Briefings in Bioinformatics, 14*, 162–171.

Sutherland, O. (1969). The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum. Journal of Insect Physiology, 15*, 1385–1410.

Tagu, D., Dugravot, S., Outreman, Y., Rispe, C., Simon, J.-C., & Colella, S. (2010). The anatomy of an aphid genome: From sequence to biology. *Comptes Rendus Biologies, 333*, 464–473.

Tagu, D., Le Trionnaire, G., Tanguy, S., Gauthier, J.-P., & Huynh, J.-R. (2014a). EMS Mutagenesis in the Pea Aphid Acyrthosiphon pisum. *G3: Genes|Genomes|Genetics, 4*, 657–667.

Tagu, D., Prunier-Leterme, N., Legeai, F., Gauthier, J.-P., Duclert, A., Sabater-Muñoz, B., Bonhomme, J., & Simon, J.-C. (2004). Annotated expressed sequence tags for studies of the regulation of reproductive modes in aphids. *Insect Biochemistry and Molecular Biology, 34*, 809–822.

Tagu, D., Colbourne, J. K., & Nègre, N. (2014b). Genomic data integration for ecological and evolutionary traits in non-model organisms. *BMC Genomics, 15*(1), 490.

The International HapMap Consortium. (2007). A second generation human haplotype map of over 3.1 million SNPs. *Nature, 449*, 851–861.

Tian, L., & Zhou, X. (2014). The soldiers in societies: Defense, regulation, and evolution. *International Journal of Biological Sciences, 5*, 296–308.

Tsuchida, T., Koga, R., Horikawa, M., Tsunoda, T., Maoka, T., Matsumoto, S., Simon, J.-C., & Fukatsu, T. (2010). Symbiotic bacterium modifies aphid body color. *Science, 330*, 1102–1104.

van Munster, M., Dullemans, A., Verbeek, M., van den Heuvel, J., Reinbold, C., Brault, V., Clerivet, A., & van der Wilk, F. (2003). Characterization of a new densovirus infecting the green peach aphid *Myzus persicae. Journal of Invertebrate Pathology, 84*, 6–14.

Walsh, T. K., Brisson, J. A., Robertson, H. M., Gordon, K., Jaubert-Possamai, S., Tagu, D., & Edwards, O. R. (2010). A functional DNA methylation system in the pea aphid, Acyrthosiphon pisum. *Insect Molecular Biology, 19*, 215–228.

Wang, S. H., & Elgin, S. C. (2011). Drosophila Piwi functions downstream of piRNA production mediating a chromatin-based transposon silencing mechanism in female germ line. *Proceedings of the National Academy of Sciences, 108*, 21164–21169.

Wang, X., & Kang, L. (2014). Molecular mechanisms of phase change in locusts. *Annual Review of Entomology, 59*, 225–244.

Whyard, S., Singh, A. D., & Wong, S. (2009). Ingested double-stranded RNAs can act as species-specific insecticides. *Insect Biochemistry and Molecular Biology, 39*, 824–832.

Wucher V., Tagu D., & Nicolas J. (2015). Edge selection in a noisy graph by concept analysis: Application to a Genomic Network. In B. Lausen, S. Krolak-Schwerdt, & M. Böhmer (Eds.), *Data science, learning by latent structures, and knowledge discovery* (pp. 353–364). Berlin/ Heidelberg: Springer. 978-3-662-44982-0. I–331.

Xu, L., Duan, X., Lv, Y., Zhang, X., Nie, Z., Xie, C., Ni, Z., & Liang, R. (2014). Silencing of an aphid carboxylesterase gene by use of plant-mediated RNAi impairs Sitobion avenae tolerance of Phoxim insecticides. *Transgenic Research, 1–8*(2), 389–396.

Yang, M., Wei, Y., Jiang, F., Wang, Y., Guo, X., He, J., & Kang, L. (2014a). MicroRNA-133 inhibits behavioral aggregation by controlling dopamine synthesis in locusts. *PLoS Genetics, 10*, e1004206.

Yang, X., Liu, X., Xu, X., Li, Z., Li, Y., Song, D., Yu, T., Zhu, F., Zhang, Q., & Zhou, X. (2014b). Gene expression profiling in winged and wingless cotton Aphids, Aphis gossypii (Hemiptera: Aphididae). *International Journal of Biological Sciences, 10*, 257.

Hormonal Regulation of Development and Reproduction

Subba Reddy Palli

Abstract The two major insect hormones ecdysterioids (the most active form is the 20-hydroxyecdysone, 20E), sequiterpenoids (juvenile hormones, JH) regulate almost every aspect of insect life including embryogenesis, molting, metamorphosis and reproduction. The physiological and biological processes regulated by these hormones have been studied over the last century and well documented in several reviews. After the advent of genomic technologies, the molecular processes that are regulated by these hormones and the genes targeted by receptors and transcription factors that transduce signals of these hormones are being actively investigated. With the sequencing of genomes and transcriptomes of many pest insects, we began to identify key players involved in action of these hormones in pest insects. Insulin-like peptides have been identified in Drosophila melanogaster and shown to play key roles in regulation of growth, development of nervous system, reproduction and lifespan. Recently, insulin-like peptides have been identified in several insect pests and disease vectors and shown to play key roles in regulation of growth, development and reproduction.

In this review, I will focus on the hormonal regulation of embryogenesis, metamorphosis and reproduction. I shall focus on systems and physiological processes that are amenable for development of methods including double-stranded RNA or small molecules to interfere with these processes with a goal to develop novel pest and disease vector control strategies.

1 Introduction

The two major insect hormones ecdysterioids (the most active form is the 20-hydroxyecdysone, 20E), sequiterpenoids (juvenile hormones, JH) regulate almost every aspect of insect life including embryogenesis, molting, metamorphosis and reproduction. The physiological and biological processes regulated by these

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hormones have been studied over the last century and well documented in several reviews. After the advent of genomic technologies, the molecular processes that are regulated by these hormones and the genes targeted by receptors and transcription factors that transduce signals of these hormones are being actively investigated. With the sequencing of genomes and transcriptomes of many pest insects, we began to identify key players involved in action of these hormones in pest insects.

Insulin-like peptides have been identified in *Drosophila melanogaster* and shown to play key roles in regulation of growth, development of nervous system, reproduction and lifespan. Recently, insulin-like peptides have been identified in several insect pests and disease vectors and shown to play key roles in regulation of growth, development and reproduction.

In this review, I will focus on the hormonal regulation of embryogenesis, metamorphosis and reproduction. I shall focus on systems and physiological processes that are amenable for development of methods including double-stranded RNA or small molecules to interfere with these processes with a goal to develop novel pest and disease vector control strategies.

2 Embryogenesis

Ecdysteriods are synthesized in the prothoracic glands during the embryonic and imaginal stages. Prothoracic glands degenerate during the adult development and the reproductive tissues including ovary, testes and accessory glands participate in synthesis of ecdysteriods in adults. Ecdysteriods have been detected in the embryos of *D. melanogaster* (Maroy et al. 1988). Mutations in the genes coding for enzymes involved in ecdysteroid biosynthesis cause embryonic lethality. Phenotypes observed include defects in head involution, dorsal closure, gut development and cuticle deposition. These observations suggest that ecdysteriods play key roles in embryonic development (Chavez et al. 2000; Ono et al. 2006). Ecdysterioids are also thought to be involved in embryonic development of *Locusta migratoria* (Tawfik et al. 2002) and the cricket *allonemobius socius* (Reynolds and Hand 2009).

In *D. melanogaster*, the critical players involved in 20E action including ecdysone receptor (EcR), ultraspiracle (USP), E74, E75, hormone receptor 3 (HR3) and βFTZ-F1 are sequentially expressed during embryogenesis beginning at 6 h after egg laying and the sequential expression of these genes in embryos is similar to that observed during larval-pupal metamorphosis (Maroy et al. 1988; Sullivan and Thummel 2003). Analysis of fruit fly mutants for some of these genes showed that these genes are required for successful embryonic development (Bender et al. 1997; Carney et al. 1997; Chavoshi et al. 2010; Yamada et al. 2000). For example, EcR null mutations cause embryonic lethality and show phenotypes such as aberrant germ band retraction (Bender et al. 1997; Kozlova and Thummel 2003). EcR is also required for organ morphogenesis in *D. melanogaster* embryos (Chavoshi et al. 2010). EcR mRNAs have been detected during the embryonic stages of silkworm

Bombyx mori (Maeda et al. 2008). DHR3 deficient embryos die at the end of embryogenesis and show some defects in peripheral nervous system (Carney et al. 1997). βFTZ-F1 null mutants also are unable to complete embryonic development (Yamada et al. 2000). DHR3 and βFTZ-F1 null mutants exhibit common phenotypes that include defects in tracheal system function, the size and pigmentation of denticles and ventral nerve cord condensation (Ruaud et al. 2010). Broad complex (BR-C) is expressed during the second half of the embryogenesis in *D. melanogaster* (Sullivan and Thummel 2003). However, in *D. melanogaster*, disruption of BR-C function by mutagenesis, does not affect embryogenesis as well as larval development, the BR-C null mutants die at pupariation (Belyaeva et al. 1980; Kiss et al. 1988). These data suggest that BR-C in fruit flies is required for larval-pupal metamorphosis but not for embryonic or larval development. In contrast, in *Oncopeltus fasciatus* knockdown in the expression of gene coding for BR-C causes defects ranging from posterior truncations to completely fragmented embryonic tissues depending on the RNAi efficiency (Erezyilmaz et al. 2009). Similarly, in German cockroach, *Blattella germanica*, a short germ band insect, knockdown in the expression of BR-C causes developmental defects in embryos (Piulachs et al. 2010). BR-C is constitutively expressed during the embryonic development of the ametabolous firebrat, *Thermobia domestica* (Erezyilmaz et al. 2009). These observations suggest that BR-C plays an important role during the embryonic development of ametabolous and hemimetabolous insects but this role may have been lost in the holometabolous insects.

In holometabolous insects, a peak of JH was detected in embryos at about 50 % development coinciding with the formation of second (first larval) cuticle (Bergot et al. 1981). In hemimetabolous insect, *Locusta migratoria* JH appears at about 70 % of embryonic development at the time of formation of first nymphal cuticle (Temin et al. 1986). Treatment of the locust, *Schistocerca gregaria* embryos with JH analogs cause defects in embryonic development (Novak 1969). Juvenile hormones have been detected in the embryos of the cockroaches *Nauphoeta cinerea* (Lanzrein et al. 1984, 1985), *Diploptera punctata* (Holbrook et al. 1996, 1998; Stay et al. 2002) and Blattella germanica (Maestro et al. 2010). In cockroaches, low JH titers were detected until dorsal closure, the titers increased and reached the maximum levels between 60 and 80 % embryonic development and then the titers decrease to undetectable levels. Treatment of the cricket, *Acheta domesticus* embryos with JH III or pyriproxyfen caused stage dependent effects on pronymphal cuticle formation. In addition, the effect of JH seems to be confined to embryonic molts in the presence of ecdysteriods (Erezyilmaz et al. 2004). These studies on hormonal regulation of embryonic development suggest that both ecdysteroids and JH play critical roles in developing embryo. Therefore, it is possible to control insects by interfering with the embryonic development using ecdysteroid and JH analogs.

3 Molting and Metamorphosis

Molting and metamorphosis are regulated by several important hormones. Among them, ecdysteroids and juvenile hormones play critical roles. When insects reach the critical weight for molting, signals to brain induce the production of prothoracicotrophic hormone (PTTH), which in turn induces the production of ecdysteriods in the prothoracic glands. Ecdysterioids induce molting and the type of molt is determined by JH. Increase in ecdysterioids in the presence of JH induces larval-larval molts. Increase in ecdysterioids in the absence of JH induces metamorphosis.

Ecdysteroid signals are transduced by a cascade of gene expression and repression events starting with the binding of 20E to a heterodimer of nuclear receptors, ecdysone receptor (EcR) and ultraspiracle (USP). This hormone-receptor complex then binds to ecdysone response elements present in the promoters of genes coding for ecdysone induced transcription factors such as E74 and E75 and induces their expression. EcR, USP and E75 are members of nuclear receptor superfamily. Members of the nuclear receptor superfamily are characterized by the presence of a well-conserved 66 amino acid DNA binding domain and a conserved ligand binding domain. Typically, about 20 members of nuclear receptor superfamily have been identified in the genomes of insects sequenced so far. Some of the members of nuclear receptor family are activated by ligands. For example, EcR/USP heterodimer binds to ecdysteroids. The E75 nuclear receptor contains Heme and is nitric oxide responsive (Reinking et al. 2005). The EcR/USP/20E complex also induces expression of an early-late gene HR3, also a member of nuclear receptor superfamily (Koelle et al. 1992). HR3 protein binds to response elements present in the promoter and induces expression of the gene coding for βFTZ-F1, another member of the nuclear receptor superfamily (Kageyama et al. 1997; Lam et al. 1997; White et al. 1997). The βFTZ-F1 protein function as a competence factor and is involved in transduction of ecdysteriod signals during prepupal stage. Mutagenesis experiments showed that βFTZ-F1 protein is required for expression of E74A, E75A and E93, ecdysone-induced transcription factors during prepupal to pupal transition (Broadus et al. 1999; Woodard et al. 1994; Yamada et al. 2000). The cascade of gene expression discovered in the fruit fly *D. melanogaster* has been found to be operating in other insect species including *Manduca sexta*, *Bombyx mori*, *Aedes aegypti* and *Tribolium castaneum* (Kamimura et al. 1996; Margam et al. 2006; Riddiford et al. 1999, 2001; Tan and Palli 2008a) Besides, five nuclear receptors, EcR, USP, E75, DHR3 and βFTZ-F1 already discussed other nuclear receptors such as HR4, HR38, HR39, HR51, SVP also play important roles in regulation of molting and metamorphosis. For example, 19 canonical and 2 Knirps family nuclear receptors were identified in the genome of the red flour beetle, *T. castaneum*. Functional characterization using RNA interference which works very well in this insect revealed that knockdown in the expression of genes coding for 10 out of the 19 nuclear receptors (TcE75, TcHR3, TcHR4, TcEcR, TcUSP, TcFTZ-F1, TcHR51, SVP, TcHR38 and TcHR39) during larval stage by injecting dsRNA targeting these genes caused problems in larval-pupal or pupal-adult metamorphosis (Tan and Palli

2008b). These data showed that 10 nuclear receptors play important roles in regulation of gene expression during larval-pupal metamorphosis (Tan and Palli 2008b).

Recent studies showed that JH signals are transduced through a member of bHLH-PAS family transcription factors, methoprene tolerant (Met). The Met was first discovered in *D. melanogaster* mutant flies that are resistant to JH analog, methoprene (Wilson and Turner 1992). The Met mutant flies showed resistance to JH III as well JH analogs including methoprene. However, mutations to Met did not cause lethality due to the presence of a duplicated paralog, germ cell expresser (gce). Therefore, Met was not widely accepted as a JH receptor until the function of Met was demonstrated in *T. castaneum*. RNAi–aided knockdown in the expression of gene coding for Met in *T. castaneum* caused precocious metamorphosis and exhibited typical phenotypes cause by JH deficiency (Konopova and Jindra 2007). This was made possible by the absence of gce in *T. castaneum*. The RNAi of Met during the last instar larval stage caused premature development of adult characters revealing important function for JH in suppression of adult characters during larval-pupal metamorphosis (Parthasarathy et al. 2008b). Met has been shown to bind to JH III with high affinity. JH-Met complex recruits steroid receptor co-activator homolog, SRC/taiman/FISC (Charles et al. 2011; Li et al. 2011; Zhang et al. 2011). This protein-hormone complex then binds to JH response elements present in the promoters of JH-response genes such as kruppel homolog 1 (Kr-h1) and regulate its expression.

Juvenile hormone response elements containing canonical E box motif have been identified in the promoter of Kr-h1 gene *in D. melanogaster*, *B. mori* (Kayukawa et al. 2012), *T. castaneum* (Kayukawa et al. 2013) and *Aedes aegypti* (Cui et al. 2014). Multiple copies of JHRE and a minimal promoter cloned upstream to the Luicferase reporter gene show dose-dependent induction of this reporter gene by JH and its analogs (Kayukawa et al. 2012, 2013). This has been developed as an assay to identify JH agonists and antagonists that could be used for controlling insects pests. JH agonists have been used for a long time controlling insects that damage crops and food grains during adult stage as well as insects such as mosquitoes that transmit disease during adult stage (Parthasarathy et al. 2012).

Broad complex proteins (BR-C) containing Broad-Tramtrack-Bric-a-bac (BTB) and zinc finger domains is one the early genes induced by 20E (Zollman et al. 1994). Null mutants of BR-C failed to undergo larval-pupal transformation suggesting that BR-C plays critical roles in metamorphosis in *D. melanogaster* (Kiss et al. 1988). Extensive studies on BR-C in *D. melanogaster*, *M. sexta* and *B. mori* defined BR-C as a pupal specifier i.e. to ensure that the larva molts into the pupal stage rather than to the adult stage in holometabolous insects (Uhlirova et al. 2003; Zhou et al. 1998; Zhou and Riddiford 2001, 2002). The application of methoprene mimicked BR-C mutant phenotypes in multiple tissues that failed to undergo metamorphic changes (Restifo and Wilson 1998). This anti-metamorphic effect of JH was overcome in *T. castaneum* larvae that had Met knockdown by RNAi (Konopova and Jindra 2007). In *T. castaneum* BR-C is predominantly expressed during the quiescent stage prior to larval-pupal metamorphosis in the presence of both 20E and JH (Fig. 1). This expression is essential for larval-pupal metamorphosis, because, RNAi-aided

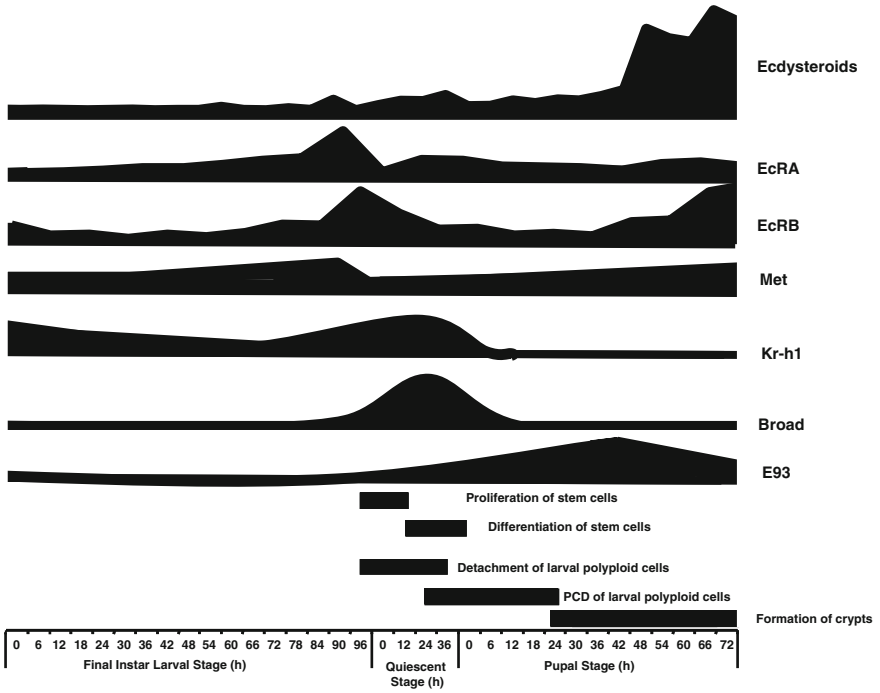

Fig. 1 Expression profiles of genes involved in signal transduction of ecdysteroids and juvenile hormones during larval and pupal stages are shown. The events during midgut remodeling are also shown

knockdown of BR-C during final instar larval stage affected larval-pupal metamorphosis and produced insects that showed a mosaic of larval, pupal and adult structures (Parthasarathy et al. 2008a). Knockdown of BR-C during the final instar larval stage affected midgut remodeling during larval-pupal metamorphosis. These data suggest that the expression of BR-C during the final instar larval stage promotes pupal metamorphosis while suppressing adult metamorphosis ensuring a transitory pupal stage in holometabolous insects. Recent studies in *T. castaneum* also showed that Kr-h1 transcription factor induced by JH-Met complex is also required for induction of BR-C (Minakuchi et al. 2009). Thus, BR-C expression appears to be regulated by both 20E and JH pathways (Fig. 2).

E93 is another 20E induced early gene and transcription factor identified in *D. melanogaster* (Baehrecke and Thummel 1995). E93 has been proposed to direct ecdysteriod induced cell death of larval tissues in *D. melanogaster* (Baehrecke and Thummel 1995; Lee et al. 2000; Liu et al. 2014; Mou et al. 2012). Recent studies in the cockroach, the fruit fly and the red flour beetle showed that E93 plays key roles in regulation of adult metamorphosis (Belles and Santos 2014; Urena et al. 2014). During pupal stage, absence of JH, Met and Kr-h1 allow expression of E93 which then regulates genes involved in tissue remodeling and adult metamorphosis (Fig. 2).

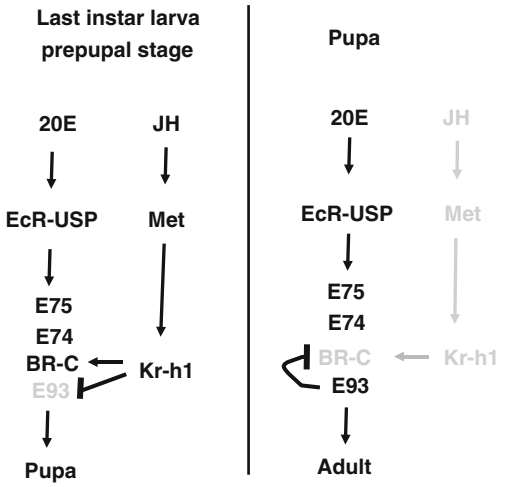

Fig. 2 A proposed model for gene expression and repression events mediated by key players involved in transduction of ecdysteroid and juvenile hormone signals during metamorphosis

E93 which promotes destruction of larval tissues and promotes adult metamorphosis could be an adult specifier similar to BR-C's role in specifying pupal stage.

4 Female Reproduction

4.1 Vitellogenin Synthesis

In insects, vitellogenin (Vg) proteins are synthesized primarily in the fat body and secreted into the hemolymph. The developing oocytes take up Vg from the hemolymph. In some insects Vg is also synthesized in the ovary. Two major hormones ecdysteroids and Juvenile hormones that regulate embryogenesis and metamorphosis also play important roles in regulation of viltellogenesis. The hormonal regulation of Vg synthesis varies quite a bit in insects. In some insects, 20E induces Vg synthesis while in others JH induces Vg synthesis (Hagedorn and Kunkel 1979; Raikhel and Dhadialla 1992). For example, in locusts and cockroaches JH regulates expression of Vg gene (Wyatt and Davey 1996). In addition, application of JH or its analogs induce Vg production in the fat body of these insects (Glinka and Wyatt 1996; Keeley and Mckercher 1985; Wyatt 1988; Zhang et al. 1993). Both 20E and JH induce yolk protein production in *D. melanogaster* (Richard et al. 2001). In *Ae. aegypti*, soon after adult emergence, JH titers increase and prepare fat body for massive Vg synthesis and after blood meal, 20E induces expression of Vg gene (Raikhel et al. 2002). Both JH and 20E appear to induce Vg synthesis in insects belonging to Hymenoptera (Amdam et al. 2004; Brent et al. 2006; Pinto et al. 2000). However, a few exceptions such as in the endoparastic wasp, where 20E induces Vg synthesis in the fat body and JH accelerates Vg sequestration by the oocytes have been reported (Dong et al. 2009). In lepidopteran insects, the hormonal regulation of Vg

synthesis is more variable, in some species ecdysteroids regulate Vg synthesis while in other species JH is required for induction of Vg synthesis in the fat body (Ramaswamy et al. 1997). In *T. castaneum* JH induces Vg synthesis, microarray and quantitative real-time PCR analyses showed that the genes coding for proteins involved in JH biosynthesis and action, but not those involved in ecdysteroid biosynthesis and action had similar expression patterns as the genes coding for Vg (Parthasarathy et al. 2010b). Knockdown in the expression of these genes coding for proteins involved in biosynthesis and action of JH and ecdysteroids showed that both JH and 20E were required for Vg gene expression. However, Vg mRNA was induced by the application of JH III but not by the injection of 20E into the previtellogenic females suggesting that JH is required for Vg synthesis in the fat body (Parthasarathy et al. 2010b). However, JH does not act directly on the promoters of Vg genes in this insect, JH induces synthesis of two insulin-like peptides, ILP2 and ILP3 which in turn work through insulin signaling pathway and affect phosphorylation status and nuclear localization of FOXO (Sheng et al. 2011). FOXO binding sites have been identified in the Vg promoter of *T. castaneum* (Sheng et al. 2011). Similar indirect regulation of Vg gene expression by JH has been proposed in other insects such as locust and cockroach. In *T. castaneum* double-sex transcription factor is also required for full expression of Vg gene (Shukla and Palli 2012). *T. castaneum* starved adults do not produce Vg, nutritional signals induce expression of ILP3 which likely functions through insulin signaling pathway and induce expression of Vg gene. Thus, in this insect, expression of Vg genes is regulated by multiple factors including nutrition, juvenile hormone, insulin signaling pathway and sex-specific transcription factor, double-sex. In lidenbug, *Pyrrhocoris apterus* JH regulates Vg synthesis through Met and Taiman (Smykal et al. 2014).

4.2 Oogenesis

Most female insects have a pair of ovaries and each ovary in turn contains a few to many ovarioles. Each ovariole contains developing follicles arranged in a linear array of progressive developmental stages. Oogenesis includes formation of follicles in the germarium, its previtellogenic and postvitellogenic development resulting in formation of egg that moves into the uterus where it is fertilized by a sperm. Germline and somatic cells reside in the germarium, the terminal region of the ovariole. These cells divide and differentiate to form follicles. The follicles are arranged like beads on a string. Each follicle consists of 16 germline cells (1 oocyte and 15 nurse cells) that are surrounded by few 100 epithelial cells often referred to as follicles cells. The nurse cells produce cytoplasm of the oocyte. The follicular epithelial cells play important roles including oocyte patterning, synthesis and transport of hormones, yolk proteins and egg shell proteins. All these processes in oogenesis are regulated by hormones including ecdysterioids, juvenile hormones and insulin-like peptides.

Regulation of oogenesis by ecdysteriods has been shown in several insects. Ovarian development in *B. mori* adults is induced by ecdysteriods. Ovarian development is arrested when the spinning larvae are ligated between thorax (where prothoracic glands that secrete ecdysteroids are located) and abdomen (where oocytes develop). A single injection of ecdysteroids into the abdomens induces ovarian development suggesting that ecdysteroids regulate ovarian development in this insect (Swevers and Iatrou 1999; Tsuchida et al. 1987). Interestingly, the same gene expression cascade that mediates ecdysteriod action during metamorphosis and embryonic development also functions in ecdysteroid regulation of oogenesis. Both heterodimeric partners of ecdysone receptor complex, EcR and USP are expressed in the follicular epithelium and other cells of developing follicle (Swevers et al. 1995) suggesting that follicles are direct target of ecdysteroids. Other nuclear receptors E75, HR3, βFTZ-F1 and HNF4 have been identified as ecdysone-response genes in *B. mori* ovaries (Swevers and Iatrou 2003). The expression of E75 isoforms in follicles in a stage dependent manner has been reported (Swevers et al. 2002).

Studies on EcR mutants of *D. melanogaster* showed that ecdysteriod action is required for oogenesis (Carney and Bender 2000). Expression profiles of E75, E74 and BR-C in *D. melanogaster* ovary suggest that ecdysteriods regulate oogenesis (Buszczak et al. 1999). All three isoforms of E75 are induced after a blood meal and are highly expressed in the ovary of *Ae. aegypti* suggesting their involvement in the regulation of oogenesis (Pierceall et al. 1999). In honeybee, the expression of E74 is localized to the ovary suggesting a role for ecdysteroids in oogenesis of this insect (Paul et al. 2005).

In the red flour beetle, *T. castaneum*, increase in expression of genes coding for proteins involved in ecdysteroid action was detected by microarray analysis of RNA isolated from female adults on the 4th day after their eclosion (Parthasarathy et al. 2010a). Knockdown in expression of genes involved in JH and ecdysteriod biosynthesis and action blocked ovarian growth and primary oocyte maturation (Parthasarathy et al. 2010a). RNAi studies also showed that the heterodimer of ecdysone receptor complex, EcR and USP are required for the ovarian growth, primary oocyte maturation and the growth and migration of the follicle cells. The phenotypes observed for EcR knockdown are most severe with follicles stuck at early stage of development and appear like a bunch of grapes. RNAi studies also showed that JH does not play a critical role in regulation of oogenesis in *T. castaneum*. RNAi studies in *T. castaneum* showed that seven nuclear receptors [E75, HR3, EcR, USP, seven-up (SVP), βFTZ-F1] and hormone receptor 4 (HR4) are required for vitellogenesis and oogenesis (Xu et al. 2010). In *T. castaneum*, Knockdown in the expression of bHLH transcription factors (Trachealess, Myc, Max, Emc, HLH106 and AP-4) caused severe defects in oocyte maturation. The oocytes in adults injected with dsRNA targeting these genes were blocked at the early stages of development (Bitra and Palli 2010). Whether or not some of these transcription factors mediate action of 20E, JH, Insulin-like peptides or any other hormones is not known yet.

4.3 Nutrition Regulation

Reproduction in insects is regulated by nutritional signals. Nutritional signals are transduced through the target of rapamycin (TOR) protein (Hansen et al. 2004, 2005) and insulin/insulin-like growth factor signaling (ISS) (Garofalo 2002). Nutritional regulation of female reproduction has been studied well in the anautogenous mosquito, *Ae. aegypti* where blood meals trigger the initiation of egg production (Attardo et al. 2006; Brown et al. 2008; Hansen et al. 2004, 2005, 2006, 2007, 2011; Riehle and Brown 2002). In *D. melanogaster* the nutrients affect yolk protein synthesis and egg production (Bownes and Blair 1986; Bownes and Reid 1990; Schwartz et al. 1985; Sondergaard et al. 1995; Terashima and Bownes 2004). In *T. castaneum*, we studied the effect ISS and the amino acid signaling pathway (TOR) on vitellogenesis and oogenesis. Starvation of female beetles resulted in a block in Vg synthesis but the growth of primary oocyte progressed to the resting stage (Parthasarathy and Palli 2011). Feeding after starvation induced Vg synthesis as well as growth of primary oocytes that progressed from the resting stage to the vitellogenic stage. Microarray and by RNAi studies showed that nutritional signals play key roles in regulation of both vitellogenesis and oogenesis (Parthasarathy and Palli 2011). In the German cockroach, FOXO, the terminal transcription factor in ISS pathway plays an important role in nutritional regulation of female reproduction. RANi was used to show that FOXO inhibits production of JH III and Vg during nutrient shortage (Suren-Castillo et al. 2012). Knockdown in expression of gene coding for insulin receptor also showed similar phenotypes in blocking JH biosynthesis and Vg production suggesting that ISS pathway mediates nutritional regulation of reproduction in this insect (Abrisqueta et al. 2014). The ISS/TOR pathways are also involved in JH biosynthesis in the mosquito *Ae. aegypti* (Pérez-Hedo et al. 2013).

5 Male Reproduction

Compared to hormonal regulation of female reproduction in insects, the hormonal regulation of male reproduction has not been well studied. Ecdysteroid regulation of development of male reproductive system as well as differentiation of spermatocytes was shown in some insects (Dumser 1980). In *Spodoptera littoralis*, ecdysteroids control testicular sperm release in a daily rhythm (Polanska et al. 2009). Expression of six genes coding for known enzymes involved in ecdysteroid biosynthesis was detected in the testes of last instar larvae of this insect (Iga et al. 2013). Production of ecdysteroids in testes of *Heliothis virescens* (Loeb et al. 1984) and *B. mori* (Fugo et al. 1996) have been reported. Ecdysteroids also regulate growth and maturation of MAG during the pupal and early adult stages in most of the insects (Gallois 1989; Shinbo and Happ 1989; Sridevi et al. 1989).

Male accessory glands of some insects are capable of synthesizing JH de novo (Borovsky et al. 1994). In some cases MAG converts corpora allata-produced JH acid into JH (Shirk et al. 1976). Transfer of JH through seminal fluids has been shown in some insects (Cusson et al. 1999). JH was shown to be involved in growth and maturation of MAG in many insects (Couche et al. 1985; Gold and Davey 1989; Regis et al. 1985). In *D. melanogaster*, exposure of MAG to JH analogs stimulated Acp secretion (Yamamoto et al. 1988). Use of Met mutant showed the involvement of JH in Acp synthesis in MAG of *D. melanogaster* (Shemshedini et al. 1990; Wilson et al. 2003). Exploiting the availability of *T. castaneum* genome sequence, custom microarrays, hormone analog treatments and well functioning RNAi (Parthasarathy et al. 2009) identified 112 genes that were highly expressed in MAG of *T. castaneum*. The bioinformatics approaches identified 59 out of 112 genes as putative secretory Acps. The 59 secretory proteins showed both sequence and functional similarity with Acps identified in other insects. Expression analyses, hormone analog treatments and RNAi showed that JH, but not ecdysteroids play an important role in male reproduction by influencing the growth of MAG and regulating Acp synthesis in *T. castaneum* (Parthasarathy et al. 2009). Nutrition functioning through TOR/IIS pathways likely regulates production of Acp in MAG. However, not much is known on the mechanisms and players involved in nutritional regulation of male reproduction.

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References

Abrisqueta, M., Suren-Castillo, S., & Maestro, J. L. (2014). Insulin receptor-mediated nutritional signalling regulates juvenile hormone biosynthesis and vitellogenin production in the German cockroach. *Insect Biochemistry and Molecular Biology, 49*, 14–23.

Amdam, G. V., Simoes, Z. L. P., Hagen, A., Norberg, K., Schroder, K., Mikkelsen, O., Kirkwood, T. B. L., & Omholt, S. W. (2004). Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. *Experimental Gerontology, 39*, 767–773.

Attardo, G. M., Hansen, I. A., Shiao, S. H., & Raikhel, A. S. (2006). Identification of two cationic amino acid transporters required for nutritional signaling during mosquito reproduction. *Journal of Experimental Biology, 209*, 3071–3078.

Baehrecke, E. H., & Thummel, C. S. (1995). The *Drosophila* E93 gene from the 93 F early puff displays stage- and tissue-specific regulation by 20-hydroxyecdysone. *Developmental Biology, 171*, 85–97.

Belles, X., & Santos, C. G. (2014). The MEKRE93 (Methoprene tolerant-Kruppel homolog 1-E93) pathway in the regulation of insect metamorphosis, and the homology of the pupal stage. *Insect Biochemistry and Molecular Biology, 52C*, 60–68.

Belyaeva, E. S., Aizenzon, M. G., Semeshin, V. F., Kiss, I. I., Koczka, K., Baritcheva, E. M., Gorelova, T. D., & Zhimulev, I. F. (1980). Cytogenetic analysis of the 2b3-4-2b11 region of the x-chromosome of Drosophila melanogaster. 1. Cytology of the region and mutant complementation groups. *Chromosoma, 81*, 281–306.

Bender, M., Imam, F. B., Talbot, W. S., Ganetzky, B., & Hogness, D. S. (1997). Drosophila ecdysone receptor mutations reveal functional differences among receptor isoforms. *Cell, 91*, 777–788.

Bergot, B., Baker, F., Cerf, D., Jamieson, G., & Schooley, D. A. (1981). Qualitative and quantitative aspects of juvenile hormone titers in developing embryos of several insect species: discovery of a new JH-like substance extracted from eggs of *Manduca sexta*. In G. E. Pratt & G. T. Brooks (Eds.), *Juvenile hormone biochemistry* (pp. 33–45). Amsterdam: Elsevier.

Bitra, K., & Palli, S. R. (2010). The members of bHLH transcription factor superfamily are required for female reproduction in the red flour beetle, *Tribolium castaneum. Journal of Insect Physiology, 56*, 1481–1489.

Borovsky, D., Carlson, D. A., Hancock, R. G., Rembold, H., & Vanhandel, E. (1994). De-novo biosynthesis of juvenile hormone III and hormone-i by the accessory glands of the male mosquito. *Insect Biochemistry and Molecular Biology, 24*, 437–444.

Bownes, M., & Blair, M. (1986). The effects of a sugar diet and hormones on the expression of the *Drosophila* yolk protein genes. *Journal of Insect Physiology, 32*, 493–501.

Bownes, M., & Reid, G. (1990). The role of the ovary and nutritional signals in the regulation of fat-body yolk protein geneexpression in *Drosophila melanogaster. Journal of Insect Physiology, 36*, 471–479.

Brent, C., Peeters, C., Dietmann, V., Crewe, R., & Vargo, E. (2006). Hormonal correlates of reproductive status in the queenless ponerine ant, *Streblognathus peetersi. Journal of Comparative Physiology A, 192*, 315–320.

Broadus, J., McCabe, J. R., Endrizzi, B., Thummel, C. S., & Woodard, C. T. (1999). The *Drosophila* beta FTZ-F1 orphan nuclear receptor provides competence for stage-specific responses to the steroid hormone ecdysone. *Molecular Cell, 3*, 143–149.

Brown, M. R., Clark, K. D., Gulia, M., Zhao, Z., Garczynski, S. F., Crim, J. W., Sulderman, R. J., & Strand, M. R. (2008). An insulin-like peptide regulates egg maturation and metabolism in the mosquito Aedes aegypti. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 5716–5721.

Buszczak, M., Freeman, M. R., Carlson, J. R., Bender, M., Cooley, L., & Segraves, W. A. (1999). Ecdysone response genes govern egg chamber development during mid-oogenesis in *Drosophila. Development, 126*, 4581–4589.

Carney, G. E., & Bender, M. (2000). The Drosophila ecdysone receptor (EcR) gene is required maternally for normal oogenesis. *Genetics, 154*, 1203–1211.

Carney, G. E., Wade, A. A., Sapra, R., Goldstein, E., & Bender, M. (1997). DHR3, an ecdysone-inducible early-late gene encoding a Drosophila nuclear receptor, is required for embryogenesis. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 12024–12029.

Charles, J. P., Iwema, T., Epa, V. C., Takaki, K., Rynes, J., & Jindra, M. (2011). Ligand-binding properties of a juvenile hormone receptor, Methoprene tolerant. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 21128–21133.

Chavez, V. M., Marques, G., Delbecque, J. P., Kobayashi, K., Hollingsworth, M., Burr, J., Natzle, J. E., & O'Connor, M. B. (2000). The *Drosophila* disembodied gene controls late embryonic morphogenesis and codes for a cytochrome P450 enzyme that regulates embryonic ecdysone levels. *Development, 127*, 4115–4126.

Chavoshi, T. M., Moussian, B., & Uv, A. (2010). Tissue-autonomous EcR functions are required for concurrent organ morphogenesis in the *Drosophila* embryo. *Mechanisms of Development, 127*, 308–319.

Couche, G. A., Gillott, C., Tobe, S. S., & Feyereisen, R. (1985). Juvenile-hormone biosynthesis during sexual-maturation and after mating in the adult male migratory grasshopper, Melanoplus sanguinipes. *Canadian Journal of Zoology, 63*, 2789–2792.

Cui, Y., Sui, Y., Xu, J., Zhu, F., & Palli, S. R. (2014). Juvenile hormone regulates aedes aegypti kruppel homolog 1 through a conserved E box motif. *Insect Biochemistry and Molecular Biology, 52C*, 23–32.

Cusson, M., Delisle, J., & Miller, D. (1999). Juvenile hormone titers in virgin and mated *Choristoneura fumiferana* and *C. rosaceana* females: Assessment of the capacity of males to produce and transfer JH to the female during copulation. *Journal of Insect Physiology, 45*, 637–646.

Dong, S. Z., Ye, G. Y., Guo, J. Y., & Hu, C. (2009). Roles of ecdysteroid and juvenile hormone in vitellogenesis in an endoparasitic wasp, *Pteromalus puparum* (Hymenoptera: Pteromalidae). *General and Comparative Endocrinology, 160*, 102–108.

Dumser, J. B. (1980). Regulation of spermatogenesis in insects. *Annual Review of Entomology, 25*, 341–369.

Erezyilmaz, D. F., Riddiford, L. M., & Truman, J. W. (2004). Juvenile hormone acts at embryonic molts and induces the nymphal cuticle in the direct developing cricket. *Development Genes and Evolution, 214*, 313–323.

Erezyilmaz, D. F., Rynerson, M. R., Truman, J. W., & Riddiford, L. M. (2009). The role of the pupal determinant broad during embryonic development of a directdeveloping insect. *Development Genes and Evolution, 219*, 535–544.

Fugo, H., Yamauchi, M., & Dedos, S. G. (1996). Testicular ecdysteroids in the silkmoth, Bombyx mori. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences, 72*, 34–37.

Gallois, D. (1989). Control of cell-differentiation in the male accessory reproductive glands of *Locusta migratoria* acquisition and reversal of competence to imaginal secretion. *Journal of Insect Physiology, 35*, 189–195.

Garofalo, R. S. (2002). Genetic analysis of insulin signaling in *Drosophila. Trends in Endocrinology and Metabolism, 13*, 156–162.

Glinka, A. V., & Wyatt, G. R. (1996). Juvenile hormone activation of gene transcription in locust fat body. *Insect Biochemistry and Molecular Biology, 26*, 13–18.

Gold, S. M. W., & Davey, K. G. (1989). The effect of juvenile hormone on protein synthesis in the transparent accessory gland of male *Rhodnius prolixus. Insect Biochemistry and Molecular Biology, 19*, 139–143.

Hagedorn, H. H., & Kunkel, J. G. (1979). Vitellogenin and vitellin in insects. *Annual Review of Entomology, 24*, 475–505.

Hansen, I. A., Attardo, G. M., Park, J. H., Peng, Q., & Raikhel, A. S. (2004). Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 10626–10631.

Hansen, I. A., Attardo, G. M., Roy, S. G., & Raikhel, A. S. (2005). Target of rapamycin-dependent activation of S6 kinase is a central step in the transduction of nutritional signals during egg development in a mosquito. *The Journal of Biological Chemistry, 280*, 20565–20572.

Hansen, I. A., Sieglaff, D. H., Shiao, S. H., Munro, J. B., Knox, J., & Raikhel, A. S. (2006). The forkhead box gene family of transcription factors of the yellow fever mosquito Aedes aegypti and its role in mosquito reproduction. *The American Journal of Tropical Medicine and Hygiene, 75*, 262.

Hansen, I. A., Sieglaff, D. H., Munro, J. B., Shiao, S. H., Cruz, J., Lee, I. W., Heraty, J. M., & Raikhel, A. S. (2007). Forkhead transcription factors regulate mosquito reproduction. *Insect Biochemistry and Molecular Biology, 37*, 985–997.

Hansen, I. A., Boudko, D. Y., Shiao, S. H., Voronov, D. A., Meleshkevitch, E. A., Drake, L. L., Aguirre, S. E., Fox, J. M., Attardo, G. M., & Raikhel, A. S. (2011). AACAT1 of the yellow fever mosquito, *Aedes aegypti* a novel histidine-specific amino acid transporter from the SLC7 family. *The Journal of Biological Chemistry, 286*, 10803–10813.

Holbrook, G. L., Chiang, A. S., & Schal, C. (1996). Allatostatin inhibition and farnesol stimulation of corpus allatum activity in embryos of the viviparous cockroach, *Diploptera punctata. Archives of Insect Biochemistry and Physiology, 32*, 341–352.

Holbrook, G. L., Chiang, A. S., Lee, Y. J., Lin, C. Y., & Schal, C. (1998). Juvenile hormone synthesis in relation to corpus allatum development in embryos of the viviparous cockroach *Diploptera punctata. Invertebrate Reproduction & Development, 33*, 69–79.

Iga, M., Blais, C., & Smagghe, G. (2013). Study on ecdysteroid levels and gene expression of enzymes related to ecdysteroid biosynthesis in the larval testis of *Spodoptera littoralis. Archives of Insect Biochemistry and Physiology, 82*, 14–28.

Kageyama, Y., Masuda, S., Hirose, S., & Ueda, H. (1997). Temporal regulation of the mid-prepupal gene FTZ-F1: DHR3 early late gene product is one of the plural positive regulators. *Genes to Cells, 2*, 559–569.

Kamimura, M., Tomita, S., & Fujiwara, H. (1996). Molecular cloning of an ecdysone receptor (B1 isoform) homologue from the silkworm, *Bombyx mori*, and its mRNA expression during wing disc development. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 113*, 341–347.

Kayukawa, T., Minakuchi, C., Namiki, T., Togawa, T., Yoshiyama, M., Kamimura, M., Mita, K., Imanishi, S., Kiuchi, M., Ishikawa, Y., & Shinoda, T. (2012). Transcriptional regulation of juvenile hormone-mediated induction of Kruppel homolog 1, a repressor of insect metamorphosis. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 11729–11734.

Kayukawa, T., Tateishi, K., & Shinoda, T. (2013). Establishment of a versatile cell line for juvenile hormone signaling analysis in *Tribolium castaneum. Science Reports, 3*, 1570.

Keeley, L. L., & Mckercher, S. R. (1985). Endocrine regulations of ovarian maturation in the cockroach *Blaberus discoidalis. Comparative Biochemistry and Physiology. Part A, 80*, 115–121.

Kiss, I., Beaton, A. H., Tardiff, J., Fristrom, D., & Fristrom, J. W. (1988). Interactions and developmental effects of mutations in the broad-complex of *Drosophila melanogaster. Genetics, 118*, 247–259.

Koelle, M. R., Segraves, W. A., & Hogness, D. S. (1992). Dhr3 – a *Drosophila* steroid-receptor homolog. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 6167–6171.

Konopova, B., & Jindra, M. (2007). Juvenile hormone resistance gene Methoprene-tolerant controls entry into metamorphosis in the beetle *Tribolium castaneum. Proceedings of the National Academy of Sciences of the United States of America, 104*, 10488–10493.

Kozlova, T., & Thummel, C. S. (2003). Essential roles for ecdysone signaling during Drosophila mid-embryonic development. *Science, 301*, 1911–1914.

Lam, G. T., Jiang, C. A., & Thummel, C. S. (1997). Coordination of larval and prepupal gene expression by the DHR3 orphan receptor during *Drosophila* metamorphosis. *Development, 124*, 1757–1769.

Lanzrein, B., Imboden, H., Burgin, C., Bruning, E., & Gfeller, H. (1984). On titers, origin, and functions of juvenile hormone III, methylfarnesoate and ecdysteroids in embryonic development of the ovoviviparous cockroach *Nauphoeta cinerea*. In J. Hoffmann & M. Porchet (Eds.), *Biosynthesis, metabolism and mode of action of invertebrate hormones*. Heidelberg: Springer.

Lanzrein, B., Gentinetta, V., Abegglen, H., Baker, F. C., Miller, C. A., & Schooley, D. A. (1985). Titers of ecdysone, 20-hydroxyecdysone and juvenile hormone-III throughout the life-cycle of a hemimetabolous insect, the ovoviviparous cockroach *Nauphoeta cinerea. Experientia, 41*, 913–917.

Lee, C. Y., Wendel, D. P., Reid, P., Lam, G., Thummel, C. S., & Baehrecke, E. H. (2000). E93 directs steroid-triggered programmed cell death in Drosophila. *Molecular Cell, 6*, 433–443.

Li, M., Mead, E. A., & Zhu, J. (2011). Heterodimer of two bHLH-PAS proteins mediates juvenile hormone-induced gene expression. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 638–643.

Liu, H., Wang, J., & Li, S. (2014). E93 predominantly transduces 20-hydroxyecdysone signaling to induce autophagy and caspase activity in Drosophila fat body. *Insect Biochemistry and Molecular Biology, 45*, 30–39.

Loeb, M. J., Brandt, E. P., & Birnbaum, M. J. (1984). Ecdysteroid production by testes of the tobacco budworm, *Heliothis virescens*, from last larval instar to adult. *Journal of Insect Physiology, 30*, 375–381.

Maeda, S., Nakashima, A., Yamada, R., Hara, N., Fujimoto, Y., Ito, Y., & Sonobe, H. (2008). Molecular cloning of ecdysone 20-hydroxylase and expression pattern of the enzyme during embryonic development of silkworm Bombyx mori. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology, 149*, 507–516.

Maestro, J. L., Pascual, N., Treiblmayr, K., Lozano, J., & Belles, X. (2010). Juvenile hormone and allatostatins in the German cockroach embryo. *Insect Biochemistry and Molecular Biology, 40*, 660–665.

Margam, V. M., Gelman, D. B., & Palli, S. R. (2006). Ecdysteroid titers and developmental expression of ecdysteroid-regulated genes during metamorphosis of the yellow fever mosquito, *Aedes aegypti* (Diptera : Culicidae). *Journal of Insect Physiology, 52*, 558–568.

Maroy, P., Kaufmann, G., & Dubendorfer, A. (1988). Embryonic ecdysteroids of *Drosophila melanogaster. Journal of Insect Physiology, 34*, 633–637.

Minakuchi, C., Namiki, T., & Shinoda, T. (2009). Kruppel homolog 1, an early juvenile hormone-response gene downstream of Methoprene-tolerant, mediates its anti-metamorphic action in the red flour beetle *Tribolium castaneum. Developmental Biology, 325*, 341–350.

Mou, X., Duncan, D. M., Baehrecke, E. H., & Duncan, I. (2012). Control of target gene specificity during metamorphosis by the steroid response gene E93. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 2949–2954.

Novak, V. J. A. (1969). Morphogenetic analysis of effects of juvenile hormone analogues and other morphogenetically active substances on embryos of schistocerca GREGARIA (forskal). *Journal of Embryology and Experimental Morphology, 21*, 1–21.

Ono, H., Rewitz, K. F., Shinoda, T., Itoyama, K., Petryk, A., Rybczynski, R., Jarcho, M., Warren, J. T., Marques, G., Shimell, M. J., Gilbert, L. I., & O'Connor, M. B. (2006). Spook and Spookier code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. *Developmental Biology, 298*, 555–570.

Parthasarathy, R., & Palli, S. R. (2011). Molecular analysis of nutritional and hormonal regulation of female reproduction in the red flour beetle, *Tribolium castaneum. Insect Biochemistry and Molecular Biology, 41*, 294–305.

Parthasarathy, R., Tan, A., Bai, H., & Palli, S. R. (2008a). Transcription factor broad suppresses precocious development of adult structures during larval-pupal metamorphosis in the red flour beetle, *Tribolium castaneum. Mechanisms of Development, 125*, 299–313.

Parthasarathy, R., Tan, A. J., & Palli, S. R. (2008b). bHLH-PAS family transcription factor methoprene-tolerant plays a key role in JH action in preventing the premature development of adult structures during larval-pupal metamorphosis. *Mechanisms of Development, 125*, 601–616.

Parthasarathy, R., Tan, A., Sun, Z., Chen, Z., Rankin, M., & Palli, S. R. (2009). Juvenile hormone regulation of male accessory gland activity in the red flour beetle, *Tribolium castaneum. Mechanisms of Development, 126*, 563–579.

Parthasarathy, R., Sheng, Z., Sun, Z., & Palli, S. R. (2010a). Ecdysteroid regulation of ovarian growth and oocyte maturation in the red flour beetle, *Tribolium castaneum. Insect Biochemistry and Molecular Biology, 40*, 429–439.

Parthasarathy, R., Sun, Z., Bai, H., & Palli, S. R. (2010b). Juvenile hormone regulation of vitellogenin synthesis in the red flour beetle, *Tribolium castaneum. Insect Biochemistry and Molecular Biology, 40*, 405–414.

Parthasarathy, R., Farkas, R., & Palli, S. R. (2012). Recent progress in Juvenile Hormone Analogs (JHA) Research. In T. S. Dhadialla (Ed.), *Advances in insect physiology* (Insect growth disruptors, Vol. 43, pp. 353–436). London: Academic Press Ltd/Elsevier Science Ltd.

Paul, R. K., Takeuchi, H., Matsuo, Y., & Kubo, T. (2005). Gene expression of ecdysteroid-regulated gene E74 of the honeybee in ovary and brain. *Insect Molecular Biology, 14*, 9–15.

Pérez-Hedo, M., Rivera-Perez, C., & Noriega, F. G. (2013). The insulin/TOR signal transduction pathway is involved in the nutritional regulation of juvenile hormone synthesis in Aedes aegypti. *Insect Biochemistry and Molecular Biology, 43*, 495–500.

Pierceall, W. E., Li, C., Biran, A., Miura, K., Raikhel, A. S., & Segraves, W. A. (1999). E75 expression in mosquito ovary and fat body suggests reiterative use of ecdysone-regulated hierarchies in development and reproduction. *Molecular and Cellular Endocrinology, 150*, 73–89.

Pinto, L. Z., Bitondi, M. M. G., & Simoes, Z. L. P. (2000). Inhibition of vitellogenin synthesis in Apis mellifera workers by a juvenile hormone analogue, pyriproxyfen. *Journal of Insect Physiology, 46*, 153–160.

Piulachs, M. D., Pagone, V., & Belles, X. (2010). Key roles of the broad-complex gene in insect embryogenesis. *Insect Biochemistry and Molecular Biology, 40*, 468–475.

Polanska, M. A., Maksimiuk-Ramirez, E., Ciuk, M. A., Kotwica, J., & Bebas, P. (2009). Clock-controlled rhythm of ecdysteroid levels in the haemolymph and testes, and its relation to sperm release in the Egyptian cotton leafworm, *Spodoptera littoralis. Journal of Insect Physiology, 55*, 426–434.

Raikhel, A. S., & Dhadialla, T. S. (1992). Accumulation of yolk proteins in insect oocytes. *Annual Review of Entomology, 37*, 217–251.

Raikhel, A. S., Kokoza, V. A., Zhu, J. S., Martin, D., Wang, S. F., Li, C., Sun, G. Q., Ahmed, A., Dittmer, N., & Attardo, G. (2002). Molecular biology of mosquito vitellogenesis: From basic studies to genetic engineering of antipathogen immunity. *Insect Biochemistry and Molecular Biology, 32*, 1275–1286.

Ramaswamy, S. B., Shu, S. Q., Park, Y. I., & Zeng, F. R. (1997). Dynamics of juvenile hormone-mediated gonadotropism in the lepidoptera. *Archives of Insect Biochemistry and Physiology, 35*, 539–558.

Regis, L., Gomes, Y. D., & Furtado, A. F. (1985). Factors influencing male accessory-gland activity and 1st mating in *Triatoma infestans* and *Panstrongylus megistus* (Hemiptera, Reduviidae). *Insect Science and Its Application, 6*, 579–583.

Reinking, J., Lam, M. M. S., Pardee, K., Sampson, H. M., Liu, S., Yang, P., Williams, S., White, W., Lajoie, G., Edwards, A., & Krause, H. M. (2005). The *Drosophila* nuclear receptor E75 contains heme and is Gas responsive. *Cell, 122*, 195–207.

Restifo, L. L., & Wilson, T. G. (1998). A juvenile hormone agonist reveals distinct developmental pathways mediated by ecdysone-inducible broad complex transcription factors. *Developmental Genetics, 22*, 141–159.

Reynolds, J. A., & Hand, S. C. (2009). Embryonic diapause highlighted by differential expression of mRNAs for ecdysteroidogenesis, transcription and lipid sparing in the cricket al*onemobius socius. Journal of Experimental Biology, 212*, 2074–2083.

Richard, D. S., Jones, J. M., Barbarito, M. R., Cerula, S., Detweiler, J. P., Fisher, S. J., Brannigan, D. M., & Scheswohl, D. M. (2001). Vitellogenesis in diapausing and mutant *Drosophila melanogaster*: Further evidence for the relative roles of ecdysteroids and juvenile hormones. *Journal of Insect Physiology, 47*, 905–913.

Riddiford, L. M., Hiruma, K., Lan, Q., & Zhou, B. H. (1999). Regulation and role of nuclear receptors during larval molting and metamorphosis of lepidoptera. *American Zoologist, 39*, 736–746.

Riddiford, L. M., Cherbas, P., & Truman, J. W. (2001). Ecdysone receptors and their biological actions. *Vitamins and Hormones – Advances in Research and Applications, 60*, 1–73.

Riehle, M. A., & Brown, M. R. (2002). Insulin receptor expression during development and a reproductive cycle in the ovary of the mosquito *Aedes aegypti. Cell and Tissue Research, 308*, 409–420.

Ruaud, A. F., Lam, G., & Thummel, C. S. (2010). The Drosophila nuclear receptors DHR3 and beta FTZ-F1 control overlapping developmental responses in late embryos. *Development, 137*, 123–131.

Schwartz, M. B., Kelly, T. J., Imberski, R. B., & Rubenstein, E. C. (1985). The effects of nutrition and methoprene treatment on ovarian ecdysteroid synthesis in Drosophila melanogaster. *Journal of Insect Physiology, 31*, 947.

Shemshedini, L., Lanoue, M., & Wilson, T. G. (1990). Evidence for a juvenile hormone receptor involved in protein synthesis in *Drosophila melanogaster. The Journal of Biological Chemistry, 265*, 1913–1918.

Sheng, Z. T., Xu, J. J., Bai, H., Zhu, F., & Palli, S. R. (2011). Juvenile hormone regulates vitellogenin gene expression through insulin-like peptide signaling pathway in the red flour beetle, *Tribolium castaneum. The Journal of Biological Chemistry, 286*, 41924–41936.

Shinbo, H., & Happ, G. M. (1989). Effects of ecdysteroids on the growth of the post-testicular reproductive organs in the silkworm, Bombyx mori. *Journal of Insect Physiology, 35*, 855–864.

Shirk, P. D., Dahm, K. H., & Roller, H. (1976). Accessory sex glands as repository for juvenile hormone in male cecropia moths. *Zeitschrift Fur Naturforschung C-A Journal of Biosciences, 31*, 199–200.

Shukla, J. N., & Palli, S. R. (2012). Doublesex target genes in the red flour beetle, *Tribolium castaneum. Science Reports, 2*, 948.

Smykal, V., Bajgar, A., Provaznik, J., Fexova, S., Buricova, M., Takaki, K., Hodkova, M., Jindra, M., & Dolezel, D. (2014). Juvenile hormone signaling during reproduction and development of the linden bug, Pyrrhocoris apterus. *Insect Biochemistry and Molecular Biology, 45*, 69–76.

Sondergaard, L., Mauchline, D., Egetoft, P., White, N., Wulff, P., & Bownes, M. (1995). Nutritional response in a *Drosophila* yolk protein gene promoter. *Molecular Genetics and Genomics, 248*, 25–32.

Sridevi, R., Bajaj, P., & Duttagupta, A. (1989). Hormonal regulation of macromolecular-synthesis in testes and accessory reproductive glands of *Spodoptera litura* during post-embryonic and adult development. *Indian Journal of Experimental Biology, 27*, 699–703.

Stay, B., Zhang, J. R., & Tobe, S. S. (2002). Methyl farnesoate and juvenile hormone production in embryos of Diploptera punctata in relation to innervation of corpora allata and their sensitivity to allatostatin. *Peptides, 23*, 1981–1990.

Sullivan, A. A., & Thummel, C. S. (2003). Temporal profiles of nuclear receptor gene expression reveal coordinate transcriptional responses during *Drosophila* development. *Molecular Endocrinology, 17*, 2125–2137.

Suren-Castillo, S., Abrisqueta, M., & Maestro, J. L. (2012). FoxO inhibits juvenile hormone biosynthesis and vitellogenin production in the German cockroach. *Insect Biochemistry and Molecular Biology, 42*, 491–498.

Swevers, L., & Iatrou, K. (1999). The ecdysone agonist tebufenozide (RH-5992) blocks the progression into the ecdysteroid-induced regulatory cascade and arrests silkmoth oogenesis at mid-vitellogenesis. *Insect Biochemistry and Molecular Biology, 29*, 955–963.

Swevers, L., & Iatrou, K. (2003). The ecdysone regulatory cascade and ovarian development in lepidopteran insects: Insights from the silkmoth paradigm. *Insect Biochemistry and Molecular Biology, 33*, 1285–1297.

Swevers, L., Drevet, J. R., Lunke, M. D., & Iatrou, K. (1995). The silkmoth homolog of the *Drosophila* ecdysone receptor (BI Isoform): Cloning and analysis of expression during follicular cell differentiation. *Insect Biochemistry and Molecular Biology, 25*, 857–866.

Swevers, L., Eystathioy, T., & Iatrou, K. (2002). The orphan nuclear receptors BmE75A and BmE75C of the silkmoth Bombyx mori: Hornmonal control and ovarian expression. *Insect Biochemistry and Molecular Biology, 32*, 1643–1652.

Tan, A., & Palli, S. R. (2008a). Edysone receptor isoforms play distinct roles in controlling molting and metamorphosis in the red flour beetle, *Tribolium castaneum. Molecular and Cellular Endocrinology, 291*, 42–49.

Tan, A., & Palli, S. R. (2008b). Identification and characterization of nuclear receptors from the red flour beetle, *Tribolium castaneum. Insect Biochemistry and Molecular Biology, 38*, 430–439.

Tawfik, A. I., Tanaka, Y., & Tanaka, S. (2002). Possible involvement of ecdysteroids in embryonic diapause of *Locusta migratoria. Journal of Insect Physiology, 48*, 743–749.

Temin, G., Zander, M., & Roussel, J. P. (1986). Physicochemical (Gc-Ms) Measurements of juvenile hormone III titers during embryogenesis of *Locusta migratoria. International Journal of Invertebrate Reproduction and Development, 9*, 105–112.

Terashima, J., & Bownes, M. (2004). Translating available food into the number of eggs laid by *Drosphila melanogaster. Genetics, 167*, 1711–1719.

Tsuchida, K., Nagata, M., & Suzuki, A. (1987). Hormonal control of ovarian development in the silkworm, *Bombyx mori. Archives of Insect Biochemistry and Physiology, 5*, 167–177.

Uhlirova, M., Foy, B. D., Beaty, B. J., Olson, K. E., Riddiford, L. M., & Jindra, M. (2003). Use of Sindbis virus-mediated RNA interference to demonstrate a conserved role of Broad-Complex in insect metamorphosis. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 15607–15612.

Urena, E., Manjon, C., Franch-Marro, X., & Martin, D. (2014). Transcription factor E93 specifies adult metamorphosis in hemimetabolous and holometabolous insects. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 7024–7029.

White, K. P., Hurban, P., Watanabe, T., & Hogness, D. S. (1997). Coordination of *Drosophila* metamorphosis by two ecdysone-induced nuclear receptors. *Science, 276*, 114–117.

Wilson, T. G., & Turner, C. (1992). Molecular analysis of methoprene-tolerant, a gene in Drosophila involved in resistance to juvenile hormone analog insect growth-aegulators. *ACS Symposium Series, 505*, 99–112.

Wilson, T. G., DeMoor, S., & Lei, J. (2003). Juvenile hormone involvement in *Drosophila melanogaster* male reproduction as suggested by the Methoprene-tolerant(27) mutant phenotype. *Insect Biochemistry and Molecular Biology, 33*, 1167–1175.

Woodard, C. T., Baehrecke, E. H., & Thummel, C. S. (1994). A molecular mechanism for the stage specificity of the *Drosophila* prepupal genetic response to ecdysone. *Cell, 79*, 607–615.

Wyatt, G. R. (1988). Vitellogenin synthesis and the analysis of juvenile hormone action in locust fat body. *Canadian Journal of Zoology, 66*, 2600–2610.

Wyatt, G. R., & Davey, K. G. (1996). Cellular and molecular actions of juvenile hormone. 2. Roles of juvenile hormone in adult insects. *Advances in Insect Physiology, 26*, 1–155.

Xu, J. J., Tan, A. J., & Palli, S. R. (2010). The function of nuclear receptors in regulation of female reproduction and embryogenesis in the red flour beetle, *Tribolium castaneum. Journal of Insect Physiology, 56*, 1471–1480.

Yamada, M., Murata, T., Hirose, S., Lavorgna, G., Suzuki, E., & Ueda, H. (2000). Temporally restricted expression of transcription factor beta FTZ-F1: significance for embryogenesis, molting and metamorphosis in *Drosophila melanogaster. Development, 127*, 5083–5092.

Yamamoto, K., Chadarevian, A., & Pellegrini, M. (1988). Juvenile-hormone action mediated in male accessory-glands of *Drosophila* by calcium and kinase-c. *Science, 239*, 916–919.

Zhang, J. Z., Mccracken, A., & Wyatt, G. R. (1993). Properties and sequence of a female-specific, juvenile hormone-induced protein from *Locust* Hemolymph. *The Journal of Biological Chemistry, 268*, 3282–3288.

Zhang, Z. L., Xu, J. J., Sheng, Z. T., Sui, Y. P., & Palli, S. R. (2011). Steroid Receptor Co-activator Is Required for Juvenile Hormone Signal Transduction through a bHLH-PAS Transcription Factor, Methoprene Tolerant. *The Journal of Biological Chemistry, 286*, 8437–8447.

Zhou, B. H., & Riddiford, L. M. (2001). Hormonal regulation and patterning of the broad-complex in the epidermis and wing discs of the tobacco hornworm, *Manduca sexta. Developmental Biology, 231*, 125–137.

Zhou, X. F., & Riddiford, L. M. (2002). Broad specifies pupal development and mediates the 'status quo' action of juvenile hormone on the pupal-adult transformation in *Drosophila* and *Manduca. Development, 129*, 2259–2269.

Zhou, B. H., Hiruma, K., Shinoda, T., & Riddiford, L. M. (1998). Juvenile hormone prevents ecdysteroid-induced expression of broad complex RNAs in the epidermis of the tobacco hornworm, *Manduca sexta. Developmental Biology, 203*, 233–244.

Zollman, S., Godt, D., Prive, G. G., Couderc, J. L., & Laski, F. A. (1994). The BTB domain, found primarily in zinc-finger proteins, defines an evolutionarily conserved family that includes several developmentally-regulated genes in *Drosophila. Proceedings of the National Academy of Sciences of the United States of America, 91*, 10717–10721.

Revelations on the Regulatory Mechanisms in Moth Sex-Pheromone Signals

Ada Rafaeli

Abstract The understanding of chemical communication in Lepidoptera, particularly in moths, has advanced greatly over the last half-century including sex-pheromone identification and synthesis, but the application of this knowledge in pest management has had only marginal success, possibly due to the complexity of the biosynthetic cascades that result in the production of the pheromone components. Sexual encounters in moths are initiated by the release of a unique blend of volatile organic compounds, the sex pheromones, by one sex, to attract conspecifics and signal receptivity for mating. After mating, pheromone biosynthetic activity in females is reduced, calling behavior ceases and oviposition is enhanced. Both post-mating responses i.e. reduced receptivity and increased oviposition, can be theoretically visualized as systems that could be manipulated to the advantage for pest management. This review examines the research trend concerning mating behavior in moths by appraising the available information revealed by molecular, genomic, phylogenetic and transcriptomic studies on the mechanisms that up-regulate sex-pheromone production in receptive females and down-regulate after mating. The review concludes by examining future research directions needed to enhance our present-day knowledge concerning these regulatory mechanisms so as to reach a level of understanding that will facilitate its utilization for pest management.

1 Overview

The understanding of chemical communication in Lepidoptera, particularly in moths, has advanced greatly over the last half-century including sex-pheromone identification and synthesis, but the application of this knowledge in pest management has had only marginal success, possibly due to the complexity of the biosynthetic cascades that result in the production of the pheromone components. Sexual encounters in moths are initiated by the release of a unique blend of volatile organic

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compounds, the sex pheromones, by one sex, to attract conspecifics and signal receptivity for mating. After mating, pheromone biosynthetic activity in females is reduced, calling behavior ceases and oviposition is enhanced. Both post-mating responses i.e. reduced receptivity and increased oviposition, can be theoretically visualized as systems that could be manipulated to the advantage for pest management. This review examines the research trend concerning mating behavior in moths by appraising the available information revealed by molecular, genomic, phylogenetic and transcriptomic studies on the mechanisms that up-regulate sex-pheromone production in receptive females and down-regulate after mating. The review concludes by examining future research directions needed to enhance our present-day knowledge concerning these regulatory mechanisms so as to reach a level of understanding that will facilitate its utilization for pest management.

2 Moth Behavior and Regulation of Pheromone Signals

Insects as a group have demonstrated evolutionary resilience through their phenomenal reproductive success. Understanding the behavioral adaptations of their mating and post-mating responses would contribute considerably to our knowledge concerning the evolutionary significance of these processes and how we can intervene to disrupt mating for use in pest management. The understanding of chemical communication in Lepidoptera, particularly in moths, has advanced greatly over the last half-century including sex-pheromone identification and synthesis but the application of this knowledge in pest management has had only marginal success, mostly for the use in population monitoring and perception-disruption, the latter only for a select number of pests (Welter et al. 2005). In many of the species, sexual encounters are initiated by the release of a unique blend of volatile organic compounds, the sex pheromones, to attract conspecifics and signal receptivity for mating. In Lepidoptera, pheromone release is characterized by calling behavior in which the female extrudes the ovipositor tip exposing the pheromone gland to release the sex pheromone blend. In most moths this blend is derived from downstream products of fatty acid biosynthesis in the pheromone gland which is located between the ultimate and penultimate terminal segments of the abdomen (see reviews Rafaeli 2002, 2009, 2011).

The driving force behind reproductive isolation and species differentiation in insects, particularly those highly dependent on sex-pheromone components for mate location, lies within the variation in pheromone components. This diversity is reflected in a variation of the biosynthetic pathways and the enzymes that are involved. In addition, further complexity and diversification of sex-pheromone communication is attained through multiple component systems integrated with stereoisomer composition (Bjostad et al. 1987; Abad et al. 2001). A major class of sex-pheromones produced by female moths is the C_{10}–C_{18} unsaturated, acyclic, aliphatic compounds that contain an oxygenated functional group, such as aldehydes, alcohols or acetate esters. These pheromone components are synthesized *de novo* in

the pheromone gland, from acetyl-CoA involving acetyl-CoA carboxylase (ACCase) and fatty acid synthetase producing fatty acids (Jurenka 2003; Rafaeli and Jurenka 2003; Tsfadia et al. 2008). The production of fatty acids is followed with the double bond positioning as a result of the action of unique desaturases to make mono- and di-unsaturated fatty acids (Tillman et al. 1999). Chain shortening through chain-shortening enzymes that make the specific chain-length fatty acid and, depending on the functional group of the pheromone, a fatty-acyl reductase, an acetyl-transferase or an alcohol oxidase will produce the final pheromone blend. The order in which these enzymes act and the stereo-specificity of the enzymes involved determines the final pheromone components produced (Jurenka 2003). Using comparative transcriptomics and EST development from cDNA libraries, followed by BLAST searches (NR and Swissprot databases), a large number of candidate genes in the Lepidopteran pheromone biosynthetic pathways have been identified but many await further exploration by functional expression studies and/or RNAi technology (Strandh et al. 2008; Vogel et al. 2010; Gu et al. 2013; Jung and Kim 2014).

After mating, pheromone biosynthetic activity in females is reduced and calling behavior ceases. Females do not re-mate for the remainder of the night in some cases, and permanently in others (see reviews Rafaeli 2011; Hanin and Rafaeli 2014). In addition, oogenesis and the rate of ovulation and oviposition increase in mated females (Soller et al. 1999; Jin and Gong 2001). The absence of released pheromone effectively terminates male orientation to females, indicating female non-receptivity. Both responses i.e. reduced receptivity and increased oviposition, can be theoretically visualized as systems that could be manipulated to the advantage for pest management. If females receive the signal for non-receptivity or oviposition prematurely and permanently, mating will not occur and viable eggs will not be laid, in effect unfertilized eggs will be aborted and for those insects with one vitellogenic cycle, essentially the affected female will not be able to produce progeny.

3 Revelations from Molecular, Genomic, Phylogenetic and Transcriptomic Studies

3.1 PBAN & PBAN-Receptors: Up-Regulation

Regulatory mechanisms of sex-pheromone biosynthesis involving endocrine and gene regulation have progressed relatively slowly due to the complexity of the biosynthetic cascades that result in the production of the pheromone components. Over 20 years ago, a neurohormone, Pheromone Biosynthesis Activating Neuropeptide (PBAN) was identified, using classical endocrine methodology, as a regulatory hormone in the biosynthetic pathway of some moth sex-pheromones (Raina and Klun 1984). Since the discovery of PBAN, a steep rise in publications on regulation of pheromone production was witnessed for the next 10 years but after that it has

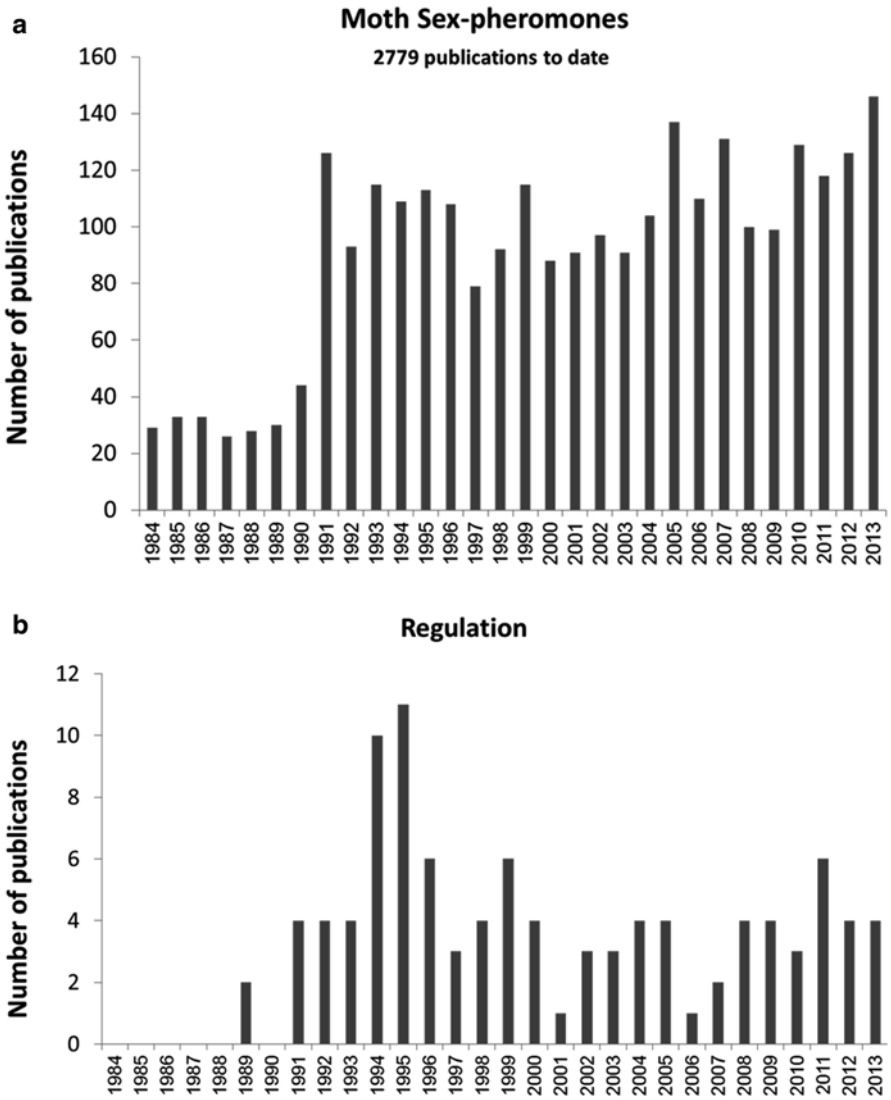

Fig. 1 Number of publications in the past 30 years, after the discovery of PBAN, on moth sex pheromones (**a**) and regulation of moth pheromones (**b**) (Search performed via ISI Web of Science, 2014)

declined to a steady state despite rapid advances in moth genomics. Such a decline however, was not observed in publications concerning sex pheromones in moths in general (Fig. 1) and probably reflects the complex nature of these regulatory mechanisms.

In *Helicoverpa armigera*, PBAN up-regulates the production of malonyl coenzyme A from acetate by the action of acetyl coenzyme A carboxylase (ACCase)

(Eliyahu et al. 2003; Tsfadia et al. 2008; Hanin et al. 2008). Additionally, ACCase inhibitors and commercial, grass-selective herbicides such as 2-aryloxyphenoxypropionate (e.g., diclofop) and cyclohexandione oxime (e.g., tralkoxydim) inhibit the PBAN-stimulation of pheromone production in several moth species (*H. armigera*: Eliyahu et al. 2003; Hanin et al. 2008; *H. armigera & Plodia interpunctella*: Tsfadia et al. 2008; *Cydia pomonella*: Kleinman 2008; Fig. 2), particularly the stimulation of malonyl-CoA incorporation into the main pheromone component (Tsfadia et al. 2008).

The above studies provide irrefutable support to the hypothesis that the PBAN-induced rate-limiting step for sex-pheromone biosynthesis lies within the activation of this enzyme. Moreover, the results indicate that moth pheromone biosynthesis may also be targeted by these herbicides and suggest that they may be used as a novel means of manipulating moth pest populations of important agricultural crops. Future research, directed at formulating methods of dissemination of these compounds amongst targeted pest populations, will have to be undertaken.

PBAN is photoperiodically released from the corpora cardiaca into the hemolymph during scotophase in response to circadian cues (Fabrias et al. 1994; Jacquin et al. 1994; Nagalakshmi et al. 2007; Bloch et al. 2013). Circulating PBAN stimulates pheromone gland cells directly to produce and release sex pheromone. Its pheromonotropic role has been well elucidated in many Lepidoptera (Rafaeli 2011). PBAN, a 33-amino acid *C*-terminal amidated peptide was subsequently shown to be

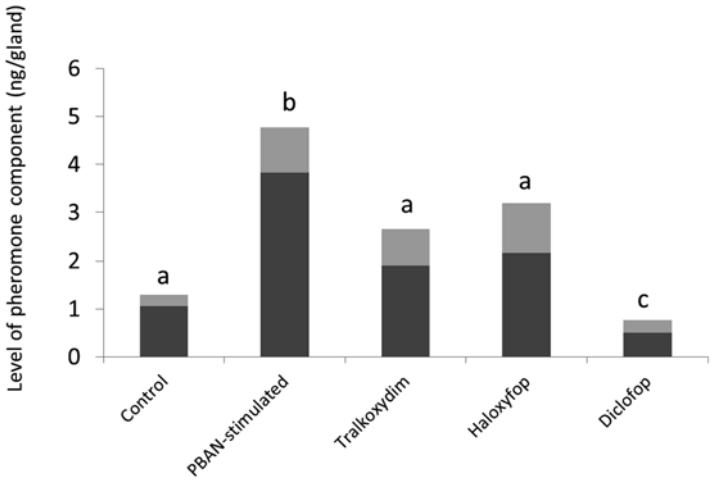

Fig. 2 Reduction in levels of the main pheromone component of *Cydia pomonella* females, (E,E)-8,10 dodecadienol (codlemone), after inhibition with various herbicides. Decapitated (24 h) females were injected with either Control = water; PBAN-stimulated = synthetic HezPBAN (5 μM); or PBAN + herbicide (100 μM Tralkoxydim, Haloxyfop or Diclofop). Pheromone titers were quantified using GC and tridecenyl acetate as internal standard. *Light shaded areas* show extent of SEM, n = 4–27 replicates. *Different letters* indicate a statistically significant difference (two tailed ANOVA, Fisher protected least significance difference test $P \leq 0.05$) (Unpublished data, Kleinman 2008)

a member of the pyrokinin (PK)/PBAN family of peptides characterized by a common *C*-terminal FXPRL amide motif (X = G/S/T/V), the minimum sequence necessary to elicit activity. It is produced by neurosecretory cells within the subesophageal ganglion and the gene encoding PBAN has post-translational processing sites that could produce four additional PBAN-gene neuropeptides: PGN-24 (pyrokinin-like/diapause hormone), PGN-18, PGN-8 and PGN-7 all having the FXPRLamide motif. Moreover, it was shown to be widespread throughout insects where it has diverse functions (Rafaeli 2011). In the moth *H. armigera*, in addition to its presence in pheromone glands, PBAN-R protein and gene transcript are detected in membranes of neural tissues and therefore indicate a possible neural function, perhaps as neurotransmitter (Rafaeli et al. 2007).

PBAN activity on pheromone gland cells causes an influx of extracellular calcium that promotes the production of intracellular cyclic-AMP through the involvement of G proteins, indicating its association with a G-protein coupled receptor (GPCR) (Rafaeli and Gileadi 1996; Rafaeli 2002). GPCRs belong to the largest superfamily of membrane-bound receptors and have in common a topology based on seven-transmembrane α-helical domains, coupling to heterotrimeric G proteins (Gαβγ). We were the first to identify a PBAN-receptor from moths (HezPBAN-R) (Choi et al. 2003). At that time, due to the lack of genomic information on moth model species, our strategy involved homology comparisons of the ligand PBAN, assuming that ligand and receptor co-evolve. PBAN's similarity to the vertebrate neurohormone, neuromedin U suggested that the vertebrate neuromedin U receptor could be used as a basis for the search for the PBAN-R in moths. The gene was thus identified based on sequence identity to a group of GPCRs from the *Drosophila* genome that is homologous to neuromedin U receptors in vertebrates.

The full-length PBAN receptor was subsequently cloned and expressed in Sf9 insect cells and shown to mobilize calcium in response to PBAN. Subsequent RNAi silencing of the PBAN-R gene transcript in *H. armigera* females significantly reduced the level of pheromone produced by females during their peak pheromone production (7th hour during the scotophase) (Fig. 3), however, preliminary tests performed in small cages did not successfully demonstrate a reduction in mating (Hanin and Rafaeli, unpublished). This may be the result of close encounters between males and females in the small cages. Thus, demonstration of the effectiveness of RNAi technology on mating disruption in the field is yet to be resolved.

Since its discovery, PBAN was known to be present in the suboesophageal ganglion of both males and females but its role in the males remained a mystery. The identification of the gene transcript for the PBAN-R in the aedeagus of males, a tissue homologous in position to the female pheromone gland initiated a study aimed at elucidating PBAN's role in the male moth. The aedeagus is connected to lateral valvae containing hair-pencils that are displayed during courtship and are implicated in the dissemination of male putative sex-pheromone components (Birch 1974). Male hair-pencil complexes contain fatty-acid components and alcohol components that bear similarity to the female sex-pheromone components. We demonstrated a distinct diel periodicity in a number of these components and showed the influence of PBAN on their levels (Bober and Rafaeli 2010). In addition, gene

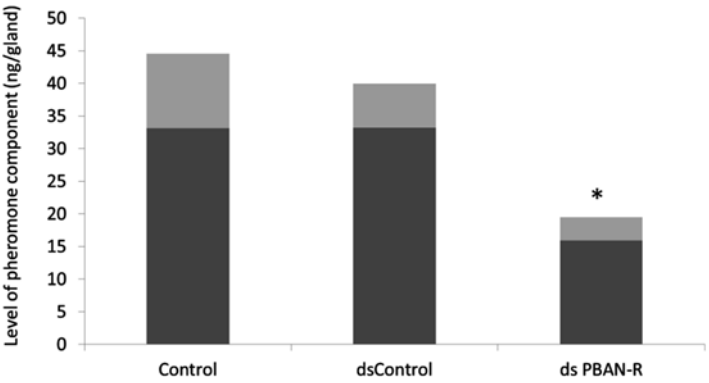

Fig. 3 Reduction in levels of the main pheromone component, Z-11 hexadecenal of *H. armigera* females after silencing of the PBAN-R gene transcript. Females were injected with either Control = diethyl polycarbonate-treated water injected females; dsControl = λphage DE3ea59 dsRNA; dsPBAN-R = PBAN-R dsRNA during the first photophase (photophase after emergence). Pheromone titers were quantified during the 7th hour of the second scotophase as published previously (Hanin et al. 2012). *Light shaded areas* show extent of SEM, n = 7–12 replicates. *Asterix* indicates a statistically significant difference (two tailed ANOVA, Fisher protected least significance difference test $P \leq 0.05$) (Unpublished data, Hanin and Rafaeli)

expression of the PBAN-R transcript in male hair-pencil complexes was shown to be differentially up-regulated after emergence of the adult moth from the pupal stage (Bober et al. 2010). Subsequently RNAi knockdown of the PBAN-R gene transcript, revealed its function in regulating the levels of key male sex-pheromone components (Bober and Rafaeli 2010) thus associating PBAN as a regulatory neuropeptide of male hair-pencil sex-pheromone components, as was shown in females.

Since the identification of the HezPBAN-R, a considerable number of additional receptors in this family have been sequenced based on homology studies or identified from genome sequencing projects (Bober and Rafaeli 2009). Thus, with the advances in sequence technologies and the emergence of Lepidoptera as model organisms, the physiological role of PBAN; its target site; its G-protein coupled receptor and the signal transduction involved in the induction of downstream enzymatic events have been elucidated. Although GPCRs in insects are regulators in many essential functions involved in survival and propagation and are therefore an attractive target for insecticide design, the rational designing of potent antagonists has been hampered by the lack of structural information on receptor-bound ligands. Some progress has been attained on the development of peptidomimetic analogs of the PK family of peptides with enhanced biostability and bioavailability and with the potential to disrupt the reproductive process in insect pests of agricultural importance (Nachman 2014). However, the peptides in the PK family do not exhibit species specificity and experiments have shown that all of the functions listed can be stimulated by more than one peptide. Therefore, due to their ubiquitous and multifunctional actions, the PK family of peptides and their receptors are unlikely candidates for future targeted application against insect pests since they bear the possibility of also affecting beneficial insects.

3.2 Sex-Peptide (SP) and SP-Receptors: Down-Regulation

During copulation male insects transfer sperm and seminal fluid to females. Within the seminal fluid are peptides (Acps) produced by the male accessory glands that are implicated in influencing the behavior of females after mating (Kingan et al. 1995; Wolfner 2009; Kubli 2003; Gillott 2003). In *Drosophila*, where this aspect of reproductive biology has been most thoroughly investigated, mating-induced changes in females, from sperm and seminal fluid transfer during copulation, induce both short- and long-term effects (Ottiger et al. 2000). Of particular importance in eliciting post-mating non-receptivity and increased fecundity in *Drosophila* is sex-peptide (DrmSP or Acp70A) (Chen et al. 1988).

DrmSP is a 36-amino-acid peptide produced in the male *Drosophila* accessory gland. During mating the peptide is transferred with the seminal fluid and passes from the female reproductive tract into the hemolymph, ultimately acting directly on targets in the nervous system of females (Kubli 2003; Hasemeyer et al. 2009; Yang et al. 2009). Ectopic expression of DrmSP and injection of purified peptide into virgin females decreased receptivity to males and stimulated egg production for 1–2 days in *Drosophila melanogaster*, indicating its major role in both short- and long-term post-mating effects (Chen et al. 1988; Nakayama et al. 1997; Aigaki et al. 1991). The long-term actions of DrmSP require the presence of sperm and involve binding to sperm via *N*-terminal sequences and subsequent cleavage, releasing the *C*-terminus of the peptide that then enters the hemolymph (Chapman et al. 2003; Liu and Kubli 2003; Peng et al. 2005). The mode of action of DrmSP has been partially determined and has been shown to be allatotropic in *D. melanogaster*, stimulating the release of juvenile hormone (JH-III-bisepoxide) from the corpora allata thereby modulating oocyte maturation and egg-laying (Moshitzky et al. 1996; Soller et al. 1997, 1999).

Similar to its allatotropic effect in *D. melanogaster*, synthetic DrmSP also stimulates JH-II production in isolated corpora allata from virgin female *H. armigera in vitro* in a dose dependent manner (Fan et al. 1999, 2000). These observations are significant in light of previous reports demonstrating the increase in JH titers in females after mating and the involvement of JH in mating-induced suppression of pheromone production (Ramaswamy et al. 1997; Delisle et al. 2000). Consistent with these findings is the observation that JH as well as fenoxycarb (a JH analog) inhibit pheromone production in female *H. armigera* and reduce transcript and protein levels of the PBAN-receptor (PBAN-R) (Rafaeli and Bober 2005; Bober et al. 2010). Nagalakshmi et al. (2007) showed that PBAN levels in the hemolymph of virgin *H. armigera* females are drastically reduced after mating. Significantly, synthetic DrmSP and truncated fragments of DrmSP injected into the hemolymph of virgin female *H. armigera* lead to the termination of PBAN-stimulated pheromone production (Fan et al. 1999, 2000) and inhibition of calling behavior by female moths (Hanin et al. 2012).

The male accessory glands of *H. armigera* contain proteins that result in the termination of pheromone production when injected into the hemolymph of virgin

females as crude extracts. Some of these accessory gland proteins are immunoreactive with antibodies against DrmSP and have been shown to be pheromonostatic in *H. armigera* (Nagalakmish et al. 2004). Despite the presence of DrmSP-like activity and immunoreactivity in the moth and after many attempts to identify the SP-like factor from the moth accessory glands, SP has only been identified in *Drosophila*, notwithstanding the large body of genomic data available to date in several species of insects.

Nagalakshmi et al. (2007) reported that different sets of DrmSP-like immunoreactive peptides (HeaSP) in the moth are up-regulated during scotophase in male accessory glands and the central nervous system (CNS) of mated females. These findings suggested that target receptors for these seminal-peptides reside in the pheromone gland as well as the CNS of females. Indeed, a receptor for DrmSP (SP-R, CG16752) was identified in the reproductive tract and nervous system of *D. melanogaster* (Yapici et al. 2008). Mutants that lacked this receptor failed to respond to DrmSP and continued to show virgin behavior even after mating. Comparative genomic studies that followed the latter discovery, have revealed the presence of SP-R genes in several *Drosophila* species, *Aedes* and *Anopheles* mosquitoes, *Tribolium* and the moth *Bombyx*. On the basis of sequence homologies deposited in the GenBank, we identified a putative SP-R in *H. armigera* (HeaSP-R) with 99 % homology to the *Bombyx mori* SP-R (Hanin et al. 2011).

To determine whether this receptor is involved in mating behavior, we conducted a differential expression study of this receptor comparing gene expression levels in relation to different photoperiods, sex and mating status of the moth. Photoperiod and mating influence SP-R gene expression levels and sexual dimorphic changes were observed in neural tissues due to the different physiological states. After mating SP-R transcript levels in female neural tissues and pheromone glands are up-regulated. Physiological studies *in vivo* confirm the up-regulation of gene expression levels in pheromone glands isolated from mated females (Hanin et al. 2011). Thus, these studies confirmed that the SP-R in the moth plays a role in mating behavior.

Recent studies (Kim et al. 2010; Poels et al. 2010), showed that heterologously expressed *D. melanogaster* SP-R responded to *D. melanogaster* myoinhibitory peptides (MIPs). MIPs are pleiotropic peptides that belong to the $W(X)_6W$ amide peptide family that were initially named based on their ability to inhibit spontaneous muscle contractions of insect gut and oviduct but have also been shown to suppress ecdysteroid production in the prothoracic gland, and they control salivary gland activity in ticks (see review Hanin and Rafaeli 2014). Due to the response to MIPs, Kim et al. (2010) claim that the SP-R is the ancestral receptor for MIPs whilst SP, that contains a similar conserved sequence of tryptophan residues but with different spacing and positioning ($W(X)_8W$), adopted this receptor in the course of evolution. However, MIPs failed to mimic the SP post-mating responses *in vivo* when tested either through generating a transgene or through the injection of varying concentrations of synthetic MIPs into the hemocoel of virgin females (Kim et al. 2010).

Utilizing RNAi technology we were able to silence the moth HeaSP-R expression by 50–60 % which effectively prevented *in vivo Drm*SP-suppression of pheromone production and calling behavior. However, sex pheromone production by

mated, silenced females remained low, comparable to mated, normal females, thereby indicating the probable involvement of additional factors in the suppression of sex pheromone production after mating in the moth. None-the-less, mated, silenced females failed to increase their oviposition rates as is normally observed in mated females, and their behavior in that respect did not differ from that of virgin females indicating that the SR-R plays a crucial role in the post-mating behavioral changes in the female moth that influence oviposition.

4 Future Research Directions and Applications in Pest Management

As discussed above, after mating the female moth undergoes drastic behavioral changes in particularly pertaining to reproductive behavioral changes. In this context, pheromone biosynthesis, receptivity and calling behavior are terminated and newly mated females actively reject subsequent attempts of males to mate with them. Concurrently, oogenesis and the rate of ovulation and oviposition increase in mated females. As observed in many insect species during copulation, males transfer seminal peptides (Acps) that are implicated in these post-mating responses. It is probable that these processes are regulated by more than one factor (Rafaeli and Hanin 2013) but it is unclear how they are associated. Several Acps have been identified to various degrees in diverse insect species. A recent study demonstrated that allatotropic activity in males influences the production and consequent transfer of juvenile hormone to females during copulation (Hassanien et al. 2014). Not many studies have been undertaken focusing on defining the physiological, biochemical and molecular effects of these Acps and their precise roles in the manifestation of the observed behavioral changes in the mated female. Moreover, the interactions of seminal peptides with the regulatory peptides controlling receptivity and the biosynthesis of pheromones have not been studied. I believe that with the present day rapid progress in genomic techniques, we will be able to identify and elucidate the roles of several of these seminal peptides in model insects that are serious agricultural pests.

A number of well-established strategies for insect population suppression such as the Sterile Insect Technique (SIT) are based on understanding and exploiting aspects of the reproductive behavior of the target insect (Knipling 1979). The SIT is based on introducing large numbers of sterile males to reduce the overall growth of a target population. Wild, fertile females, once mated to sterile males will have reduced reproductive output depending upon aspects of their reproductive behavior. The impact of mating with a sterile male is most effective when females mate only once, which effectively removes the female permanently from the population. Where females mate multiple times, implementing the SIT becomes more demanding since a single sterile mating only temporarily reduces the effective population size and subsequent mating with wild fertile males will result in progeny. Some of

the most successful SIT programs are directed against insects whose females have low re-mating frequencies, e.g. *Ceratitis capitata* and *Pectinophora gossypiella*. If the frequency of female re-mating can be reduced or eliminated in polyandrous species then the SIT could be applied and would be more effective and efficient. Understanding the molecules and mechanisms responsible for these post-mating behaviors is therefore important if reproduction-based control strategies such as the SIT are to find more widespread use.

Exploitation of the mating process for transmitting desired control molecules into females could be envisioned as the "next generation" of insect pest strategies. Transgenic insect technology is a mature and robust technology whose application is limited largely only by the amenability of insects to embryo-microinjection and post-injection manipulation (O'Brochta and Handler 2008). With current transgenic technologies there are few, if any, genetic and biochemical barriers to integrate genes into insect genomes (O'Brochta and Handler 2008). Given the maturity of transgenic insect technology, it is not surprising to find multiple examples of the technology being applied to insect population control and eradication efforts. For example, the control of *P. gossypiella* by the SIT is being enhanced through the release of sterilized insects containing a dominant visible transgene (Simmons et al. 2007) and transgenic *Aedes aegypti* are being used to suppress wild populations of this species (Enserink 2010; Miller 2011). Improved transgenic technologies to facilitate the creation of males that have a potent mixture of accessory gland proteins (and conceptually transmitting other physiologically active specific compounds) capable of strongly inhibiting female mating behaviors or female fitness will provide solutions for the manipulation of pest species in the future, once transgenic methods are accepted and validated.

5 Concluding Remarks

This review endeavored to assess the available literature concerning up- and down-regulation of Lepidopteran sex-pheromone production. Targeting up-regulatory pathways involves the possible use of herbicides that will influence enzymatic function; the use of RNAi silencing or peptide mimics. Difficulties in the success rate for RNAi silencing have been reported, particularly for the Lepidoptera, although in our research we have not encountered such difficulties. This could be attributed to our preliminary developmental expression studies in order to determine the ideal timing for silencing. On the whole, targeting PBAN or its receptor, is unlikely to produce specific effects on pest species alone due to the fact that it is multifunctional, it is present in most insect species, and its presence in both females and males. On the other hand, the unique presence of SP in *Drosophila* that shows activity in moths, whilst contrasting with the widespread presence of its receptor, presents a unique opportunity for its exploitation as a target against moth pest species. In this review, I have suggested the consideration of integrating insect transgenesis,

the sterile insect technique and SP for the development of an effective method to down-regulate the reproductive potential of pest moths.

References

Abad, J. L., Camps, F., & Fabriàs, G. (2001). Stereospecificity of the (Z)-9 desaturase that converts (E)-11-tetradecenoic acid into (Z, E)-9,11-tetradecadienoic acid in the biosynthesis of spodoptera littoralis sex pheromone. *Insect Biochemistry and Molecular Biology, 31*, 799–803.

Aigaki, T., Fleischmann, I., Chen, P. S., & Kubli, E. (1991). Ectopic expression of sex peptide alters reproductive behavior of female *D. melanogaster. Neuron, 7*, 557–563.

Birch, M. C. (Ed.). (1974). *Pheromones* (pp. 115–134). Amsterdam: North-Holland Pub. Co.

Bjostad, L. B., Wolf, W., & Roelofs, W. L. (1987). *Pheromone biochemistry* (G. J. Blomquist & G. D. Prestwich, Eds.) (pp. 77–120). New York: Academic.

Bloch, G., Hazan, E., & Rafaeli, A. (2013). Circadian rhythms and endocrine functions in adult insects. *Journal of Insect Physiology, 59*, 56–69.

Bober, R., & Rafaeli, A. (2009). Pheromone Biosynthesis Activating Neuropeptide (PBAN) and its G-protein coupled receptor. In M. Krishnan & K. Chandrasekar (Eds.), *Short views on insect molecular biology* (chap. 7, pp. 131–145). South India: International Book Mission, Academic Publisher.

Bober, R., & Rafaeli, A. (2010). Gene-silencing reveals the functional significance of pheromone biosynthesis activating neuropeptide receptor (PBAN-R) in a male moth. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 16858–16862.

Bober, R., Azrielli, A., & Rafaeli, A. (2010). Developmental regulation of the Pheromone Biosynthesis Activating Neuropeptide-Receptor (PBAN-R): Re-evaluating the role of juvenile hormone. *Insect Molecular Biology, 19*, 77–86.

Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M. F., Smith, H. K., & Partridge, L. (2003). The sex peptide of *Drosophila melanogaster*: Female post-mating responses analyzed by using RNA interference. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 9923–9928.

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., & Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster. Cell, 54*, 291–298.

Choi, M. Y., Fuerst, E. J., Rafaeli, A., & Jurenka, R. A. (2003). Identification of a pheromone biosynthesis-activating neuropeptide G protein-coupled receptor in pheromone glands of *Helicoverpa zea. Proceedings of the National Academy of Sciences of the United States of America, 100*, 9721–9726.

Delisle, J., Picimbon, J. F., & Simard, J. (2000). Regulation of pheromone inhibition in mated females of *Choristoneura fumiferana* and *C. rosaceana. Journal of Insect Physiology, 46*, 913–921.

Eliyahu, D., Applebaum, S. W., & Rafaeli, A. (2003). Moth sex-pheromone biosynthesis is inhibited by the herbicide diclofop. *Pesticide Biochemistry and Physiology, 77*, 75–81.

Enserink, M. (2010). GM mosquito trial strains ties in gates-funded project. *Science, 330*, 1030–1031.

Fabrias, G., Marco, M. P., & Camps, F. (1994). Effect of the pheromone biosynthesis activating neuropeptide on sex pheromone biosynthesis in *Spodoptera littoralis* isolated glands. *Advances Insect Biochemistry and Physiology, 27*, 77–87.

Fan, Y., Rafaeli, A., Gileadi, C., Kubli, E., & Applebaum, S. W. (1999). *Drosophila melanogaster* sex peptide stimulates JH-synthesis and depresses sex pheromone production in *Helicoverpa armigera. Journal of Insect Physiology, 45*, 127–133.

Fan, Y., Rafaeli, A., Moshitzky, P., Kubli, E., Choffat, Y., & Applebaum, S. W. (2000). Common functional elements of Drosophila melanogaster-seminal peptides involved in reproduction of Drosophila melanogaster and Helicoverpa armigera. *Insect Biochemistry and Molecular Biology, 30*, 805–812.

Gillott, C. (2003). Male accessory gland secretions: Modulators of female reproductive physiology and behavior. *Annual Review of Entomology, 48*, 163–184.

Gu, S.-H., Wu, K.-M., Guo, Y.-Y., Pickett, J. A., Field, L. M., Zhou, J.-J., & Zhang, Y.-J. (2013). Identification of genes expressed in the sex pheromone gland of the black cutworm Agrotis ipsilon with putative roles in sex pheromone biosynthesis and transport. *BMC Genomics, 14*, 636.

Hanin, O., & Rafaeli, A. (2014). Understanding the functions of sex peptide receptors? In R. Chandrasekar, B. K. Tyagi, Z. Z. Gui, & G. Reeck (Eds.), *Short views on insect molecular biology* (Vol. 2, pp. 371–383). Manhattan: Kansas State University.

Hanin, O., Rubin, B., Applebaum, S. W., & Rafaeli, A. (2008). Structure-activity relationships of pheromonostasis induced by ACCase-inhibitor herbicides in the moth *Helicoverpa armigera. Pesticide Biochemistry and Physiology, 91*, 153–159.

Hanin, O., Azrielli, A., Zakin, V., Applebaum, S., & Rafaeli, A. (2011). Identification and differential expression of a sex-peptide receptor in *Helicoverpa armigera. Insect Biochemistry and Molecular Biology, 41*, 537–544.

Hanin, O., Azrielli, A., Applebaum, S., & Rafaeli, A. (2012). Functional impact of silencing the *Helicoverpa armigera* sex-peptide receptor on female reproductive behavior. *Insect Molecular Biology, 21*, 161–167.

Hasemeyer, M., Yapici, N., Heberlein, U., & Dickson, B. J. (2009). Sensory neurons in the *Drosophila* genital tract regulate female reproductive behavior. *Neuron, 61*, 511–518.

Hassanien, I. T. E., Grötzner, M., Meyering-Vos, M., & Hoffmann, K. H. (2014). Neuropeptides affecting the transfer of juvenile hormones from males to females during mating in Spodoptera frugiperda. *Journal of Insect Physiology, 66*, 45–52.

Jacquin, E., Jurenka, R. A., Ljungberg, H., Nagnan, P., Lofstedt, C., Descoins, C., & Roelofs, W. L. (1994). Control of sex pheromone biosynthesis in the moth *Mamestra brassicae* by the pheromone biosynthesis activating neuropeptide. *Insect Biochemistry and Molecular Biology, 24*, 203–211.

Jin, Z. Y., & Gong, H. (2001). Male accessory gland derived factors can stimulate oogenesis and enhance oviposition in Helicoverpa armigera (Lepidoptera: Noctuidae). *Archives of Insect Biochemistry and Physiology, 46*, 175–185.

Jung, C. R., & Kim, Y. (2014). Comparative transcriptome analysis of sex pheromone glands of two sympatric lepidopteran congener species. *Genomics, 103*, 308–315.

Jurenka, R. A. (2003). Biochemistry of female moth sex pheromones. In R. Vogt & G. Blomquist (Eds.), *Insect pheromone biochemistry and molecular biology* (pp. 53–80). New York: Academic.

Kim, Y. J., Bartalska, K., Audsley, N., Yamanaka, N., Yapici, N., Lee, J. Y., Kim, Y. C., Markovic, M., Isaac, E., Tanaka, Y., & Dickson, B. J. (2010). MIPs are ancestral ligands for the sex peptide receptor. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 6520–6525.

Kingan, T. G., Bodnar, W. M., Raina, A. K., Shabanowitz, J., & Hunt, D. F. (1995). The loss of female sex pheromone after mating in the corn earworm moth *Helicoverpa zea*: identification of a male pheromonostatic peptide. *Proceedings of the National Academy of Sciences of the United States of America, 92*, 5082–5086.

Kleinman, Z. (2008). *Reproductive behavior and pheromone production of the Codling Moth Cydia pomonella (Lepidptera: Tortricidae): Stimulatory and inhibitory influences*. M.Sc. thesis, The Hebrew University (In Hebrew with English Abstract).

Knipling, E. F. (1979). *The basic principles of insect population suppression management* (U.S.D.A. Agric. Handbook No. 512, 659 p). Washington, DC.

Kubli, E. (2003). Sex-peptides: seminal peptides of the *Drosophila* male. *Cellular and Molecular Life Sciences, 60*, 1689–1704.

Liu, H., & Kubli, E. (2003). Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster. Proceedings of the National Academy of Sciences of the United States of America, 100*, 9929–9933.

Miller, T. A. (2011). Genetically modifies insects as used in SIT should not require regulation. *Phytoparasitica, 39*, 415–418.

Moshitzky, P., Fleischmann, I., Chaimov, N., Saudan, P., Klauser, S., Kubli, E., & Applebaum, S. W. (1996). Sex-peptide activates juvenile hormone biosynthesis in the *Drosophila melanogaster* corpus allatum. *Archives of Insect Biochemistry and Physiology, 32*, 363–374.

Nachman, R. (2014). Peptidomics applied: A new strategy for development of selective antagonists/agonists of insect pyrokinin (FXPRLamide) family using a novel conformational-mimetic motif. *European Proteomics Association Open Proteomics, 3*, 138–142.

Nagalakmish, V., Applebaum, S. W., Kubli, E. C., Choffat, Y., & Rafaeli, A. (2004). The presence of *Drosophila melanogaster* sex Peptide-like immunoreactivity in the accessory glands of male *Helicoverpa armigera. Journal of Insect Physiology, 50*, 241–248.

Nagalakshmi, V. K., Applebaum, S. W., Azrielli, A., & Rafaeli, A. (2007). Female sex pheromone suppression and the fate of sex-peptide like peptides in mated moths of *Helicoverpa armigera. Advances Insect Biochemistry and Physiology, 64*, 142–155.

Nakayama, S., Kaiser, K., & Aigaki, T. (1997). Ectopic expression of sex-peptide in a variety of tissues in *Drosophila* females using the P (GAL4) enhancer-trap system. *Molecular & General Genetics, 254*, 449–455.

O'Brochta, D. A., & Handler, A. M. (2008). Perspectives on the state of insect transgenics. *Advances in Experimental Medicine and Biology, 627*, 1–18.

Ottiger, M., Soller, M., Stocker, R. F., & Kubli, E. (2000). Binding Sites of *Drosophila melanogaster* sex peptide pheromones. *Journal of Neurobiology, 44*, 57–71.

Peng, J., Chen, S., Büsser, S., Liu, H., Honegger, T., & Kubli, E. (2005). Gradual release of sperm bound sex-peptide controls female post-mating behavior in *Drosophila. Current Biology, 15*, 207–213.

Poels, J., Van Loy, T., Vandersmissen, H. P., Van Hiel, B., Van Soest, S., Nachman, R. J., & Vanden Broeck, J. (2010). Myoinhibiting peptides are the ancestral ligands of the promiscuous Drosophila sex peptide receptor. *Cellular and Molecular Life Sciences, 67*, 3511–3522.

Rafaeli, A. (2002). Neuroendocrine control of pheromone biosynthesis in moths. *International Review of Cytology, 213*, 49–92.

Rafaeli, A. (2009). Pheromone Biosynthesis Activating Neuropeptide (PBAN): Regulatory role and mode of action. *General and Comparative Endocrinology, 162*, 69–78.

Rafaeli, A. (2011). Moth sex-pheromone production: Biosynthetic pathways, regulatory physiology, inhibitory processes and disruption. In L. Cauterruccio (Eds.), *Moths: Types, ecological significance and control* (chap. 4, pp. 115–143). Huntington: Nova Science Publishers, Inc.

Rafaeli, A., & Bober, R. (2005). The effect of the juvenile hormone analog, fenoxycarb on the PBAN-receptor and pheromone production in adults of the moth *Helicoverpa armigera*: an "aging" hormone in adult females? *Journal of Insect Physiology, 51*, 401–410.

Rafaeli, A., & Gileadi, C. (1996). Multi-signal transduction of moth pheromone biosynthesis-activating neuropeptide (PBAN) and its modulation: Involvement of G-proteins? In B. Kirsch & R. Mentlein (Eds.), *The peptidergic neuron* (pp. 239–244). Basel: Birkhauser.

Rafaeli, A., & Hanin, O. (2013). The influence of photoperiod and mating on the profiles of seminal fluid peptides from male accessory glands of *Helicoverpa armigera. Israel Journal of Entomology, 43*, 51–79.

Rafaeli, A., & Jurenka, R. A. (2003). PBAN regulation of pheromone biosynthesis in female moths. In G. J. Blomquist & R. Vogt (Eds.), *Insect Pheromone biochemistry & molecular biology* (pp. 107–136). London: Elsevier Academic Press.

Rafaeli, A., Bober, R., Becker, L., Choi, M.-Y., Fuerst, E.-J., & Jurenka, R. A. (2007). Spatial distribution and differential expression of the receptor for pheromone-biosynthesis-activating

neuropeptide (PBAN-R) at the protein and gene levels in tissues of adult *Helicoverpa armigera* (*Lepidoptera*: *Noctuidae*). *Insect Molecular Biology, 16*, 287–293.

Raina, A. K., & Klun, J. A. (1984). Brain factor control of sex pheromone production in the female corn earworm moth. *Science, 225*, 531–532.

Ramaswamy, S. B., Shu, S., Park, Y. I., & Zeng, F. (1997). Dynamics of Juvenile Hormone-mediated gonadotropism in the Lepidoptera. *Archives of Insect Biochemistry and Physiology, 35*, 539–558.

Simmons, G. S., Alphey, L. S., Vasquez, T., Morrison, N. I., Epton, M. J., Miller, E., Miller, T. A., & Staten, R. T. (2007). Potential use of a conditional lethal transgenic pink bollworm Pectinophora gossypiella in area-wide eradication or suppression programmes. In M. J. B. Vreysen, A. S. Robinson, & J. Hendrichs (Eds.), *Area-wide control of insect pests from research to field implementation* (XV, 789 p). Dordrecht: Springer.

Soller, M., Bownes, M., & Kubli, E. (1997). Mating and sex peptide stimulate the accumulation of yolk in oocytes of *D. melanogaster. European Journal of Biochemistry, 243*, 732–738.

Soller, M., Bownes, M., & Kubli, E. (1999). Control of oocyte maturation in sexually mature *Drosophila* females. *Developmental Biology, 208*, 337–351.

Strandh, M., Johansson, T., Ahrén, D., & Löfstedt, C. (2008). Transcriptional analysis of the pheromone gland of the turnip moth, *Agrotis segetum* (Noctuidae) reveals candidate genes involved in pheromone production. *Insect Molecular Biology, 17*, 73–85.

Tillman, J. A., Seybold, S. J., Jurenka, R. A., & Blomquist, G. J. (1999). Insect pheromones – an overview of biosynthesis and endocrine regulation. *Insect Biochemistry and Molecular Biology, 29*, 481–514.

Tsfadia, O., Azrielli, A., Falach, L., Zada, A., Roelofs, W., & Rafaeli, A. (2008). Pheromone biosynthetic pathways: PBAN-regulated rate-limiting steps and differential expression of desaturase genes in moth species. *Insect Biochemistry and Molecular Biology, 38*, 552–567.

Vogel, H., Heidel, A. J., Heckel, D. G., & Groot, A. T. (2010). Transcriptome analysis of the sex pheromone gland of the noctuid moth *Heliothis virescens. BMC Genomics, 11*, 29.

Welter, S. C., Pickel, C., Millar, J. G., Cave, F., Van Steenwyk, R. A., & Dunley, J. (2005). Pheromone mating disruption offers selective management options for key pests. *California Agriculture, 59*, 16–22.

Wolfner, M. F. (2009). Battle and ballet: Molecular interactions between the sexes in *Drosophila. The Journal of Heredity, 100*, 399–410.

Yang, C. H., Rumpf, S., Xiang, Y., Gordon, M. D., Song, W., Jan, L. Y., & Jan, Y. N. (2009). Control of the postmating behavioral switch in *Drosophila* females by internal sensory neurons. *Neuron, 61*, 519–526.

Yapici, N., Kim, Y. J., Ribeiro, C., & Dickson, B. J. (2008). A receptor that mediated the post-mating switch in *Drosophila* reproductive behaviour. *Nature, 451*, 33–37.

Part III
Virus-Vector Interactions

Interactions Between Insect Vectors and Propagative Plant Viruses

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Abstract Plant-infecting viruses that are transmitted in a persistent-propagative manner must persist and replicate in two divergent hosts, plants and insects. There are five groups of persistent-propagative plant viruses: rhabdoviruses, reoviruses, bunyaviruses, marafiviruses, and tenuiviruses. Throughout the transmission cycle of a persistent-propagative virus, there is a close association between virus and vector that is dependent on specific interactions. The –omic technologies that are now widely used for simultaneous examination of thousands of genes (genomics), mRNAs (transcriptomics), and proteins (proteomics) combined with high-throughput bioinformatic tools to extract a vast amount of information have become a popular approach to better understand virus-vector interactions. The integration of the available datasets that result from these –omic studies is contributing to the identification of host factors that are required for the viral replication cycle. Current knowledge of the vector components that function in viral infection is still limited for the majority of persistent-propagative viruses. However, the emerging information on genomes, transcriptomes, and proteomes for insect vectors of plant viruses provides unique opportunities for studying the function of genes involved in virus attachment, acquisition, and transmission in different vector species. In this chapter we discuss the major groups of plant viruses transmitted in a persistent-propagative manner, the biology of these viruses, the interactions with their vectors, and the –omic technologies applied to study these virus-vector pathosystems.

1 Overview

Most plant viruses rely largely on arthropod vectors as a mean of spread between plants due to the sessile nature of their hosts. The majority of the arthropods that are efficient vectors of plant viruses have the capacity to pierce the leaf epidermis with their stylets and delicately deposit virus particles in the cytoplasm of plant cells. Using their specialized feeding structures they are able to overcome a primary plant

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defense, the cell wall, without destroying the integrity of the plant cell. Plant viruses that are transmitted by arthropods in a persistent-propagative manner are vectored by insects with piercing-sucking mouthparts in the orders Hemiptera and Thysanoptera. There are five groups of persistent-propagative plant viruses (Table 1); three of these virus groups, rhabdoviruses, reoviruses and bunyaviruses, have members that infect vertebrate animals while the remaining two virus groups, marafiviruses and tenuiviruses, do not have animal-infecting members. During persistent-propagative transmission, the virus invades insect tissues and replicates in the vector. In the insect, the virus moves from the gut epithelia into the hemolymph or other tissues and finally to the salivary glands from which they can be inoculated to initiate infection of a new plant host when the insect feeds. This virus pathway in the insect vector is similar to the dissemination pathway taken by animal viruses in mosquitoes and other blood-feeding vectors (Gray and Banerjee 1999). Throughout the transmission cycle of a persistent-propagative virus, there is a close association between virus-encoded proteins and vector constituents that mostly rely on specific protein-protein interactions. Current knowledge of the vector components that function in viral infection is still limited for persistent-propagative viruses. However, the emerging information on genomes, transcriptomes, and

Table 1 Plant viruses transmitted in a persistent-propagative manner by various arthropod vectors

Virus family and genus	Type species	Acronym	Vector family	Vector genus/spp.
Rhabdoviridae				
Cytorhabdovirus	*Lettuce necrotic yellows*	LNYV	Aphididae	*Hyperomyzus lactucae*
Nucleorhabdovirus	*Potato yellow dwarf**	PYDV	Cicadellidae	*Nephotettix apicalis*
Reoviridae				
Phytoreovirus	*Rice dwarf**	RDV	Cicadellidae	*Nephotettix cincticeps*
Oryzavirus	*Rice ragged stunt*	RRSV	Delphacidae	*Nilaparvata lugens*
Fijivirus	*Fiji disease**	FDV	Delphacidae	*Perkinsiella saccharicida*
				Perkinsiella vastatrix
Unassigned				
Tenuivirus	*Rice stripe**	RSV	Delphacidae	*Laodelphax striatellus*
Tymoviridae				
Marafivirus	*Maize rayado fino*	MRFV	Cicadellidae	*Dalbulus maidis*
Bunyaviridae				
Tospovirus	*Tomato spotted wilt*	TSWV	Thripidae	*Frankliniella occidentalis*

Adapted from Ammar and Nault (2002), Mann and Dietzgen (2014).
*Indicates that transovarial transmission has been document for this virus.

proteomes for insect vectors of plant viruses provide unique opportunities for studying the function of genes involved in virus attachment, acquisition, and transmission in different vector species. This knowledge might allow comparisons on the role of genes in different insect groups, thereby providing a more comprehensive insight into the interactions of a plant virus with its specific insect vector. In this chapter we discuss the major groups of plant viruses transmitted in a persistent-propagative manner, the biology of these viruses, the interactions with their vectors, and the –omic technologies applied to study these virus-vector pathosystems.

2 Rhabdoviruses

2.1 *The* Rhabdoviridae *Family, Virus Structure, and Genome Organization*

The *Rhabdoviridae* is a large family of viruses containing genera that infect a wide range of hosts including humans. There are nine recognized genera in this family (Table 2): *Vesiculovirus, Lyssavirus, Novirhabdovirus, Ephemerovirus, Perhabdovirus, Sigmavirus, Cytorhabdovirus*, *Nucleorhabdovirus*, and *Tibrovirus*.

Table 2 Recognized genera of the family *Rhabdoviridae*, order *Mononegavirales*

Genus	Type species	Host	Means of transmission
Cytorhabdovirus	*Lettuce necrotic yellows virus*	Plants	Aphids
Ephemerovirus	*Bovine ephemeral fever virus*	Cattle	Culicoids (midges) and mosquitoes
Lyssavirus	*Rabies virus*	Bats and terrestrial carnivores	Bites, scratches or saliva by infected mammals
Novirhabdovirus	*Infectious hematopoietic necrosis virus*	Fish (salmonid)	Water
Nucleorhabdovirus	*Potato yellow dwarf virus*	Plants	Leafhoppers, planthoppers, aphids
Perhabdovirus	*Perch rhabdovirus*	Fish	Water
Sigmavirus	*Drosophila melanogaster sigmavirus*	Fruit fly (*Drosophila* spp.)	Vertical transmission
Tibrovirus	*Tibrogargan virus*	Cattle and water buffalo	Midges
Vesiculovirus	*Vesicular stomatitis Indiana virus*	Mammals (cattle, pigs and horses), fish and insects	Sandflies, blackflies and midges (mammals); water (fish)

Adapted from Mann and Dietzgen (2014)

The plant-infecting rhabdoviruses belong to the genera *Cytorhabdovirus* and *Nucleorhabdovirus* which are classified based on the intracellular localization of virus maturation and the genome sequence (Ammar et al. 2005; Bourhy et al. 2005; Mann and Dietzgen 2014). Nucleorhabdoviruses mature in the nucleus and bud through the nuclear membrane into the perinuclear space of plant cells while cytorhabdoviruses mature in the cytoplasm of plant cells specifically on viroplasms in the endoplasmic reticulum (Ammar et al. 2005; Jackson et al. 2005). Most plant-infecting rhabdoviruses are transmitted by hemipteran insects from the Cicadellidae and Delphacidae families (Table 2). In addition to the viruses classified as members of this family, recent discoveries have revealed related viruses. A new genus, *Dichorhavirus*, was recently proposed to classify rhabdo-like viruses with bipartite genomes (Dietzgen et al. 2013). The dichorhaviruses have significant sequence similarity with plant-infecting rhabdoviruses and Orchid fleck virus is considered the type species of this virus group (Dietzgen et al. 2013; Kondo et al. 2013).

All members of the family *Rhabdoviridae* have bullet or bacilliform virions with a spiked surface and a striated capsid core. In general, the virion length and width are about 130–350 nm and 45–100 nm, respectively (Hogenhout et al. 2008; Jackson et al. 1999; Redinbaugh and Hogenhout 2005). Complete genome sequences are available for several cytorhabdoviruses and nucleorhabdoviruses (Mann and Dietzgen 2014). The negative-sense RNA genome of rhabdoviruses ranges in size from 11 to 16 kb and it is fully encapsidated by the nucleoprotein (N) and surrounded by a lipid bilayer derived from plant or insect host cell membranes. A single glycoprotein (G) is embedded in the lipid membrane and several G molecules are exposed as spikes in the surface of the virion (Jackson et al. 1999). The matrix (M) protein interacts with the lipid bilayer components and N while the phosphoprotein (P) and large polymerase (L) protein are required for synthesis of viral mRNAs (Redinbaugh and Hogenhout 2005). There is at least one ancillary protein encoded by plant rhabdoviruses between the viral P and M genes in addition to the five structural genes (Ammar et al. 2009). The MMV P3, MFSV P4, RYSV P3, TaVCV P3, and SNYV sc4 proteins have secondary structure similar to the movement proteins (MPs) of the 30 K superfamily of virus MPs proposed to facilitate the intracellular movement of the virus between plant cells through the plasmodesmata (Huang et al. 2005; Jackson et al. 2005). With the exception of the proposed rhabdovirus MPs, all the viral proteins are thought to function similarly during infection of plant and insect hosts. Plant rhabdoviruses infect a large number of monocotyledonous and dicotyledonous species and most of these rhabdoviruses are completely dependent on insect vectors for their spread and transmission to susceptible plant hosts.

2.2 Vectors of Rhabdoviruses

The primary vectors of rhabdoviruses are insects in the order Hemiptera including planthoppers, leafhoppers, and aphids (Jackson et al. 2005; Redinbaugh and Hogenhout 2005). Insect vectors of plant rhabdoviruses transmit them in a

persistent-propagative manner and some can also be transovarially transmitted like the aphid-transmitted nucleorhabdovirus *Coriander feathery red-vein virus* (CFRVV). The honeysuckle aphid, *Hyadaphis foeniculi*, remains infective for life once it acquires CFRVV and transovarial passage occurs at about 55 % maternal rate (Misari and Sylvester 1983). Another nucleorhabdovirus, PYDV, can be occasionally transmitted through eggs of *A. constricta* (Black 1943). The recently discovered bipartite dichorhaviruses are transmitted by *Brevipalpus* mites (Acari: Tenuipalpidae) (Dietzgen et al. 2013). A high degree of specificity has been described for plant rhabdoviruses and the insect vectors that transmit them. For example, *Maize mosaic virus* (MMV) is transmitted only by *Peregrinus maidis*, *Wheat American striate mosaic virus* (WASMV) by *Endria inimica* and *Graminella nigrifrons*, *Maize fine streak virus* (MFSV) by Elymana virescens, *Maize Iranian mosaic virus* (MIMV) by *Ribautodelphax notabilis*, and *Sorghum stunt mosaic virus* (SSMV) by *Graminella sonora* (Jackson et al. 2005). When multiple insects are identified as vectors of a plant rhabdovirus, one insect is frequently a more efficient vector than the other. For example, *Rice yellow stunt virus* (RYSV, also known as *Rice transitory yellows virus*) can be transmitted efficiently by both *Nephotettix cincticeps* and *N. nigropictus* but lower efficiency was observed in *N. virescens* (Hibino 1996; Inoue 1979). When 4th instar nymphs were allowed a 2-day acquisition access period (AAP) on infected plants, 25–59 % and 35–75 % of *N. cincticeps* and *N. nigropictus* transmitted RYSV, respectively, but *N. virescens* transmitted the same virus at 0–5 % only (Inoue 1979). The leafhopper *E. virescens* can transmit WASMV but less efficiently compared to *E. inimica* (Sinha 1970). Similarly, LNYV is transmitted primarily by *Hyperomyzus lactucae* but can also be transmitted by another aphid species, *H. carduellinus*. A low rate of transovarial passage, however, occurs in its main vector, *H. lactucae* (Sylvester 1980). This vector specificity emphasizes the intimate relationships between plant rhabdoviruses and their insect vectors and might explain, at least in part, why the geographical distribution of some of these plant viruses is restricted and appear to be closely linked to the distribution of their vectors (Kormelink et al. 2011).

2.3 Vector-Plant Rhabdovirus Interactions

Plant rhabdoviruses multiply in their vectors as it has been demonstrated by transmission studies where the virus is serially passed from one insect to either a plant host or to its progeny by serial dilution transmission, transovarial passage experiments, injection of purified virus, electron microscopy, immunohistochemistry, serological analyses, or replication in vector cell monolayers (VCMs) (Black 1979; Ammar and Nault 1985; Ammar et al. 2007; Ammar and Hogenhout 2008; Redinbaugh et al. 2012; Yao et al. 2013). Virus accumulation and distribution has been characterized in detail in different tissues of viruliferous *P. maidis, G. nigrifrons*, and *Drosophila* infected with *Sigma virus* (Fig. 1).

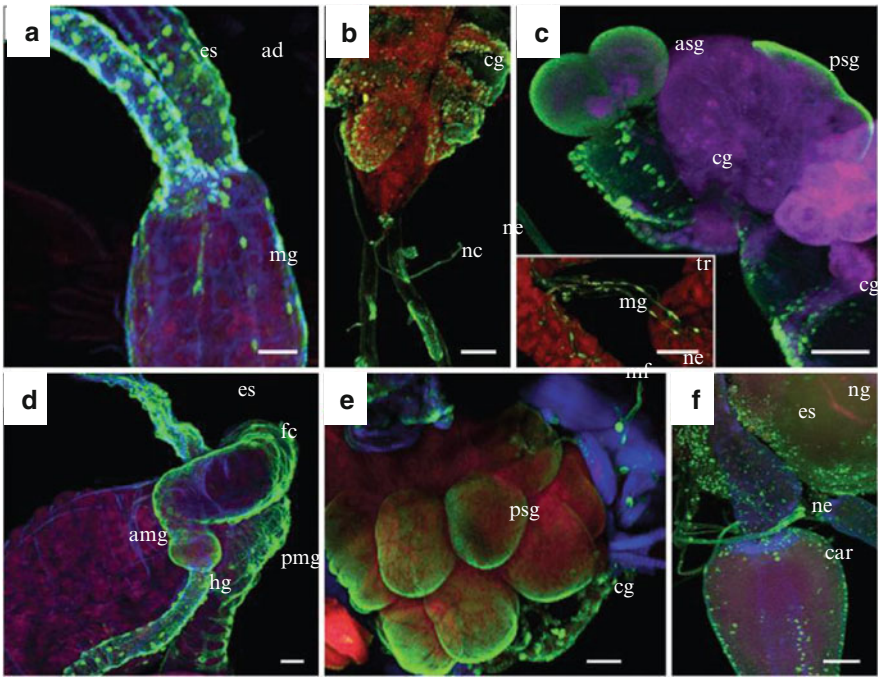

Fig. 1 Immunofluorescence localization of some rhabdoviruses in their insect hosts. (**a**–**c**) *Maize mosaic virus* (*MMV*) localization in the midgut (*mg*), anterior diverticulum (*ad*), esophagus (*es*), compound ganglion (*cg*), nerve cord (*nc*), nerves (*ne*), trachea (*tr*), and the accessory (*asg*) and principal salivary glands (*psg*) of the planthopper vector *Peregrinus maidis*. (**d**, **e**) Maize fine streak virus (*MFSV*) localization in the esophagus (*es*), filter chamber (*fc*), anterior (*amg*) and posterior midgut (*pmg*), hindgut (*hg*), lobes of the principal salivary glands (*psg*), compound ganglion (*cg*), and nerves (*ne*) of the leafhopper vector *Graminella nigrifrons*. (**f**) *Sigma virus* localization in the esophagus (*es*), cardia (*car*), nerve ganglia (*ng*), and nerves (*ne*) of an infected *Drosophila*. Additional abbreviations: *ep* epidermis, *me* mesophyll, *mf* muscle fibers, *vb* vascular bundle. Scale bars: 40 μm. (**a**–**f**) were incubated with virus-specific antibodies, then with a secondary antibody conjugated to Alexa Fluor 488 (*green*), the nuclear stain propidium iodide (*red*), and with an actin stain, Phalloidin (*blue/purple*) (Adapted from Ammar et al. (2009))

The dissemination route of MMV in its vector *P. maidis* was studied in detail using immunofluorescence labelling following acquisition of the virus by feeding on MMV-infected corn plants (Ammar and Hogenhout 2008). In this study, Ammar and Hogenhout were able to show that a plant rhabdovirus follows a neurotropic route in its insect vector similar to *Rabies virus* in mammalian host. The early and prominent infection of the nerves, nerve cord and compound ganglionic mass, compared to other tissues strongly suggest MMV to be neurotropic in its insect vector. A similar study was conducted by Todd et al. (2010) where they looked at the infection of different organs of *G. nigrifrons* infected with MFSV. The authors observed a similar pattern of infection as the one seen for MMV in *P. maidis*, where MFSV was first detected in the gut, nerves, and visceral muscle cells, and then progressed to the salivary glands at 4 weeks after virus acquisition from infected plants. In the

same study, Todd et al. (2010) also linked virus dissemination with transmission efficiency by the insect vector. MFSV was detected in about 20 % of insects that fed on infected plants but <10 % of the insects transmitted the virus. In the majority of non-transmitting insects, MFSV was found only in the midgut or in other organs but not in the salivary glands. These results suggest that a salivary gland infection barrier may be present in *G. nigrifrons* for MFSV and that not all the insects from the same population will have the same reaction upon virus infection.

During virus entry and dissemination in the insect vector, it is hypothesized that rhabdoviruses use a similar entry and exit strategy as the one observed in animal hosts. Rhabdoviruses enter the cell via the endocytic pathway through receptor recognition and fusion with a host cellular membrane that is mediated by the viral glycoprotein. The vertebrate-infecting rhabdovirus *Vesicular stomatitis virus* (VSV) is endocytosed in clathrin-based, dynamin-2-dependent manner where the virions are able to induce *de novo* formation of clathrin-coated pits for their uptake (Johannsdottir et al. 2009). Several putative receptors have been identified for animal-infecting rhabdoviruses. For example, *Rabies virus* is thought to initiate infection in the host by binding to a nicotinic acetylcholine receptor (nAChR) that is a transmembrane receptor-ion channel protein (Gastka et al. 1996). In the analysis of MMV-infected *P. maidis* gut transcriptome, a nAChR has been identified (Whitfield et al. 2011). Additionally, a fibronectin-like protein which is known to participate in virus entry and replication in the host of *Viral hemorrhagic septicemia virus* (VHSV), a *Novirhabdovirus*, was also identified in *P. maidis* (Bearzotti et al. 1999; Whitfield et al. 2011). Currently, the identification and characterization of receptors or interacting proteins from insect vectors with specific plant rhabdoviral components has not been pursued. An attractive target to study is the viral G that has important functions in virus entry into host cells, virion assembly, and escape from host cells. These functions are mediated by specific protein-protein interactions between vector molecules and G. In our laboratory, we have successfully constructed a *P. maidis* cDNA library and screened it with MMV G utilizing a modified yeast two hybrid screen (Y2H) called split-ubiquitin membrane-based Y2H (MbY2H). Using this technique, we were able to identify vector proteins that interacted with MMV G which are putatively involved in intracellular trafficking and the endocytosis and exocytosis pathways (Barandoc-Alviar et al. 2014). Additional studies comparing plant and insect host proteins that interact with rhabdoviral proteins would enable identification of conserved and unique steps during the viral replication cycle in the two disparate hosts.

2.4 Genomic and Transcriptomic Studies of Insect Vectors of Plant Rhabdoviruses

The partial transcriptomes of three important rhabdovirus vectors, *P. maidis*, *G. nigrifrons*, and *L. striatellus*, have been sequenced. The rhabdovirus-vector interaction studies conducted thus far have focused on nucleorhabdoviruses that infect

cereal crops (Cassone et al. 2014; Chen et al. 2012; Whitfield et al. 2011). Notably, important aphid vectors of cytorhabdoviruses are understudied but the transcriptomic resources that are available for *L. striatellus* enables further characterization of cytorhabdovirus-vector interactions. The transcriptomes of these three vectors have enabled the identification of transcripts that may play a role in the viral infection cycle such as immune pathways, endocytosis and exocytosis, and replication. The corn planthopper, *P. maidis*, is a major pest of agronomically important crops and it transmits MMV and *Maize stripe tenuivirus* (MStV). *P. maidis* has emerged as a model insect for understanding rhabdovirus-vector interactions due to the availability of sequence resources and that functional tools (*i.e.*, RNAi) have been developed for this vector (Yao et al. 2013). Initial transcriptome sequencing efforts were focused on the gut of *P. maidis* with 20,771 expressed sequence tags (ESTs) generated and deposited into dbEST (Whitfield et al. 2011). Comparison of *P. maidis* gut ESTs with other insect amino acid sequences revealed 202 *P. maidis* transcripts with putative homology to proteins associated with insect innate immunity, including those implicated in the Toll, Imd, JAK-STAT, Jnk, and the small interfering RNA-mediated pathways. Expression analysis of four putative innate immune responsive genes (ATG 3, PI3K, Jnk, TPPii) revealed that the average normalized expression of each of these target genes to actin tended to be lower for MMV-infected guts compared to non-infected guts (Whitfield et al. 2011). Subsequent experiments with phosphoinositol-4, 5-bisphosphate-3-kinase (PI3K) have validated that this gene is a MMV-responsive gene and knock-down experiments indicate that reduction in PI3K abundance is associated with higher MMV titers in *P. maidis* (Yao and Whitfield unpublished). Additional mRNA sequencing of male and female planthoppers in our laboratory has yielded 68,003 contigs (Barandoc-Alviar et al. 2014). With sequence resources and functional genomics assays in place, *P. maidis* is a good candidate insect for comparing insect response to multiple propagative viruses.

The transcriptome of the black-faced leafhopper, *G. nigrifrons*, the vector of MFSV, has been generated by RNA sequencing (RNA-Seq) of two separate cDNA libraries consisting of virus acquirers or transmitters (Chen et al. 2012). This study generated 38,240 ESTs from these two libraries and 194 *G. nigrifrons* sequences were predicted to be components of the insect immune response (Chen et al. 2012). Expression analysis of a subset of innate immunity genes revealed that three transcripts for peptidoglycan recognition proteins (PGRP-SB1, -SD, and -LC) showed significant down-regulation in transmitters versus acquirers leafhoppers suggesting that the insect mounts a response to MFSV infection (Chen et al. 2012). In further studies with *G. nigrifrons*, Cassone et al. (2014) conducted experiments aimed at understanding leafhopper response to *Maize fine streak virus* (MFSV) and *Maize chlorotic dwarf virus* (MCDV) that are persistent-propagative and semi-persistent transmitted viruses, respectively. These RNA-seq experiments revealed that *G. nigrifrons* fed on MFSV- or MCDV-infected plants showed changes in transcript accumulation in the vector in early and late virus infection (Cassone et al. 2014). *Laodelphax striatellus* or the small brown planthopper is the vector of *Barley yellow striate mosaic virus* (BYSMV) and Northern cereal mosaic virus (NCMV). It is also

a vector of the tenuivirus *Rice stripe virus* (RSV) and the fijivirus *Rice black-streaked dwarf virus* (RBSDV). The interactions between *L. striatellus* with tenuiviruses and reoviruses will be discussed later in the chapter. The transcriptome for this insect vector has been assembled utilizing 454–FLX high-throughput sequencing to determine differences between viruliferous (for RSV) and naïve *L. striatellus* (Zhang et al. 2010). Furthermore, its mitochondrial genome has been sequenced and discussed later in the Sect. 5 of this book chapter. Thus, *L. striatellus* is a good candidate for additional studies of virus-vector interactions because it is a vector of two cytorhabdoviruses and two other persistent-propagative viruses.

The cytorhabdoviruses, LNYV and SCV and the nucleorhabdoviruses SYNV, SYVV and CRFVV are all transmitted by aphids. CRFVV is transmitted by the honeysuckle aphid, *Hyadaphis foeniculi*, and 1 % transmission was recorded for the green peach aphid, *Myzus persicae* (Mann and Dietzgen 2014; Misari and Sylvester 1983). There are no sequence resources for *H. foeniculi* but there are transcriptomic and forthcoming genomic resources for *M. persicae.* Moreover, sequencing of 16 cDNA libraries from four different aphid lineages representing different developmental stages and tissues such as the gut and salivary glands has been done by Ramsey et al. (2007). These sequencing produced 26,669 ESTs with 2423 genes that are potentially aphid-specific. Comparison of the cDNA data among the four aphid lineages identified 67 high confidence single nucleotide polymorphisms that can be used as potential genetic markers. Furthermore, an oligonucleotide (60-mer probe) microarray was designed for *M. persicae* representing 10,478 unique genes. With the current resources available for *M. persicae*, this insect vector is a good candidate for characterizing aphid-rhabdovirus interactions.

3 Tospoviruses

3.1 The Tospovirus *Genus, Virus Structure, and Genome Organization*

Tospoviruses belong to the genus *Tospovirus*, the only plant-infecting group within the virus family *Bunyaviridae*, which is primarily composed of animal-infecting viruses classified in four genera (*Orthobunyavirus*, *Hantavirus*, *Nairovirus*, and *Phlebovirus*). *Tomato spotted wilt virus* (TSWV), the type species of the *Tospovirus* genus, is considered one of the ten most devastating plant viruses known due to the extremely wide host range of the virus and the ubiquitous nature of its thrips vectors (Scholthof et al. 2011). Since the original description and characterization of TSWV in Australia in the early 1900s (Brittlebank 1919; Samuel et al. 1930), the number of tospoviruses has increased to 11 approved species and 18 tentative species (Table 3). The virions of tospoviruses are pleomorphic in shape with a diameter that ranges from 80 to 120 nm in size (Gonzalez-Scarano and Nathanson 1996; Shope 1985). A

Table 3 *Tospovirus* species (approved and tentative)

Approved *Tospovirus* species	**Acronym**	**Reference**
Groundnut bud necrosis virus	GBNV	Reddy et al. (1968)
Groundnut ringspot virus	GRSV	de Avila et al. (1993)
Groundnut yellow spot virus	GYSV	Satyanarayana et al. (1996)
Impatiens necrotic spot virus	INSV	de Avila et al. (1992)
Polygonum ringspot virus	PolRSV	Ciuffo et al. (2008)
Tomato chlorotic spot virus	TCSV	de Avila et al. (1990)
Tomato spotted wilt virus	TSWV	Samuel et al. (1930)
Watermelon silver mottle virus	WSMV	Yeh and Chang (1995)
Zucchini lethal chlorosis virus	ZLCV	Rezende et al. (1997)
Tentative *Tospovirus* species	**Acronym**	**Reference**
Alstroemeria necrotic streak virus	ANSV	Hassani-Mehraban et al. (2010)
Bean necrotic mosaic virus	BNMV	de Oliveira et al. (2011)
Calla lily chlorotic spot virus	CCSV	Chen et al. (2005)
Capsicum chlorosis virus	CaCV	McMichael et al. (2002)
Chrysanthemum stem necrosis virus	CSNV	Duarte et al. (1995)
Hippeastrum chlorotic ringspot virus	HCRV	Dong et al. (2013)
Groundnut chlorotic fan-spot virus	GCFV	Chu et al. (2001)
Groundnut ringspot virus/Tomato chlorotic spot virus reassortant	GRSV/TCSV	Webster et al. (2011)
Iris yellow spot virus	IYSV	Cortes et al. (1998)
Melon severe mosaic virus	MeSMV	Ciuffo et al. (2009)
Melon yellow spot virus	MYSV	Kato et al. (2000)
Mulberry vein banding virus	MuVBV	Meng et al. (2013)
Pepper necrotic spot virus	PNSV	Torres et al. (2012)
Physalis severe mottle virus	PhySMV	Cortez et al. (2001)
Soybean vein necrosis virus	SVNV	Zhou et al. (2011)
Tomato necrosis virus	TNeV	Reported in Plyusnin et al. (2012)
Tomato necrotic ringspot virus	TNRV	Seepiban et al. (2011)
Tomato yellow (fruit) ring virus	TYRV	Winter et al. (2006)
Tomato zonate spot virus	TZSV	Dong et al. (2008)
Watermelon bud necrosis virus	WBNV	Jain et al. (1998)

single virion contains all the genome segments enclosed in a host-derived membrane that is decorated with two viral glycoproteins (G_N and G_C). The genome segments are encapsidated by the nucleocapsid (N) protein to assemble the ribonucleocapsid particles (RNPs) that form a panhandle structure each associated with few copies of the RNA-dependent RNA-polymerase (RdRp).

Tospoviruses have a multipartite genome that consists of three negative-sense, single-stranded RNA (ssRNA) segments designated small (S RNA), medium (M RNA), and large (L RNA) (Gonzalez-Scarano and Nathanson 1996). Each segment

forms a panhandle structure due to the complementarity of their 5′ and 3′ ends, which are thought to contain recognition sites for the viral polymerase within important regulatory signals such as replication promoters (Strauss and Strauss 1988). Sequence and translational analysis of TSWV has shown that the S (de Haan et al. 1990) and M (Kormelink et al. 1992) RNAs are ambisense, while the L RNA is negative-sense only (de Haan et al. 1991). The S RNA encodes a nonstructural (NSs) protein in the viral (v) sense and the N protein in the viral complementary (vc) sense (de Haan et al. 1990). The NSs protein of TSWV and other tospoviruses have been shown to be necessary and sufficient for the silencing suppression activity in plants and insects by binding double-stranded and short interfering RNAs (dsRNAs and siRNAs, respectively) (Bucher et al. 2003; Garcia et al. 2006; Oliveira et al. 2011; Schnettler et al. 2010; Takeda et al. 2002). The N protein binds to ssRNA and other N protein units to encapsidate the viral RNA segments (Richmond et al. 1998; Uhrig et al. 1999). A nonstructural (NSm) protein and the polyprotein precursor to the G_N and G_C glycoproteins are encoded in the v sense and vc sense, respectively, of the M RNA (Kormelink et al. 1992). The NSm protein is involved in virus movement of the RNPs in plants as it localizes to and alters the size exclusion limit of plasmodesmata and forms aggregated tubules to aid in the cell-to-cell movement of tospoviruses in their plant host (Kormelink et al. 1994; Lewandowski and Adkins 2005; Li et al. 2009; Storms et al. 1995; Strauss and Strauss 1988). The G_N/G_C polyprotein precursor is cleaved by a yet unknown host protease to generate the two mature viral glycoproteins (G_N and G_C) which are involved in virion assembly and transmission by thrips vectors (Adkins et al. 1996; Kikkert et al. 1999, 2001; Whitfield et al. 2004, 2005, 2008). The L RNA encodes the L protein, which is the RdRp, in the vc sense (de Haan et al. 1991). Purified L protein synthesizes both the v sense and the vc sense strands and highjacks the 5′ caps from host mRNAs by cap snatching for translation of the viral proteins (Adkins et al. 1995; Duijsings et al. 1999, 2001; van Knippenberg et al. 2005; van Poelwijk et al. 1996).

3.2 Vectors of Tospoviruses

Tospoviruses can be artificially transmitted to plants in the laboratory using mechanical inoculation. In nature, however, tospoviruses are exclusively transmitted from plant-to-plant by a limited but specific number of thrips vector species. *Thrips tabaci* Linden was the first thrips species determined to be vector of TSWV (Pittman 1927). Currently, 15 thrips species belonging to the genera *Frankliniella*, *Thrips*, *Ceratothripoides*, *Dictyothrips*, *Neohydatothrips*, and *Scirtothrips* are known vectors of tospoviruses (Table 4) from the 7400 described species of thrips that make up the insect order Thysanoptera. Thrips vectors differ in the number of tospovirus species they transmit, which reflects their differences in feeding habits and geographical distribution (Table 5). For example, *T. tabaci* and *F. occidentalis* are polyphagous, have a worldwide distribution and transmit several tospovirus species, while others like *F. zucchini* and *D. betae* have a narrow host range, a limited

Table 4 Thrips vectors of *Tospoviruses*

Thrips vectors	Common name	*Tospovirus* species	Reference
Ceratothripoides claratis	Oriental tomato thrips	Capsicum chlorosis virus[a]	Premachandra et al. (2005)
Dictyothrips betae		*Polygonum ring spot virus*	Ciuffo et al. (2010)
Frankliniella bispinosa	Florida flower thrips[b]	*Tomato spotted wilt virus*	Avila et al. (2006)
Frankliniella cephalica		*Tomato spotted wilt virus*	Ohnishi et al. (2006)
Frankliniella fusca	Tobacco thrips	*Impatiens necrotic spot virus*	Naidu et al. (2001)
		Tomato spotted wilt virus	Sakimura (1963)
Frankliniella gemina		Chrysanthemum stem necrosis virus[a]	Reviewed in Ullman et al. (1997)
		Groundnut ringspot virus	de Borbon et al. (1999)
		Tomato spotted wilt virus	de Borbon et al. (1999)
Frankliniella intonsa	European flower thrips	*Groundnut ringspot virus*	Wijkamp et al. (1995)
		Impatiens necrotic spot virus	Sakurai et al. (2004)
		Tomato chlorotic spot virus	Wijkamp et al. (1995)
		Tomato spotted wilt virus	Wijkamp et al. (1995)
Frankliniella occidentalis	Western flower thrips	Alstroemeria necrotic streak virus[a]	Hassani-Mehraban et al. (2010)
		Chrysanthemum stem necrosis virus[a]	Nagata and Avila (2000)
		Groundnut ringspot virus	Wijkamp et al. (1995)
		Impatiens necrotic spot virus	De Angelis et al. (1993)
		Tomato chlorotic spot virus	Nagata et al. (2004)
		Tomato spotted wilt virus	Wijkamp et al. (1995)
Frankliniella schultzei	Common blossom thrips[b], tomato thrips[b]	Chrysanthemum stem necrosis virus[a]	Nagata and Avila (2000)
		Groundnut bud necrosis virus	Meena et al. (2005)
		Groundnut ringspot virus	Wijkamp et al. (1995)
		Impatiens necrotic spot virus	Reviewed in Ullman et al. (1997)
		Tomato chlorotic spot virus	Wijkamp et al. (1995)
		Tomato spotted wilt virus	Sakimura (1969)

Frankliniella zucchini		*Zucchini lethal chlorosis virus*	Nakahara and Monteiro (1999)
Neohydatothrips variabilis	Soybean thrips	Soybean vein necrosis virus[a]	Zhou et al. (2011)
Scirtothrips dorsalis	Chilli thrips[b], yellow tea thrips[b]	*Groundnut bud necrosis virus*	Meena et al. (2005)
		Groundnut chlorotic fan-spot virus[a]	Chen et al. (1996)
		Groundnut yellow spot virus	Gopal et al. (2010)
Thrips palmi	Melon thrips	Calla lily chlorotic spot virus[a]	Chen et al. (2005)
		Capsicum chlorosis virus	Reviewed in Ullman et al. (1997)
		Groundnut bud necrosis virus	Reddy et al. (1992)
		Melon yellow spot virus[a]	Kato et al. (2000)
		Watermelon bud necrosis virus	Reviewed in Ullman et al. (1997)
		Watermelon silver mottle virus	Iwaki et al. (1984)
Thrips setosus	Light-brown soybean thrips[b], Japanese flower thrips[b]	*Tomato spotted wilt virus*	Tsuda et al. (1996)
Thrips tabaci	Onion thrips	*Iris yellow spot virus*	Cortes et al. (1998)
		Tomato spotted wilt virus	Wijkamp et al. (1995)
		Tomato yellow (fruit) ring virus[a]	Ghotbi et al. (2005)

Virus names in italics indicate *Tospovirus* species approved by the International Committee for the Taxonomy of Viruses
[a]Tentative *Tospovirus* species
[b]Thrips common name is not recognized by the Entomological Society of America
Adapted from Montero-Astúa et al. 2016.

Table 5 Feeding habits and geographical distribution of thrips vectors of *Tospoviruses*

Vector species	Feeding habits	Geographical distribution
Ceratothripoides claratis	Polyphagous	Australia, India, and Thailand
Dictyothrips betae	Specialist (on *Polygonum convolvulus*, *P. dumetorum*, and *Beta vulgaris*)	Paleartic (including Czechoslovakia, Hungary, Romania, Ukraine, Germany, The Netherlands, and Italy)
Frankliniella bispinosa	Polyphagous	Southeastern USA, Bermuda, and Bahama Islands
Frankliniella cephalica	Polyphagous	Widespread between Bermuda, Trinidad, Mexico, and Colombia; also found in Japan (Okinawa) and Taiwan
Frankliniella fusca	Polyphagous	USA, Canada, Puerto Rico, Martinique, and Mexico
Frankliniella gemina	Highly polyphagous	Brazil and Argentina
Frankliniella intonsa	Highly polyphagous	Washington, British Columbia; widespread across western Europe to Vietnam, Japan, and Taiwan
Frankliniella occidentalis	Highly polyphagous	Worldwide
Frankliniella schultzei	Highly polyphagous	Pantropical
Frankliniella zucchini	Polyphagous (within Cucurbitaceae)	Brazil to Mexico
Neohydatothrips variabilis	Specialist (within Fabaceae)	USA (including New Jersey, Georgia, Illinois, Nebraska, Oklahoma, Tennessee, Arizona, Utah, California, and British Columbia)
Scirtothrips dorsalis	Highly polyphagous	Asia; widespread between Pakistan, Japan, Australia, Israel, Florida, and the Caribbean
Thrips palmi	Highly polyphagous	Asia, northern Australia, widespread in the Tropics (including the Caribbean and southern Florida)
Thrips setosus	Polyphagous	Japan
Thrips tabaci	Highly polyphagous	Worldwide

Adapted from Montero-Astúa et al. 2016.

geographical distribution and only transmit one species each. Furthermore, potentially new thrips species might be identified as vectors of recently discovered tospovirus species such as MuVBV infecting mulberry trees in China. TSWV is the best characterized tospovirus with respect to interactions with its most efficient thrips vector, *F. occidentalis* Pergande. Virus transmission by adult thrips occurs exclusively if TSWV acquisition takes place during the larval stages, with the first instar larvae being the most efficient at acquiring the virus (Lindord 1932; van de Wetering et al. 1996). Early second instar larvae can also acquire TSWV but to a lower extent than the first instar larvae (Chatzivassiliou et al. 2002). Adult thrips that feed on virus-infected plants can acquire TSWV as well, however, this does not result in a productive infection and transmission does not occur (de Assis Filho et al. 2004;

Ullman et al. 1992). The latent period for TSWV is completed during the larval stages before pupation begins (Peters et al. 1995) as the virus must persists the replacement of internal organs and tissues that occur during the prepupal and pupal stages as thrips molt, which ultimately results in the transtadial transmission of tospoviruses (Moritz 1997). TSWV does not infect the reproductive tissues of their thrips vectors, thus, the virus is not transovarially transmitted from viruliferous adult thrips to their progeny (Wijkamp et al. 1996). Finally, TSWV is transmitted by adult male and female thrips to a susceptible plant host from where the transmission cycle must begin again.

3.3 Vector-Tospovirus Interactions

The dynamics of tospovirus transmission are the result of complex interactions between the virus and the insect vector. TSWV acquisition can occur in feeding periods as short as 5 min (Razvyazkina 1953; Wijkamp et al. 1996), but continuous feeding and longer AAP may be required for a thrips vector to ingest and acquire enough virus particles to become viruliferous (Peters et al. 1995). The AAP for thrips to acquire TSWV can vary depending on the plant host from which the virus is acquired and also the distribution and availability of the virus within the plant tissue (Peters et al. 1995). A latent period is required before TSWV transmission by the thrips vector can occur, which length decreases with increasing temperatures (Wijkamp and Peters 1993). Finally, an inoculation access period (IAP) to deliver TSWV virions into plant cells of 5–130 min have been reported and might depend on the feeding behavior of individual thrips (Peters et al. 1995; Wijkamp et al. 1996). Virus inoculation by thrips occurs more readily during brief probes involving little to no ingestion (Sakimura 1962, 1963), as viruses require intact cells to initiate the infection process. TSWV particles are delivered to plants through the saliva of thrips (Hunter and Ullman 1992; Ullman et al. 1997). Salivation is a major component of brief probes, while ingestion is not extensive or even null and plant cells are maintained intact during these shallow probes. Van de Wetering and associates showed that female thrips produced more feeding scars (a cluster of dead cells emptied of their contents) than male thrips, while males transmitted TSWV with a higher efficiency than females (van de Wetering et al. 1998). Using electrical penetration graph, it has been demonstrated that TSWV modifies the feeding behavior of its thrips vector as virus-infected males fed more than non-infected males and they made almost three times more ingestion probes (probes in which they salivate but left the cells undamaged) than their non-infected conspecifics (Stafford et al. 2011).

Tospoviruses possess a dual tropism, since they replicate in animal (thrips vectors) and plant cells. A successful acquisition, multiplication, dissemination, and transmission of TSWV by thrips involve the digestive system, the salivary glands, and several different physiological barriers within these tissues (German et al. 1992; Moritz 1997; Nagata et al. 1999; Tsuda et al. 1996; Ullman et al. 1992). TSWV transmission is dependent on virus uptake in the thrips midgut (mg), which is divided in three sections designated mg1, mg2, and mg3. Using immunolabeling of

serial sections from thrips larval midguts, Ullman and associates showed that TSWV envelope glycoproteins bind to the brush border plasmalemma of thrips midgut (Ullman et al. 1995). Several studies have reported that the thrips midgut epithelium is indeed the initial site of TSWV entry and multiplication during virus acquisition (Nagata et al. 1999; Ullman et al. 1993a, b, 1995; Wijkamp and Peters 1993). A time-course of infection by TSWV of its main thrips vector, *F. occidentalis*, determined that the virus infection began in and is initially restricted to the epithelial cells of mg1 (de Assis Filho et al. 2002). A dose-dependent process seems to regulate the virus accumulation in the midgut, where virus particles subsequently become available upon TSWV replication to further spread the viral infection to other tissues and organs of the thrips vector (Nagata et al. 1999; Montero-Astúa 2012). Once TSWV uptake and replication in the midgut epithelium takes place, it is followed by infection of the visceral and longitudinal muscle cells surrounding the mg1 region of the alimentary canal (Wijkamp and Peters 1993). Subsequently, TSWV disseminates laterally only through the visceral and longitudinal muscle cells from the mg1 into the mg2 and mg3 regions during the late larval stage (de Assis Filho et al. 2002). At this time the viral infection can also be found in the foregut of the late larval stage (Montero-Astúa 2012).

The time-course of infection by TSWV in *F. occidentalis* identified that the cardiac valve (the junction between the foregut and the midgut) and the ligaments are also infected (de Assis Filho et al. 2002). More recently, the infection of the tubular salivary glands by TSWV has also been observed (Montero-Astúa 2012), which provides together with the ligaments a possible conduit for the virus to spread from the midgut to the principal salivary glands. A second round of replication takes place in the principal salivary glands (Nagata et al. 1999) from where TSWV virions have been visualized budding across the apical salivary gland membranes into the lumen of the salivary ducts of viruliferous thrips (Ullman et al. 1991, 1992), which must occur for virus transmission into a new plant host. Moreover, TSWV was never found infecting the hindgut, pyloric valve (the junction between the midgut and the hindgut), and the Malpighian tubules of its thrips vector (de Assis Filho et al. 2002).

Three putative models for TSWV movement within thrips have been proposed. In the first one, the virus traverses the thrips midgut epithelial and muscle cells and once it reaches the hemocoel it circulates within the hemocytes to finally infect the principal salivary glands (Ullman et al. 1991). The second model is based on thrips ontogeny. In the first instar larvae the principal salivary glands and the visceral muscle cells of the midgut are in direct contact and are all compressed into one area of the thorax, while spatial separation occurs thereafter as thrips development proceeds to adulthood (Moritz et al. 2004). This second model proposes that TSWV could move from the midgut to the primary salivary glands when these organs are in direct contact only (at the first and early second instar larval stage) but not once the principal salivary glands have moved forward into the cephalic case and are spatially separated from the midgut (during late second larval stage through adulthood). Both the tubular salivary glands and ligament structures connect the midgut with the

principal salivary glands. In the third model, these two anatomical structures form a physical bridge that could enable virus spread from the initial place of virus entry with the final place, which infection is a prerequisite for transmission to occur (Montero-Astúa 2012; Nagata et al. 1999).

The viral determinants for thrips transmissibility have been determined and studied in detail for the type species of the *Tospovirus* genus. The viral glycoproteins of TSWV, G_N and G_C, were initially proposed to be the viral attachment and fusion proteins, respectively, that bind to a cellular receptor(s) in the thrips midgut and lead to receptor-mediated endocytosis entry into the midgut epithelial cells. Several experimental approaches have enabled definition of the role of the G_N glycoprotein in virus attachment and entry into the thrips vectors. First, TSWV isolates that were unable to assemble virions were readily mechanically passaged between plant hosts but were not thrips transmissible (Nagata et al. 2000). Second, the G_N glycoprotein was implicated as the viral determinant involved in the attachment to the thrips midgut using immunolocalization of this viral protein in the thrips digestive track and overlay assays (Bandla et al. 1998; Kikkert et al. 1998; Ullman et al. 1995). Third, reassortant viruses were used to demonstrate that virus transmission determinants map to the M RNA that encodes the viral glycoproteins (Sin et al. 2005). Sin and associates also showed that a single nonsynonymous nucleotide substitution in the open reading frame encoding G_N/G_C abrogate thrips transmissibility without abolishing virion assembly. The candidate amino acid residue is a proline in the C-terminus of the G_N protein, which is present in G_N proteins of 16 different tospoviruses (Zheng et al. 2011). Fourth, G_N was found to be involved in virus acquisition and transmission of TSWV by thrips, when a truncated and soluble form of this viral glycoprotein, designated G_N-S, was found to bind larval thrips midguts and inhibited acquisition and transmission of TSWV *in vitro* and *in vivo* (Montero-Astúa et al. 2014; Whitfield et al. 2004, 2008). The combined analyses provide strong evidence that G_N plays an essential role in binding and entry into thrips guts and is likely the viral attachment protein.

TSWV G_C glycoprotein is hypothesized to be the viral fusion protein. Computational analysis of G_C proteins from different bunyaviruses suggests that this glycoprotein is a type II fusion protein based on sequence comparisons (Garry and Garry 2004). Functional analyses of the La Crosse virus G_C protein conducted by Plassmeyer and colleagues showed that this viral glycoprotein mediates the release of viral RNPs into the cell after fusion of the viral envelope with a host vesicle membrane (Plassmeyer et al. 2005, 2007). Biochemical evidence indicates that TSWV G_C glycoprotein undergoes a conformational change at low pH that is consistent with changes necessary for virus fusion (Whitfield et al. 2005). Collectively, these results of function and the conservation of fusion domains among members of the *Bunyaviridae* family suggest that G_C is most likely the viral fusion protein. However, despite the importance of the thrips vectors on the intricate interaction with tospoviruses, little is known about the insect determinants for virus transmission (*e.g.* insect molecules involved in the direct interaction or response to tospovirus infection).

3.4 *Genomic, Transcriptomic, and Proteomic Studies of Insect Vectors of Tospoviruses*

Advances in genomics, transcriptomics, and proteomics of thrips and tospovirus-thrips interactions have been limited to only a few studies using *F. occidentalis* and in some instances the response of this insect vector to TSWV infection. Rotenberg and Whitfield conducted the first transcriptomic analysis of a thrips that generated 12,700 nonredundant EST contigs using a normalized cDNA library from the first instar larvae of *F. occidentalis* from which only 35 % had significant similarities to protein sequences available in NCBI (Rotenberg and Whitfield 2010). This transcriptomic analysis specifically identified 74 sequences with putative homology to proteins associated to innate immunity genes of insects. For example, 16 sequences had significant similarity to proteins associated with the RNA interference (RNAi) pathway such as Dicer, RNA binding proteins, Argonaute, Armitage, Vasa-like protein, DEAD box-helicases, and PIWI proteins. A second transcriptomic analysis using 454 pyrosequencing from all the insect life stages (first instar larvae, second instar larvae, prepupae, pupae, and adult males and females) infected and non-infected by TSWV was conducted and combined with the ESTs sequences generated previously in order to amplify the genomic tools available for *F. occidentalis* (Badillo-Vargas et al. 2012). This hybrid transcriptome was then used to identify proteins from naïve first instar larvae of *F. occidentalis* and differentially expressed proteins due to TSWV infection of the insect vector (Badillo-Vargas et al. 2012). Forty seven percent of the resolved protein spots from naïve thrips were identified using the thrips hybrid transcriptome compared to only twenty three percent matching sequences from the Metazoan non-redundant (nr) protein database from NCBI suggesting that thrips are very different from insects with sequenced genomes. In relation to insect defense response, 15 protein spots comprising six different proteins were functionally annotated as proteins associated with cell killing or immune system processes in naïve thrips. These proteins were tubulin alpha-1 chain, beta tubulin, glutaredoxin 5, heat shock protein, cysteine protease, and lethal giant larvae homologue. In this study, Badillo-Vargas and associates also identified 26 protein spots that were differentially expressed between *F. occidentalis* first instar larvae that were infected and non-infected by TSWV from which 62 % were down-regulated by the viral infection (Badillo-Vargas et al. 2012). Electrospray ionization mass spectrometry resulted in the identification of 37 proteins within the 26 spots. Among the 14 differentially expressed proteins that were ascribed to the response to stimuli category, 9 proteins were clearly associated with innate immune defenses (Skp1, 40S ribosomal protein S3, mitochondrial ATP synthase α subunit, actin, lysozyme C, thioredoxin-dependent peroxidase, pyruvate dehydrogenase kinase 3, stress-induced phosphoprotein 1, and heat shock cognate 71 protein). This represents the first proteomic study of the response of a thrips vector to the infection by a tospovirus and provided a suite of candidate genes that can be potentially involved in TSWV replication and spread within *F. occidentalis* and in the antiviral defense responses as well.

The first salivary gland transcriptomic analysis of *F. occidentalis* have been conducted and it generated 31,392 high quality contigs from which only 39 % had

significant sequence similarity to known proteins in NCBI (Stafford-Banks et al. 2014). This sialotranscriptome revealed that the majority of the sequences matched proteins involved in metabolism. Several genes potentially involved in detoxification and inhibition of plant defense responses (aldehyde dehydrogenases, glucose oxidase, glucose dehydrogenase, regulacin, and proteases), extra-oral digestion of plant structural tissues (β-glucosidase, endo-beta-glucanase, and pectin lyase), and extra-oral digestion of sugars (maltase, sucrase, α-glucosidase, and α-amylase) were identified in the salivary glands of *F. occidentalis*.

The genome of *F. occidentalis* has been sequenced by the Baylor College of Medicine (https://www.hgsc.bcm.edu/arthropods/western-flower-thrips-genome-project) as part of the i5K Initiative (Evans et al. 2013). This represents the first genome of any member of the insect order Thysanoptera to be sequenced and it will aid in understanding the biology of this cosmopolitan insect pest and its interaction with tospoviruses. The genome of thrips is also an important tool for biologists to better understand the evolution of this insect order. Furthermore, a thrips genome might allow the identification of thrips transcripts and proteins that otherwise seem to have no sequence similarities to other well-characterized insects or arthropods. Recently, an RNAi injection method has been developed to directly deliver dsRNA into the hemocoel of female thrips (Badillo-Vargas et al. 2015). The knockdown of vacuolar ATP synthase subunit B (V-ATPase-B) at the transcript and protein level resulted in increased mortality (number of live insects) and reduced fecundity (number of viable offspring produced) of *F. occidentalis* female thrips (Badillo-Vargas et al. 2015). The establishment of RNAi for thrips provides functional tools that can be used to designate specific roles to thrips genes in the insect life cycle and in the infection cycle of the plant viruses they transmit (tospoviruses and ilarviruses). The development of these tools will help to uncover the thrips molecules that are important during tospovirus infection of the insect vector and provide a suite of targets for the design of novel approaches to control tospoviruses and thrips pests of agricultural importance.

4 Tenuiviruses

4.1 The Tenuivirus *Genus, Virus Structure, and Genome Organization*

Tenuiviruses are an unusual group of plant viruses that infect monocotyledonous plants in the family Poaceae such as corn, rice, wheat, sorghum, barley, and oat. These viruses, which were officially recognized as a plant-infecting virus group in 1983 (Gingery 1988), belong to the genus *Tenuivirus* that is currently not assigned to a family. *Rice stripe virus* (RSV) is the type species of the genus *Tenuivirus* that contains six accepted species (*Echinochloa hoja blanca virus* (EHBV), *Maize stripe virus* (MSpV), *Rice hoja blanca virus* (RHBV), *Rice grassy stunt virus* (RGSV), RSV, and *Urochloa hoja blanca virus* (UHBV)) and eight tentative species (Barley

dubia virus (BDV), European wheat striate mosaic virus (EWSMV), Iranian wheat stripe virus (IWSV), Maize yellow stripe virus (MYSV), Oat pseudorosette virus (OPRV), Phleum green stripe virus (PGSV), Rice wilted stunt virus (RWSV), and Winter wheat mosaic virus (WWMV)). Additionally, Wheat yellow head virus (WYHV) (Seifers et al. 2005) and two viruses isolated from black spruce (Castello et al. 2000) have been reported to have amino acid sequences similar to plant viruses in this genus. Tenuiviruses have non-enveloped, filamentous particles of 500–2100 nm long and 8 nm wide that form pseudocircular structures due to the complementarity of the 5′ and 3′ ends of their genome segments (Ishikawa et al. 1989). All genome segments are encapsidated by nucleocapsid (N) protein that form the ribonucleocapsid particles (RNPs) each containing a single genomic RNA segment.

Tenuiviruses have a segmented genome that consists of four to six genome segments depending on the virus species. For example, RSV and RHBV have four genome segments, while MStV, EHBV, and some RSV isolates have five genome segments (de Miranda et al. 1996a, b, c; Falk and Tsai 1984; Ishikawa et al. 1989; Ramirez, et al. 1992; Toriyama and Watanabe 1989). The genome of RGSV is the only one composed of six genome segments identified so far (Toriyama et al. 1997). All genome segments are individually encapsidated by N protein to form the RNPs that are associated with the RdRp (Barbier et al. 1992; Ramirez and Haenni 1994; Toriyama 1986, 1987). The RNPs may appear circular, branched, filamentous, flexuous, or spiral after extensive processing of the virus preparations (Gingery et al. 1981; Lastra 1985; Morales and Niessen 1983; Takahashi et al. 1993). However, Hibino and colleagues showed that tenuivirus RNPs were mostly circular and that their differences in circumference correspond to their sedimentation coefficients in rate-zonal gradients (Hibino et al. 1985; Ishikawa et al. 1989). The circular conformation of tenuivirus genome segments are formed due to the complementarity of their 5′ and 3′ ends, which is similar to viruses in the family *Bunyaviridae* and more specifically to those in the genus *Phlebovirus* (Falk and Tsai 1984; Ishikawa et al. 1989; Kakutani et al. 1990; Takahashi et al. 1990). The fact that tenuiviruses and phleboviruses share the exact same 8 nucleotides at the ends of their genome segments and have very similar nucleotide composition of their intergenic regions have led to the proposition that they have likely evolved from a common ancestor and retained a number of common molecular characteristics (Falk and Tsai 1998).

Tenuiviruses have one of the largest genomes among plant viruses, which are approximately 18–19 kb (Ramirez and Haenni 1994; Toriyama et al. 1998). Genomic analysis of MSpV showed that molecules of both polarities are encapsidated for each genome segment, although, not to the same ratio (Falk and Tsai 1984). Tenuiviruses, similar to members of the *Bunyaviridae* family, use a negative and ambisense conding strategy to express their viral proteins. RNA1 is of complete negative polarity and possesses a single ORF that encodes an RdRp in the viral complementary RNA1 (vcRNA1) (Toriyama et al. 1994). Sequence analysis of RNA2, RNA3, RNA4, and RNA5 showed that they are all ambisense segments coding for two viral proteins, one in the vcRNA and the other in the viral RNA (vRNA) (de Miranda et al. 1996a, b, c; Kakutani et al. 1990, 1991). The viral protein p2

encoded by the vRNA2 is a small hydrophobic protein that has been proposed to be a cell-to-cell movement protein in plants (Chomchan et al. 2003) and pvc2 is a polyprotein encoded from vcRNA2 that is proteolytically processed to yield two mature glycoproteins, designated NSvc2-N and NSvc2-C, which are targeted to the Golgi bodies in plants upon co-expression (Estabrook et al. 1996; Yao et al. 2014). The nucleocapsid or pc3 is encoded on the vcRNA3 (de Miranda et al. 1994, 1996a, b, c) and p3, also called NS3, is a silencing suppressor functional in both plant and insect cells (Hemmes et al. 2007). The major noncapsid protein (NCP also known as NS4) or p4 forms inclusion bodies that associate with RNPs involved in virus dissemination within the insect vector is encoded by vRNA4 (Huiet et al. 1990, 1992; Wu et al. 2014) while pc4 that is encoded from the vcRNA4 is a viral movement protein that localizes to the plasmodesmata (Xiong et al. 2008; Zhang et al. 2013a). The p5 protein encoded by the vRNA5 seems to have an essential role in virus infection in both plants and insects (Chomchan et al. 2003) and pc5 encoded by the vcRNA5 is a hydrophilic protein encoded by the vcRNA5 that has no similar homology to any known protein so far (de Miranda et al. 1996a, b, c).

4.2 Vectors of Tenuiviruses

Tenuiviruses are not seed-borne and are usually not mechanically transmitted to plants except by vascular puncture inoculation (Louie 1995). In nature, the transmission of tenuiviruses is completely dependent on their insect vectors. All tenuiviruses are transmitted by planthoppers in the family Delphacidae within the insect order Hemiptera in a persistent-propagative manner (Falk et al. 1987; Nault and Gordon 1988). However, MYSV, a tentative member of the genus *Tenuivirus*, is also transmitted propagatively by a leafhopper vector in the family Cicadellidae also within the insect order Hemiptera (Ammar et al. 2007). Due to the low sequence similarity between MYSV and MSpV (Mahmoud et al. 2007), it has been suggested that MYSV should be the type species of a new genus, *Cicatenuivirus*, in a new family *Tenuiviridae* that will contain the planthopper-borne tenuiviruses in the genus *Tenuivirus* (Ammar and Peterschmitt 2004). No insect vectors have been identified so far for WYHV and the tenui-like viruses from black spruce. Thus, identification of planthoppers, leafhoppers, or insects in other groups that efficiently transmit these viruses might shed light in the evolution of tenuiviruses with their vectors and may aid in their organized classification.

The timing of events necessary for virus acquisition and transmission have been described previously (Falk and Tsai 1998; Hogenhout et al. 2008) and are briefly summarized here. Tenuiviruses can be acquired by their insect vectors from infected plants in periods ranging from 10 min to 4 h. Latent periods, during which the virus replicates and disseminates throughout the insect vector before transmission can occur, range from 1 to 36 days. Tenuivirus inoculation thresholds usually range from as short as 30 s up to 1 h. Although, vectors may remain viruliferous for up to

84 days after virus acquisition and most likely throughout their entire life span, their ability to transmit often declines as vectors age (Falk and Tsai 1998; Heydarnejad et al. 2007). Moreover, transovarial transmission to the offspring of infectious female planthoppers has been reported for most tenuiviruses (Falk and Tsai 1998; Heydarnejad et al. 2007). RHBV also appears to be paternally transmitted to the offspring by its vector *Sogatodes oryzicola* (Zeigler and Morales 1990). However, no transovarial transmission has been reported for MYSV in its leafhopper vector *Cicadulina chinai* (Ammar et al. 2007) or for RGSV or RWSV in their planthopper vector *Nilaparvata lugens* (Chen and Chiu 1989). Transovarial transmission of RHBV and RSV can be attained for 10–40 generations, respectively, of the planthopper vector (Falk and Tsai 1998). Unlike the outcome of infection of insect vectors by other plant viruses transmitted in a persistent-propagative manner, tenuivirus infection on their insect vectors can have effects that vary from mild to lethal. These infections can impair the longevity and fecundity of viruliferous females (Ammar 1975; Brcak 1979; Hirao et al. 1987; Jennings and Pineda 1971; Nasu 1963) and in extreme cases, RSV can be lethal to eggs and early first instar nymphs (Fukushi 1969).

4.3 Vector-Tenuivirus Interactions

The dissemination route of RGSV and RSV in their brown planthopper and small brown planthopper vectors, respectively, have been described in precise detail. Initial studies found that tenuiviruses can infect various organs of their planthopper vectors including the digestive and respiratory tracts, Malpighian tubules, leg muscles, fat bodies, brain, salivary glands, and reproductive tracts of both sexes (Hibino 1996; Nault and Gordon 1988). A sequential infection study revealed that RGSV initially infected the midgut epithelium, then crossed the basal lamina into the midgut and hindgut visceral muscles from where it spread to the entire alimentary canal (including the esophagus and anterior diverticulum), to the hemolymph and subsequently into both the principal and accessory salivary glands of *N. lugens* (Zheng et al. 2014). Wu and associates studied the route of infection of RSV in the small brown planthopper and found that this tenuivirus also infected the midgut epithelial cells from where it progressed into the visceral muscles of both the midgut and the hindgut and then the entire alimentary canal to finally reach the salivary glands and the reproductive organs from both sexes (Wu et al. 2014). A high number of inclusion bodies with high electron density were observed in the cytoplasmic matrix and vacuoles of follicular cells of ovarioles in RSV-infected small brown planthoppers, while a large number of RNPs were distributed diffusely throughout the eggshell and interior of the ovum (Deng et al. 2013). Later these inclusion bodies were found to be mostly composed of the nonstructural protein NS4, which were in close association with RSV RNPs through the direct interaction of NS4 with N proteins (Wu et al. 2014). Functional genomics studies of NS4

using RNAi resulted in a significant reduction of RSV spread in the bodies of the insect vector without impairing viral replication in VCMs (Wu et al. 2014). Recently, the RNPs of RSV were found to accumulate in the terminal filaments and pedicel areas of the ovaries prior to vitellogenin expression after which the N protein was found to bind vitellogenin and colocalize in the germarium and nurse cells right after vitellogenin expression (Huo et al. 2014). Furthermore, knockdown of vitellogenin expression resulted in inhibition of RSV invasion of the ovarioles of *L. striatellus* (Huo et al. 2014) suggesting that this plant virus uses the vitellogenin trafficking pathway to infect the embryos of its vector and be transovarially transmitted.

4.4 Genomic, Transcriptomic, and Proteomic Studies of Insect Vectors of Tenuiviruses

The brown planthopper, *N. lugens* Stal, is a destructive insect pest of rice that transmits the reovirus *Rice ragged stunt virus* (RRSV) and the tenuivirus RGSV. More than 37,000 high-quality expressed sequence tags (ESTs) were obtained from libraries of various tissues and stages of the brown planthopper (Noda et al. 2008). Among the top ten most abundantly expressed genes identified in these EST libraries, three had no sequence similarities to known genes in BLAST searches. One of these genes (AA0383) was only found in libraries that contained the female gonads and later showed to be specifically expressed in the lateral oviduct of the ovaries by *in-situ* hybridization (Noda et al. 2008). A second transcriptomic analysis using six digital gene expression (DGE) libraries coupled with Illumina sequencing generated 85,526 unigenes, including 13,102 clusters and 72,424 singletons (Xue et al. 2010). In this study, a total of 11 genes showed no homology amongst the ten most differentially up-regulated and ten most differentially down-regulated genes between the brachypterous adult female and macropterous adult female libraries. A comparative analysis between the macropterous adult female and macropterous adult male libraries revealed that there were 9 genes with no homology amongst the ten most differentially up-regulated and ten most differentially down-regulated genes. Additionally, Bass and associates used a total of 78,959 ESTs generated by 454 pyrosequencing combined with 37,392 publically available ESTs sequences to assemble a larger transcriptome that was then mined to identify detoxification enzymes (Bass et al. 2012). This hybrid transcriptome allowed the identification of sequences encoding 31 cytochrome P450s, 9 glutathione S-transferases, and 26 carboxyl/cholinesterases which are putatively involved in the detoxification of xenobiotic compounds (*e.g.* plant secondary metabolites and synthetic insecticides) by this insect species. Furthermore, this transcriptomic sequencing effort was used to construct an oligonucleotide microarray containing probes for ~19,000 unigene sequences (Bass et al. 2012).

In an intestine-specific transcriptomic analysis, Peng and associates found that sugar hydrolases and transporters, proteases, and detoxification-related genes are highly abundant in the midgut of the brown planthopper (Peng et al. 2011). Similarly, a different study of the brown planthopper revealed a number of digestion-, defense-, and detoxification-related genes that were abundant in the insect's gut (Bao et al. 2012). This study reported a suite of novel genes, including 33 digestion-related genes, 25 immune responsive genes, and 27 detoxification-related genes many of which were specifically expressed in the digestive track of the brown planthopper as shown by real-time quantitative PCR (qPCR) (Bao et al. 2012). An antenna transcriptome of the brown planthopper identified 10 odorant-binding proteins (OBPs), including 7 previously unidentified, and 11 chemosensory proteins (CSPs), including 2 new members (Zhou et al. 2014). Using real-time qPCR, OBP6, OBP7, OBP8, OBP9, and CSP10 were found to have antenna-specific expression in the brown planthopper. In a separate transcriptomic study, 48 candidate genes were found to encode all known insect neuropeptides and neurohormones (with the exception of neuropeptide-like precursor 2 and trissin), while 57 putative neuropeptide G-protein coupled receptors (GPCRs) were identified (Tanaka et al. 2014). The authors suggested that *N. lugens* possess the most comprehensive neuropeptide system yet found in insects as two vertebrate hormone receptors (thyrotropin-releasing hormone receptor and parathyroid hormone receptor) were identified in the brown planthopper.

A recent genome- and transcriptome-wide analysis of innate immunity in the brown planthopper has identified a number of pattern recognition proteins, modulation proteins in the prophenoloxidase activating cascade, immune effectors, and molecules involved in signaling transduction pathways, including the Toll, Imd, and JAK-STAT pathways (Bao et al. 2013). Among the effector genes present in the brown planthopper genome are defensin, reeler, lysozyme, and NOS. Immune challenge of the brown planthopper by heat killed bacteria showed that some of these genes were significantly up-regulated in the gut while others were unresponsive or highly expressed in other tissues, such as the carcass, fat body, or the salivary glands (Bao et al. 2013). Furthermore, an in depth study of the serine protease gene family and their expression profile analysis has also been performed in the brown planthopper (Bao et al. 2014). A total of 90 serine protease-like genes were identified in this study which had tissue-, development-, or sex-specific expression patterns as shown by real-time qPCR (Bao et al. 2014). For example, two transmembrane serine protease genes showing high sequence similarity to ovarian serine protease and ovochymase 2 from *Tribolium castaneum* were highly expressed in adult females and eggs (Bao et al. 2014). Ultimately, the accomplishment of sequencing the entire *N. lugens* genome (Bao et al. 2013) is enabling a better understanding of the molecular bases of biological and physiological processes as illustrated by the examples discussed below.

Two comparative transcriptomic analysis experiments from two populations of the brown planthopper differing in their virulence level in rice have been conducted. In the first study, genes related to metabolism, digestion and adsorption, and sali-

vary secretion were differentially expressed in the salivary glands of these two populations of the brown planthopper (Ji et al. 2013). Sixty seven genes encoding putative secreted proteins were differentially expressed from which 43 and 24 were up- and down-regulated, respectively, in the virulent population when compared to its less virulent counterpart, suggesting that these secreted proteins might play important roles in the virulence of the brown planthopper in rice (Ji et al. 2013). In the second study, most differentially expressed transcripts in the fat bodies of these two brown planthopper populations were genes related to metabolism and innate immunity (Yu et al. 2014). Components of the cellular and humoral immune responses (*e.g.* lysosomes, phagosomes, and coagulation) as well as some involved in signaling transduction (*e.g.* Toll and JAK-STAT signal transduction pathways) were identified as having higher transcript level in the virulent population of brown planthoppers suggesting that virulence might be more complicated than previously believed and that innate immune responses may play a yet unidentified role in insect virulence on its plant hosts (Yu et al. 2014). Moreover, 57 differentially expressed genes in this comparative analysis of the fat body from the brown planthopper corresponded to yeast-like symbionts and *Wolbachia* (Yu et al. 2014). These findings poses the question about what role symbionts could play that may account, at least in part, for the differences observed in virulence amongst these two brown planthopper populations in rice plants. In a different study, a transcriptomic analysis of fecundity in the brown planthopper identified a suite of mRNA transcripts that were up-regulated in a high fecundity population compared to a low fecundity one of a second day fifth instar nymphs and second day brachypterous adult females (Zhai et al. 2013). Among 30 selected up-regulated transcripts further analyzed by real-time qPCR analysis, 28 showed higher expression levels in the high fecundity population (Zhai et al. 2013). These suite of genes represent valuable candidates to further characterize virulence and fecundity in *N. lugens* and are potential targets for the control of this insect pest.

The small brown planthopper vector of the tenuivirus RSV, *Laodelphax striatellus* Fallén, has also been the subject of genomic studies but to a lesser extent than the brown planthopper. Seventeen enzymes contributing to amino acid biosynthesis were identified from the small brown planthopper using sequence homology coupled with reverse transcription-PCR (RT-PCR); three genes originated from symbionts while the remainder were from insect origin (Yang et al. 2012). A comparative transcriptome analysis of RSV-infected and naïve small brown planthopper identified components of the RNAi, JAK-STAT, and Imd pathways, although, there expression was not altered by RSV infection of the insect vector (Zhang et al. 2010). On the other hand, a number of transcripts were differentially expressed between naïve and virus-infected insects. For example, vitellogenin was most abundant in viruliferous *L. striatellus*, suggesting that RSV might exploit the vitellogenin traffic pathway to overcome the physical barrier between follicle cells and oocytes necessary for the transovarial transmission of this virus (Zhang et al. 2010), which has recently been found to be the case (Huo et al. 2014). Interestingly, viral transcripts from the NS3 were the most abundant viral derived ESTs from virus-infected plan-

thoppers while viral transcripts from NSvc4 were not detected at all in the insect vector (Zhang et al. 2010). This finding is in agreement with the roles of these two viral proteins; NS3 is a viral suppressor of RNAi that is functional in plants and insects and NSvc4 is the movement protein predicted to play a role in cell-to-cell movement of the viral RNPs in plants through the plasmodesmata. RSV-derived small interfering RNAs (vsiRNAs) generated in the insect vector during virus infection were found to be uniformly distributed throughout the entire viral genome (Xu et al. 2012b). Moreover, silencing of Argonaute 2 in *L. striatellus* enhanced RSV accumulation in its insect vector (Xu et al. 2012a, b) suggesting that RNAi is an antiviral mechanism used by the small brown planthopper to modulate the viral infection in the insect vector. Thus, RNAi can be exploited to ascribe the role of virtually any gene in this insect pest with regards to its biology and its interaction with RSV.

Currently, proteomics studies with planthopper/leafhoppers vectors of tenuiviruses are scarce. Sharma and associates used two-dimensional (2-D) electrophoresis and gas-phase protein sequencing to assess the toxicity of *o-sec*-butylphenyl methylcarbamate compound (BPMC) in the brown planthopper that resulted in the modulation of 22 proteins (Sharma et al. 2004). Protein expression of serine/threonine protein kinase, paramyosin, heat shock protein 90, β-tubulin, calreticulin, ATP synthase, actin and tropomyosin was up-regulated while that of β-mitochondrial processing peptidase, dihydrolipoamide dehydrogenase, enolase and ayl-coA dehydrogenase was down-regulated due to the BPMC exposure. Konishi and associates conducted an analysis of salivary gland proteins from the brown planthopper in which they identified several proteins involved in energy metabolism, protein synthesis, folding, and modification (Konishi et al. 2009). Three unidentified proteins from the sialoproteome (*i.e.* proteome of salivary glands) possess an EF-hand domain, which is a helix-loop-helix structural motif found in a large family of calcium-binding proteins, suggesting a possible role during phloem feeding by the brown planthopper (Konishi et al. 2009). In a proteomic analysis of fecundity using high- and low-fecundity populations of the brown planthopper, a total of 54 and 75 proteins were significantly changed in the third and sixth day brachypterous females, respectively, from which 39 and 54 proteins were identified (Zhai et al. 2013). Silencing of one of these up-regulated proteins, glutamine synthetase, resulted in reduced fecundity, disrupted ovary development, and inhibited vitellogenin expression suggesting that this protein may regulate ovary development by controlling the accumulation of vitellogenin in the ovaries of *N. lugens* (Zhai et al. 2013). Additional genomic, transcriptomic, and proteomic analyses of several planthopper/leafhopper and tenuivirus combinations are needed to shed light on the molecular bases underpinning these virus-vector interactions. Ultimately, this knowledge will aid in the development of novel strategies to control this group of plant viruses and their insect vectors in ways that are more consonant with the environment and human health.

5 Reoviruses

5.1 *The* Reovirus *Family, Virus Structure, and Genome Organization*

The members of the family *Reoviridae* have genomes composed of multiple (9–12) segments of linear double-stranded RNA (dsRNA) that are encased in a nonenveloped particle (Attoui et al. 2012). Reoviruses have icosahedral symmetry with a diameter of approximately 60–85 nm made up of one or more layers of capsid protein. The *Reoviridae* is the most diverse of the dsRNA virus families with 2 subfamilies, 15 genera, and hosts ranging from plants to humans (Attoui et al. 2012; Bragard et al. 2013). There are three plant-infecting reovirus genera: *Fijivirus, Phytoreovirus*, and *Oryzavirus* that are all transmitted in a persistent-propagative manner by planthoppers (Hemiptera: Delphacidae) and leafhoppers (Hemiptera: Cicadellidae). The animal-infecting reoviruses within the genera *Orbivirus*, *Coltivirus* and *Seadornavirus* also replicate in both their animal and insect hosts. The viruses belonging to the genera *Aquareovirus* infect aquatic vertebrates and crustaceans. Members of the *Cypovirus* (cytoplasmic polyhedrosis viruses) genus infect lepidopteran, hymenopteran and dipteran insects (Hill et al. 1999). *Idnoreovirus* (derived from 'Insect derived non-occluded reovirus'), *Mycoreovirus* (fungus-infecting reoviruses), *Orthoreovirus* (infects mammals, avians and reptiles) and *Rotavirus* (infects humans) are thought to be vertically or horizontally transmitted between individual host organisms often by fecal or oral route (Attoui et al. 2006; Mertens et al. 2005). The recently accepted genera *Mimoreovirus, Cardoreovirus*, and *Dinovernavirus* include viruses isolated from marine protists, crabs and mosquitoes, respectively (Attoui et al. 2006). A recently discovered reovirus vectored by an aphid (*Amphorophora agathonica* Hottes) and isolated from red raspberry plants, Raspberry latent virus, is classified into a new genus *Raslavirus*, based on transmission and sequence analysis studies (Quito-Avila et al. 2012). Also, a mosquito-infecting reovirus, Cimodo virus (CMDV), does not group with existing genera and represents a new unassigned genus (Hermanns et al. 2014).

5.2 *Vectors of Reoviruses*

Plant-infecting reoviruses are dependent on insects for plant-to-plant spread in nature. The acquisition and inoculation periods of plant reoviruses range from a few minutes to several days with shorter time for viruses found in the mesophyll of infected leaves and several hours for viruses that can be found in the phloem (Ammar and Nault 2002). Some plant-infecting reoviruses are transmitted transovarially (vertical transmission) and the virions can be transmitted to the progenies for many generations. Transovarial transmission of a plant virus by its insect vector was first described by Fukushi (1933) for *Rice dwarf virus* (RDV) by infective

females of *Nephotettix apicalis*. This was also observed for the vectors *Agallia constricta* and *N. nigropictus* transmitting *Wound tumor virus* (WTV) and *Rice gall dwarf virus* (RGDV), respectively, which are both members of the *Phytoreovirus* genus. Fijiviruses like *Fiji disease virus* (FDV), *Oat sterile dwarf virus* (OSDV), *Maize rough dwarf virus* (MRDV), and *Nilaparvata lugens virus* (NLV) can be transovarially transmitted by *Perkinsiella saccharicida* Kirkaldy, *Javesella pellucid*, and *Laodelphax striatellus*, respectively. Transovarial transmission rate for phytoreoviruses is higher (1.8–100 %) than for fijiviruses (0.2–17 %). In a laboratory environment, RDV was found to persist in experimental *N. cincticeps* colonies for multiple generations over a period of 6 years (Honda et al. 2007) demonstrating the importance of transovarial transmission in the persistence of the virus.

The plant-infecting reoviruses utilize different strategies for replication and movement in their plant and insect hosts. In plants, they exploit movement proteins (MPs) to facilitate cell-to-cell movement via a mechanism involving the plasmodesmata and a viral movement protein, Pns6 for RDV (Matthews 1982; Wei et al. 2009). In its insect hosts, Chen et al. (2011) have shown the role of tubular structures formed by the Pns10 protein of RDV to facilitate its spread within its insect vector, *N. cincticeps* (Chen et al. 2011).

5.3 Vector-Plant Reovirus Interactions

5.3.1 Phytoreovirus

Phytoreovirus is the most studied genus amongst the members of the plant-infecting reoviruses. The phytoreoviruses WTV, RDV and RGDV resemble each other biologically, morphologically, and biochemically but have distinct characteristics in terms of plant hosts (dicotyledonous or graminaceous), plant symptomatology, and plant tissue tropism. The structure of phytoreoviruses is icosahedral virions which are about 70 nm in diameter with a double-capsid structure. The outer capsid which has a T = 13 icosahedral symmetry is composed of two proteins, P2 and P8. The inner capsid is composed of P3 proteins and the icosahedron has a modified T = 1 structure that is commonly termed T = 2 icosahedral symmetry (Artimo et al. 2012). The RDV minor outer capsid protein, P2, proposed to interact with receptors on insect vector cells is essential for the infection of insect vectors by RDV (Omura et al. 1998; Wei et al. 2009) and is also the viral fusion protein (Zhou et al. 2007). The P8 protein has also been reported to play a role in viral infectivity within the vector (Omura and Jan 1999). RDV is transmitted in nature by the cicadellid leafhoppers, *N. cincticep*s, *Recilia dorsalis, N. nigropictus*, and *N. virescens* and is the best characterized phytoreovirus. Insects acquire the virus by feeding on RDV-infected rice plants for a few minutes to several days. RDV replicates in the vector and the insect becomes viruliferous after a latent period of 2–3 weeks. RDV interactions with *N. cincticeps* have been studied extensively. Chen et al. (2011) have shown that RDV accumulates in the epithelial cells of the filter chamber of

N. cincticeps 2 days after acquisition on diseased plants. RDV accumulation progresses to the anterior midgut and then spreads to the nervous system before infection of other organs can occur at later stages of infection. RDV accumulates in the alimentary canal, salivary glands and the follicular cells of the ovarioles in viruliferous insects. The viral determinants of insect transmission have been studied extensively for RDV. P2 is the minor outer capsid protein and is encoded by viral genome segment 2. This protein is a multifunctional protein that is essential for virus infection of insect vectors and contributes to the development of the dwarf phenotype in rice plants infected with RDV by interfering with the gibberellic acid synthesis (Omura et al. 1998). Intact RDV virions infect insect VCMs but lose viral infectivity when the P2 protein is removed by chemical treatment (Yan et al. 1996). Virions without P2 failed to infect insects and consequently failed to be transmitted to plants suggesting that P2 may have a role in recognition and attachment of the virus to the insect vector. P2 has been postulated to be involved in entry into insect cells as it has similar features of type I fusion proteins of enveloped viruses. When transiently expressed in insect cells, P2 caused syncytium formation indicative of membrane fusion (Zhou et al. 2007). The N-terminal hydrophobic peptide and 2 heptad repeats in P2 contributed to its fusogenic activity. This membrane fusion capability suggests that P2 interacts with receptors on insect vector cells and then mediates the release of the virus into the cytoplasm of insect cells for infection of the vector (Omura et al. 1998; Zhou et al. 2007).

Virus movement in vectors through tubule-like structures is an emerging trend for persistent-propagative plant viruses that lack envelopes and this strategy was first described for RDV dissemination in *N. cincticeps* (Chen et al. 2011; Wei et al. 2009). Chen et al. (2012) demonstrated that virus-containing tubules composed of nonstructural viral protein Pns10 are used by RDV to traffic along actin-based microvilli of the epithelial cells in viruliferous leafhoppers. Through transmission electron microscopy, the authors have seen Pns10 tubules in the epithelial cells of the filter chamber and midgut. Furthermore, they have observed the same structures using immunoflourescence microscopy in which the virus-containing tubules appeared to be passing from inside the infected cells into the lumen.

5.3.2 Oryzavirus and Fijivirus

The other plant-infecting reovirus genera, *Oryzavirus* and *Fijivirus*, are in the subfamily *Spinareovirinae* and have relatively large spikes at the 12 icosahedral vertices of the virus particle (Milne et al. 2005). Fijiviruses like phytoreoviruses are non-enveloped plant-infecting viruses with an icosahedral structure about 65–70 nm in diameter and a double-capsid shell. All known fijiviruses contain 10 linear genomic segments of dsRNA coding for 12 proteins (Milne et al. 2005). There are currently eight species in this genera and they are divided into five groups according to their plant and insect hosts: Group 1 and 3–5 have a single member each, which are *Fiji disease virus* (FDV), *Oat sterile dwarf virus* (OSDV), *Garlic dwarf virus* (GDV) and *Nilaparvata lugens virus* (NLV), respectively; Group 2 has four

members namely *Mal de Río Cuarto virus* (MRCV), *Pangola stunt virus* (PaSV), *Maize rough dwarf virus* (MRDV) and *Rice black-streaked dwarf virus* (RBSDV). FDV, MRCV and RBSDV are transmitted by planthoppers and replicate in both insect and plant hosts, while the more distantly related *Nilaparvata lugens* virus (NLV) replicates only in insects (Milne et al. 2005). Complete genomic sequence for NLV, FDV, MRCV and RBSDV are available as well as for the new proposed member, *Southern rice black-streaked dwarf virus* (SRBSDV). SRBSDV is transmitted by the white-backed planthopper *Sogatella furcifera.* Similar to RDV that is transmitted by *N. cincticeps* utilizing tubular structures containing virus particles for viral movement inside the vector, SRBSDV exploits virus-containing tubules composed of a nonstructural protein P7-1 to cross directly at the basal lamina from the initially infected epithelium towards the visceral muscles in the gut of *S. furcifera* (Marzachi et al. 1995; Wang et al. 2014). It has been found that through the interaction of P7-1 with actin, the tubules can spread along visceral muscle tissues. Indeed, tubule assembly was disrupted and viral spread was inhibited both *in vitro* and *in vivo* when RNAi was used to deplete P7-1 (Liu et al. 2011; Mar et al. 2014). In virus-infected insect VCMs, the viroplasms were found to have the nonstructural viral protein P9-1, viral RNA, outer-capsid protein P10, and viral particles. Expression of P9-1 in non-host insect cells suggested that the matrix of the viroplasms observed in the infected cells was basically made of P9-1 only. Using RNAi to knock-down P9-1 expression inhibited formation of viroplasms and viral infection suggesting that P9-1 is essential for these two processes during the virus replication cycle (Jia et al. 2012). Using an Y2H system, Mar and colleagues (2014) identified 153 insect proteins to be potential interacting protein partners of the viral P7-1 protein (Mar et al. 2014). Eighteen proteins (neuronglian, E3 ubiquitin–protein ligase, MARCH5, MLC2, polyubiquitin, ribophorin ii, profilin, chitin bind 4 [cuticular-RR2], elongation factor 1-alpha, vacuolar ATP synthase subunit E, vitellogenin, atlastin, synaptobrevin, vitellogenin receptor, GTP-binding protein, mitochondrial ATP synthase lipid binding protein, B-cell receptor-associated protein, and sec61 alpha 1 subunit) have been confirmed to be true interactors of P7-1 through co-immunoprecipitation (Mar et al. 2014).

RBSDV is transmitted by the small brown planthopper, *Laodelphax striatellus*, which is also a vector of the tenuivirus RSV and the cytorhabdoviruses BYSMV and NCMV. The RBSDV genome contains 10 segments of dsRNA encoding six putative structural proteins, P1, P2, P3, P4, P8 and P10 and five nonstructural proteins P6, P7-1, P7-2, P9-1 and P9-2. Similar to SRBSDV, RBSDV P7-1 has been reported to be a major component of the tubules observed by electron microscopy in both plants and the insect vector while P9-1 has been confirmed to accumulate in intracellular viroplasms in infected plants and insect cells and it has been found to be the only viral component required for viroplasm formation (Liu et al. 2011; Zhang et al. 2008).

Mal de Río Cuarto virus (MRCV) is transmitted by the planthopper *Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae). Efficiency of transmission of MRCV is positively correlated to the amount of virus titer in the vector and the developmental

stage when the virus was first acquired (Argüello-Caro et al. 2013). First instar nymphs with a 17d AAP transmitted the virus more efficiently (28.88 %) than third instars (20 %). Through real-time qPCR, transmitting insects were shown to have significantly higher MRCV titers ($P < 0.0127$) than non-transmitting insects indicating a strong association between viral titer and the MRCV transmission capacity of the planthopper vector (Argüello-Caro et al. 2013). MRCV is similar to other reoviruses where viroplasms are the site for viral replication. MRCV P9-1 was shown to establish cytoplasmic inclusion bodies resembling viroplasms after transfection of Sf9 cells and the carboxy-terminal half of P9-1 contains critical domains required for establishing cytoplasmic VIB-like structures (Maroniche et al. 2012). In MRCV, the major capsid protein P10 was found to co-localize with tubulin and P6 in Sf9 cells. MRCV P6 is identical to RBSDV P6 which gives rise to viroplasm-like inclusions in plant cells and is able to recruit RBSDV P9-1 to these structures (Wang et al. 2010). P9-2 is located at the plasma membrane and has been observed to be associated with superficial filopodia-like formations containing actin sometimes connecting neighboring cells (Wang et al. 2010).

Rice ragged stunt virus (RRSV) is the type species of the Oryzavirus genus which is transmitted by *Nilaparvata lugens* (Jia et al. 2012). The infection route of the virus in the insect vector was determined by Jia et al. (2012) who found that RRSV accumulates in the midgut epithelium from which it proceeds to infect the visceral muscles surrounding the midgut and hindgut and finally spread into the salivary glands (Jia et al. 2012). During infection with RRSV, viroplasms are formed in the alimentary canal and salivary glands of the insect vector. Viral non-structural proteins have been found to be essential in the formation of these viroplasms as is the case with other phytoreoviruses like RDV, SRBSDV and RBSDV. For example, RRSV Pns10 is a nonstructural protein which expression in non-host Sf9 cells showed it to be involved in the formation of viroplasms where viral particles accumulated and co-localize with Pns6. The filament-like structures protruding from the plasmamembrane formed by Pns7 strongly resembled the structures formed by P7-1 of SRBSDV and Pns10 of RDV (Liu et al. 2011). It will be interesting to investigate whether RRSV Pns7 plays a similar role as Pns10 of RDV in the spread of the virus in the cells of its insect vector *N. lugens*.

5.4 Genomic, Transcriptomic, and Proteomic Studies of Insect Vectors of Reoviruses

Despite being the best characterized plant-infecting reovirus, the genome, transcriptome, or proteome of RDV vectors have not been generated but a transcriptomic analysis BioProject has been recently submitted to NCBI for *N. cincticeps*. Given the importance of this pathosystem and the availability of RNAi tools for studying *N. cincticeps*-RDV interactions, this vector is a good candidate for

additional studies using –omic tools. Moreover, the complete mitochondrial genome (mitogenome) for *L. striatellus* has been sequenced and shown to be 16,431 bp (Zhang et al. 2013a, b). Together with the mitogenome of *N. lugens*, Zhang et al. found that mitochondrial genome arrangement patterns found in the two planthopper species was most likely involved in rearrangements of both tRNA genes and protein-coding genes (PCGs) suggesting that the rearrangement was conserved in the Delphacidae family. The information of this mitogenomic studies could be used for further studies on population genetics, phylogeographics, and phylogenetic evolution of these important rice pests in relation to virus transmission and disease control (Zhang et al. 2013a, b). RNAi technology has been developed to be used as a pest control strategy for *L. striatellus.* Feeding of chitinase (Chi) dsRNA resulted in knockdown of the corresponding gene which had significant lethal effects in *L. striatellus* (Lu et al. 2013). The transcriptome of *N. lugens* vector of RRSV has been determined by Xue et al. (2010) encompassing different developmental stages, sexes and wing phenotypes from adult insects which was previously discussed in the Sect. 4 (Xue et al. 2010). Additional genomic, transcriptomic, and proteomic studies are needed in order to obtain a more detailed understanding of the insect molecules involved in the direct interaction and response to reovirus infection of the vectors.

6 Marafiviruses

6.1 The Marafivirus *Genus, Virus Structure, and Genome Organization*

Marafivirus is one of the three genera from the *Tymoviridae* family. The longstanding members of the genus *Marafivirus* are *Maize rayado fino virus* (MRFV), *Oat blue dwarf virus* (OBDV) and *Bermuda grass etched line virus* (BELV). Marafiviruses have non-enveloped icosahedral particles of about 30 nm in diameter with a single-stranded positive-sense RNA genome of 6–7 kb (Gamez and Leon 1988). The complete nucleotide sequence for MRFV and OBDV has been determined to be 6.3 kb and 6.5 kb, respectively (Edwards et al. 1997; Hammond and Ramirez 2001). The distinct feature of the genome of marafiviruses is the possession of a single large ORF encoding a polyprotein of 221–227 kDa containing conserved signature motifs of the replication-associated proteins such as RdRp, methyltransferase and helicase; a papain-like protease domain and the "marafibox" which is a conserved 16 nucleotide stretch similar to the "tymobox" of tymoviruses only differing by two or three residues (Izadpanah et al. 2002). A new virus detected in switchgrass (*Panicum virgatum*) has been proposed to be a member of the genus *Marafivirus* to which the name *Switchgrass mosaic virus* (SwMV) has been given. Its complete genome sequence was found to be 6.4 kb long and it shares 76 % sequence identity with MRFV (Agindotan et al. 2012).

6.2 Vectors of Marafiviruses

Marafiviruses are transmitted by leafhoppers in a persistent-propagative manner. OBDV and BELV are transmitted by *Macrosteles fascifrons* Stål and *Aconurella prolix*, respectively. The newest member of this genus is *Citrus sudden death-associated virus* (CSDaV) which has no known leafhopper vector but can be transmitted by the aphids *Toxoptera citricida*, *Aphis spiraecola*, and *A. gossypii* (Maccheroni et al. 2005). MRFV is transmitted by *Dalbulus maidis* and other *Dalbulus* species but can also be transmitted by *Stirellus bicolor* and *G. nigrifrons* (Nault and Gordon 1988). The most efficient vector of MRFV is *D. maidis* and the range of acquisition and inoculation lasts from several minutes to several hours with longer acquisition periods resulting in higher transmission (Nault and Gordon 1988). When the virus is acquired from plants, the mean latent period in the vector is 16 days and the rate of transmission for an individual *D. maidis* is 15 %. The latent period is reduced to just 7 days if the virus is injected directly into the hemocoel of the vector. Nymphs acquire and transmit the virus more efficiently than adults (Nault and Gordon 1988). Three leafhopper species identified in switchgrass fields near Champaign, Illinois – *Graminella aureovittata*, *Graminella mohri* and *Flexamia atlantica* were thought to be vectors of SwMV, which was detected in 100 % of both *G. mohri* and *F. atlantica* and in 95 % of *G. aureovittata*. From these three leafhoppers species, *G. aureovittata* transmitted the virus to 'Cave in Rock' switchgrass plants in a growth chamber assay (Agindotan et al. 2012). Marafiviruses are confined to the phloem of infected plant hosts and cannot be transmitted mechanically or through seeds. Despite the complete dependency of marafiviruses on their insect vectors for transmission there are very limited studies describing marafivirus-vector interactions at any level. Genomic, transcriptomic, or proteomic studies will aid in understanding the interaction of these single-stranded positive-sense plant viruses with their leafhopper or aphid vectors.

7 Conclusions and Future Directions

Management of vector-borne plant viruses to mitigate losses has been a challenge over the years even when using preventive strategies like models that can forecast plant disease outbreaks, integrated pest management, and pest-tolerant or resistant plants. Furthermore, the demand for environmentally friendly pest control strategies has become a very important factor to consider as developments in biotechnology that can be applied to deter insect feeding and plant virus transmission are undergoing rapid improvements. The –omic technologies that are now widely and more commonly used for simultaneous examination of thousands of genes (genomics), mRNAs (transcriptomics), and proteins (proteomics) combined with high-throughput bioinformatic tools to extract a vast amount of information have become a popular approach to better understand virus-vector interactions. The integration of

the available datasets that result from these –omic studies may help to identify host cellular or molecular factors that are required for the viral replication cycle. Following the identification of these host factors, in depth biological characterization can be done using RNAi to determine the exact function of the insect genes involved in virus acquisition, replication, and transmission. Development of appropriate cell culture systems, in particular of VCM, has been established for reoviruses and it has helped to advance the understanding of the mechanisms by which these viruses interact with their vector cells. On the other hand, marafivirus-vector studies to understand the virus interactions with either their leafhopper or aphid vectors using –omic technologies are currently lacking but greatly need it. Thus, developing VCM and RNAi methods for insect vectors of persistent-propagative viruses is of crucial importance to unravel the intricacies of these complex virus-vector interactions.

References

Adkins, S., Quadt, R., Choi, T.-J., Ahlquist, P., & German, T. (1995). An RNA-dependent RNA polymerase activity associated with virions of *Tomato spotted wilt virus*, a plant- and insect-infecting Bunyavirus. *Virology, 207*, 308–311.

Adkins, S., Choi, T.-J., Israel, B. A., & Bandla, M. D. (1996). Baculovirus expression and processing of *Tomato spotted wilt tospovirus* glycoproteins. *Phytopathology, 86*, 849–855.

Agindotan, B. O., Gray, M. E., Hammond, R. W., & Bradley, C. A. (2012). Complete genome sequence of switchgrass mosaic virus, a member of a proposed new species in the genus *Marafivirus*. *Archives of Virology, 157*, 1825–1830.

Ammar, E. D. (1975). Effect of European wheat striate mosaic, acquired by feeding on diseased plants, on biology of its planthopper vector *Javesella pellucida*. *Annals of Applied Biology, 79*, 195–202.

Ammar, E. D., & Hogenhout, S. A. (2008). A neurotropic route for *Maize mosaic virus* (*Rhabdoviridae*) in its planthopper vector *Peregrinus maidis*. *Virus Research, 131*, 77–85.

Ammar, E.-D., & Nault, L. R. (1985). Assembly and accumulation sites of *Maize mosaic virus* in its planthopper vector. *Phytopathology, 84*, 1054–1060.

Ammar, E., & Nault, L. R. (2002). Virus transmission by leafhoppers, planthoppers and treehoppers (Auchenorrhyncha, Homoptera). *Advances in Botanical Research, 36*, 141–167.

Ammar, E. D., & Peterschmitt, M. (2004). Maize yellow stripe. In H. Lapierre & P.-A. Signoret (Eds.), *Viruses and virus diseases of Poaceae (Graminae)* (pp. 682–685). Versailles: INRA Editions.

Ammar, E. D., Gomez-Luengo, R. G., Gordon, D. T., & Hogenhout, S. A. (2005). Characterization of Maize Iranian mosaic virus and comparison with Hawaiian and other isolates of Maize mosaic virus (*Rhabdoviridae*). *Journal of Phytopathology, 153*, 129–136.

Ammar, E. D., Khlifa, E. A., Mahmoud, A., Abol-Ela, S. E., & Peterschmitt, M. (2007). Evidence for multiplication of the leafhopper-borne *Maize yellow stripe virus* in its vector using ELISA and dot-blot hybridization. *Archives of Virology, 152*, 489–494.

Ammar, E. D., Tsai, C. W., Whitfield, A. E., Redinbaugh, M. G., & Hogenhout, S. A. (2009). Cellular and molecular aspects of rhabdovirus interactions with insect and plant hosts. *Annual Review of Entomology, 54*, 447–468.

Argüello-Caro, E. B., Maroniche, G. A., Dumón, A. D., Sagadín, M. B., Del Vas, M., & Truol, G. (2013). High viral load in the planthopper vector *Delphacodes kuscheli* (Hemiptera:

Delphacidae) is associated with successful transmission of Mal de Río Cuarto Virus. *Annals of the Entomological Society of America, 106*, 93–99.

Artimo, P., Jonnalagedda, M., Arnold, K., Baratin, D., Csardi, G., de Castro, E., Duvaud, S., Flegel, V., Fortier, A., Gasteiger, E., Grosdidier, A., Hernandez, C., Ioannidis, V., Kuznetsov, D., Liechti, R., Moretti, S., Mostaguir, K., Redaschi, N., Rossier, G., Xenarios, I., & Stockinger, H. (2012). ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Research, 40*, W597–W603.

Attoui, H., Jaafar, F. M., Belhouchet, M., de Micco, P., de Lamballerie, X., & Brussard, C. P. (2006). Micromonas pusilla reovirus: A new member of the family *Reoviridaea* assigned to a novel proposed genus (*Mimoreovirus*). *Journal of General Virology, 87*, 1375–1383.

Attoui, H., Becnel, J., Belaganahalli, S., Bergoin, M., Brussaard, C. P., Chappell, J. D., Ciarlet, M., del Vas, M., Dermody, T. S., Dormitzer, P. R., Duncan, R., Fang, Q., Graham, R., Guglielmi, K. M., Harding, R. M., Hillman, B., Makkay, A., Marzachi, A. C., Matthijnssens, J., Mertens, P. P. C., Milne, R. G., Mohd Jaafar, F., Mori, H., Noordeloos, A. A., Omura, T., Patton, J. T., Rao, S., Maan, M., Stoltz, D., Suzuki, N., Upadhyaya, N. M., Wei, C., & Zhou, H. (2012). Part II: The viruses – The double stranded RNA viruses – Family Reoviridae. In A. M. Q. King, M. J. Adams, E. B. Carstens, & E. J. Lefkowitz (Eds.), *Virus taxonomy ninth report of the international committee on taxonomy of viruses* (pp. 541–637). USA: Academic Press.

Avila, Y., Stavisky, J., Hague, S., Funderburk, J., Reitz, S., & Momol, T. (2006). Evaluation of *Frankliniella bispinosa* (Thysanoptera: Thripidae) as a vector of the *Tomato spotted wilt virus* in pepper. *Florida Entomologist, 89*, 204–207.

Badillo-Vargas, I. E., Rotenberg, D., Schneweis, D., Hiromasa, Y., Tomich, J. M., & Whitfield, A. E. (2012). Proteomic analysis of Frankliniella occidentalis and differentially expressed proteins in response to Tomato spotted wilt virus infection. *Journal of Virology, 86*, 8793–8809.

Badillo-Vargas, I., Rotenberg, D., Schneweis, B. A., & Whitfield, A. E. (2015). RNA interference tools for the western flower thrips, Frankliniella occidentalis. *Journal of Insect Physiology, 76*, 36–46.

Bandla, M. D., Campbell, L. R., Ullman, D. E., & Sherwood, J. L. (1998). Interaction of Tomato spotted wilt tospovirus (TSWV) glycoproteins with a thrips midgut protein, a potential cellular receptor for TSWV. *Phytopathology, 88*, 98–104.

Bao, Y., Wang, Y., Wu, W., Zhao, D., Xue, J., Zhang, B., Shen, Z., & Zhang, C. (2012). *De novo* intestine-specific transcriptome of the brown planthopper *Nilaparvata lugens* revealed potential functions in digestion, detoxification and immune response. *Genomics, 99*, 256–264.

Bao, Y., Qu, L., Zhao, D., Chen, L., Jin, H., Xu, L., Cheng, J., & Zhang, C. (2013). The genome- and transcriptome-wide analysis of innate immunity in the brown planthopper, *Nilaparvata lugens. BMC Genomics, 14*, 160.

Bao, Y., Qin, X., Yu, B., Chen, L., Wang, Z., & Zhang, C. (2014). Genomic insights into the serine protease gene family and expression profile analysis in the planthopper, *Nilaparvata lugens. BMC Genomics, 15*, 507.

Barandoc-Alviar, K., Rotenberg, D., & Whitfield, A. E. (2014). Identifying novel interacting proteins of Maize mosaic rhabdovirus glycoprotein using the split-ubiquitin membrane-based yeast two hybrid system. *Phytopathology, 104*(Suppl 3), S3.11.

Barbier, P., Takahashi, M., Nakamura, I., Toriyama, S., & Ishihama, A. (1992). Solubilization and promoter analysis of RNA-polymerase from *Rice stripe virus. Journal of Virology, 66*, 6171–6174.

Bass, C., Hebsgaard, M., & Hughes, J. (2012). Genomic resources for the brown planthopper, *Nilaparvata lugens*: Transcriptome pyrosequencing and microarray design. *Insect Science, 19*, 1–12.

Bearzotti, M., Delmas, B., Lamoureux, A., Loustau, A., Chilmonczyk, S., & Bremont, M. (1999). Fish rhabdovirus cell entry is mediated by fibronectin. *Journal of Virology, 73*, 7703–7709.

Black, L. M. (1943). Genetic variation ion the clover leafhopper's ability to transmit potato yellow-dwarf virus. *Genetics, 28*, 200–209.

Black, L. M. (1979). Vector cell monolayers and plant viruses. *Advances in Virus Research, 25*, 191–271.

Bourhy, H., Cowley, J. A., Larrous, F., Holmes, E. C., & Walker, P. J. (2005). Phylogenetic relationships among rhabdoviruses inferred using the L polymerase gene. *Journal of General Virology, 86*, 2849–2858.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J. J., MacFarlane, S., Peters, D., Susi, P., & Torrance, L. (2013). Status and prospects of plant virus control through interference with vector transmission. *Annual Review of Phytopathology, 51*, 177.

Brcak, J. (1979). Leafhopper and planthopper vectors of plant disease agents in central and southern Europe. In K. Maramorosch & K. F. Harris (Eds.), *Leafhopper vectors and plant disease agents* (pp. 97–154). New York: Academic Press.

Brittlebank, C. C. (1919). Tomato diseases. *Journal of Agricultural, 17*, 231–235.

Bucher, E., Sijen, T., de Haan, P., Goldbach, R., & Prins, M. (2003). Negative-strand Tospoviruses and Tenuiviruses carry a gene for a suppressor of gene silencing at analogous genomic positions. *Journal of Virology, 77*, 1329–1336.

Cassone, B. J., Wijeratne, S., Michel, A. P., Stewart, L. R., Chen, Y., Yan, P., & Redinbaugh, M. G. (2014). Virus-independent and common transcriptome responses of leafhopper vectors feeding on maize infected with semi-persistently and persistent propagatively transmitted viruses. *BMC Genomics, 15*, 133.

Castello, J. D., Rogers, S. O., Bachand, G. D., Fillhart, R. C., Murray, J. S., Weidemann, K., Bachand, M., & Almond, M. A. (2000). Detection and partial characterization of tenuiviruses from black spruce. *Plant Diseases, 84*, 143–147.

Chatzivassiliou, E. K., Peters, D., & Katis, N. I. (2002). The efficiency by which Thrips tabaci populations transmit Tomato spotted wilt virus depends on their host preference and reproductive strategy. *Phytopathology, 92*, 603–609.

Chen, C. C., & Chiu, R. J. (1989). Transmission of *Rice wilted stunt virus* by the planthopper (*Nilaparvata lugens*). *Bulletin of Taichung District Agricultural Improvement Station, 23*, 3–10.

Chen, C. C., Chao, C. H., & Chiu, R. J. (1996). Studies on host range, transmission and electron microscopy of Peanut chlorotic fan-spot virus in Taiwan. *Bulletin of Taichung District Agricultural Improvement Station, 52*, 59–68.

Chen, C. C., Chen, T. C., Lin, Y. H., Yeh, S. D., & Hsu, H. T. (2005). A chlorotic spot disease on calla lilies (*Zantedeschia* spp.) is caused by a tospovirus serologically but distantly related to *Watermelon silver mottle virus. Plant Diseases, 89*, 440–445.

Chen, H., Chen, Q., Omura, T., Uehara-Ichiki, T., & Wei, T. (2011). Sequential infection of *RIce dwarf virus* in the internal organs of its insect vector after ingestion of virus. *Virus Research, 160*, 389–394.

Chen, Y., Cassone, B. J., Bai, X., Redinbaugh, M. G., & Michel, A. P. (2012). Transcriptome of the plant virus vector *Graminella nigrifrons* and the molecular interactions of *Maize fine streak rhabdovirus* transmission. *PLoS One, 7*, e40613.

Chomchan, P., Li, S. F., & Shirako, Y. (2003). *Rice grassy stunt tenuivirus* nonstructural protein p5 interacts with itself to form oligomeric complexes *in vitro* and *in vivo. Journal of Virology, 77*, 769–775.

Chu, F. H., Chao, C. H., Peng, Y. C., Lin, S. S., Chen, C. C., & Yeh, S. D. (2001). Serological and molecular characterization of Peanut chlorotic fan-spot virus, a new species of the genus *Tospovirus. Phytopathology, 91*, 856–863.

Ciuffo, M., Tavella, L., Pacifico, D., Masenga, V., & Turina, M. (2008). A member of a new Tospovirus species isolated in Italy from wild buckwheat (*Polygonum convolvulus*). *Archives of Virology, 153*, 2059–2068.

Ciuffo, M., Kurowski, C., Vivoda, E., Copes, B., Masenga, V., Falk, B. W., & Turina, M. (2009). A new Tospovirus sp. in cucurbit crops in Mexico. *Plant Diseases, 93*, 467–474.

Ciuffo, M., Mautino, G. C., Bosco, L., Turina, M., & Tavella, L. (2010). Identification of *Dictyothrips betae* as the vector of Polygonum ring spot virus. *Annals of Applied Biology, 157*, 299–307.

Cortes, I., Livieratos, I. C., Derks, A., Peters, D., & Kormelink, R. (1998). Molecular and serological characterization of Iris yellow spot virus, a new and distinct Tospovirus species. *Phytopathology, 88*, 1276–1282.

Cortez, I., Saaijer, J., Wongjkaew, K. S., Pereira, A. M., Goldbach, R., Peters, D., & Kormelink, R. (2001). Identification and characterization of a novel tospovirus species using a new RT-PCR approach. *Archives of Virology, 146*, 265–278.

De Angelis, J. D., Sether, D. M., & Rossignol, P. A. (1993). Survival, development, and reproduction in western flower thrips (Thysanoptera: Thripidae) exposed to *Impatiens necrotic virus. Environmental Entomology, 22*, 1308–1312.

de Assis Filho, F. M., Naidu, R. A., Deom, C. M., & Sherwood, J. L. (2002). Dynamics of Tomato spotted wilt virus replication in the alimentary canal of two thrips species. *Phytopathology, 92*, 729–733.

de Assis Filho, F. M., Deom, C. M., & Sherwood, J. L. (2004). Acquisition of Tomato spotted wilt virus by adults of two thrips species. *Phytopathology, 94*, 333–336.

de Avila, A. C., Huguenot, C., Resende, R. D., Kitajima, E. W., Goldbach, R. W., & Peters, D. (1990). Serological differentiation of 20 isolates of *Tomato spotted wilt virus. Journal of General Virology, 71*, 2801–2807.

de Avila, A. C., De Haan, P., Kitajima, E. W., Kormelink, R., Resende, R. D., Goldbach, R. W., & Peters, D. (1992). Characterization of a distinct isolate of *Tomato spotted wilt virus* (TSWV) from *Impatiens* Sp. in the Netherlands. *Journal of Phytopathology, 134*, 133–151.

de Avila, A. C., De Haan, P., Kormelink, R., Resende, R. D., Goldbach, R. W., & Peters, D. (1993). Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. *Journal of General Virology, 74*, 153–159.

de Borbon, C. M., Gracia, O., & De Santis, L. (1999). Survey of Thysanoptera occurring on vegetable crops as potential Tospovirus vectors in Mendoza, Argentina. *Revista De Sociedad Entomologica Argentina, 58*, 59–66.

de Haan, P., Wagemakers, L., Peters, D., & Goldbach, R. (1990). The S RNA segment of Tomato spotted wilt virus has an ambisense character. *Journal of General Virology, 71*, 1001–1007.

de Haan, P., Kormelink, R., de Oliveira Resende, R., van Poelwijk, F., Peters, D., & Goldbach, R. (1991). Tomato spotted wilt virus L RNA encodes a putative RNA polymerase. *Journal of General Virology, 72*, 2207–2216.

de Miranda, J., Hernandez, M., Hull, R., & Espinoza, A. M. (1994). Sequence analysis of Rice hoja blanca virus RNA-3. *Journal of General Virology, 75*, 2127–2132.

de Miranda, J. R., Munoz, M., Madriz, J., Wu, R., & Espinoza, A. M. (1996a). Sequence of Echinochloa hoja blanca tenuivirus RNA-3. *Virus Genes, 13*, 65–68.

de Miranda, J. R., Munoz, M., Wu, R., & Espinoza, A. M. (1996b). Sequence of Echinochloa hoja blanca tenuivirus RNA-5. *Virus Genes, 12*, 131–134.

de Miranda, J. R., Munoz, M., Wu, R., Hull, R., & Espinoza, A. M. (1996c). Sequence of Rice hoja blanca tenuivirus RNA-2. *Virus Genes, 12*, 231–237.

de Oliveira, A. S., Machado-Bertran, A. G., Inoue-Nagata, A. K., Nagata, T., Kitajima, E. W., & Resende, R. O. (2011). An RNA-dependent RNA-polymerase gene of a distinct Brazilian tospovirus. *Virus Genes, 43*, 385–389.

Deng, J., Li, S., Hong, J., Ji, Y., & Zhou, Y. (2013). Investigation on subcellular localization of *Rice stripe virus* in its vector small brown planthopper by electron microscopy. *Virology Journal, 10*, 310.

Dietzgen, R. G., Kuhn, J. H., Clawson, A. N., Freitas-Astua, J., Goodin, M. M., Kitajima, E. W., Wetzel, T., & Whitfield, A. E. (2013). Dichoravirus: A proposed new genus for Brevipalpus mite-transmitted, nuclear, bacilliform, bipartite, negative-strand RNA plant virus. *Archives of Virology, 159*, 607–619.

Dong, J. H., Cheng, X. F., Yin, Y. Y., Fang, Q., Ding, M., Li, T. T., Zhang, L. Z., Su, X. X., McBeath, J. H., & Zhang, Z. K. (2008). Characterization of Tomato zonate spot virus, a new tospovirus in China. *Archives of Virology, 153*, 855–864.

Dong, J. H., Yin, Y. Y., Fang, Q., Mcbeath, J. H., & Zhang, Z. K. (2013). A new tospovirus causing chlorotic ringspot on *Hippeastrum* sp. in China. *Virus Genes, 46*, 567–570.

Duarte, L. M. L., Rivas, E. B., Alexandre, M. A. V., de Avila, A. C., Nagata, T., & Chagas, C. M. (1995). Chrysanthemum stem necrosis caused by a possible novel tospovirus. *Journal of Phytopathology, 143*, 569–571.

Duijsings, D., Kormelink, R., & Goldbach, R. (1999). Alfalfa mosaic virus RNAs serve as cap donors for Tomato spotted wilt virus transcription during coinfection of Nicotiana benthamiana. *Journal of Virology, 73*, 5172–5175.

Duijsings, D., Kormelink, R., & Goldbach, R. (2001). In vivo analysis of the TSWV capsnatching mechanism: Single base complementarity and primer length requirements. *EMBO Journal, 20*, 2545–2552.

Edwards, M. C., Zhijun, Z., & Weiland, J. J. (1997). Oat blue dwarf marafivirus resembles the tymoviruses in sequence, genome organization, and expression strategy. *Virology, 232*, 217–229.

Estabrook, E. M., Suyenaga, K., Tsai, J. H., & Falk, B. W. (1996). *Maize stripe tenuivirus* RNA2 transcripts in plant and insect hosts and analysis of pvc2, a protein similar to the Phlebovirus virion membrane glycoproteins. *Virus Genes, 12*, 239–247.

Evans, J. D., Brown, S. J., Hackett, K. J., Robinson, G., Richards, S., Lawson, D., Elsik, C., Coddington, J., Edwards, O., Emrich, S., Gabaldon, T., Goldsmith, M., Hanes, G., Misof, B., Munoz-Torres, M., Niehuis, O., Papanicolaou, A., Pfrender, M., Poelchau, M., Purcell-Miramontes, M., Robertson, H. M., Ryder, O., Tagu, D., Torres, T., Zdobnov, E., Zhang, G., Zhou, X., & i5K Consortium. (2013). The i5K initiative: Advancing arthropod genomics for knowledge, human health, agriculture, and the environment i5K CONSORTIUM. *Journal of Heredity, 104*, 595–600.

Falk, B. W., & Tsai, J. H. (1984). Identification of single-stranded and double-stranded RNAs associated with *Maize stripe virus. Phytopathology, 74*, 909–915.

Falk, B. W., & Tsai, J. H. (1998). Biology and molecular biology of viruses in the genus *Tenuivirus. Annual Review of Phytopathology, 36*, 139–163.

Falk, B. W., Tsai, J. H., & Lommel, S. A. (1987). Differences in levels of detection for the *Maize stripe virus* capsid and major non-capsid proteins in plant and insect hosts. *Journal of General Virology, 68*, 1801–1811.

Fukushi, T. (1933). Transmission of the virus through the eggs of an insect vector. *Proceedings of the Imperial Academy Tokyo, 9*, 457–460.

Fukushi, T. (1969). Relationships between propagative rice viruses and their vectors. In K. Maramorosch (Ed.), *Viruses, vectors and vegetation* (pp. 279–301). New York: John Wiley & Sons, Inc.

Gamez, R., & Leon, P. (1988). Maize rayado fino and related viruses. In R. Koenig (Ed.), *The plant viruses* (pp. 213–233). New York: Plenum Press.

Garcia, S., Billecocq, A., Crance, J. M., Prins, M., Garin, D., & Bouloy, M. (2006). Viral suppressors of RNA interference impair RNA silencing induced by a Semliki Forest virus replicon in tick cells. *Journal of General Virology, 87*, 1985–1989.

Garry, C., & Garry, R. (2004). Proteomics computational analyses suggest that the carboxyl terminal glycoproteins of Bunyaviruses are class II viral fusion protein (beta-penetrenes). *Theoretical Biology and Medical Modelling, 1*, 10.

Gastka, M., Horvath, J., & Lentz, T. L. (1996). Rabies virus binding to the nicotinic acetylcholine receptor subunit demonstrated by virus overlay protein binding assay. *Journal of General Virology, 77*, 2437–2440.

German, T. L., Ullman, D. E., & Moyer, J. W. (1992). Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. *Annual Review of Phytopathology, 30*, 315–348.

Ghotbi, T., Shahraeen, N., & Winter, S. (2005). Occurrence of tospoviruses in ornamental and weed species in Markazi and Tehran provinces in Iran. *Plant Diseases, 89*, 425–429.

Gingery, R. E. (1988). The rice stripe virus group. In R. G. Milne (Ed.), The plant viruses (pp. 297–329). Springer: New York.

Gingery, R. E., Nault, L. R., & Bradfute, O. E. (1981). *Maize stripe virus*: Characteristics of a new virus class. *Virology, 182*, 99–108.

Gonzalez-Scarano, F., & Nathanson, N. (1996). Bunyaviridae. In B. N. Fields, D. M. Knipe, & P. M. Howley (Eds.), *Fields Virology* (pp. 1473–1504). Philadelphia: Lippincott-Raven.

Gopal, K., Reddy, M. K., Reddy, D. V. R., & Muniyappa, V. (2010). Transmission of *Peanut yellow spot virus* (PYSV) by thrips, *Scirtothrips dorsalis* Hood in groundnut. *Archives of Phytopathology and Plant Protection, 43*, 421–429.

Gray, S. M., & Banerjee, N. (1999). Mechanisms of arthropod transmission of plant and animal viruses. *Microbiology and Molecular Biology Reviews, 63*, 128–148.

Hammond, R. W., & Ramirez, P. (2001). Molecular characterization of the genome of *Maize rayado fino virus*, the type member of the genus Marafivirus. *Virology, 282*, 338–347.

Hassani-Mehraban, A., Botermans, M., Verhoeven, J. T. J., Meekes, E., Saaijer, J., Peters, D., Goldbach, R., & Kormelink, R. (2010). A distinct tospovirus causing necrotic streak on *Alstroemeria* sp. in Colombia. *Archives of Virology, 155*, 423–428.

Hemmes, H., Lakatos, L., Goldbach, R., Burgyan, J., & Prins, M. (2007). The NS3 protein of *Rice hoja blanca tenuivirus* suppresses RNA silencing in plant and insect hosts by efficiently binding both siRNAs and miRNAs. *RNA, 13*, 1079–1089.

Hermanns, K., Zirkel, F., Kurth, A., Drosten, C., & Junglen, S. (2014). Cimodo virus belongs to a novel lineage of reovirus isolated from African mosquitoes. *Journal of General Virology, 95*, 905–909.

Heydarnejad, J., Izadpanah, K., Hunter, F. R., & Gooding, M. J. (2007). Transmission properties of Iranian wheat stripe virus. *Australasian Plant Pathology, 36*, 354–357.

Hibino, H. (1996). Biology and epidemiology of rice viruses. *Annual Review of Phytopathology, 34*, 249–274.

Hibino, H., Usugi, T., Omura, T., Tsuchizaki, T., Shohara, K., & Iwasaki, M. (1985). *Rice grassy stunt virus* – A planthopper-borne circular filament virus. *Phytopathology, 75*, 894–899.

Hill, C. L., Booth, T. F., Prasad, B. V. V., Grimes, J. M., Mertens, P., Sutton, G. C., & Stuart, D. I. (1999). The structure of a cypovirus and the functional organization of dsRNA viruses. *Nature Structural and Molecular Biology, 6*, 565–568.

Hirao, J., Oya, S., & Inoue, H. (1987). Transmission of rice grassy stunt virus (RGSV) by the brown planthopper, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae). *Bulletin of the Kyushu National Agricultural Experiment Station, 24*, 307–337.

Hogenhout, S. A., Ammar, E. D., Whitfield, A. E., & Redinbaugh, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology, 46*, 327–359.

Honda, K., Wei, T., Hagiwara, K., Higashi, T., Kimura, I., Akutsu, K., & Omura, T. (2007). Retention of *Rice dwarf virus* by descendants of pairs of viruliferous vector insects after rearing for 6 Years. *Phytopathology, 97*, 712–716.

Huang, Y. W., Geng, Y. F., Ying, X. B., Chen, C. Y., & Fang, R. X. (2005). Identification of a movement protein of rice yellow stunt rhabdovirus. *Journal of Virology, 79*, 2108–2114.

Huiet, L., Klaassen, V., Tsai, J. H., & Falk, B. W. (1990). Identification and sequence-analysis of the Maize Stripe Virus major noncapsid protein gene. *Virology, 179*, 862–866.

Huiet, L., Tsai, J. H., & Falk, B. W. (1992). Complete sequence of Maize Stripe Virus Rna4 and mapping of its subgenomic RNAs. *Journal of General Virology, 73*, 1603–1607.

Hunter, W. B., & Ullman, D. E. (1992). Anatomy and ultrastructure of the piercing-sucking mouthparts and paraglossal sensilla of Frankliniella occidentalis (Pergande) (Thysanoptera, Thripidae). *International Journal of Insect Morphology and Embryology, 21*, 17–35.

Huo, Y., Wenwen, L., Fujie, Z., Xiaoying, C., Li, L., Qifei, L., Yijun, Z., Taiyun, W., Rongxiang, F., & Wang, X. (2014). Transovarial transmission of a plant virus is mediated by vitellogenin of its insect vector. *PLoS Pathogen, 10*, e1003949.

Inoue, H. (1979). Transmission efficiency of rice transitory yellowing virus by the green rice leafhoppers, Nephotettix spp. (Hemiptera: Cicadellidae). *Applied Entomology and Zoology, 14*, 123–125.

Ishikawa, K., Omura, T., & Hibino, H. (1989). Morphological-characteristics of *Rice Stripe Virus. Journal of General Virology, 70*, 3465–3468.

Iwaki, M., Honda, Y., Hanada, K., Tochihara, H., Yonaha, T., Hokama, K., & Yokoyama, T. (1984). Silver mottle disease of watermelon caused by *Tomato spotted wilt virus. Plant Diseases, 68*, 1006–1008.

Izadpanah, K., Zhang, Y. P., Daubert, S., & Rowhani, A. (2002). Sequence of the coat protein gene of *Bermuda grass etched-line virus* and of the adjacent "marafibox" motif. *Virus Genes, 24*, 131–134.

Jackson, A. O., Goodin, M., Moreno, I., Johnson, J., & Lawrence, D. M. (1999). Rhabdoviruses *(Rhabdoviridae)*: Plant rhabdoviruses. In A. Granoff & R. G. Webster (Eds.), *Encyclopedia of virology* (pp. 1531–1541). San Diego: Academic Press.

Jackson, A. O., Dietxgen, R. G., Goodin, M. M., Bragg, J. N., & Deng, M. (2005). Biology of plant rhabdoviruses. *Annual Review of Phytopathology, 43*, 623–660.

Jain, R. K., Pappu, H. R., Pappu, S. S., Krishna Reddy, M., & Vani, A. (1998). Watermelon bud necrosis tospovirus is a distinct virus species belonging to serogroup IV. *Archives of Virology, 143*, 1637–1644.

Jennings, P. R., & Pineda, A. T. (1971). The effect of the *Hoja blanca virus* on its insect vector. *Phytopathology, 61*, 142–143.

Ji, R., Yu, H., Fu, Q., Chen, H., Ye, W., Li, S., & Lou, Y. (2013). Comparative transcriptome analysis of salivary glands of two populations of rice brown planthopper, *Nilaparvata lugens*, that differ in virulence. *PLoS One, 8*, e79612.

Jia, D., Chen, H., Zheng, H., Chen, Q., Liu, Q., Xie, L., Wu, Z., & Wei, T. (2012). Development of an insect vector cell culture and RNA interference system to investigate the functional role of Fijivirus replication protein. *Journal of Virology, 86*, 5800–5807.

Johannsdottir, H. K., Mancini, R., Kartenbeck, J., Amato, L., & Helenius, A. (2009). Host cell factors and functions involved in *Vesicular stomatitis virus* entry. *Journal of Virology, 83*, 440–453.

Kakutani, T., Hayano, Y., Hayashi, T., & Minobe, Y. (1990). Ambisense segment-4 of *Rice Stripe Virus* – Possible evolutionary relationship with *Phleboviruses* and *Uukuviruses* (Bunyaviridae). *Journal of General Virology, 71*, 1427–1432.

Kakutani, T., Hayano, Y., Hayashi, T., & Minobe, Y. (1991). Ambisense segment-3 of *Rice Stripe Virus* – The 1st Instance of a virus containing 2 ambisense segments. *Journal of General Virology, 72*, 465–468.

Kato, K., Hanada, K., & Kameya-Iwaki, M. (2000). Melon yellow spot virus: A distinct species of the genus *Tospovirus* isolated from melon. *Phytopathology, 90*, 422–426.

Kikkert, M., Meurs, C., van de Wetering, F., Dorfmuller, S., Peters, D., Kormelink, R., & Goldbach, R. (1998). Binding of *Tomato spotted wilt virus* to a 94-kDa thrips protein. *Phytopathology, 88*, 63–69.

Kikkert, M., Van Lent, J., Storms, M., Bodegom, P., Kormelink, R., & Goldbach, R. (1999). *Tomato spotted wilt virus* particle morphogenesis in plant cells. *Journal of Virology, 73*, 2288–2297.

Kikkert, M., Verschoor, A., Kormelink, R., Rottier, P., & Goldbach, R. (2001). *Tomato spotted wilt virus* glycoproteins exhibit trafficking and localization signals that are functional in mammalian cells. *Journal of Virology, 75*, 1004–1012.

Kondo, H., Chiba, S., Andika, I. B., Maruyama, K., Tamda, T., & Suzuki, N. (2013). *Orchid Fleck Virus* structural proteins N and P form intranuclear viroplasm-like structures in the absence of viral infection. *Journal of Virology, 87*, 7423–7434.

Konishi, H., Noda, H., Tamura, Y., & Hattori, M. (2009). Proteomic analysis of the salivary glands of the rice brown planthopper, *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae). *Applied Entomology and Zoology, 44*, 525–534.

Kormelink, R., de Haan, P., Meurs, C., Peters, D., & Goldbach, R. (1992). The nucleotide sequence of the M RNA segment of *Tomato spotted wilt virus*, a Bunyavirus with two ambisense RNA segments. *Journal of General Virology, 73*, 2795–2804.

Kormelink, R., Storms, M., Van Lent, J., Peters, D., & Goldbach, R. (1994). Expression and subcellular location of the NSm protein of *Tomato spotted wilt virus* (TSWV), a putative viral movement protein. *Virology, 200*, 56–65.

Kormelink, R., Garcia, M. L., Goodin, M., Sasaya, T., & Haenni, A.-L. (2011). Negative-strand RNA viruses: The plant-infecting counterparts. *Virus Research, 162*, 184–202.

Lastra, R. (1985). Mechanical transmission, purification and properties of an isolate of *Maize Stripe Virus* from Venezuela. *Journal of Phytopathology, 114*, 168–179.

Lewandowski, D. J., & Adkins, S. (2005). The tubule-forming NSm protein from *Tomato spotted wilt virus* complements cell-to-cell and long-distance movement of Tobacco mosaic virus hybrids. *Virology, 342*, 26–37.

Li, W., Lewandowski, D. J., Hilf, M. E., & Adkins, S. (2009). Identification of domains of the Tomato spotted wilt virus NSm protein involved in tubule formation, movement and symptomatology. *Virology, 390*, 110–121.

Lindord, M. B. (1932). Transmission of the Pineapple yellow-spot virus by *Thrips tabaci. Phytopathology, 22*, 301–324.

Liu, Y., Jia, D., Chen, H., Chen, Q., Xie, L., Wu, Z., & Wei, T. (2011). The P7-1 protein of *Southern rice black-streaked dwarf virus*, a fijivirus, induces the formation of tubular structures in insect cells. *Archives of Virology, 156*, 1729–1736.

Louie, R. (1995). Vascular puncture of Maize Kernels for the mechanical transmission of Maize White Line Mosaic-Virus and other viruses of Maize. *Phytopathology, 85*, 139–143.

Lu, D., Wu, M., Pu, J., Feng, A., Zhang, Q., & Han, Z. (2013). A functional study of two dsRNA binding protein genes in *Laodelphax striatellus. Pest Management Science, 69*, 1034–1039.

Maccheroni, W., Alegria, M. C., Greggio, C. C., Piazza, J. P., Kamla, R. F., Zacharias, P. R. A., Bar-Joseph, M., Kitajima, E. W., Assumpção, L. C., Camarotte, G., Cardozo, J., Casagrande, E. C., Ferrari, F., Franco, S. F., Giachetto, P. F., Girasol, A., Jordão, H., Jr., Silva, V. H. A., Souza, L. C. A., Aguilar-Vildoso, C. I., Zanca, A. S., Arruda, P., Kitajima, J. P., Reinach, F. C., Ferro, J. A., & da Silva, A. C. R. (2005). Identification and genomic characterization of a new virus (Tymoviridae family) associated with citrus sudden death disease. *Journal of Virology, 79*, 3028–3037.

Mahmoud, A., Royer, M., Granier, M., Ammar, E.-D., Thouvenel, J.-C., & Peterschmitt, M. (2007). Evidence for a segmented genome and partial nucleotide sequences of maize yellow stripe virus, a proposed new tenuivirus. *Archives of Virology, 152*, 1757–1762.

Mann, K. S., & Dietzgen, R. G. (2014). Plant rhabdoviruses: New insights and research needs in the interplay of negative-strand RNA viruses with plant and insect hosts. *Archives of Virology, 159*, 1889–1900.

Mar, T. T., Wenwen, L., & Xifeng, W. (2014). Proteomic analysis of interaction between P7-1 of *Southern rice black-streaked dwarf virus* and the insect vector reveals diverse insect proteins involved in successful transmission. *Journal of Proteomics, 102*, 83–97.

Maroniche, G. A., Mongelli, V. C., Llauger, G., Alfonso, V., Taboga, O., & del Vas, M. (2012). In vivo subcellular localization of *Mal de Río Cuarto virus* (MRCV) non-structural proteins in insect cells reveals their putative functions. *Virology, 430*, 81–89.

Marzachi, C., Boccardo, G., Milne, R., Isogai, M., & Uyeda, I. (1995). Genome structure and variability of Fijiviruses. *Seminars in Virology, 6*, 103–108.

Matthews, R. E. F. (1982). Classification and nomenclature of viruses. *Intervirology, 17*, 1–199.

McMichael, L. A., Persley, D. M., & Thomas, J. E. (2002). A new tospovirus serogroup IV species infecting capsicum and tomato in Queensland. *Australasian Plant Pathology, 31*, 231–239.

Meena, R. L., Venkatesan, T. R. S., & Mohankumar, S. (2005). Molecular characterization of tospovirus transmitting thrips populations from India. *American Journal of Biochemistry and Biotechnology, 1*, 167–172.

Meng, J. R., Liu, P. P., Zou, C. W., Wang, Z. Q., Liao, Y. M., Cai, J. H., Qin, B. X., & Chen, B. S. (2013). First report of a *Tospovirus* in mulberry. *Plant Disease, 97*, 1001.

Mertens, P. P. C., Attoui, H., Duncan, R., & Dermody, T. S. (2005). Reoviridae. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, & L. A. Ball (Eds.), *Virus taxonomy: Eight report of the international committee on taxonomy of viruses* (pp. 447–454). London: Elsevier/ Academic Press.

Milne, R. G., del Vas, M., Harding, R. M., Marzachi, R., & Mertens, P. P. C. (2005). Fijivirus. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, & L. A. Ball (Eds.), *Virus taxonomy: Eight report of the international committee on taxonomy of viruses* (pp. 534–542). London: Elsevier/Academic Press.

Misari, S. M., & Sylvester, E. S. (1983). Coriander feathery red-vein virus, a propagative plant rhabdovirus and its transmission by the aphid *Hyadaphis foeniculi* Passerini. *Hilgardia, 51*, 1–38.

Montero-Astúa, M. (2012). *Unveiling and blocking the interaction between Tomato spotted wilt virus and its insect vector, Frankliniella occidentalis* (Doctoral dissertation). Kansas State University in Manhattan.

Montero-Astúa, M., Rotenberg, D., Leach-Kieffaber, A., Schneweis, B. A., Park, S., Park, J. K., German, T. L., & Whitfield, A. E. (2014). Disruption of vector transmission by a plant-expressed viral glycoprotein. *Molecular Plant-Microbe Interactions Journal, 27*, 296–304.

Montero-Astúa, M., Stafford, C., Badillo-Vargas, I., Rotenberg, D., Ullman, D. E., & Whitfield, A. E. (2011). Tospovirus -thrips biology. In J. K. Brown, (Ed.), *Vector-mediated transmission of plant pathogens*. St. Paul, MN: APS Press.

Morales, F. J., & Niessen, A. I. (1983). Association of spiral filamentous virus like particles with Rice Hoja Blanca. *Phytopathology, 73*, 971–974.

Moritz, G. (1997). Structure, growth, and development. In T. Lewis (Ed.), *Thrips as crop pests* (pp. 15–63). Oxon: CAB International.

Moritz, G., Kumm, S., & Mound, L. (2004). Tospovirus transmission depends on thrips ontogeny. *Virus Research, 100*, 143–149.

Nagata, T., & Avila, A. C. (2000). Transmission of Chrysanthemum stem necrosis virus, a recently discovered tospovirus, by two thrips species. *Journal of Phytopathology, 148*, 123–125.

Nagata, T., Inoue-Nagata, A. K., Smid, H. M., Goldbach, R., & Peters, D. (1999). Tissue tropism related to vector competence of *Frankliniella occidentalis* for *Tomato spotted wilt tospovirus. Journal of General Virology, 80*, 507–515.

Nagata, T., Nagata-Inoue, A. K., Prins, M., Goldbach, R., & Peters, D. (2000). Impeded thrips transmission of defective Tomato spotted wilt virus isolates. *Phytopathology, 90*, 454–459.

Nagata, T., Almeida, A. C. L., Resende, R. O., & de Avila, A. C. (2004). The competence of four thrips species to transmit and replicate four tospoviruses. *Plant Pathology, 53*, 136–140.

Naidu, R. A., Deom, C. M., & Sherwood, J. L. (2001). First report of *Frankliniella fusca* as a vector of *Impatiens necrotic spot tospovirus. Plant Diseases, 85*, 1211.

Nakahara, S., & Monteiro, R. C. (1999). *Frankliniella zucchini* (Thysanoptera: Thripidae), a new species and vector of tospovirus in Brazil. *Proceedings of the Entomological Society of Washington, 101*, 290–294.

Nasu, S. (1963). Studies on some leafhoppers and planthoppers which transmit diseases of rice plant in Japan. *Bulletin of the Kyushu Agricultural Experiment Station, 8*, 153–340.

Nault, L. R., & Gordon, D. T. (1988). Multiplication of *Maize stripe virus* in *Peregrinus maidis. Phytopathology, 78*, 991–995.

Noda, H., Kawai, S., Koizumi, Y., Matsui, K., Zhang, Q., Furukawa, S., Shimomura, M., & Mita, K. (2008). Annotated ESTs from various tissues of the brown planthopper *Nilaparvata lugens*: A genomic resource for studying agricultural pests. *BMC Genomics, 9*, 117.

Ohnishi, J., Katsuzaki, H., Tsuda, S., Sakurai, T., Akutsu, K., & Murai, T. (2006). *Frankliniella cephalica*, a new vector for *Tomato spotted wilt virus. Plant Diseases, 90*, 685.

Oliveira, V. C., Bartasson, L., Batista de Castro, M. E., Correa, J. R., Ribeiro, B. M., & Oliveira Resende, R. (2011). A silencing suppressor protein (NSs) of a tospovirus enhances baculovirus replication in permissive and semipermissive insect cell lines. *Virus Research, 155*, 259–267.

Omura, T., & Jan, J. (1999). Role of outer capsid proteins in transmission of Phytoreovirus by insect vectors. *Advances in Virus Research, 54*, 15–43.

Omura, T., Yan, J., Zhong, B., Wada, M., & Zhu, Y. (1998). The P2 protein of *Rice dwarf virus* is required for adsorption of the virus to cells of the insect vector. *Journal of Virology, 72*, 9370–9373.

Peng, X., Zha, W., He, R., Lu, T., Zhu, L., Han, B., & He, G. (2011). Pyrosequencing the midgut transcriptome of the brown planthopper, *Nilaparvata lugens. Insect Molecular Biology, 20*, 745–762.

Peters, D., Wijkamp, I., van de Wetering, F., & Goldbach, R. (1995). Vector relations in the transmission and epidemiology of tospoviruses. *International Symposium on Tospoviruses and Thrips of Floral and Vegetable Crops Acta Hort, 431*, 29–43.

Pittman, H. A. (1927). Spotted wilt of tomatoes. *Journal of Australian Council for Scientific and Industrial Research, 1*, 74–77.

Plassmeyer, M. L., Soldan, S. S., Stachelek, K. M., Martin-Garcia, J., & Gonzalez-Scarano, F. (2005). California serogroup, G_C (Gl) glycoprotein is the principal determinant of pH-dependent cell fusion and entry. *Virology, 338*, 121–132.

Plassmeyer, M. L., Soldan, S. S., Stachelek, K. M., Roth, S. M., Martin-Garcia, J., & Gonzalez-Scarano, F. (2007). Mutagenesis of the La Crosse Virus glycoprotein supports a role for Gc (1066–1087) as the fusion peptide. *Virology, 358*, 273–282.

Plyusnin, A., Beaty, B. J., Elliott, R. M., Goldbach, R., Kormelink, R., Lundkvist, A., Schmaljohn, C. S., & Tesh, R. B. (2012). Bunyaviridae. In A. M. Q. King, E. Lefkowitz, M. J. Adams, & E. B. Carstens (Eds.), *Virus taxonomy: Ninth report of the international committee on taxonomy of viruses* (pp. 725–741). New York: Academic Press.

Premachandra, W. T., Borgemeister, C., Maiss, E., Knierim, D., & Poehling, H. M. (2005). *Ceratothripoides claratris*, a new vector of a Capsicum chlorosis virus isolate infecting tomato in Thailand. *Phytopathology, 95*, 659–663.

Quito-Avila, D. F., Lightle, D., Lee, J., & Martin, R. R. (2012). Transmission biology of Raspberry latent virus, the First Aphid-Borne Reovirus. *Phytopathology, 102*, 547–553.

Ramirez, B. C., & Haenni, A. L. (1994). Molecular biology of Tenuiviruses, a remarkable group of Plant-Viruses. *Journal of General Virology, 75*, 467–475.

Ramirez, B. C., Macaya, G., Calvert, L. A., & Haenni, A. L. (1992). Rice Hoja Blanca Virus genome characterization and expression in vitro. *Journal of General Virology, 73*, 1457–1464.

Ramsey, J. S., Wilson, A. C. C., de Vos, M., Sun, Q., Tamborindeguy, C., Winfield, A., Malloch, G., Smith, D. M., Fenton, B., Gray, S. M., & Jander, G. (2007). Genomic resources for Myzus persicae: EST sequencing, SNP identification, and microarray design. *BMC Genomics, 8*, 423.

Razvyazkina, G. M. (1953). The importance of the tobacco thrips in the development of outbreaks of tip chlorosis of Makhorka. *The Review of Applied Entomology, A42*, 146.

Reddy, M., Reddy, D. V. R., & Appa, R. (1968). A new record of virus disease on peanut. *The Plant Disease Reporter, 52*, 494–495.

Reddy, D. V. R., Ratna, A. S., Sudarshana, M. R., Poul, F., & Kumar, I. K. (1992). Serological relationships and purification of Bud necrosis virus, a tospovirus occurring in peanut (*Arachis hypogaea* L.) in India. *Annals of Applied Biology, 120*, 279–286.

Redinbaugh, M. G., & Hogenhout, S. A. (2005). Plant Rhabdoviruses. *Current Topics in Microbiology and Immunology, 292*, 143–163.

Redinbaugh, M. G., Whitfield, A. E., & Ammar, E. D. (2012). Insect vector interaction and transmission of cereal-infecting rhabdoviruses. In R. G. Dietzgen & I. V. Kuzmin (Eds.), *Rhabdoviruses: Molecular taxonomy, evolution, genomics, ecology, host-vector interactions, cytopathology and control* (pp. 147–164). Norfolk: Caister Academic Press.

Rezende, J. A. M., Galleti, S. R., Pozzer, L., Resende, R. O., Avila, A. C., & Scagliusi, S. M. M. (1997). Incidence, biological and serological characteristics of a tospovirus in experimental fields of zucchini in Sao Paulo State, Brazil. *Fitopatologia Brasileira, 22*, 92–95.

Richmond, K. E., Chenault, K., Sherwood, J. L., & German, T. L. (1998). Characterization of the nucleic acid binding properties of *Tomato spotted wilt virus* nucleocapsid protein. *Virology, 248*, 6–11.

Rotenberg, D., & Whitfield, A. E. (2010). Analysis of expressed sequence tags from *Frankliniella occidentalis*, the western flower thrips. *Insect Molecular Biology, 19*, 537–551.

Sakimura, K. (1962). The present status of thrips-borne viruses. In K. Maramorosch (Ed.), *Biological transmission of disease agents* (pp. 33–40). New York: Academic Press.

Sakimura, K. (1963). *Frankliniella fusca*, an additional vector for *Tomato spotted wilt virus* with notes on *Thrips tabaci*, another vector. *Phytopathology, 53*, 412–415.

Sakimura, K. (1969). A comment on color forms of *Frankliniella schultzei* (Thysanoptera-Thripidae) in relation to transmission of *Tomato spotted wilt virus. Pacific Insects, 11*, 761–762.

Sakurai, T., Inoue, T., & Tsuda, S. (2004). Distinct efficiencies of *Impatiens necrotic spot virus* transmission by five thrips vector species (Thysanoptera: Thripidae) of tospoviruses in Japan. *Applied Entomology and Zoology, 39*, 71–78.

Samuel, G., Bald, J. G., & Pittman, H. A. (1930). Investigations on "spotted wilt" of tomatoes in Australia. *Commonwealth Council for Scientific and Industrial Research Bulletin, 44*, 8–11.

Satyanarayana, T., Reddy, K. L., Ratna, A. S., Deom, C. M., Gowda, S., & Reddy, D. V. R. (1996). Peanut yellow spot virus: A distinct tospovirus species based on serology and nucleic acid hybridisation. *Annals of Applied Biology, 129*, 237–245.

Schnettler, E., Hemmes, H., Huismann, R., Goldbach, R., Prins, M., & Kormelink, R. (2010). Diverging affinity of tospovirus RNA silencing suppressor proteins, NSs, for various RNA duplex molecules. *Journal of Virology, 84*, 11542–11554.

Scholthof, K. B. G., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P., Hemenway, C., & Foster, G. D. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology, 12*, 938–954.

Seepiban, C., Gajanandana, O., Attathom, T., & Attathom, S. (2011). Tomato necrotic ringspot virus, a new tospovirus isolated in Thailand. *Archives of Virology, 156*, 263–274.

Seifers, D. L., Harvey, T. L., She, Y.-M., Ens, W., Standing, K. G., Salomon, R., & Gera, A. (2005). Association of a virus with wheat displaying yellow head disease symptoms in the great plains. *Plant Disease, 89*, 888–895.

Sharma, R., Komatsu, S., & Noda, H. (2004). Proteomic analysis of the brown planthopper: Application to the study of carbamate toxicity. *Insect Biochemistry and Molecular Biology, 34*, 425–432.

Shope, R. E. (1985). Bunyaviruses. In B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roezman, & R. E. Shope (Eds.), *Virology* (pp. 1055–1082). New York: Raven Press.

Sin, S.-H., McNulty, B. C., Kennedy, G. G., & Moyer, J. W. (2005). Viral genetic determinants for thrips transmission of *Tomato spotted wilt virus. Proceedings of the National Academy of Sciences of the United States of America, 102*, 5168–5173.

Sinha, R. C. (1970). *Ellymana virescens*, a newly described vector of wheat striate mosaic virus. *Canadian Plant Disease Survey, 50*, 118–120.

Stafford, C. A., Walker, G. P., & Ullman, D. E. (2011). Infection with a plant virus modifies vector feeding behavior. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 9350–9355.

Stafford-Banks, C. A., Rotenberg, D., Johnson, B. R., Whitfield, A. E., & Ullman, D. E. (2014). Analysis of the salivary gland transcriptome of *Frankliniella occidentalis. PLoS One, 9*, e94447.

Storms, M. H., Kormelink, R., Peters, D., Van Lent, J. W. M., & Goldbach, R. W. (1995). The nonstructural NSm protein of *Tomato spotted wilt virus* induces tubular structures in plant and insect cells. *Virology, 214*, 485–493.

Strauss, J. H., & Strauss, E. G. (1988). Evolution of RNA viruses. *Annual Review of Microbiology, 42*, 657–683.

Sylvester, E. S. (1980). Circulative and propagative virus transmission by aphids. *Annual Review of Entomology, 25*, 257–286.

Takahashi, M., Toriyama, S., Kikuchi, Y., Hayakawa, T., & Ishihama, A. (1990). Complementarity between the 5′ and 3′ terminal sequences of *Rice strip virus* RNAs. *Journal of General Virology, 71*, 2817–2821.

Takahashi, M., Toriyama, S., Hamamatsu, C., & Ishihama, A. (1993). Nucleotide sequence and possible ambisense coding strategy of *Rice stripe virus* RNA segment-2. *Journal of General Virology, 74*, 769–773.

Takeda, A., Sugiyama, K., Nagano, H., Mori, M., Kaido, M., Mise, K., Okuno, S., & Tsuda, T. (2002). Identification of a novel RNA silencing suppressor. NSs protein of *Tomato spotted wilt virus. FEBS Letters, 532*, 75–79.

Tanaka, Y., Suetsugu, Y., Yamamoto, K., Noda, H., & Shinoda, T. (2014). Transcriptome analysis of neuropeptides and G-protein coupled receptors (GPCRs) for neuropeptides in the brown planthopper *Nilaparvata lugens. Peptides, 53*, 125–133.

Todd, J. C., Ammar, E.-D., Redinbaugh, M. G., Hoy, C., & Hogenhout, S. A. (2010). Plant host range and leafhopper transmission of *Maize fine streak virus. Phytopathology, 100*, 1138–1145.

Toriyama, S. (1986). An RNA-dependent RNA-polymerase associated with the filamentous nucleoproteins of *Rice stripe virus. Journal of General Virology, 67*, 1247–1255.

Toriyama, S. (1987). Ribonucleic acid polymerase activity in filamentous nucleoproteins of *Rice grassy stunt virus. Journal of General Virology, 68*, 925–929.

Toriyama, S., & Watanabe, Y. (1989). Characterization of single-stranded and double-stranded RNAs in particles of *Rice stripe virus. Journal of General Virology, 70*, 505–511.

Toriyama, S., Akahashi, M., Sano, Y., Shimizu, T., & Ishihama, A. (1994). Nucleotide sequence of RNA-1, the largest genomic segment of *Rice stripe virus*, the prototype of the tenuiviruses. *Journal of General Virology, 75*, 3569–3579.

Toriyama, S., Kimishima, T., & Takahashi, M. (1997). The proteins encoded by rice grassy stunt virus RNA5 and RNA6 are only distantly related to the corresponding proteins of other members of the genus *Tenuivirus. Journal of General Virology, 78*, 2355–2363.

Toriyama, S., Kimishima, T., Takahashi, M., Shimizu, T., Minaka, N., & Akutsu, K. (1998). The complete nucleotide sequence of the rice grassy stunt virus genome and genomic comparisons with viruses of the genus *Tenuivirus. Journal of General Virology, 79*, 2051–2058.

Torres, R., Larenas, J. A., Fribourg, C., & Romero, J. (2012). Pepper necrotic spot virus, a new tospovirus infecting solanaceous crops in Peru. *Archives of Virology, 157*, 609–615.

Tsuda, S., Fujisawa, I., Ohnishi, J., Hosokawa, D., & Tomaru, K. (1996). Localization of *Tomato spotted wilt tospovirus* in larvae and pupae of the insect vector *Thrips setosus. Phytopathology, 86*, 1199–1203.

Uhrig, J. F., Soellick, T. R., Minke, C. J., Philipp, C., Kellmann, J. W., & Schreier, P. H. (1999). Homotypic interaction and multimerization of nucleocapsid protein of *Tomato spotted wilt tospovirus*: Identification and characterization of two interacting domains. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 55–60.

Ullman, D. E., Westcot, D. M., Mau, R. F. L., Cho, J. J., & Cluster, D. M. (1991). *Tomato spotted wilt virus* and one thrips vector: *Frankliniella occidentalis* (Pergande) internal morphology and virus location. *USDA Agricultural Research Service, 87*, 127–136.

Ullman, D. E., Cho, J. J., Mau, R. F. L., Wescot, D. M., & Custer, D. M. (1992). A midgut barrier to *Tomato spotted wilt virus* acquisition by adult western flower thrips. *Phytopathology, 82*, 1333–1342.

Ullman, D. E., Wescot, D. M., Cantone, F. A., Sherwood, J. L., & German, T. L. (1993a). Immunocytochemical evidence for *Tomato spotted wilt virus* (TSWV) replication in cells of the western flower thrips, *Frankliniella occidentalis* (Pergrande). *Phytopathology, 83*, 456–463.

Ullman, D. E., Sherwood, J. L., German, T. L., Westcot, D. M., Chenault, K. D., & Cantone, F. A. (1993b). Location and composition of cytoplasmic inclusions in thrips cells infected with *Tomato spotted wilt tospovirus* (TSWV). *Phytopathology, 83*, 1374.

Ullman, D. E., Westcot, D. M., Chenault, K. D., Sherwood, J. L., German, T. L., Bandla, M. D., Cantone, F. A., & Duer, H. L. (1995). Compartmentalization, intracellular transport, and autophagy of *Tomato spotted wilt tospovirus* proteins in infected thrips cells. *Phytopathology, 85*, 644–654.

Ullman, D. E., Sherwood, J. L., & German, T. L. (1997). Thrips as vectors of plant pathogens. In T. Lewis (Ed.), *Thrips as crop pests* (pp. 539–565). New York: CAB International.

van de Wetering, F., Goldbach, R., & Peters, D. (1996). *Tomato spotted wilt tospovirus* ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. *Phytopathology, 86*, 900–905.

van de Wetering, F., Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R., & Peters, D. (1998). Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females. *Entomologia Experimentalis et Applicata, 88*, 9–15.

van Knippenberg, I., Lamine, M., Goldbach, R., & Kormelink, R. (2005). Tomato spotted wilt virus transcriptase *in vitro* displays a preference for cap donors with multiple base complementarity to the viral template. *Virology, 335*, 122–130.

van Poelwijk, F., Kolkman, J., & Goldbach, R. (1996). Sequence analysis of the 5′ ends of Tomato spotted wilt virus N mRNAs. *Archives of Virology, 141*, 177–184.

Wang, Q., Yang, J., Zhou, G. H., Zhang, H. M., Chen, J. P., & Adams, M. J. (2010). The complete genome sequence of two isolates of *Southern rice black-streaked dwarf virus*, a new member of the genus *Fijivirus. Journal of Phytopathology, 158*, 733–737.

Wang, H., Xu, D., Pu, L., & Zhou, G. (2014). *Southern rice black-streaked dwarf virus* alters insect vectors' host orientation preferences to enhance spread and increase *Rice ragged stunt virus* co-infection. *Phytopathology, 104*, 196–201.

Webster, C. G., Reitz, S. R., Perry, K. L., & Adkins, S. (2011). A natural M RNA reassortant arising from two species of plant- and insect-infecting bunyaviruses and comparison of its sequence and biological properties to parental species. *Virology, 413*, 216–225.

Wei, T., Hibino, H., & Omura, T. (2009). Release of *Rice dwarf virus* from insect vector cells involves secretory exosomes derived from multivesicular bodies. *Communicative and Integrative Biology, 2*, 324–326.

Whitfield, A. E., Ullman, D. E., & German, T. L. (2004). Expression and characterization of a soluble form of *Tomato spotted wilt virus glycoprotein* G_N. *Journal of Virology, 78*, 13197–13206.

Whitfield, A. E., Ullman, D. E., & German, T. L. (2005). *Tomato spotted wilt virus* glycoprotein $G_{(C)}$ is cleaved at acidic pH. *Virus Research, 110*, 183–186.

Whitfield, A. E., Kumar, N. K. K., Rotenberg, D., Ullman, D. E., Wyman, E. A., Zietlow, C., Willis, D. K., & German, T. L. (2008). A soluble form of the *Tomato spotted wilt virus* (TSWV) glycoprotein G_N (G_N-S) inhibits transmission of TSWV by *Frankliniella occidentalis*. *Phytopathology, 98*, 45–50.

Whitfield, A. E., Rotenberg, D., Aritua, V., & Hogenhout, S. A. (2011). Analysis of expressed sequence tags from *Maize mosaic rhabdovirus*-infected gut tissues of P*eregrinus maidis* reveals the presence of key components of insect innate immunity. *Insect Molecular Biology, 20*, 225–242.

Wijkamp, I., & Peters, D. (1993). Determination of the median latent period of two tospoviruses in *Frankliniella occidentalis*, using a novel leaf disk assay. *Phytopathology, 83*, 986–991.

Wijkamp, I., Almarza, N., Goldbach, R., & Peters, D. (1995). Distinct levels of specificity in thrips transmission of tospoviruses. *Phytopathology, 85*, 1069–1074.

Wijkamp, I., Goldbach, R., & Peters, D. (1996). Propagation of *Tomato spotted wilt virus* in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. *Entomologia Experimentalis et Applicata, 81*, 285–292.

Winter, S., Shahraeen, N., Koerbler, M., & Lesemann, D. E. (2006). Characterization of Tomato fruit yellow ring virus: A new Tospovirus species infecting tomato in Iran. *Plant Pathology, 55*, 287.

Wu, W., Zheng, L., Chen, H., Jia, D., Li, F., & Wei, T. (2014). Nonstructural protein NS4 of *Rice stripe virus* plays a critical role in viral spread in the body of vector insects. *PLoS One, 9*, e88636.

Xiong, R., Wu, J., Zhou, Y., & Zhou, X. (2008). Identification of a movement protein of the tenuivirus *Rice stripe virus. Journal of Virology, 82*, 12304–12311.

Xu, Y., Zhou, W., Zhou, Y., Wu, J., & Zhou, X. (2012a). Transcriptome and comparative gene expression analysis of *Sogatella furcifera* (Horvath) in response to *Southern rice black-streaked dwarf virus. PLoS One, 7*, e36238.

Xu, Y., Huang, L., Fu, S., Wu, J., & Zhou, X. (2012b). Population diversity of *Rice stripe virus-derived* siRNAs in three different hosts and RNAi-based antiviral immunity in *Laodelphax striatellus. PLoS One, 7*, e46238.

Xue, J., Bao, Y.-Y., Li, B.-L., Cheng, Y.-B., Peng, Z.-Y., Liu, H., Xu, H.-J., Zhu, Z.-R., Lou, Y.-G., Cheng, J.-A., & Zhang, C.-X. (2010). Transcriptome analysis of the brown planthopper *Nilaparvata lugens. PLoS One, 5*, e14233.

Yan, J., Tomaru, M., Takahashi, A., Kimura, I., Hibino, H., & Omura, T. (1996). P2 protein encoded by genome segment S2 of rice dwarf phytoreovirus is essential for virus infection. *Virology, 224*, 539–541.

Yang, L., Fu, Q., Hu, W.-B., Li, F., & Li, G. (2012). Transcriptome-based identification of enzymes involved in amino acid biosynthesis in the small brown plamthopper, *Laodelphax striatellus. Open Access Insect Physiology, 4*, 19–29.

Yao, J., Rotenberg, D., Afsharifar, A., Barandoc-Alviar, K., & Whitfield, A. E. (2013). Development of RNAi methods for *Peregrinus maidis*, the corn planthopper. *PLoS One, 8*, 70243.

Yao, M., Liu, X., Li, S., Xu, Y., Zhou, Y., Zhou, X., & Tao, X. (2014). Rice stripe tenuivirus NSvc2 glycoproteins targeted to the Golgi body by the N-terminal transmembrane domain and adjacent cytosolic 24 amino acids via the COP I- and COP II-dependent secretion pathway. *Journal of Virology, 88*, 3223–3234.

Yeh, S. D., & Chang, T. F. (1995). Nucleotide sequence of the N gene of Watermelon silver mottle virus, a proposed new member of the genus *Tospovirus. Phytopathology, 85*, 58–64.

Yu, H., Ji, R., Ye, W., Chen, H., Lai, W., Fu, Q., & Lou, Y. (2014). Transcriptome analysis of fat bodies from two brown planthopper (*Nilaparvata lugens*) populations with different virulence levels in rice. *PLoS One, 9*, e88528.

Zeigler, R. S., & Morales, F. J. (1990). Genetic determination of replication of *Rice hoja blanca virus* within its planthopper vector, *Sogatodes oryzicola. Phytopathology, 80*, 559–566.

Zhai, Y., Zhang, J., Sun, Z., Dong, X., He, Y., Kang, K., Liu, Z., & Zhang, W. (2013). Proteomic and transcriptomic analyses of fecundity in the brown planthopper *Nilaparvata lugens* (Stal). *Journal of Proteome Research, 12*, 5199–5212.

Zhang, C., Liu, Y., Liu, L., Lou, Z., Zhang, H., Miao, H., Hu, X., Pang, Y., & Qiu, B. (2008). Rice black streaked dwarf virus p 9–1, an a-helical protein, self-interacts and forms viroplasms in vivo. *Journal of General Virology, 89*, 1770–1776.

Zhang, F., Guo, H., Zheng, H., Zhou, T., Zhou, Y., Wang, S., Fang, R., Qian, W., & Chen, X. (2010). Massively parallel pyrosequencing-based transcriptome analyses of small brown planthopper (*Laodelphax striatellus*), a vector insect transmitting *Rice stripe virus* (RSV). *BMC Genomics, 11*, 303.

Zhang, C., Pei, X., Wang, Z., Jia, S., Guo, S., Zhang, Y., & Li, W. (2013a). The Rice stripe virus pc4 functions in movement and foliar necrosis expression in *Nicotiana benthamiana. Virology, 425*, 113–121.

Zhang, K. J., Zhu, W. C., Rong, X., Zhang, Y. K., Ding, X. L., Liu, J., Chen, D. S., Du, Y., & Hong, X. Y. (2013b). The complete mitochondrial genomes of two rice planthoppers, *Nilaparvata lugens* and *Laodelphax striatellus*: Conserved genome rearrangement in Delphacidae and discovery of new characteristics of atp8 and tRNA genes. *BMC Genomics, 14*, 417.

Zheng, Y. X., Chen, C. C., & Jan, F. J. (2011). Complete nucleotide sequence of Capsicum chlorosis virus isolated from Phalaenopsis orchid and the prediction of the unexplored genetic information of tospoviruses. *Archives of Virology, 156*, 421–432.

Zheng, L., Mao, Q., Xie, L., & Wei, T. (2014). Infection route of *Rice grassy stunt virus*, a tenuivirus, in the body of its brown planthopper vector, *Nilaparvata lugens* (Hemiptera: Delphacidae) after ingestion of virus. *Virus Research, 188*, 170–173.

Zhou, F., Pu, Y., Wei, T., Liu, H., Deng, W., Wei, C., Ding, B., Omura, T., & Li, Y. (2007). The P2 capsid protein of the nonenveloped *Rice dwarf phytoreovirus* induces membrane fusion in insect host cells. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 19547–19552.

Zhou, J., Kantartzi, S., Wen, R. H., Newman, M., Hajimorad, M., Rupe, J., & Tzanetakis, I. (2011). Molecular characterization of a new tospovirus infecting soybean. *Virus Genes, 43*, 289–295.

Zhou, S.-S., Sun, Z., Ma, W., Chen, W., & Wang, M. (2014). De novo analysis of the *Nilaparvata lugens* (Stal) antenna transcriptome and expression patterns of olfactory genes. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 9*, 31–39.

Interactions Between the Whitefly *Bemisia tabaci* and Begomoviruses: Biological and Genomic Perspectives

Murad Ghanim and Henryk Czosnek

Abstract Begomoviruses are an emerging group of plant viruses, exclusively transmitted by the whitefly *Bemisia tabaci* in a persistent-circulative manner. Despite the economic importance of both, very little is known about begomovirus-whitefly interactions. Specific topics of interest that have been a subject of intensive research during the last decade include the route of the virus in the insect organs and cells, the influence of the virus on the insect's behavior and transcriptome, the proteins that mediate begomovirus translocation and the role of bacterial symbionts in this phenomenon. These topics are summarized and discussed in this chapter.

1 Introduction

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an insect that causes tremendous damages to agricultural crops and ornamentals, because of its feeding habits and because it transmits many plant viruses (Stansley and Naranjo 2010). *B. tabaci* was first reported to be a serious insect pest in the late 1920s in Northern India (Misra and Lamba 1929) and is now globally distributed in all continents, except in Antarctica (Martin et al. 2000). *B. tabaci* develops into several stages before adult emerge within 3–4 weeks. A single female may lay 200–400 eggs during her lifetime. The eggs hatch in 5–10 days and first instar nymphs called crawlers, stroll a short distance until they cease moving. Then, they mature into three nymphal stages (2nd, 3rd and 4th instar) until the pupal stage. Within a week,

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young adult emerge, which may live up to 5 weeks. Adults are 1–2 mm long and have opaque shaped wings and a body dusted with wax. Unfertilized eggs give raise to males (haploid) and fertilized eggs to females (diploid), a phenomenon called Arrhenotoky.

B. tabaci can damage plants in several ways. Because they feed on sap, whiteflies may reduce vigor and yield of mature plants and even can cause death of seedlings. Adult insects excrete honeydew, which sticks to cotton bolls and serve as nutrient for sooty mold, reducing the value of the fibers. Feeding by immature *B. tabaci* has been associated with tomato irregular ripening and squash silverleaf disorder. Nonetheless, the most serious damages caused by *B. tabaci* is due to its ability to vector viruses, begomoviruses being the virus family with the highest economic impact by far. Among them the *Tomato yellow leaf curl virus* (TYLCV) complex is culminating (Czosnek 2007). TYLCV (Begomovirus, Geminiviridae) is the causative agent of tomato yellow leaf curl disease (TYLCD), which thrives in tropical and subtropical regions, resulting in crop losses of up to 100 %. In susceptible tomatoes, symptoms include severe stunting, marked reduction in leaf size, upward cupping, chlorosis of leaf margins, mottling and flower abscission and significant yield reduction. The severity of the viral epidemic correlates with the proportion of the whitefly population that vectors TYLCV (Czosnek and Ghanim 2011). Application of insecticides against *B. tabaci* populations in the field and greenhouses is the most commonly used strategy to manage TYLCD. The first TYLCV tolerant tomato cultivars were developed in the late 1970s (Pilowski and Cohen 1974), by introgressing resistant traits from wild tomato species into susceptible cultivars.

Although *B. tabaci* has been known for many years as a virus vector, only recently have the tools been developed that allowed to open a broad window and shed light on the interactions between the insect, the virus it vectors and the host plant on the whole genome scale. Indeed, the last decade has witnessed the development of tools that allow analyzing the *B. tabaci* genome (including its endosymbiotic bacteria) and transcriptome, and the expression of selected genes. They include the construction of cDNA libraries from *B. tabaci* adults and from developmental stages, the use of microarrays with spotted cDNA and oligonucleotides, the sequencing of the transcriptome and proteome sequencing, and gene silencing. In this chapter, we and others in this Book (see Wang and Liu), are summarizing and discussing the current knowledge on the interactions between the *B. tabaci* vector, TYLCV, and the virus host plant.

2 *Bemisia tabaci* and Its Status as a Global Agricultural Pest

2.1 Discovery of an Insect Pest

B. tabaci was first described in 1889 as a tobacco pest in Greece and named *Aleyrodes tabaci* in 1889 (Gennadius 1889) and *Bemisia tabaci* in 1957 (Russell 1957). Comprehensive recent reviews of the *B. tabaci* history and its cryptic species

status are available (Gill and Brown 2010; Firdaus et al. 2013; Boykin et al. 2013). *B. tabaci* is a highly polyphagous insect that feeds on over 700 plant species from 86 families, including a large number of agriculturally and industrially important crops (Jones 2003). The whitefly's major impact on agriculture is due to the fact that this insect serves as a vector of more than 110 plant viruses, many of which have great impact on plants and agricultural production, including *Begomovirus, Crinivirus, Closterovirus, Carlavirus* and *Torradovirus* (Navas-Castillo et al. 2011). Among whitefly transmitted viruses, 90 % belong to the *Begomovirus* genera, which include approximately 200 species (Brown et al. 2014), and which are considered as the most threatening to agricultural crops. It was estimated that 20 million hectares of crops and 15 million farmers are directly affected by this insect (Anderson and Morales 2005).

2.2 Bemisia tabaci*, a Cryptic Species Complex*

B. tabaci is considered as a cryptic species complex (or biotypes), with individuals differing in their adaptability to hosts, resistance to chemicals, and in their ability to transmit begomoviruses (Brown 2000; Jiu et al. 2007; Crowder et al. 2010; Gorman et al. 2010). As early as the 1950s, biotypes were proposed to characterize the morphologically indistinguishable populations of *B. tabaci* on the basis of host range, host-plant adaptability and plant-virus transmission capabilities (Bird and Maramorosch 1978; Costa and Russel 1975). During the 1980s, the A biotype became a serious problem in cotton and cucurbits in the southwestern US and Mexico. In 1991, the introduced B biotype displaced the A biotype in the Southwestern US. In the same year, it was suggested that A and B biotypes were separate species; the new B was classified as a separate species, coined *B. argentifolii* (Perring et al. 1993). Allozymes and random amplified polymorphic cDNA-polymerase chain reaction (RAPD-PCR) were used to differentiate A and B biotypes (Perring et al. 1993; Gawel and Bartlett 1993).

As the different members of *B. tabaci* species complex are morphologically indistinguishable, various molecular methods have been applied over the past two decades to delimit the members of this species complex. The most popular techniques and the types of DNA markers used to study *B. tabaci* include sequence characterized amplified regions (SCAR) (Chu et al. 2004), cleaved amplified polymorphic sequences or restriction fragment length polymorphisms (CAPS/RFLP) (Ma et al. 2009), amplified fragment length polymorphisms (AFLP) (Cervera et al. 2000), mitochondrial cytochrome oxidase 1 (mtCO1) (Frohlich et al. 1999; Liu et al. 2012; Lee et al. 2013), nuclear ribosomal internal transcribe spacer 1 (ITS1) (De Barro et al. 2000), and microsatellites (De Barro et al. 2003; Wang et al. 2014).

Using CO1based Bayesian phylogenetic analysis and sequence divergence, a speciation system on the basis of a demarcation criterion of a 3.5 % divergence threshold was proposed (Dinsdale et al. 2010), which was raised to 4 % (Lee et al. 2013). Following the above criteria, 37 morphologically indistinguishable species

(Africa, Asia I, Asia II 1, Asia II 2, Asia II 3, Asia II 4, Asia II 5, Asia II 6, Asia II 7, Asia II 8, Asia II 9, Asia II 10, Asia II 11, Asia II 12, Asia III, Asia IV, Australia, Australia/Indonesia, China 1, China 2, China 3, China 4, Indian Ocean, Middle East Asia Minor (MEAM) I, Middle East Asia Minor II, Mediterranean (MED), New World 1, New World 2, Japan 1, Japan 2, Uganda, Italy 1, Sub Saharan Africa 1, Sub Saharan Africa 2, Sub Saharan Africa 3, Sub Saharan Africa 4, Sub Saharan Africa 5) have been currently delimited at the global level (Dinsdale et al. 2010; Boykin et al. 2013). Among the *B. tabaci* species complex, the most important biotypes worldwide are B and Q, recently termed as the MEAM1 and MED species, respectively (Brown et al. 1995; De Barro et al. 2011).

Comparative studies on whitefly biotypes/cryptic species and the begomoviruses they transmit (based on the virus coat protein sequence) showed a clear parallel grouping indicating that begomoviruses and their whitefly vector are grouped in similar patterns according to their geographic origin (Brown 2007; Brown and Czosnek 2002).

3 Tomato Yellow Leaf Curl Virus (TYLCV)

3.1 Tomato Yellow Leaf Curl Disease

TYLCV causes one of the most devastating diseases affecting tomato cultures, worldwide. Besides tomato, TYLCV is capable of infecting more than thirty plant species, including vegetables, ornamentals, weeds and wild plant species. In nature, the virus is transmitted exclusively by the whitefly *B. tabaci*. The TYLCV disease is usually managed by frequent applications of insecticides to contain the whitefly populations in fields and greenhouses. Breeding tomato cultivars resistant to TYLCV has started in the late 1970s. It has consisted in introgressing resistant traits found in wild tomato species into cultivated varieties (Vidavski et al. 2008). Several tomato cultivars are commercially available, which present excellent levels of resistance, satisfactory yields and good fruit quality. Breeding for TYLCV resistance has been aided by the use of molecular markers (Zamir et al. 1994) and the discovery of the first TYLCV resistance gene (Verlaan et al. 2013).

3.2 Tomato Yellow Leaf Curl, a Family of Begomoviruses

Geminiviruses constitute the most important class of pathogens transmitted by *B. tabaci.* They are small plant viruses characterized by a 22×38 nm geminate particle consisting of two joined incomplete icosahedra encapsidating a single-stranded genome of approximately 2800 nucleotides (Zhang et al. 2001). *Tomato yellow leaf curl virus* (TYLCV) is the generic name given to a begomovirus that devastates

tomato cultures worldwide. The TYLCV complex includes several species and numerous isolates distinguishable by their sequence (Brown et al. 2014). Unlike most begomoviruses, which possess two genomic components denominated DNA-A and DNA-B (bipartite), the TYLCV species have a single DNA-A-like genome component (monopartite) (Navot et al. 1991). The virion-sense strand comprises two genes, V1 and V2, while the complementary-sense strand comprises four genes, C1 to C4). The role of the proteins encoded by the TYLCV genome has been summarized elsewhere (Diaz-Pendon et al. 2010). V1 encodes the coat protein (CP), which is essential for cell-to-cell movement, systemic infection and transmission by the whitefly vector. V2 encodes a multi-functional protein involved in virus movement, in the suppression of post-transcriptional gene silencing (PTGS) and in the suppression of methylation-mediated transcriptional gene silencing (TGS). C1 encodes a replication-associated protein (Rep) which initiates viral replication. C2 is the transcriptional activator protein (TrAP), which interferes with transcriptional gene silencing and PTGS. C3 encodes the replication enhancer protein (REn) involved in viral replication. C4 counteracts PTGS. A non-coding 200–300 nucleotide-long intergenic region (IR) located upstream the V2 and C1 genes contains a conserved stem-loop structure embedding the origin of replication and signals necessary for the replication and transcription of the viral genome in host plants.

4 *B. tabaci*-TYLCV Biological Interactions

4.1 Circulation of TYLCV in Its B. tabaci *Vector*

Young leaves and apices are the best target for whitefly-mediated inoculation (Ber et al. 1990). In these tissues, the viral DNA replicates at the site of inoculation and is transported first to the roots then to the shoot apex, and finally to the neighboring leaves. Inoculation of the oldest leaves and cotyledons is inefficient. With the development of techniques such as PCR and quantitative PCR (qPCR), it is now possible to detect and quantify viral molecules in individual insects. It is also possible to visualize the virus in the insect using anti-CP antibodies and fluorescent Cy-3-labeled second antibody.

While feeding on a tomato plant, the stylets of *B. tabaci* follow a convoluted path before reaching the phloem from where begomoviruses are acquired (Pollard 1955). During begomovirus circulation in the vector, the viral CP is hypothesized to interact with insect receptors and chaperons present along the viral path. Once acquired, virions pass along the food canal in the stylet and reach the esophagus, and reach the guts. Virions can cross to the haemolymph via the filter chamber, a structure that combines tissue from the midgut, hindgut, and the caeca. It is hypothesized that most TYLCV virions are absorbed from the filter chamber into the haemolymph, while a minority of the virions circulate into the descending then the ascending midguts, and cross the midgut epithelial cells to the haemolymph. Microscopic

studies have shown extensive location of TYLCV virions in the filter chamber area, and their concentration decreases toward the descending and the ascending midguts (Medina et al. 2006; Ghanim and Medina 2007; Skaljac and Ghanim 2010; Cicero and Brown 2012). Unlike aphids and luteoviruses, TYLCV virions cross the epithelial cells in the midgut and not hindgut, and the specificity resides in this area of the digestive system (Czosnek et al. 2002). In the haemolymph, TYLCV virions interact with a 63 KDa GroEL protein produced by the primary endosymbiotic bacteria of *B. tabaci,* which protects the virions from proteolysis by the insect's immune system (Morin et al. 1999, 2000; Gottlieb et al. 2010). Virions cross the first barrier of the digestive system into the haemolymph (Ghanim et al. 2001a). A second recognition barrier is thought to reside on the apical membrane of the primary salivary gland of *B. tabaci* (Fig. 1) (Brown and Czosnek 2002), unlike the aphid-luteovirus system in which recognition resides in the accessory salivary glands (Gildow and Gray 1993). Specific secretory cells around the *B. tabaci* primary salivary gland secretory region determine the recognition, retention and transmission of begomoviruses (Wei et al. 2014).

4.2 *Parameters of TYLCV Acquisition, Retention and Transmission in Its* B. tabaci *Vector*

Based on biological tests, the parameters of acquisition, retention, and transmission of a begomovirus were first defined for TYLCV from Israel (Cohen and Harpaz 1964). The reported minimum acquisition access period (AAP) and inoculation access period (IAP) of Middle Eastern TYLCV isolates varied from 15–60 min and from 15–30 min, respectively (reviewed by Czosnek and Ghanim 2011). Similar values were reported for *Tomato yellow leaf curl Sardinia virus* TYLCSV from Italy

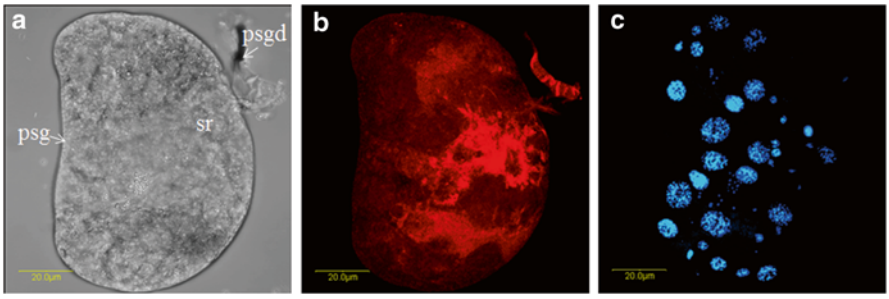

Fig. 1 Immunolocalization of TYLCV in the primary salivary glands (*psg*) of *Bemisia tabaci* following acquisition of the virus from infected plants for several days. (**a**) Light micrograph showing the one psg, the secretory region (*sr*) and the primary salivary gland duct (*psgd*). Immunolocalization was performed using first antibody against the virus coat protein and secondary antibody attached to Cy3 dye (*red*). (**b**) Dark field showing TYLCV localization to the secretory region of the glands and (**c**) DAPI staining of the nuclei (*blue*)

(Caciagli et al. 1995) and *Tomato leaf curl Bangalore virus* (ToLCBV-In) from India (Muniyappa et al. 2000). Southern blot hybridization showed that that whiteflies (B biotype) that had access to the same tissues for the same period of time could acquire variable amounts of viral DNA (Zeidan and Czosnek 1991). PCR allowed detecting TYLCV DNA in 20 % of the individuals collected 5 min after the beginning of the AAP and in all insects 5 min thereafter (Atzmon et al. 1998). Analysis of the electronic waveforms produced during insect feeding indicated that following a short probing period, the minimum phloem contact threshold period was 1.8 min for successful inoculation of TYLCV (Jiang et al. 2000).

A single insect is able to infect a tomato plant with TYLCV following a 24 h AAP. Gender and age affect transmission ability (Czosnek et al. 2001). Nearly all the 1–2 week-old adult females from synchronized populations of adult *B. tabaci* were able to infect tomato plants after a 24 h IAP, following a 48 h AAP. In comparison, only around 20 % of the males of the same age under the same conditions were able to infect plants. Infection capability decreased with age. While 60 % of the 3 week-old females infected plants, the males were totally unable to infect tomato plants. Only 20 % of the 6 week-old females were able to infect tomato plants. Aging insects acquire fewer viruses than younger individuals (Rubinstein and Czosnek 1997). Transmission efficiency of the Q biotype is not essentially different from that of the B. Transmission of a TYLCSV isolate from Murcia, Spain (TYLCSV-ES) was studied using the B, Q and S biotypes of *B. tabaci* (Jiang et al. 2004). Both B and Q-biotypes of *B. tabaci w*ere able to transmit TYLCSV-ES from infected tomato plants to *Solanum nigrum* and *Datura stramonium* and *vice versa.* No significant difference was found in transmission efficiency from infected tomato plants to weed plants between the B- and Q-biotypes. The S-biotype could not survive on tomato long enough to acquire or transmit TYLCSV-ES. In these studies, the age and gender of the whiteflies was not taken into account.

After 48 h AAP, begomoviruses are retained in their whitefly vector for several weeks and sometimes for the entire life of the insect. SLCV and TYLCV remain associated with *B. tabaci* during the entire life of the vector (Rubinstein and Czosnek 1997) while TYLCSV is undetectable after approximately 20 days (Caciagli and Bosco 1997). Investigation on viral transmission and retention suggest that the viral DNA remained associated with the insects for much longer than transmission ability. For instance, TYLCSV DNA was detectable up to 20 days after the end of the 48 h AAP whereas transmission could occur only for up to 8 days (Caciagli et al. 1995). TYLCV DNA and CP are not retained in *B. tabaci* for the same time periods. Following the end of the 48 h AAP, TYLCV DNA was detected throughout the 5 week life span of the insect while the amount of TYLCV CP steadily decreased until it was undetectable at day 12 (Rubinstein and Czosnek 1997). The disappearance of the virus CP was associated with a rapid decrease in the whitefly infectivity. Besides, TYLCV was retained for much shorter time in the non-vector *Trialeurodes vaporariorum* than in the *B. tabaci* vector (Czosnek et al. 2002). TYLCV DNA was detected in *T. vaporariorum* only during the first 6 h that followed the end of the AAP, and the CP for up to 4 h. In a recent study, the parameters of acquisition, retention and transmission of TYLCV from infected tomatoes by male and female

whiteflies (B and Q from China) were revisited (Ning et al. 2015). Q females acquired and transmitted TYLCV more efficiently than Q males, and more efficiently than B females and males. TYLCV acquisition and transmission efficacy of B males and females was similar. Electrical penetration graphs showed that B and Q females fed better on phloem sap than B and Q males. The epidemics of TYLCV in China was related to the high capacity of Q whiteflies to transmit TYLCV (Ning et al. 2015).

4.3 The Path of TYLCV in Its B. tabaci *Vector*

TYLCV is vectored by *B. tabaci* in a persistent circulative manner (Ghanim et al. 2001a). Once ingested by whiteflies, begomoviruses translocate in the insect digestive tract, penetrate the gut membranes into the haemolymph and cross the epithelial cells of the whitefly digestive tract which bridge between the gut lumen and the haemolymph (Ghanim et al. 2001b). From there, begomoviral particles reach the salivary systems and finally enter the salivary duct from where they are egested with the saliva. Translocation of begomoviruses from the digestive tract to the haemolymph and from the haemolymph to the salivary gland is thought to be mediated by still un-identified receptors. Rate of translocation of TYLCV in the circulative transmission has been reported (Ghanim et al. 2001a) by using PCR on dissected organs. TYLCV DNA was first detected in the head of *B. tabaci* after a 10-min AAP. The virus was present in the midgut after 40 min and was first detected in the haemolymph after 90 min. TYLCV was found in the salivary glands 5.5 h after it was first detected in the haemolymph. The velocity of *Squash leaf curl virus* SLCV (a bipartite begomovirus) translocation in *B. tabaci* was similar (Rosell et al. 1999). The TYLCV CP was immunodetected in the stylets, associated mainly with the food canal all along the lumen. Similarly, TYLCV was immunolocalized to the proximal part of the descending midgut, the filter chamber and the distal part of the descending midgut and in the primary salivary glands, suggesting that at least part of the virus is moving as a virion (Brown and Czosnek 2002; Czosnek et al. 2002) (Figs. 1 and 2). Similarly, TYLCSV has been detected in the midgut, microvilli, and in the cytoplasm of the primary salivary gland cells (Ghanim and Medina 2007).

4.4 The Role of Whitefly Endosymbiotic Bacteria in TYLCV Transmission

Whitefly bacterial endosymbiont proteins are involved in begomovirus circulative transmission in the whitefly body (Morin et al. 1999). Like other phloem-feeder insects, *B. tabaci* harbors a diverse fauna of endosymbionts (Baumann 2005), including the primary endosymbiont *Portiera aleyrodidarum*, and several other facultative

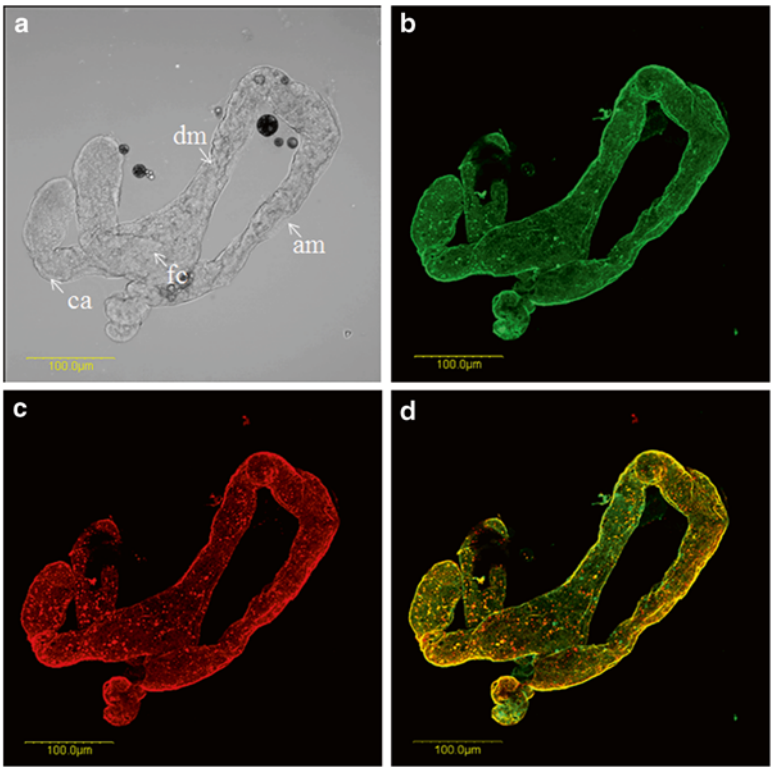

Fig. 2 Co-immunolocalization of TYLCV and HSP70 in the midgut of *Bemisia tabaci* following acquisition of the virus from infected plants for several days. (**a**) Light micrograph showing the midgut with the filter chamber (*fc*), ascending (*am*) and descending (*dm*) midguts and the caeca (*ca*). (**b**–**d**) Co-immunolocalization of TYLCV CP and HSP70 using two-step immunostaining. First antibody against HSP70 protein and a Monkey anti-Rabbit secondary antibody attached to Cy2 (**b**, *green signal*) were used in the first step, and then anti-TYLCV CP first antibody followed by a Monkey anti-Rabbit secondary antibody attached to Cy3 (**c**, *red signal*) were used in the second step. (**d**) combined signals from (**b**, **c**) showing *yellow spots*, indicating co-localization of TYLCV CP and HSP70

secondary symbionts, including *Rickettsia*, *Hamiltonella*, *Wolbachia*, *Arsenophonus*, *Cardinium*, and *Fritschea* (Chiel et al. 2007). Nearly all secondary symbionts co-localize with the primary endosymbiont inside whitefly specialized cells called bacteriocytes, ensuring their vertical transmission. Whiteflies in different locations harbor different secondary symbionts. In Israel, the B biotype harbors *Hamiltonella,* and the Q biotype harbors *Wolbachia* and *Arsenophonus.* Both biotypes harbor *Rickettsia* and *Portiera* (the obligatory primary symbionts (Chiel et al. 2007).

A chaperone GroEL protein produced by *Hamiltonella* in *B. tabaci* B biotype was shown to be essential for the transmission of TYLCV to tomato test plants. This GroEL interacted with the TYLCV CP in the yeast two hybrid system and the interaction was confirmed by immuno-capture. Disturbing this interaction by feeding

insects with a GroEL antibody markedly reduced the ability of the treated whiteflies to infect tomato test plants (Morin et al. 1999). In contrast, other GroEL proteins produced by other secondary endosymbionts in both B and Q biotypes do not interact with the TYLCV CP, indicating that the secondary endosymbionts play a minor role, if at all, in TYLCV transmission (Gottlieb et al. 2010).

In contrast to the symbionts housed in the bacteriocytes, the facultative *B. tabaci* endosymbiont *Rickettsia* can be found free in the insect digestive tract. By comparing *B. tabaci* (B biotype) population infected and non-infected with *Rickettsia* (with otherwise the same endosymbiotic bacterial fauna), it was found that the infected *B tabaci* acquired more TYLCV than their non-infected counterparts. Fluorescence *in situ* hybridization (FISH) analysis of *Rickettsia*-infected midguts showed that high levels of the bacterium in the midgut resulted in higher virus concentrations in the filter chamber, a favored site for virus translocation in the circulative transmission pathway, whereas low levels of *Rickettsia* resulted in an even distribution of the virus in the midgut (Kliot et al. 2014). Hence, *Rickettsia*, by infecting the midgut, increases TYLCV transmission efficacy, adding further insights into the complex association between persistent plant viruses, their insect vectors, and microorganism tenants that reside within these insects.

4.5 Transovarial Transmission and Transmission During Mating

TYLCV DNA was detected by PCR in dissected ovaries and eggs of viruliferous whiteflies and in the insect progeny (Ghanim et al. 1998). The closely related TYLCSV was also found to be transmitted transovarially: the virus was detected in eggs and nymphs as well as in adults of the first generation progeny (Bosco et al. 2004). The vertical transmission of TYLCV and *Tomato yellow leaf curl china virus* (TYLCCNV) by the B and Q biotypes of *B. tabaci* was studied using virus isolates and whitefly colonies established in China (Wang et al. 2009). Virus DNA was detected in eggs and nymphs but not in the adults of the first generation progeny, except in the combination of TYLCV and Q biotype whitefly where about 3 % of the adults contained the virus DNA. These adults did not transmit the viruses to test plants.

TYLCV can be transmitted between *B. tabaci* B biotype males and females in a gender-dependent manner, in the absence of any other source of the virus (Ghanim and Czosnek 2000). TYLCV was transmitted from viruliferous males to non-viruliferous females and from viruliferous females to non-viruliferous males, but not between insects of the same sex. Following mating, TYLCV was first detected in the haemolymph of the recipient insects about 1.5 h after caging, but was detected neither in the midgut nor in the head at this time. From there, TYLCV followed the pathway associated with acquisition from infected plants and did not cross the gut membranes back into the digestive system. Hence it seems that TYLCV passes from one insect to another via the open blood circulative system of the sexual partners. Interestingly, TYLCV was not transmitted when individuals from the B biotypes

where caged with individuals from the Q biotype (Ghanim et al. 2007a), confirming that B and Q biotypes do not mate (Pascual and Callejas 2004). In addition, caging together *B. tabaci* and *T. vaporariorum*, two whitefly species that do not mate, confirmed that mating is obligatory for TYLCV transmission. The horizontal transmission of TYLCV and TYLCCNV by the B and Q biotypes of *B. tabaci* was studied (Wang et al. 2009). Both TYLCV DNA and TYLCCNV DNA were shown to be transmitted horizontally by each of the two biotypes of the whitefly, however the overall percentage of horizontal transmission for either TYLCCNV or TYLCV in each of the two whitefly biotypes was below 5 %.

4.6 TYLCV Replication in B. tabaci

Previous reports have suggested begomoviral transcription and replication in the insect. The increasing amounts of TYLCV DNA in *B. tabaci* fed on TYLCV-infected plants then on a virus non-host plant suggested that the virus is able to replicate in the insect (Czosnek et al. 2001). Transcripts from TYLCV and *Tomato mottle virus* (ToMoV) genes encoded by the virion sense strand and by the complementary-sense strand (the latter synthesized only during replication) where quantified in whiteflies feeding on cotton plants (a non-host) following virus acquisition on infected tomato plants (Sinisterra et al. 2005). While the levels of ToMoV transcripts rapidly decreased, the TYLCV transcripts steadily increased even after 7 d. There was no attempt to detect viral proteins in the insect vector. These results supported the hypothesis that TYLCV (but not ToMoV) may replicate in *B. tabaci*.

A recent study has shown that manipulating the physiological status of *B. tabaci* after acquisition and retention of TYLCV resulted in a continuous accumulation of viral DNA. After virus acquisition, stress conditions were imposed on the whitefly, and the levels of three viral gene sequences were measured over time. When whiteflies were exposed to TYLCV and treatment with two different pesticides, the virus levels continuously increased. Upon exposure to heat stress, the virus levels gradually decreased. Switching whiteflies between pesticide, heat-stress and control treatments caused fluctuating increases and decreases in virus levels. These results were confirmed using FISH analysis (Pakkianathan et al. 2015 in press).

5 *B. tabaci* Gene Expression

5.1 The Genome of B. tabaci *and Its Endosymbiotic Bacteria*

Although *B. tabaci* is such an important pest, the sequence of its genome has not been published yet. The nuclear DNA content of adult male and female *B. tabaci* was estimated by flow cytometry using standards of known genome size such as chicken red blood cells (1C = 1.50 pg), *Drosophila melanogaster* (1C = 0.40 pg) and

Arabidopsis thaliana (1C = 0.16 pg). The haploid DNA content of *B. tabaci* was 1C = 1.04 pg, suggesting that the insect genome is made up of approximately 1000 Mbp. This value indicates that *B. tabaci* has a genome about 5.5 times larger than that of *Drosophila*, six times larger than that of the honey bee *Apis mellifera*, four times that of the mosquito *Anopheles gambiae*, three times that of the aphid *Myzus persicae*, and twice that of the silkworm moth *Bombyx mori* (Brown et al. 2005), It is likely that the whitefly has a large portion of the genome which does not encode genes, and may have a relatively high proportion of highly repetitive DNA sequences. In a recent study, flow cytometry and k-mer analyses were used to estimate the genome size of the B and Q *B. tabaci* biotypes to be between 640 and 682 Mbp (Guo et al. 2015). These estimates differ probably because differences in reference standard and dyes.

The complete mitochondrial DNA genome (mitogenome) of the Asia I member of the *B. tabaci* complex was sequenced. Its 15,210 bp genome encodes 13 protein-coding genes, 22 transfer RNAs (tRNAs), 2 ribosomal RNA (rRNAs) and a 467 bp putative control region (Tay et al. 2014). A draft genome sequence of the *Rickettsia B. tabaci* MEAM1 strain was recently published (Rao et al. 2012). The *Rickettsia* genome has about 1.24 Mbp and encodes 1247 genes. The genome of the primary endosymbiont *Portiera aleyrodidarum* of the B and Q biotypes of *B. tabaci* was also sequenced (Jiang et al. 2012). The 351 Kbp circular molecule encode 36 RNA genes and 277 (B) to 281 (Q) protein-coding genes. The genome of B and Q share 99 % homology.

5.2 *Whitefly Proteins Involved in TYLCV Circulative Transmission*

Besides GroEL produced by the *B. tabaci* endosymbiotic bacteria (Morin et al., 2009), a small number of proteins have been involved in the circulative transmission of begomoviruses in their whitefly vector. A 16 kDa small heat shock protein (coined BtHSP16) was reported to bind to TYLCSV CP (Ohnesorge and Bejarano 2009). The CP- BtHSP16 interaction domain was located within the conserved region of the N-terminal part of the TYLCSV CP (amino acids 47–66), overlapping almost completely with the nuclear localization signal described for the CP of TYLCV (Kunik et al. 1998). The region necessary for transmission of TYLCSV by *B. tabaci* (amino acids 129–152) was not directly involved in the specific interaction between the CP and BtHSP16.

Microarray, real-time PCR and western blot analyses indicated that a *B. tabaci* (B biotype) gene encoding a heat shock protein 70 (*hsp70*) specifically responded to the acquisition and retention of TYLCV. Immunocapture PCR, immuno-precipitation and virus overlay protein binding assays showed *in vitro* interaction between TYLCV and HSP70. Immuno-localization showed co-localization of TYLCV and the bipartite *Watermelon chlorotic stunt virus* (WmCSV) virions and HSP70 protein

within *B. tabaci* midgut epithelial cells (Fig. 2). Membrane feeding of whiteflies with anti-HSP70 antibodies and TYLCV virions induced an increase in TYLCV transmission, suggesting that HSP70 inhibits virus transmission (Götz et al. 2012), a role that might be related to protection against begomoviruses deleterious effects in the whitefly (Rubinstein and Czosnek 1997).

5.3 Construction and Use of a Whitefly Spotted cDNA Microarray

A genomic project was launched in 2002 and has sequenced more than 20,000 Expressed Sequence Tags (ESTs) from adult whiteflies, as well as other developmental stages including nymphs, eggs, and viruliferous adults with TYLCV and ToMoV (Leshkowitz et al. 2006). This large-scale sequencing of ESTs from *B. tabaci* led to better understanding the genetic makeup of the whitefly relative to other insect models. Following this sequencing, a spotted DNA microarray containing 6,000 unique whitefly ESTs was developed and used to study the resistance capability of the whitefly to insecticides (Ghanim and Kontsedalov 2007), the immune response to the parasitoid wasp *Eretmocerus mundus* (Mahadav et al. 2008), and the reaction to heat stress in the B and the Q biotypes (Mahadav et al. 2009). The microarray was also used to identify genes involved in the adaptability of *B. tabaci* to plant secondary defense compounds, such as phenylpropanoids (Alon et al. 2012). An advanced version of this microarray, based on Agilent's technology, was used to study *B. tabaci* response to feeding on plants, response to modified contents of nicotine in tobacco plants, and response to the presence/absence of selected symbiotic bacteria.

5.4 Application of Gene Silencing to Study the Role of Whitefly Genes

Gene silencing is an exquisite method that allows to deplete the amount of transcripts of a target gene and to examine the lack of function in the manipulated organism (Mohr et al. 2014). A first study in *B. tabaci* has demonstrated that RNA interference (RNAi) is effective in downregulating target genes identified by microarray hybridization (Ghanim et al. 2007b). By injecting into the body cavity long dsRNA molecules, specifically directed against genes uniquely expressed in the midgut and salivary glands, the targeted mRNA amounts in the different organs were depleted up to 70 % compared to whiteflies injected with buffer or with a GFP-specific dsRNA. Phenotypic effects were observed in *B. tabaci* ovaries following dsRNA targeting the whitefly *Drosophila chickadee* homologue. The siRNA machinery of *B. tabaci* has been recently described. Sequence homology and

phylogenetic analysis revealed that RNAi machinery of whitefly is close to aphids (Upadhyay et al. 2013).

The effectiveness of gene silencing in whiteflies was demonstrated by developing a high throughput method to silence whitefly genes using a leaf-mediated dsRNA feeding method. This method was applied to explore the roles of genes within the molting hormone-ecdysone synthesis and signaling pathway for the survival, reproduction and development of whiteflies. Gene silencing reduced survival and delayed development of the whitefly during nymphal stages (Luan et al. 2013). These data showed that disruption of whitefly gene expression opens the door to new strategies aimed at curbing down the deleterious effects of this insect pest to agriculture.

5.5 B. tabaci *Transcriptome Studies*

The functional genomics in the *B. tabaci* complex is discussed in this Book by Wang and Liu. In brief, the transcriptome of several *B. tabaci* species (B, Q, ZHJ1) has been sequenced using the Roche 454 and the Illumina technologies and compared. The transcriptome of the begomovirus non-vector whitefly species *T. vaporariorum* is also available for comparison and for data-mining genes that may be involved in virus transmission. The transcriptomes of different organs such as the guts and the salivary glands are also available. Gene expression changes upon acquisition of begomoviruses and upon exposure to pesticides (susceptible and resistant whitefly populations) have been studied.

The ongoing transcriptomics studies reveal that a number of highly expressed genes that belong to different and conserved metabolic pathways with other insects are involved in virus transmission, insecticide resistance and immune responses to parasitoids. Taken together, the transcriptomics results collected so far provide not only a roadmap for further functional genomic studies and extensive whitefly research in general, but also a large collection of gene and EST sequences for future genome sequencing and annotation efforts.

6 Concluding Remarks and Future Perspective

The diversity among arthropod vectors and the viruses they transmit are expanding their economic importance worldwide. In particular, begomoviruses vectored by *B. tabaci* are causing the most devastating viral diseases in agricultural crops worldwide. While new and diverse pest control strategies are adopted for controlling whiteflies, they continue to be of great economic impact. Differences in plant host-preference, host range, fecundity, dispersal behavior, vector competency, phytotoxic feeding effects, endosymbiont composition, invasiveness, and insecticide resistance, are all among the factors that directly influenced the ability of *B. tabaci* to

become a worldwide top-rated pest. Research on TYLCV- plant and TYLCV- *B. tabaci* interaction have resulted in hundreds of research papers for understanding the biological, molecular and cellular events underlying these interactions.

Whitefly genomics research is expected to open important avenues into the discovery of novel strategies for whitefly and whitefly-transmitted virus management based on an improved understanding of molecular, cellular, and biological processes. The genome sequence of *B. tabaci* will synergize projects underway to develop and sequence *B. tabaci* expressed sequence tags (EST) or cDNA libraries for functional genomics and proteomics analysis. The benefits are far reaching and include their application to identify genes that cope with abiotic and biotic stresses that often lead to invasiveness and insecticide resistance, and to understand the basis for whitefly-virus specificity. Collectively, genomics, proteomics, and functional genomics efforts will initiate further local, regional, national and international partnerships to expand present and future efforts aimed at determining the *B. tabaci* genome and proceed to undertake functional genomics aspects that are of high interest amongst a broad user community.

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References

Alon, M., Elbaz, M., Ben-Zvi, M. M., Feldmesser, E., Vainstein, A., & Morin, S. (2012). Insights into the transcriptomics of polyphagy: *Bemisia tabaci* adaptability to phenylpropanoids involves coordinated expression of defense and metabolic genes. *Insect Biochemistry and Molecular Biology, 42*, 251–263.

Anderson, P. K., & Morales, F. J. (2005). *Whitefly and whitefly-borne viruses in the tropics: building a knowledge base for global action* (p. 351). Cali: CIAT.

Atzmon, G., van Hoss, H., & Czosnek, H. (1998). PCR-amplification of tomato yellow leaf curl virus (TYLCV) from squashes of plants and insect vectors: Application to the study of TYLCV acquisition and transmission. *European Journal of Plant Pathology, 104*, 189–194.

Baumann, P. (2005). Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annual Review of Microbiology, 59*, 155–189.

Ber, R., Navot, N., Zamir, D., Antignus, Y., Cohen, S., & Czosnek, H. (1990). Infection of tomato by the Tomato yellow leaf curl virus: susceptibility to infection, symptom development and accumulation of viral DNA. *Archives of Virology, 112*, 169–180.

Bird, J., & Maramorosch, K. (1978). Viruses and virus diseases associated with whiteflies. *Advances in Virus Research, 22*, 55–110.

Bosco, D., Mason, G., & Accotto, G. P. (2004). TYLCSV DNA, but not infectivity, can be transovarially inherited by the progeny of the whitefly vector *Bemisia tabaci* (Gennadius). *Virology, 323*, 276–283.

Boykin, L. M., Bell, C. D., Evans, G., Small, I., & De Barro, P. J. (2013). Is agriculture driving the diversification of the *Bemisia tabaci* species complex (Hemiptera: Sternorrhyncha: Aleyrodidae)?: Dating, diversification and biogeographic evidence revealed. *BMC Evolutionary Biology, 13*, 228.

Brown, J. K. (2000). Molecular markers for the identification and global tracking of whitefly vector-Begomovirus complexes. *Virus Research, 71*, 233–260.

Brown, J. K. (2007). The *Bemisia tabaci* complex: Genetic and phenotypic variation and relevance to TYLCV-vector interactions. In H. Czosnek (Ed.), *Tomato yellow leaf curl virus disease* (pp. 25–56). Dordrecht: Springer.

Brown, J. K., & Czosnek, H. (2002). Whitefly transmission of plant viruses. In R. T. Plumb (Ed.), *Advances in botanical research* (pp. 65–100). New York: Academic.

Brown, J. K., Frohlich, D. R., & Rosell, R. C. (1995). The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci*or a species complex? *Annual Review of Entomology, 40*, 511–534.

Brown, J. K., Lambert, G. M., Ghanim, M., Czosnek, H., & Galbraith, D. W. (2005). Nuclear DNA content of the whitefly *Bemisia tabaci* (Genn.) (Aleyrodidae: Homoptera/Hemiptera) estimated by flow cytometry. *Bulletin of Entomological Research, 95*, 309–312.

Brown, J. K., Zerbuni, F. M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J. C. F., Fiallo-Olivé, E., Briddon, R. W., Hernández-Zepeda, C., & Idris, A. (2014). Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. *Archives of Virology, 160*, 1593–1619.

Caciagli, P., & Bosco, D. (1997). Quantitation over time of tomato yellow leaf curl geminivirus DNA in its whitefly vector. *Phytopathology, 87*, 610–613.

Caciagli, P., Bosco, D., & Al-Bitar, L. (1995). Relationships of the Sardinian isolate of tomato yellow leaf curl geminivirus with its whitefly vector *Bemisia tabaci* Gen. *European Journal of Plant Pathology, 101*, 163–170.

Cervera, M. T., Cabezas, J. A., Simon, B., Martinez-Zapater, J. M., Beitia, F., & Cenis, J. L. (2000). Genetic relationships among biotypes of *Bemisia tabaci* Hemiptera. Aleyrodidae based on AFLP analysis. *Bulletin of Entomological Research, 90*, 391–396.

Chiel, E., Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Katzir, N., Inbar, M., & Ghanim, M. (2007). Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci. Bulletin of Entomological Research, 97*, 407–413.

Chu, D., Zhang, Y. J., Cong, B., Xu, B. Y., & Wu, Q. J. (2004). Developing sequence characterized amplified regions (SCARs) to identify *Bemisia tabaci* and *Trialeurodes vaporariorum. Plant Protection, 30*, 27–30.

Cicero, J. M., & Brown, J. K. (2012). Ultrastructural studies of the salivary duct system in the whitefly vector *Bemisia tabaci* (Aleyrodidae: Hemiptera). *Annals of the Entomological Society of America, 105*, 7010717.

Cohen, S., & Harpaz, I. (1964). Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius). *Entomologia Experimentalis et Applicata, 7*, 155–166.

Costa, H. S., & Russel, M. (1975). Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera, Aleyrodidae). *Ciencia e Cultura, 27*, 388–390.

Crowder, D. W., Horowitz, A. R., & De Barro, P. J. (2010). Mating behaviour, life history and adaptation to insecticides determine species exclusion between whiteflies. *Journal of Animal Ecology, 79*, 563–570.

Czosnek, H. (Ed.). (2007). *Tomato yellow leaf curl virus disease: Management, molecular biology, breeding for resistance* (p. 420). Dordrecht: Springer.

Czosnek, H., & Ghanim, M. (2011). Bemisia tabaci – Tomato yellow leaf curl virus interaction causing worldwide epidemics. In W. M. O. Thompson (Ed.), *Bemisia tabaci interaction with geminivirus-infected host plants*. The Netherlands: Springer.

Czosnek, H. G., Ghanim, M., Rubinstein, G., Morin, S., Fridman, V., & Zeidan, M. (2001). Whiteflies: Vectors, and victims (?), of geminiviruses. In K. Maramorosch (Ed.), *Advances in virus research* (Vol. 57). New York: Academic.

Czosnek, H., Ghanim, M., & Ghanim, M. (2002). Circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci*–insights from studies with *Tomato yellow leaf curl virus. Annals of Applied Biology, 140*, 215–231.

De Barro, P. J., Driver, F., Trueman, J. W. H., & Curran, J. (2000). Phylogenetic relationship of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. *Molecular Phylogenetics and Evolution, 16*, 29–36.

De Barro, P. J., Scott, K. D., Graham, G. C., Lange, C. L., & Schutze, M. K. (2003). Isolation and characterization of microsatellite loci in *Bemisia tabaci. Molecular Ecology Notes, 3*, 40–43.

De Barro, P. J., Liu, S. S., Boykin, L., & Dinsdale, A. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology, 56*, 1–19.

Diaz-Pendon, J. A., Cañizares, M. C., Moriones, E., Bejarano, E. R., Czosnek, H., & Navas-Castillo, J. (2010). Tomato yellow leaf curl viruses: Ménage a trios between the virus complex, the plant and the whitefly vector. *Molecular Plant Pathology, 11*, 441–450.

Dinsdale, A., Cook, L., Riginos, C., Buckley, Y. M., & De Barro, P. (2010). Refined global analysis of *Bemisia tabaci*(Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. *Annals of the Entomological Society of America, 103*, 196–208.

Firdaus, S., Vosman, B., Hidayati, N., Supena, E., Visser, R. G. F., & van Heusden, A. W. (2013). The *Bemisia tabaci* species complex: Additions from different parts of the world. *Insect Sci., 20*, 723–733.

Frohlich, D. R., Torres-Jerez, I., Bedford, I. D., Markham, P. G., & Brown, J. K. (1999). A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Molecular Ecology, 8*, 1683–1691.

Gawel, N. J., & Bartlett, A. C. (1993). Characterization of differences between whiteflies using RAPD-PCR. *Insect Molecular Biology, 2*, 33–38.

Gennadius, P. (1889). Disease of tobacco plantations in the Trikonia. The aleyrodid of tobacco. *Ellenike Georgia, 5*, 1–3.

Ghanim, M., & Czosnek, H. (2000). Tomato yellow leaf curl geminivirus (TYLCV-Is) is transmitted among whiteflies (*Bemisia tabaci*) in a sex-related manner. *Journal of Virology, 74*, 4738–4745.

Ghanim, M., & Kontsedalov, S. (2007). Gene expression in pyriproxyfen resistant *Bemisia tabaci* Q biotype. *Pest Management Science, 63*, 776–783.

Ghanim, M., & Medina, V. (2007). Localization of tomato yellow leaf curl virus in its whitefly vector *Bemisia tabaci*. In H. Czosnek (Ed.), *Tomato yellow leaf curl virus disease: Management, molecular biology, breeding for resistance* (pp. 171–183). Dordrecht: Springer.

Ghanim, M., Morin, S., Zeidan, M., & Czosnek, H. G. (1998). Evidence for transovarial transmission of tomato yellow leaf curl virus by its vector, the whitefly *Bemisia tabaci. Virology, 240*, 295–303.

Ghanim, M., Morin, S., & Czosnek, H. (2001a). Rate of Tomato yellow leaf curl virus (TYLCV) translocation in the circulative transmission pathway of its vector, the whitefly *Bemisia tabaci. Phytopathology, 91*, 188–196.

Ghanim, M., Rosell, R. C., Campbell, L. R., Czosnek, H., Brown, J. K., & Ullman, D. E. (2001b). Microscopic analysis of the digestive, salivary and reproductive organs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B. *Journal of Morphology, 248*, 22–40.

Ghanim, M., Sobol, I., Ghanim, M., & Czosnek, H. (2007a). Horizontal transmission of begomoviruses between *Bemisia tabaci* biotypes. *Arthropod-Plant Interactions, 1*, 195–204.

Ghanim, M., Kontsedalov, S., & Czosnek, H. (2007b). Tissue-specific gene silencing by RNA interference in the whitefly *Bemisia tabaci* (Gennadius). *Insect Biochemistry and Molecular Biology, 37*, 732–738.

Gildow, F. E., & Gray, S. M. (1993). The aphid salivary gland basal lamina as a selective barrier associated with vector-specific transmission of barley yellow dwarf luteovirus. *Phytopathology, 83*, 1293–1302.

Gill, R. J., & Brown, J. K. (2010). Systematics of Bemisia and Bemisia relatives: can molecular techniques solve the Bemisia tabaci complex conundrum – a taxonomist's viewpoint. In P. A. Stansley & S. E. Naranjo (Eds.), *Bemisia: Bionomics and management of a global pest*. Amsterdam: Springer.

Gorman, K., Slater, R., Blande, J., Clarke, A., Wren, J., McCaffery, A., & Denholm, I. (2010). Cross-resistance relationships between neonicotinoids and pymetrozine in *Bemisia tabaci* (Hemiptera:Aleyrodidae). *Pest Management Science, 66*, 1186–1190.

Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Kontsedalov, S., Skaljac, M., Brumin, M., Sobol, I., Czosnek, H., Vavre, F., Fleury, F., & Ghanim, M. (2010). The transmission efficiency of *Tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. *Journal of Virology, 84*, 9310–7.

Götz, M., Popovski, S., Kollenberg, M., Gorovitz, R., Brown, J. K., Cicero, J., Czosnek, H., Winter, S., & Ghanim, M. (2012). Implication of *Bemisia tabaci* heat shock protein 70 in begomovirus–whitefly interactions. *Journal of Virology, 86*, 13241–13252.

Guo, L. T., Wang, S. L., Wu, Q. J., Zhou, X. G., Xie, W., & Zhang, W. J. (2015). Flow cytometry and K-mer analysis estimates of the genome sizes of *Bemisia tabaci* B and Q (Hemiptera:Aleyrodidae). *Frontiers in Physiology*. doi:10.3389/fphys.2015.00144.

Jiang, Y. X., De Blas, C., Barrios, L., & Fereres, A. (2000). A correlation between whitefly (Homoptera: Aleyrodidae) feeding behaviour and transmission of Tomato yellow leaf curl virus. *Annals of the Entomological Society of America, 93*, 573–579.

Jiang, Y. X., de Blas, C., Bedford, I. D., Nombela, G., & Muñiz, M. (2004). Effect of *Bemisia tabaci* biotype in the transmission of *tomato yellow leaf curl sardinia virus* (TYLCSV-ES) between tomato and common weeds. *Spanish Journal of Agricultural Research, 2*, 115–119.

Jiang, Z.-F., Xia, F., Johnson, K. W., Bartom, E., Tuteja, J. H., Stevens, R., Grossman, R. L., Brumin, M., White, K. P., & Ghanim, M. (2012). Genome sequences of the primary endosymbiont "*Candidatus Portiera aleyrodidarum*" in the whitefly *Bemisia tabaci* B and Q biotypes. *Journal of Bacteriology, 194*, 7778–6679.

Jiu, M., Zhou, X. P., Tong, L., Xu, J., Yang, X., Wan, F. H., & Liu, S.-S. (2007). Vector-virus mutualism accelerates population increase of an invasive whitefly. *PloS One, 2*, e182.

Jones, D. R. (2003). Plant viruses transmitted by whiteflies. *European Journal of Plant Pathology, 109*, 195–219.

Kliot, A., Cilia, M., Czosnek, H., & Ghanim, M. (2014). Implication of the bacterial endosymbiont *Rickettsia* spp. in the whitefly *Bemisia tabaci* interactions with *Tomato yellow leaf curl virus*. *Journal of Virology, 88*, 5652–5660.

Kunik, T., Palanichelvam, K., Czosnek, H., Citovsky, V., & Gafni, Y. (1998). Nuclear import of a geminivirus capsid protein in plant and insect cells: Implications for the viral nuclear entry. *The Plant Journal, 13*, 121–129.

Lee, W., Park, J., Lee, G., Lee, S., & Akimoto, S. (2013). Taxonomic status of the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) and reassessment of the number of its constituent species. *PloS One, 8*, e63817.

Leshkowitz, D., Gazit, S., Reuveni, E., Ghanim, M., Czosnek, H., McKenzie, C., Shatters, R. G., Jr., & Brown, J. K. (2006). Whitefly (*Bemisia tabaci*) genome project: analysis of sequenced clones from egg, instar, and adult (viruliferous and non-viruliferous) cDNA libraries. *BMC Genomics, 7*, 79.

Liu, S. S., Colvin, J., & De Barro, P. J. (2012). Species concepts as applied to the whitefly *Bemisia tabaci* systematics: How many species are there? *J Integr Agric, 11*, 176–186.

Luan, J.-B., Ghanim, M., Liu, S.-S., & Czosnek, H. (2013). Silencing the ecdysone (synthesis and signaling) pathway genes disrupts nymphal development in the whitefly. *Insect Biochemistry and Molecular Biology, 43*, 740–746.

Ma, D. Y., Li, X. C., Dennehy, T. J., Lei, C. L., Wang, M., et al. (2009). Utility of mtCO1 polymerase chain reaction-restriction fragment length polymorphism in differentiating between Q and B whitefly *Bemisia tabaci* biotypes. *Insect Sci Appl., 16*, 107–114.

Mahadav, A., Gerling, D., Gottlieb, Y., Czosnek, H., & Ghanim, M. (2008). Gene expression in the whitefly *Bemisia tabaci* pupae in response to parasitization by the wasp *Eretmocerus mundus*. *BMC Genomics, 9*, 342.

Mahadav, A., Kontsedalov, S., Czosnek, H., & Ghanim, M. (2009). Thermotolerance and gene expression following heat stress in the whitefly *Bemisia tabaci* B and Q biotypes. *Insect Biochemistry and Molecular Biology, 39*, 668–676.

Martin, J. H., Mifsud, D., & Rapisarda, C. (2000). The whiteflies (Hemiptera: Aleyrodidae) of Europe and the Mediterranean Basin. *Bulletin of Entomological Research, 90*, 407–448.

Medina, V., Pinner, M. S., Bedford, I. D., Achon, M. A., Gemeno, C., & Markham, P. G. (2006). Immunolocalization of tomato yellow leaf curl Sardinia virus in natural host plants and its vector *Bemisia tabaci. Journal of Plant Pathology, 88*, 299–308.

Misra, C. S., & Lamba, S. K. (1929). The cotton whitefly (*Bemisia gossypiperda* n. sp.). *Bulletin of Agriculture Research Institute, Pusa, 196*, 1–7.

Mohr, S. E., Smith, J. A., Shamu, C. R., Neumüller, R. A., & Perrimon, N. (2014). RNAi screening comes of age: Improved techniques and complementary approaches. *Nature Reviews Molecullar cell Biology, 15*, 591–600.

Morin, S., Ghanim, M., Zeidan, M., Czosnek, H., Verbeek, M., & van den Heuvel, J. F. J. M. (1999). A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of Tomato yellow leaf curl virus. *Virology, 256*, 75–84.

Morin, S., Ghanim, M., Sobol, I., & Czosnek, H. (2000). The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and non-transmissible begomoviruses in the yeast two-hybrid system. *Virology, 276*, 404–416.

Muniyappa, V., Venkatesh, H. M., Ramappa, H. K., Kulkarni, R. S., Zeidan, M., Tarba, C.-Y., Ghanim, M., & Czosnek, H. (2000). Tomato leaf curl virus from Bangalore (ToLCV-Ban4): Sequence comparison with Indian ToLCV isolates, detection in plants and insects, and vector relationships. *Archives of Virology, 145*, 1583–1598.

Navas-Castillo, J., Fiallo-Olive, E., & Sanchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology, 49*, 219–248.

Navot, N., Pichersky, E., Zeidan, M., Zamir, D., & Czosnek, H. (1991). Tomato yellow leaf curl virus: A whitefly-transmitted geminivirus with a single genomic component. *Virology, 185*, 151–161.

Ning, W., Shi, X., Liu, B., Pan, H., Wei, W., Zeng, Y., Sun, X., Xie, W., Wang, S., Wu, Q., Cheng, J., Peng, Z., & Zhang, Y. (2015). Transmission of *Tomato yellow leaf curl virus by Bemisia tabaci* as affected by whitefly sex and biotype. *Scientific Reports, 5*, 10744. doi:10.1038/srep10744.

Ohnesorge, S., & Bejarano, E. R. (2009). Begomovirus coat protein interacts with a small heat-shock protein of its transmission vector (*Bemisia tabaci*). *Insect Molecular Biology, 18*, 693–703.

Pakkianathan, B. C., Kontsedalov, S., Lebedev, G., Mahadav, A., Zeidan, M., Czosnek, H., & Ghanim, M. (2015). Replication of *Tomato yellow leaf curl* in its whitefly vector *Bemisia tabaci. Journal of Virology, 89*, 9791–9803, accepted.

Pascual, S., & Callejas, C. (2004). Intra- and interspecific competition between biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Spain. *Bulletin of Entomological Research, 94*, 369–375.

Perring, T. M., Cooper, A. D., Rodrigues, R. J., Farrar, C. A., & Bellows, T. S. J. (1993). Identification of a whitefly species by genomic and behavioural studies. *Science, 259*, 74–77.

Pilowski, M., & Cohen, S. (1974). Inheritance of resistance to tomato yellow leaf curl virus in tomato. *Phytopathology, 64*, 632–635.

Pollard, D. G. (1955). Feeding habits of the cotton whitefly. *Annals of Applied Biology, 43*, 664–671.

Rao, Q., Wang, S., Zhu, D.-T., Wang, X.-W., & Liu, S.-S. (2012). Draft genome sequence of the *Rickettsia* sp. strain MEAM1, isolated from the whitefly *Bemisia tabaci. Journal of Bacteriology, 194*, 4741–4742.

Rosell, R. C., Torres-Jerez, I., & Brown, J. K. (1999). Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, hemolymph, and honeydew. *Phytopathology, 89*, 239–246.

Rubinstein, G., & Czosnek, H. G. (1997). Long-term association of tomato yellow leaf curl virus (TYLCV) with its whitefly vector *Bemisia tabaci*: Effect on the insect transmission capacity, longevity and fecundity. *Journal of General Virology, 78*, 2683–2689.

Russell, L. M. (1957). Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae). *Bull. Brooklyn Entomol Soc, 52*, 122–123.

Sinisterra, X. H., McKenzie, C. L., Hunter, W. B., Powell, C. A., & Shatters, R. G. (2005). Differential transcriptional activity of plant-pathogenic begomoviruses in their whitefly vector (*Bemisia tabaci*, Gennadius: Hemiptera Aleyrodidae). *Journal of General Virology, 86*, 1525–1532.

Skaljac, M., & Ghanim, M. (2010). Tomato yellow leaf curl disease and plant-virus vector interactions. *Israel Journal of Plant Sciences, 58*, 103–111.

Stansley, P. A., & Naranjo, S. E. (Eds.). (2010). *Bemisia: Bionomics and management of a global pest* (p. 540). Dordrecht: Springer.

Tay, W. T., Elfekih, S., Court, L., Gordon, K. H., & DeBarro, P. J. (2014). Complete mitochondrial DNA genome of *Bemisia tabaci* cryptic pest species complex Asia I (Hemiptera: Aleyrodidae). *Mitochondrial DNA*. doi:10.3109/19401736.2014.926511.

Upadhyay, S. K., Dixit, S., Sharma, S., Singh, H., Kumar, J., Verma, P. C., & Chandrashekar, K. (2013). siRNA machinery in whitefly (*Bemisia tabaci*). *PloS One, 8*(12), e83692.

Verlaan, M. G., Hutton, S. F., Ibrahem, R. M., Kormelink, R., Visser, R. G. F., Scott, J. W., Edwards, J. D., & Bai, Y. (2013). The Tomato yellow leaf curl virus resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA–dependent RNA polymerases. *PLoS Genetics, 9*(3), e1003399.

Vidavski, F., Czosnek, H., Gazit, S., Levy, D., & Lapidot, M. (2008). Pyramiding of genes conferring resistance to *Tomato yellow leaf curl virus* from different wild tomato species. *Plant Breeding, 127*, 625–631.

Wang, J., Zhao, H., Jian, L., Jiu, M., Qian, Y.-J., & Liu, S.-S. (2009). Low frequency of horizontal and vertical transmission of two begomoviruses through whitefly *Bemisia tabaci* biotype B and Q. *Annals of Applied Biology, 157*, 125–133.

Wang, H. L., Yang, J., Boykin, L. M., Zhao, Q. Y., Wang, Y. J., Liu, S. S., & Wang, X. W. (2014). Developing conversed microsatellite markers and their implications in evolutionary analysis of the *Bemisia tabaci* complex. *Scientific Reports, 4*, 6351.

Wei, J., Zhao, J.-J., Zhang, T., Li, F.-F., Ghanim, M., Zhou, X.-P., Ye, G.-Y., Liu, S.-S., & Wang, X.-W. (2014). Specific cells in the primary salivary glands of the whitefly *Bemisia tabaci* control retention and transmission of begomoviruses. *Journal of Virology, 88*, 13460–13468.

Zamir, D., Michelson, I., Zakay, Y., Navot, N., Zeidan, N., Sarfatti, M., Eshed, Y., Harel, E., Pleban, T., Kedar, N., Rabinowitch, H., & Czosnek, H. (1994). Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *Ty-1*. *Theoretical and Applied Genetics, 88*, 141–146.

Zeidan, M., & Czosnek, H. G. (1991). Acquisition of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci. Journal of General Virology, 72*, 2607–2614.

Zhang, W., Olson, N. H., Baker, T. S., Faulkner, L., Agbandje-McKenna, M., Boulton, M. I., Davies, J. W., & McKenna, R. (2001). Structure of the *Maize streak virus* geminate particle. *Virology, 279*, 471–477.

Functional Genomics in the Whitefly *Bemisia tabaci* Species Complex

Xiao-Wei Wang and Shu-Sheng Liu

Abstract Whiteflies (Hemiptera: Aleyrodidae) include some important pests of agricultural and horticultural crops in temperate and tropical regions. The two most damaging and widespread species are the tobacco or cotton whitefly (*Bemisia tabaci*) and the greenhouse whitefly (*Trialeurodes vaporariorum*). *Bemisia tabaci* is now recognized as a species complex of at least 35 cryptic species. During the past 20 years, two species of the complex, Middle East-Asia Minor 1 (hereafter MEAM1) and Mediterranean (hereafter MED), which have been commonly referred to as the B and Q 'biotype', have risen to international prominence due to their global invasion. Despite their global prevalence, genomic sequence resources available for the whiteflies were scarce until recent years. Hence deciphering the functional genomics of whiteflies has become crucial to advance studies on their biology. The recent advancement in genomic technologies offers great opportunities for a better understanding of the complex mechanisms underlying whitefly problems. With the development of high performance sequencing technology, transcript profiling techniques allow the simultaneous examination of thousands of genes, and can be utilized to study changes in gene expression. In this review, we will summarize the recent developments in whitefly transcriptomes and gene expression profiling during virus transmission, whitefly-plant interactions and stress responses. These research efforts have provided valuable datasets for future investigations on the molecular mechanisms of whitefly biology and are expected to open important avenues into the discovery of novel strategies for whitefly management.

1 Overview

Whiteflies (Hemiptera: Aleyrodidae) include some important pests of agricultural and horticultural crops in temperate and tropical regions (Byrne and Bellows 1991; Mound and Halsey 1978). The two most damaging and widespread species are the

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tobacco or cotton whitefly (*Bemisia tabaci*) and the greenhouse whitefly (*Trialeurodes vaporariorum*). While the tobacco whitefly (*B. tabaci*) distributes mostly in tropics and subtropics, the greenhouse whitefly (*T. vaporariorum*) mainly colonizes the temperate regions (Byrne and Bellows 1991; Naranjo et al. 2010). The tobacco whitefly *B. tabaci* is now recognized as a species complex of at least 35 cryptic species (Boykin and De Barro 2014; De Barro et al. 2011; Liu et al. 2012; Xu et al. 2010). During the past 20 years, two species of the complex, Middle East-Asia Minor 1 (hereafter MEAM1) and Mediterranean (hereafter MED), which have been commonly referred to as the B and Q 'biotype', have risen to international prominence due to their global invasion (Dalton 2006; De Barro et al. 2011; Liu et al. 2007). Despite their global prevalence, genomic sequence resources available for the whiteflies were scarce until recent years (Czosnek and Brown 2010). Hence deciphering the functional genomics of whiteflies has become crucial to advance studies on their biology. The recent advancement in genomic technologies offers great opportunities for a better understanding of the complex mechanisms underlying whitefly problems (Edwards and Papanicolaou 2012). With the development of high performance sequencing technology, transcript profiling techniques allow the simultaneous examination of thousands of genes, and can be utilized to study changes in gene expression (Gibbons et al. 2009). In this review, we will summarize the recent developments in whitefly transcriptomes (Table 1) and gene expression profiling during virus transmission, whitefly-plant interactions and stress responses. These research efforts have provided valuable datasets for future investigations on

Table 1 Summary of the available whitefly transcriptome datasets

Species	Sample	Method	Number of transcripts	Reference
MEAM1 (B)	Egg, nymph, pupa, adult	EST	4,860	Leshkowitz et al. (2006)
MED (Q)	Egg, nymph, pupa, adult	Illumina	168,900	Wang et al. (2010)
T. vaporariorum	Male and female adult	Roche 454	55,000	Karatolos et al. (2011)
MEAM1 (B)	Egg, nymph, pupa, adult	Illumina	57,741	Wang et al. (2011)
Asia I	Female adult	Roche 454	3,821	Seal et al. (2012)
Asia II 3 (ZHJ1)	Egg, nymph, pupa, adult	Illumina	52,535	Wang et al. (2012)
MEAM1 (B)	Egg, nymph, pupa, adult	Roche 454	178,669	Xie et al. (2012a)
MED (Q)	Primary salivary gland	Illumina	13,615	Su et al. (2012)
MEAM1 (B)	Gut	Illumina	12,879	Ye et al. (2014)
MED (Q)	Gut	Illumina	11,246	Ye et al. (2014)
MEAM1 (B)	Male and female adult	Illumina	93,948	Xie et al. (2014)

the molecular mechanisms of whitefly biology and are expected to open important avenues into the discovery of novel strategies for whitefly management.

2 Transcriptomes of Whiteflies in the *Bemisia tabaci* Species Complex

The first endeavor in large-scale characterizing of whitefly genes started from construction of cDNA libraries and analyses of the sequences for the widespread MEAM1 species ('B biotype') (Leshkowitz et al. 2006). From the cDNA libraries of eggs, instars, non-viruliferous adults and viruliferous adults, a total of 3843 singletons and 1017 contigs were identified; and approximately half of the sequences had a match to a protein in the NCBI nr database. In fact, it was the first functional genomics project involving a hemipteran (Homopteran) insect from the subtropics/tropics and provides invaluable information for studies of whiteflies (Leshkowitz et al. 2006).

In 2010, a new short read sequencing technology (Illumina) was employed to analyze the transcriptome of the MED species ('Q biotype'). A total of 168,900 distinct sequences were assembled. Based on similarity search with known proteins, 27,290 sequences with a cut-off E-value above 10^{-5} were identified (Wang et al. 2010). The study provided the most comprehensive sequence resource available for the whitefly and demonstrated that the Illumina sequencing allows *de novo* transcriptome assembly in a species lacking genome information. Subsequently, a 454-based pyrosequencing approach was employed to generate a transcriptome for the greenhouse whitefly (*T. vaporariorum*). About 55,000 non-redundant contigs were identified and genes encoding detoxification enzymes and insecticide target proteins, such as cytochrome P450s, glutathione-S transferases (GSTs) and acetylcholinesterase enzyme were characterized in detail (Karatolos et al. 2011). For the native species in the *B. tabaci* complex, a research has been underway at the University of Greenwich, to characterize the transcriptome of the Asia 1 whitefly (Seal et al. 2012). A total of 3821 contigs have been constructed from 301,094 reads. The authors also compared a selection of these contigs with related sequences from other *B. tabaci* genetic groups. While some genes showed good alignment, misassembles were found when compared with other datasets, highlighting the need for international collaboration to obtain accurate assemblies (Seal et al. 2012).

In 2011, Wang et al. (2011) re-sequenced the transcriptome of the invasive MEAM1 whitefly using Illumina and compared it with the MED transcriptome. A total of 57,741 unique sequences were assembled for MEAM1 and 15,922 sequences were annotated. Most importantly, sequence comparison with MED transcriptome indicated the level of sequence divergence in coding, 5′ untranslated and 3′ untranslated regions were 0.83 %, 1.66 % and 1.43 %, respectively. The level of sequence divergence provides convincing support to the previous proposition

that MEAM1 and MED whiteflies are two species (Wang et al. 2011). This study further showed that 24 sequences that have evolved in response to positive selection were involved in metabolism and insecticide resistance, which might contribute to the divergence of the two whitefly species (Wang et al. 2011). To reveal the possible mechanism of whitefly invasion, 52,535 transcriptome sequences were identified from the Chinese native whitefly species Asia II 3 (Wang et al. 2012). Comparison of the sequence divergence between the transcriptomes of Asia II 3 and the invasive species MEAM1 and MED indicated that the overall divergence of coding sequences between the orthologous gene pairs of Asia II 3 and MEAM1, and that between Asia II 3 and MED, is 1.73 % and 1.84 %, respectively, which are much higher than that between MEAM1 and MED (0.83 %). The varying levels of gene divergence agree with the previous phylogenetic analyses and crossing experiments (De Barro et al. 2011). The data also demonstrated that the most divergent gene classes between the native and invasive species are related to cytochrome P450, glutathione metabolism and oxidative phosphorylation, which seemed relevant to the invasion, displacement and speciation of the species in the *B. tabaci* complex (Wang et al. 2012).

Several whitefly organs and tissues, such as salivary glands, bacteriocytes, guts and ovaries, are known to play important roles in various aspects of whitefly biology (Ghanim et al. 2001b). However due to their tiny size, the molecular constituents of these organs are almost unknown. The development of cDNA amplification technique offers a great opportunity to investigate the transcriptome with limited material, even picogram of total RNA (Tang et al. 2011). Using the cDNA amplification method in combination with llumina sequencing, Su et al. (2012) sequenced the transcriptome of the primary salivary glands of the MED species of *B. tabaci* complex. A total of 13,615 unique sequences including 3159 sequences with significant nr BLAST hits were obtained. Sequence analyses suggested that genes related to metabolism and transport are significantly enriched in the primary salivary glands. Furthermore, 295 genes were predicted to encode secretory proteins and some of them might play important roles in whitefly feeding. With similar methods, the gut transcriptomes of MEAM1 and MED whiteflies were analyzed (Ye et al. 2014). Totally 12,879 MEAM1 transcripts and 11,246 MED transcripts were annotated with a significant Blastx hit. Analyses on gut specific genes demonstrated the important roles of gut in metabolism of insecticides and secondary plant chemicals. Comparison between gut transcriptomes of the two species showed that the majority of genes evolving under positive selection are involved in metabolism and insecticide resistance. Furthermore, many genes related to detoxification were expressed at an elevated level in the gut of MED compared to MEAM1, which might be responsible for the MED's higher resistance to insecticides and environmental stresses (Ye et al. 2014).

3 Gene Expression During Whitefly Development and Sex Differentiation

To reveal the change of gene expression during whitefly development, three libraries (egg & nymph, pupa and adult) were sequenced and the gene expression patterns were analyzed (Wang et al. 2010). The sequencing results showed that the number of differentially expressed genes (DEGs) between pupa and adult are larger than that between pupa and egg & nymph. Between the adult and pupa whitefly libraries, totally 822 genes are up-regulated and 2496 genes are down-regulated. But, between pupa and egg & nymph libraries, roughly similar numbers of genes are up-regulated (1123) or down-regulated (1074). Between pupa and egg & nymph libraries, DEGs are enriched in pathways involved in energy and lipid metabolism, such as the citrate cycle, phenylpropanoid biosynthesis and fatty acid metabolism. However, between adult and pupa stages, DEGs are significantly enriched in the glycosphingolipid biosynthesis pathway (Wang et al. 2010). As a haplodiploid organism, the difference of gene expression between male and female whiteflies is also intriguing. Recently, the transcriptomes of eight *B. tabaci* MEAM1 populations (male and female whiteflies on four different host plants) were compared (Xie et al. 2014). Totally 1351 DEGs were identified between male and female whiteflies, in which 1070 are female specific and 281 are male specific. Functional analysis exhibited a gender-specific expression, including enriched translation in females, and enhanced structural constituent of cuticle in male whiteflies.

Wang et al. (2013) examined the transcriptional difference between the two invasive whitefly species, MEAM1 and MED, and one indigenous whitefly species Asia II 3. The results showed that that 2422 genes between MEAM1 and MED; 3073 genes between MEAM1 and Asia II 3; and 3644 genes between MED and Asia II 3 were differentially expressed. The DEGs were significantly enriched in the term of 'oxidoreductase activity'. Carbohydrate, amino acid and glycerolipid metabolisms were more active in MEAM1 and MED than in Asia II 3. Furthermore, the majority of genes involved in basic metabolism and detoxification were expressed at a higher level in MEAM1 and MED than in Asia II 3, which might be responsible for their higher resistance to insecticides and environmental stresses (Wang et al. 2013).

4 Whitefly-Virus Interactions

In recent years, the epidemics caused by whitefly-transmitted begomoviruses have spread worldwide, apparently due to the increased prevalence of their whitefly vectors (Moriones and Navas-Castillo 2000; Varma et al. 2011). Begomoviruses are a group of single stranded DNA viruses exclusively transmitted by the whitefly *B. tabaci* in a persistent, circulative manner (Ghanim et al. 2001a; Harrison 1985; Navas-Castillo et al. 2011). Virions are acquired by the stylet of whitefly vectors from the plant phloem, moving along the esophagus to the midgut, then crossing the

gut epithelial cells to the hemocoel, circulating with the hemolymph and reaching the salivary glands, and finally were secreted with saliva (Czosnek and Ghanim 2002). During the circulation, viruses have engaged host factors or unique strategies for replication, movement, transmission and pathogenesis. Meanwhile, the insect vectors have evolved immunologic surveillance system against viral invasion (Hogenhout et al. 2008). However, the mechanisms underlying the survival of virions in the hostile vector environment as well as responses of the insects to possible deleterious effects of the virus are largely unknown. For more details see Ghanim and Czosnek in this Book.

Suppression subtractive hybridization (SSH) is a traditional method to identify unknown transcripts that are differentially regulated among treatment groups. Li et al. (2011) investigated the DEGs in the whitefly MEAM1 feeding on healthy and *Tomato yellow leaf curl China virus* (TYLCCNV) infected tobacco by SSH. This analysis identified 124 up-regulated and 112 down-regulated genes in viruliferous whiteflies. Several genes, such as heparan sulfate proteoglycan, 26/29-kDa proteinase and *Rickettsia* 16S ribosomal RNA were up-regulated in viruliferous whiteflies; and some of them have been reported to be involved in virus transmission in other organisms (Li et al. 2011). Götz et al. (2012) analyzed the gene expression of whitefly and dissected midguts following *Tomato yellow leaf curl virus* (TYLCV) and *Squash leaf curl virus* (SLCV) infection using microarray. The authors found that TYLCV infection led the majority of the genes down-regulated, while the numbers of up- and down-regulated genes associated with SLCV infection were similar (Götz et al. 2012). Interestingly, the whitefly-encoded heat shock protein 70 (HSP70) gene was strongly up-regulated after the infection of both viruses. Moreover, the authors showed that HSP70 interacted directly with the coat proteins of these two viruses and might protect the whitefly against begomoviruses, using immunocapture PCR, virus overlay protein binding and antibody feeding assays (Götz et al. 2012).

With the development of sequencing technology, further efforts were made to decipher the complex interactions between begomoviruses and their whitefly vectors. Luan et al. (2011) investigated the transcriptional profiles of whitefly on TYLCCNV –infected and uninfected plants using Illumina sequencing. The results showed that 1606 genes were differentially regulated in the viruliferous whiteflies. Pathway analysis indicated that TYLCCNV can perturb whitefly cell cycle and primary metabolism; and activate whitefly immune responses, such as autophagy and antimicrobial peptide production. Surprisingly, TYLCCNV infection also down-regulated the expression of whitefly genes involved in immune signal transduction, revealing the relationship of coevolved adaptations between begomoviruses and whiteflies (Luan et al. 2011). However, TYLCCNV can benefit its whitefly vector indirectly, through suppression of jasmonic acid-mediated plant defense, particularly repression of terpenoid synthesis (Jiu et al. 2006; Luan et al. 2013b; Zhang et al. 2012). Interestingly, transcriptional analyses of MEAM1 whiteflies feeding on TYLCCNV-infected and uninfected tobacco plants indicated that the genes involved in the oxidative phosphorylation pathway and detoxification enzyme were down-regulated in whiteflies feeding on virus-infected plants (Luan et al. 2013a). The reduced detoxification activity is likely to attenuate energy costs, thus, enhancing the performance of whiteflies on virus-infected plants (Luan et al. 2013a).

5 Whitefly-Plant Interactions

Whiteflies can colonize more than 600 host plant species and can induce severe symptoms. To date, the majority of functional genomic studies on whitefly-plant interactions are limited to the analysis of plant gene expression in response to *B. tabaci* infestation, such as squash, tomato, tobacco and Arabidopsis. van de Ven et al. (2000) found that a number of squash genes (SLW1, a M20b peptidase-like gene and SLW3, a leaf-specific β-glucosidase-like gene) were induced systemically after whitefly feeding and the local and systemic expression of SLW1 and SLW3 differed after feeding by the closely related silver leafing and sweet potato whiteflies. McKenzie et al. (2005) studied the responses of tomato to the MEAM1 whitefly feeding using microarray. Although no discernible differences was detected between tomatoes with and without whiteflies, RNA profiling results indicated that 277 genes were up- or down-regulated in response to whitefly feeding and the regulated genes were likely to be involved in developmental regulation, stress response, wound response and ethylene production. Subsequently, a SSH strategy was used to identify genes that expressed differently in response to whiteflies infestation in tomato (*Solanum lycopersicum*) (Estrada-Hernandez et al. 2009). The results indicate that whitefly infestation in tomato lead to phase-specific expression of genes associated with photosynthesis, senescence, secondary metabolism and stress. Puthoff et al. (2010) further characterized the temporal and spatial expression of tomato wound- and defense-responsive genes to *B. tabaci* and *T. vaporariorum* feeding. The results indicate that *B. tabaci* and *T. vaporariorum* evoke similar changes in tomato gene expression and tomato plants perceive both species in a manner similar to bacterial pathogens but distinct from tissue-damaging insects (Puthoff et al. 2010).

Kempema et al. (2007) used the Affymetrix ATH1 GeneChip to monitor the *Arabidopsis thaliana* transcriptome. After feeding by MEAM1 whitefly nymphs, 700 transcripts were found to be up-regulated and 556 down-regulated. The authors also found that Arabidopsis response to whitefly feeding differed from that to chewing insects and aphids. The SA-responsive gene transcripts accumulated locally (PR1, BGL2, PR5, SID2, EDS5, PAD4) and systemically (PR1, BGL2, PR5) during whitefly feeding. In contrast, JA- and ethylene-dependent genes were either repressed or not changed after whitefly feeding (Kempema et al. 2007; Zarate et al. 2007). Dubey et al. (2013) analyzed the molecular interaction between *G. hirsutum* with *Aphis gossypii* (Aphid) and *B. tabaci*. The results showed that aphids and whiteflies affect many genes that are responsive to phytohormones and microbial infection, indicating that these pathways have complex crosstalk. The authors also observed that the expression of transcript related to photosynthesis, biotic, abiotic stresses was significantly influenced. However, some marker genes involved in phytohormonal-mediated plant resistance development, was suppressed after aphid and whitefly infestation, indicating that insects may suppress plant resistance as well (Dubey et al. 2013). Luan et al. (2013b) used a next-generation sequencing technology to identify defense genes differentially regulated in whitefly-infested

and/or virus-infected tobacco. According to the Illumina sequencing results, many of terpenoid synthesis genes were up-regulated in whitefly-infested plants, suggesting that increases in terpenoid metabolism may be associated with whitefly infestation. In contrast, in TYLCCNV-infected leaves, most terpenoid genes were unchanged and five genes were declined. Interestingly, in co-infested plants, most terpenoid genes were unchanged and only three terpenoid genes were up-regulated. This study demonstrates that virus infection can deplete the terpenoid-mediated plant defense against whiteflies, thereby favoring vector–virus mutualism (Luan et al. 2013b).

Despite its importance, few studies have addressed the molecular mechanisms of *B. tabaci* against major plant secondary defense compounds. Alon et al. (2012) compared the gene expression between *B. tabaci* adults on wild-type *Nicotiana tabacum* plants or transgenic plants constitutively activating the phenylpropanoid/flavonoids biosynthetic pathway. Both the SSH and cDNA microarray analyses indicated a complex interaction between *B. tabaci* and secondary defense metabolites produced by the phenylpropanoids/flavonoids pathway, such as expression of detoxification, immunity, oxidative stress and general stress related genes. However, the elevated transcriptional activity was not accompanied by reduction of whitefly reproductive performance, indicating high adaptability of *B. tabaci* to this large group of plant secondary defense metabolites (Alon et al. 2012). The same research group also investigated the effects of aliphatic and indolic glucosinolates on the expression of detoxification genes in MEAM1 and MED whiteflies (Elbaz et al. 2012). The result suggests that the two species use rather different strategies to cope with plant defense responses. While MEAM1 utilizes inducible defenses, MED invests significant resources in being always 'ready' for a challenge (Elbaz et al. 2012).

6 Response of *B. tabaci* to Insecticides

At present, more than 50 conventional insecticides have been employed to control the growth of *B. tabaci* populations and viral transmission (Horowitz et al. 2011). However, due to the rapidly rising resistance to insecticides, utilizing chemical agents to control *B. tabaci* is facing ever-increasing difficulties (Dennehy et al. 2010; Wang et al. 2009). During the last 20 years, a number of studies have been carried out to reveal the molecular mechanisms of whitefly resistance to insecticides. Pyriproxyfen is one of the major insecticides used to control the whitefly, however, whitefly resistance to pyriproxyfen has been observed in many regions (Crowder et al. 2007). To investigate the molecular basis underlying this resistance, a cDNA microarray was used to monitor changes in gene expression in a resistant *B. tabaci* population (Ghanim and Kontsedalov 2007). A total of 111 differentially ESTs in the resistant strain was identified. Functional analysis showed that many of the up-regulated ESTs were associated with resistance and xenobiotic

detoxification, protein, lipid and carbohydrate metabolism and JH-associated processes (Ghanim and Kontsedalov 2007).

Except pyriproxyfen, the whitefly *B. tabaci* also developed high resistance to the neonicotinoid insecticide thiamethoxam (Gorman et al. 2010). Using the SSH approach, gene profiles between the thiamethoxam-resistant and -susceptible strains were investigated. Totally 72 up-regulated and 52 down-regulated genes were obtained from the forward and reverse SSH libraries (Xie et al. 2012b). Later on, Xie et al. (2012a) sequenced the transcriptome of *B. tabaci* strains resistant to thiamethoxam, abamectin, and bifenthrin using Roche 454. In-depth transcriptome analysis also identified a number of genes potentially involved in insecticide resistance. Then, Yang et al. (2013b) analyzed the differences between resistant and susceptible stains at both transcriptional and translational levels. In total 1338 mRNAs and 52 proteins were differentially expressed; and among these genes, 118 transcripts were putatively linked to insecticide resistance. The same research group also compared gene expression in the egg, nymph and adult stages of a thiamethoxam-resistant strain with a susceptible strain using a custom whitefly microarray (Yang et al. 2013a). Gene ontology and bioinformatic analyses revealed that in all life stages many of the DEGs encoded enzymes are involved in metabolic processes and/or metabolism of xenobiotics. In addition, several ATP-binding cassette transporters were highly over-expressed in the adult stage of the TH-R strain and may play a role in resistance by active efflux (Yang et al. 2013a).

7 Response of *B. tabaci* to Biotic and Abiotic Stresses

Although the whitefly is often controlled using chemical pesticides, biological control agents constitute an important component in integrated pest management programs (Legg et al. 2014). One of these agents is the wasp *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae). Mahadav et al. (2008) investigated the physiological and molecular processes underlying *B. tabaci – E. mundus* interactions using a cDNA microarray. The results clearly indicated that genes known to be part of the defense pathways were involved in the response of *B. tabaci* to parasitization by *E. mundus*. Interestingly, *Rickettsia*, a facultative secondary symbiont, was strongly induced upon initiation of the parasitization process, suggesting that endosymbionts might be involved in the insect host's resistance to parasitoid (Mahadav et al. 2008). The fungal pathogen, *Beauveria bassiana*, is another efficient biocontrol agent against whiteflies. In 2013, next generation sequencing technology was applied to examine the expression of whitefly genes in response to the infection of *B. bassiana* (Xia et al. 2013). Compared to control, 654 and 1,681 genes were differentially expressed in whiteflies 48 and 72 h post-infection, respectively. Functional analyses indicated that the DNA damage stimulus response and drug metabolism were important anti-fungus strategies in the whitefly. By mapping the

sequencing tags to *B. bassiana* genome, a number of differentially expressed fungal genes between the early and late infection stages were also identified (Xia et al. 2013). Using RNA-seq technology, Zhang et al. (2014) investigated the whitefly's defense responses to oral infection of the *Pseudomonas aeruginosa*. Compared to uninfected controls, whiteflies 6 and 24 h post-infection showed 1348 and 1888 differentially expressed genes, respectively. The authors found that key immune elements recognized in other insect species were also important for the response of *B. tabaci* to this bacterial pathogen. The data also suggest that intestinal stem cell mediated epithelium renewal might be an important component of the whitefly's defense against oral bacterial infection.

In nature, whiteflies are continuously exposed to abiotic stresses as well. Mahadav et al. (2009) compared the expression patterns of MEAM1 and MED under 25 and 40 °C heat stress using microarray. The authors found that compared to the treatment of MED, exposure of MEAM1 to heat stress was accompanied by rapid alteration of gene expression. These differences might be due to better adaptation of one species over another and might eventually lead to change of MEAM1 and MED distribution (Mahadav et al. 2009). To reveal why females are more heat resistant than males, Lu and Wan (2008) identified the DEGs in male and female whiteflies, respectively. The authors found that difference of heat-resistance under heat-shock condition was associated with DEGs between *B. tabaci* sexes.

8 Future Perspectives

With the development of next generation sequencing technology, huge amount of sequencing data have been generated for the whitefly *B. tabaci* species complex during the last a few years. These studies have provided a solid foundation for future functional investigations into the complex molecular mechanisms of whitefly biology and evolution, such as speciation, invasion, virus transmission, and interactions with biotic and abiotic factors. Meanwhile, these studies indicate that our knowledge of whiteflies remains largely in a relatively primitive stage. Because the lack of effective genetic tools, most of the previous studies were descriptive. Future technological developments that will allow silencing or over-expression of selected genes in whiteflies will enable determining a more specific linkage between gene expression pattern and whitefly life parameters. In addition, many of the valuable datasets were not adequately annotated and analyzed. This is mainly due to the lack of whitefly genome sequences and the inexperience of the whitefly community in handing large-scale next-generation sequencing data. This situation calls for more open and intensive international collaboration on the development of informatics platforms and generation of reference genomic sequence data for the whitefly (Edwards and Papanicolaou 2012).

References

Alon, M., Elbaz, M., Ben-Zvi, M. M., Feldmesser, E., Vainstein, A., & Morin, S. (2012). Insights into the transcriptomics of polyphagy: *Bemisia tabaci* adaptability to phenylpropanoids involves coordinated expression of defense and metabolic genes. *Insect Biochemistry and Molecular Biology, 42*, 251–263.

Boykin, L. M., & De Barro, P. J. (2014). A practical guide to identifying members of the *Bemisia tabaci* species complex and other morphologically identical species. *Frontiers in Ecology and Evolution, 2*, 45. doi:10.3389/fevo.2014.00045.

Byrne, D. N., & Bellows, T. S. (1991). Whitefly biology. *Annual Review of Entomology, 36*, 431–457.

Crowder, D. W., Dennehy, T. J., Ellers-Kirk, C., Yafuso, L. C., Ellsworth, P. C., Tabashnik, B. E., & Carriere, Y. (2007). Field evaluation of resistance to pyriproxyfen in *Bemisia tabaci* (B biotype). *Journal of Economic Entomology, 100*, 1650–1656.

Czosnek, H., & Brown, J. K. (2010). The whitefly genome–white paper: A proposal to sequence multiple genomes of *Bemisia tabaci*. In P. A. Stansly & S. E. Naranjo (Eds.), *Bemisia: Bionomics and management of a global pest* (pp. 503–532). Dordrecht/London: Springer.

Czosnek, H., & Ghanim, M. (2002). The circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci* – insights from studies with *Tomato yellow leaf curl virus*. *The Annals of Applied Biology, 140*, 215–231.

Dalton, R. (2006). Whitefly infestations: The Christmas Invasion. *Nature, 443*, 898–900.

De Barro, P. J., Liu, S.-S., Boykin, L. M., & Dinsdale, A. B. (2011). *Bemisia tabaci*: A statement of species status. *Annual Review of Entomology, 56*, 1–19.

Dennehy, T. J., Degain, B. A., Harpold, V. S., Zaborac, M., Morin, S., Fabrick, J. A., Nichols, R. L., Brown, J. K., Byrne, F. J., & Li, X. (2010). Extraordinary resistance to insecticides reveals exotic Q biotype of *Bemisia tabaci* in the New World. *Journal of Economic Entomology, 103*, 2174–2186.

Dubey, N. K., Goel, R., Ranjan, A., Idris, A., Singh, S. K., Bag, S. K., Chandrashekar, K., Pandey, K. D., Singh, P. K., & Sawant, S. V. (2013). Comparative transcriptome analysis of *Gossypium hirsutum* L. in response to sap sucking insects: Aphid and whitefly. *BMC Genomics, 14*, 241.

Edwards, O., & Papanicolaou, A. (2012). A roadmap for whitefly genomics research: Lessons from previous insect genome projects. *Journal of Integrative Agriculture, 11*, 269–280.

Elbaz, M., Halon, E., Malka, O., Malitsky, S., Blum, E., Aharoni, A., & Morin, S. (2012). Asymmetric adaptation to indolic and aliphatic glucosinolates in the B and Q sibling species of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Molecular Ecology, 21*, 4533–4546.

Estrada-Hernandez, M. G., Valenzuela-Soto, J. H., Ibarra-Laclette, E., & Delano-Frier, J. P. (2009). Differential gene expression in whitefly *Bemisia tabac*i-infested tomato (*Solanum lycopersicum*) plants at progressing developmental stages of the insect's life cycle. *Physiologia Plantarum, 137*, 44–60.

Ghanim, M., & Kontsedalov, S. (2007). Gene expression in pyriproxyfen-resistant *Bemisia tabaci* Q biotype. *Pest Management Science, 63*, 776–783.

Ghanim, M., Morin, S., & Czosnek, H. (2001a). Rate of *Tomato yellow leaf curl virus* translocation in the circulative transmission pathway of its vector, the whitefly *Bemisia tabaci*. *Phytopathology, 91*, 188–196.

Ghanim, M., Rosell, R. C., Campbell, L. R., Czosnek, H., Brown, J. K., & Ullman, D. E. (2001b). Digestive, salivary, and reproductive organs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) B type. *Journal of Morphology, 248*, 22–40.

Gibbons, J. G., Janson, E. M., Hittinger, C. T., Johnston, M., Abbot, P., & Rokas, A. (2009). Benchmarking next-generation transcriptome sequencing for functional and evolutionary genomics. *Molecular Biology and Evolution, 26*, 2731–2744.

Gorman, K., Slater, R., Blande, J. D., Clarke, A., Wren, J., McCaffery, A., & Denholm, I. (2010). Cross-resistance relationships between neonicotinoids and pymetrozine in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science, 66*, 1186–1190.

Götz, M., Popovski, S., Kollenberg, M., Gorovits, R., Brown, J. K., Cicero, J. M., Czosnek, H., Winter, S., & Ghanim, M. (2012). Implication of *Bemisia tabaci* heat shock protein 70 in begomovirus-whitefly interactions. *Journal of Virology, 86*, 13241–13252.

Harrison, B. (1985). Advances in geminivirus research. *Annual Review of Phytopathology, 23*, 55–82.

Hogenhout, S. A., Ammar, E. D., Whitfield, A. E., & Redinbaugh, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology, 46*, 327–359.

Horowitz, A., Antignus, Y., & Gerling, D. (2011). *Management of Bemisia tabaci whiteflies*. Dordrecht: Springer.

Jiu, M., Zhou, X. P., & Liu, S. S. (2006). Acquisition and transmission of two begomoviruses by the B and a non – B biotype of *Bemisia tabaci* from Zhejiang. *Journal of Phytopathology, 154*, 587–591.

Karatolos, N., Pauchet, Y., Wilkinson, P., Chauhan, R., Denholm, I., Gorman, K., Nelson, D. R., Bass, C., & Williamson, M. S. (2011). Pyrosequencing the transcriptome of the greenhouse whitefly, *Trialeurodes vaporariorum* reveals multiple transcripts encoding insecticide targets and detoxifying enzymes. *BMC Genomics, 12*, 56.

Kempema, L. A., Cui, X., Holzer, F. M., & Walling, L. L. (2007). Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiology, 143*, 849–865.

Legg, J. P., Shirima, R., Tajebe, L. S., Guastella, D., Boniface, S., Jeremiah, S., Nsami, E., Chikoti, P., & Rapisarda, C. (2014). Biology and management of *Bemisia* whitefly vectors of cassava virus pandemics in Africa. *Pest Management Science, 70*, 1446–1453.

Leshkowitz, D., Gazit, S., Reuveni, E., Ghanim, M., Czosnek, H., McKenzie, C., Shatters, R. L., & Brown, J. K. (2006). Whitefly (*Bemisia tabaci*) genome project: Analysis of sequenced clones from egg, instar, and adult (viruliferous and non-viruliferous) cDNA libraries. *BMC Genomics, 7*, 79.

Li, J. M., Ruan, Y. M., Li, F. F., Liu, S. S., & Wang, X. W. (2011). Gene expression profiling of the whitefly (*Bemisia tabaci*) Middle East – Asia Minor 1 feeding on healthy and *Tomato yellow leaf curl China virus*-infected tobacco. *Instructional Science, 18*, 11–22.

Liu, S. S., De Barro, P. J., Xu, J., Luan, J. B., Zang, L. S., Ruan, Y. M., & Wan, F. H. (2007). Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science, 318*, 1769–1772.

Liu, S. S., Colvin, J., & De Barro, P. (2012). Species concepts as applied to the whitefly *Bemisia tabaci* systematics: How many species are there? *Journal of Integrative Agriculture, 11*, 176–186.

Lu, Z. C., & Wan, F. H. (2008). Differential gene expression in whitefly (*Bemisia tabaci*) B-biotype females and males under heat-shock condition. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 3*, 257–262.

Luan, J. B., Li, J. M., Varela, N., Wang, Y. L., Li, F. F., Bao, Y. Y., Zhang, C. X., Liu, S. S., & Wang, X. W. (2011). Global analysis of the transcriptional response of whitefly to *Tomato yellow leaf curl China virus* reveals the relationship of coevolved adaptations. *Journal of Virology, 85*, 3330–3340.

Luan, J. B., Wang, Y. L., Wang, J., Wang, X. W., & Liu, S. S. (2013a). Detoxification activity and energy cost is attenuated in whiteflies feeding on *Tomato yellow leaf curl China virus*-infected tobacco plants. *Insect Molecular Biology, 22*, 597–607.

Luan, J. B., Yao, D. M., Zhang, T., Walling, L. L., Yang, M., Wang, Y. J., & Liu, S. S. (2013b). Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. *Ecology Letters, 16*, 390–398.

Mahadav, A., Gerling, D., Gottlieb, Y., Czosnek, H., & Ghanim, M. (2008). Parasitization by the wasp *Eretmocerus mundus* induces transcription of genes related to immune response and symbiotic bacteria proliferation in the whitefly *Bemisia tabaci. BMC Genomics, 9*, 342.

Mahadav, A., Kontsedalov, S., Czosnek, H., & Ghanim, M. (2009). Thermotolerance and gene expression following heat stress in the whitefly *Bemisia tabaci* B and Q biotypes. *Insect Biochemistry and Molecular Biology, 39*, 668–676.

McKenzie, C. L., Sisisterra, X. H., Powell, C. A., Bausher, M., Albano, J. P., & Shatters, R. G., Jr. (2005). Deciphering changes in plant physiological response to whitefly feeding using microarray technology. *Acta Horticulturae, 695*, 347–352.

Moriones, E., & Navas-Castillo, J. (2000). *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics worldwide. *Virus Research, 71*, 123–134.

Mound, L. A., & Halsey, S. H. (1978). *Whitefly of the world*. New York: Wiley.

Naranjo, S. E., Castle, S. J., De Barro, P. J., & Liu, S. S. (2010). Population dynamics, demography, dispersal and spread of Bemisia tabaci. In P. A. Stansly & S. E. Naranjo (Eds.), *Bemisia: Bionomics and management of a global pest* (pp. 185–226). Dordrecht/London: Springer.

Navas-Castillo, J., Fiallo-Olivé, E., & Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Physical Chemistry, 49*, 219–248.

Puthoff, D. P., Holzer, F. M., Perring, T. M., & Walling, L. L. (2010). Tomato pathogenesis-related protein genes are expressed in response to *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B feeding. *Journal of Chemical Ecology, 36*, 1271–1285.

Seal, S., Patel, M., Collins, C., Colvin, J., & Bailey, D. (2012). Next generation transcriptome sequencing and quantitative real-time PCR technologies for characterisation of the Bemisia tabaci Asia 1 mtCOI phylogenetic clade. *Journal of Integrative Agriculture, 11*, 281–292.

Su, Y. L., Li, J. M., Li, M., Luan, J. B., Ye, X. D., Wang, X. W., & Liu, S. S. (2012). Transcriptomic analysis of the salivary glands of an invasive whitefly. *PLoS One, 7*, e39303.

Tang, F., Lao, K., & Surani, M. A. (2011). Development and applications of single-cell transcriptome analysis. *Nature Methods, 8*, S6–S11.

van de Ven, W. T., LeVesque, C. S., Perring, T. M., & Walling, L. L. (2000). Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding. *The Plant Cell, 12*, 1409–1423.

Varma, A., Mandal, B., & Singh, M. K. (2011). Global emergence and spread of whitefly (*Bemisia tabaci*) transmitted geminiviruses. In W.M.O. Thompson (Ed.), *The whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) interaction with geminivirus-infected host plants* (pp. 205–292). Dordrecht: Springer.

Wang, Z., Yao, M., & Wu, Y. (2009). Cross-resistance, inheritance and biochemical mechanisms of imidacloprid resistance in B-biotype *Bemisia tabaci. Pest Management Science, 65*, 1189–1194.

Wang, X. W., Luan, J. B., Li, J. M., Bao, Y. Y., Zhang, C. X., & Liu, S. S. (2010). *De novo* characterization of a whitefly transcriptome and analysis of its gene expression during development. *BMC Genomics, 11*, 400.

Wang, X. W., Luan, J. B., Li, J. M., Su, Y. L., Xia, J., & Liu, S. S. (2011). Transcriptome analysis and comparison reveal divergence between two invasive whitefly cryptic species. *BMC Genomics, 12*, 458.

Wang, X. W., Zhao, Q. Y., Luan, J. B., Wang, Y. J., Yan, G. H., & Liu, S. S. (2012). Analysis of a native whitefly transcriptome and its sequence divergence with two invasive whitefly species. *BMC Genomics, 13*, 529.

Wang, Y. L., Wang, Y. J., Luan, J. B., Yan, G. H., Liu, S. S., & Wang, X. W. (2013). Analysis of the transcriptional differences between indigenous and invasive whiteflies reveals possible mechanisms of whitefly invasion. *PLoS One, 8*, e62176.

Xia, J., Zhang, C. R., Zhang, S., Li, F. F., Feng, M. G., Wang, X. W., & Liu, S. S. (2013). Analysis of whitefly transcriptional responses to *Beauveria bassiana* infection reveals new insights into insect-fungus interactions. *PLoS One, 8*, e68185.

Xie, W., Meng, Q., Wu, Q., Wang, S., Yang, X., Yang, N., Li, R., Jiao, X., Pan, H., & Liu, B. (2012a). Pyrosequencing the *Bemisia tabac*i transcriptome reveals a highly diverse bacterial community and a robust system for insecticide resistance. *PLoS One, 7*, e35181.

Xie, W., Yang, X., Wang, S. I., Wu, Q. J., Yang, N. N., Li, R. M., Jiao, X. G., Pan, H. P., Liu, B. M., Feng, Y. T., Xu, B. Y., Zhou, X. G., & Zhang, Y. J. (2012b). Gene expression profiling in the thiamethoxam resistant and susceptible B-biotype sweetpotato whitefly, *Bemisia tabaci. Journal of Insect Science, 12*, 46.

Xie, W., Guo, L., Jiao, X., Yang, N., Yang, X., Wu, Q., Wang, S., Zhou, X., & Zhang, Y. (2014). Transcriptomic dissection of sexual differences in *Bemisia tabac*i, an invasive agricultural pest worldwide. *Scientific Reports, 4*, 4088.

Xu, J., De Barro, P. J., & Liu, S. S. (2010). Reproductive incompatibility among genetic groups of *Bemisia tabaci* supports the proposition that the whitefly is a cryptic species complex. *Bulletin of Entomological Research, 100*, 359–366.

Yang, N., Xie, W., Jones, C. M., Bass, C., Jiao, X., Yang, X., Liu, B., Li, R., & Zhang, Y. (2013a). Transcriptome profiling of the whitefly *Bemisia tabaci* reveals stage-specific gene expression signatures for thiamethoxam resistance. *Insect Molecular Biology, 22*, 485–496.

Yang, N., Xie, W., Yang, X., Wang, S., Wu, Q., Li, R., Pan, H., Liu, B., Shi, X., Fang, Y., Xu, B., Zhou, X., & Zhang, Y. (2013b). Transcriptomic and proteomic responses of sweetpotato whitefly, *Bemisia tabaci*, to thiamethoxam. *PLoS One, 8*, e61820.

Ye, X. D., Su, Y. L., Zhao, Q. Y., Xia, W. Q., Liu, S. S., & Wang, X. W. (2014). Transcriptomic analyses reveal the adaptive features and biological differences of guts from two invasive whitefly species. *BMC Genomics, 15*, 370.

Zarate, S. I., Kempema, L. A., & Walling, L. L. (2007). Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology, 143*, 866–875.

Zhang, T., Luan, J. B., Qi, J. F., Huang, C. J., Li, M., Zhou, X. P., & Liu, S. S. (2012). Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. *Molecular Ecology, 21*, 1294–1304.

Zhang, C. R., Zhang, S., Xia, J., Li, F. F., Xia, W. Q., Liu, S. S., & Wang, X. W. (2014). The immune strategy and stress response of the Mediterranean species of the *Bemisia tabaci* complex to an orally delivered bacterial pathogen. *PLoS One, 9*, e94477.

Part IV
Control of Insect Pests

Plant Immunity: Connecting the Dots Between Microbial and Hemipteran Immune Responses

Isgouhi Kaloshian and Linda L. Walling

Abstract Hemipteran insects are destructive pests worldwide and have multiple strategies for evading or coping with the host plant defenses. Innate immunity is a critical component of the plant defense against herbivory. In this chapter, we describe the status of our understanding of the three layers of defense including non-host resistance, pattern-triggered immunity and effector-triggered immunity and how they relate to immunity against hemipteran insects. We also address the gaps in our knowledge in the immune signaling against hemiptera and highlight recent advances in identification of hemipteran effectors.

1 Introduction

It is estimated that herbivorous insects destroy up to one fifth of the world's total crop production annually (Oerke 2006; FAO 2009). Insect-resistant crops that limit pre- and/or post-harvest losses have high impact on yields and are often a foundational element in integrated pest management strategies (Panda and Khush 1995; Naranjo and Ellsworth 2009; Smith and Clement 2012). Insect-resistance strategies include leveraging natural sources of genetic resistance or tolerance and construction of transgenes that confer resistance to pests (Bellotti and Arias 2001; Gatehouse 2008; Ribaut et al. 2010; Smith and Clement 2012). Independent of the resistance strategy, resistant crops have high return for growers and our environment. They significantly improve crop quality and yields, reduce pesticide use, lower production costs (including costs of machinery and fuel), improve the health and safety of farm workers, and contribute to a reduction in greenhouse gas emissions (Brookes and Barfoot 2006; Batista and Oliveira 2009). Finally, resistance to phloem-feeding

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insects can have the additional advantage of limiting virus spread and thereby curtailing losses virus-associated diseases (Kishaba et al. 1992; Tanguy and Dedryver 2009; Dogimont et al. 2014).

Several recent reviews provide a variety of discussions of resistance mechanisms to insects (Yencho et al. 2000; Powell et al. 2006; Glas et al. 2012; Last et al. 2012; Smith and Clement 2012; Kamphuis et al. 2013; Walling and Thompson 2013; Jander 2014; Kliebenstein 2014; Smith and Chuang 2014). This chapter complements these reviews and seeks to highlight the similarities and differences in the three layers of defense that provide resistance to hemipteran insects and pathogens. The first layer of defense is non-host resistance, which is active during the earliest encounters of plants with non-adapted pathogens and pests. Adapted pests and pathogens, which have developed mechanisms to circumvent non-host resistance mechanisms, induce post-invasive mechanisms of resistance that are based on induction of the host plant immune system. These mechanisms of resistance include pattern-triggered immunity (PTI) and effector-triggered immunity (ETI).

2 Non-host Resistance and Pre-invasion Strategies

Non-host resistance is a potent and multi-layered mechanism of defense that discourages insect feeding and colonization and curtails pathogen success (Heath 2000). Non-host resistance is durable and widespread, being expressed in all plants towards most pathogens and pests. Only the pests and pathogens that have adapted to a plant species can breach the formidable physical and chemical barriers associated with non-host resistance, establish residency and thrive (Stam et al. 2014). Both preformed defenses and induced responses are critical for non-host resistance (Heath 2000; Nurnberger and Lipka 2005).

2.1 Where to Land?

Unlike microbial pathogens that are transmitted by water, wind, touch, or vectors, mobile hemiptera actively choose their host plants using olfactory and/or visual cues (Gerling 1990; Powell et al. 2006; Doering 2014). The volatile emissions from plants may attract or repel an insect, reveal conspecific densities, attract natural enemies to herbivore-infested plants, or signal plant infestation with other organisms (Dicke and Baldwin 2010). To date, there is relatively little known about the olfactory receptors in hemiptera and their role in host selection or rejection (Smadja et al. 2009, 2012; Cao et al. 2014; He and He 2014). The visitations and behaviors associated with visually and odorant attractive plants determine plant suitability for feeding and supporting the growth and development of an herbivore's progeny. For many hemipterans, determining host vs. non-host status can be a life or death choice. Some hemipteran genera have immature stages that have limited or no mobility

(e.g., whiteflies, scales and psyllids) and the mother's choice is a critical one for success; instars live or die based on plant choice (Walling 2008).

2.2 Sampling and Tasting

After landing on a plant surface, hemiptera quickly determine the suitability of a plant as a host by using tactile and gustatory cues. These pre-invasion strategies include the tapping of hemipteran mouth parts on surfaces to gauge physical features and making shallow probes of the plant surface (Walker 1987; Gerling 1990; Powell et al. 2006). Accompanied by the secretion of small amounts of watery saliva to dissolve surface molecules and imbibition of these surface chemical-laced liquors (Miles 1999), whiteflies and aphids are able to evaluate the preformed chemical defenses of the leaf/stem surface. These behaviors allow hemipterans to perceive differences in the chemical milieu of the plant's exterior barriers, carbohydrate content of cell walls, as well as qualitative changes in epicuticular waxes to determine non-host or host status (Muller and Riederer 2005).

In addition, the hydrophobic cuticular waxes harbor non-volatile secondary metabolites, as well as volatile and semi-volatile compounds (e.g., monoterpenes and glucosinolate-derived volatiles), which serve to attract or repel insects (Muller and Riederer 2005; Reina-Pinto and Yephremov 2009). Finally, leaf trichomes exude secondary metabolites and proteins that deter insect settling and success (Wagner et al. 2004; Glas et al. 2012; Last et al. 2012). The metabolites of glandular trichomes often provide broad-spectrum resistance to insect pests. Assessment of preformed plant defenses allows mobile insects to escape from unsuitable hosts; the ability to move within and between plants is a critical distinction between pathogen-plant and insect-plant interactions.

2.3 Genetic and Biochemical Basis of Non-host Resistance

Non-host resistance has been genetically and biochemically dissected in plant-pathogen interactions revealing multiple parallel defense strategies. Rather surprisingly, it is not yet clear if the well-characterized pre- and post-invasion mechanisms of non-host resistance to microbes are active in non-host resistance to insects. In *Arabidopsis thaliana* (Arabidopsis), two parallel pathways for non-host resistance are operative and dependent on *PENETRATION* (*PEN*) genes. The PEN1 and PEN2/PEN3 pathways were first identified with mutants that displayed enhanced susceptibility to the non-adapted powdery mildews *Blumeria graminis* f. sp. *hordei* and *Erysiphe pisi* (Collins et al. 2003; Lipka et al. 2005; Stein et al. 2006).

The PEN1-dependent pathway in induced by non-adapted pathogens; it controls exocytosis and the delivery of anti-microbial compounds and proteins to the site of pathogen contact. PEN1 (SYP121, SYR1) is a plasma membrane-localized syntaxin

(t-SNARE) that works with the SNAP33 adaptor and the vesicle-associated membrane proteins VAMP721/722 to confer non-host resistance (Collins et al. 2003; Kwon et al. 2008). While the anti-microbial cargos that are delivered to a focal location under fungal appressoria in epidermal and mesophyll cells are not yet known, H_2O_2 and cell wall-reinforcement molecules have been proposed as a cargos (Kwon et al. 2008). To our knowledge, the importance of the PEN1 non-host resistance mechanism in deterring hemiptera or other insects has not been tested. Interestingly, PEN1 and the closely related SYP122 negatively regulate SA-controlled defenses and therefore also have important roles in post-invasive defense (Zhang et al. 2007).

The PEN2-PEN3 mechanism of non-host resistance is responsible for the targeted delivery of indole glucosinolate-derived antimicrobial metabolites into the apoplastic space (Lipka et al. 2005; Stein et al. 2006; Bednarek et al. 2009). PEN2 is induced by both non-adapted and adapted pathogens, localized to the periphery of peroxisomes and accumulates at fungal hyphae entry sites. PEN2 is an atypical myrosinase, belonging to the family 1 glucosyl hydrolases (Lipka et al. 2005). In vivo PEN2 removes the glucose residue from 4-methoxyindol-3-ylmethyl glucosinolate (4MI3M-Glc) (Bednarek et al. 2009); this product forms a conjugate with glutathione and subsequently M4I3A (4-methoxyindol-3-ylmethyl amine) is formed; this is the product that is toxic to fungi (Fig. 1). The plasma membrane-localized PEN3 (PDR8; PLEOITROPIC RESISTANCE 8) is an ATP-binding cassette multidrug transporter that conveys this toxic metabolite (and perhaps other cargos) to invasion sites (Stein et al. 2006). To date the levels of PEN2-dependent amines (e.g., M4I3A) have not been measured in aphid-infested leaves. As aphids will contact 4MI3M-Glc via phloem sap consumption and PEN2 is localized in peroxisomes of cells, this prospect seems unlikely. Accordingly, aphid fecundity on *pen2* and wild-type Arabidopsis plants was similar (De Vos and Jander 2009).

Glucosinolates are well-known repellents and anti-feedants for most adapted generalist insects, attractants and feeding stimulants for specialist insects, and key players in non-host resistance to insects (Kliebenstein et al. 2005). However, the importance of glucosinolates for adapted or non-adapted phloem-feeding generalist and specialist insects is more complex. Due to the minimal cellular damage that is caused by feeding of many phloem-feeding insects, the phloem-mobile glucosinolates rarely (if ever) contact the myrosin cell-stored myrosinases (β-thioglucoside glucohydrolase) that release more toxic aglycones. This is supported by the fact that fecundity of two aphid species, the green peach aphid *Myzus persicae* (a generalist) and the cabbage aphid *Brevicoryne brassicae* (a specialist), is not altered on the Arabidopsis *tgg1 tgg2* double mutant that abolishes myrosinase activity (Barth and Jander 2006). It should be noted that aphids will contact the glucosinolates imbedded in the epicuticular waxes. Not surprisingly, in choice tests the generalist *M. persicae* prefers transgenic *Brassica napa MINELESS* plants that have ablated myrosin cells over wild-type plants, while the specialist *B. brassicae* preferred wild-type plants (Borgen et al. 2012).

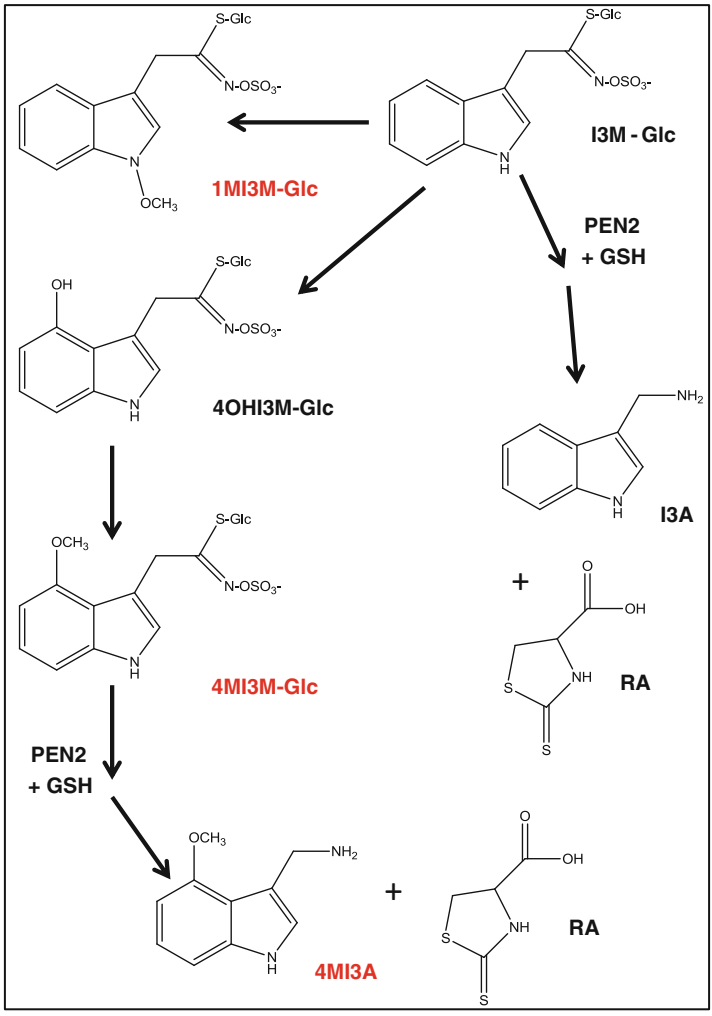

Fig. 1 PEN2- and aphid-associated indole glucosinolate metabolism. Upon aphid infestation, overall levels of the tryptophan-derived indole glucosinolates decline, However, I3M-Glc (indol-3-ylmethyl glucosinolate) is converted to the more toxic 4MI3M-Glc (4-methoxyindol-3-ylmethyl glucosinolate) and 1MI3M-Glc (1-methoxyindol-3-ylmethyl glucosinolate) that impact aphid fecundity and/or feeding (Kim and Jander 2007). In response to fungal infections, PEN2 conjugates glutathione (GSH) to I3M-Glc and 4MI3M-Glc to form a postulated unstable intermediate (not shown) that is converted to the indolic amines I3A (indol-3-ylmethylamine) and 4MI3A (4-methoxyindol-3-ylmethylamine), respectively, and the by-product RA (raphanusamic acid). 4MI3A is a putative PEN2-derived metabolite with anti-fungal properties (Bednarek et al. 2009). The production or role of I3A and 4MI3A during aphid infestation remains to be determined

2.4 Mechanisms to Survive in a Toxin-rich Environment

While the complexities of myrosin cells in hemipteran defense need further elucidation, it is clear that the adapted generalist and specialist hemipterans have developed three strategies to cope with this potent chemical defense. First, some aphids sequester glucosinolates in their bodies. The stored glucosinolates and insect-produced myrosinases serve as a potent defense against natural enemies (Kazana et al. 2007). Second, hemipterans express glucosinolate detoxification genes to inactivate these molecules. For example, two deployment strategies are used by the species *Bemisia tabaci* B (MEAM1) and Q (MED) (Elbaz et al. 2012). *B. tabaci* B induces its detoxification genes, while *B. tabaci* Q constitutively expresses many detoxification genes. In addition, aphids and whiteflies down regulate the expression of glucosinolate biosynthesis genes (De Vos et al. 2005; Kempema et al. 2007). While 2-3 days of feeding by the aphid *M. persicae* causes an overall reduction in glucosinolates in infested leaves, Arabidopsis responds by converting I3M-Glc (indol-3-ylmethyl glucosinolate) to the more toxic 4MI3M-Glc and 1MI3M-Glc (1-methoxyindol-3-ylmethyl glucosinolate) in infested leaves (Kim and Jander 2007) (Fig. 1). The importance of 4MI3M-Glc and IMI3M-Glc as an aphid deterrent in vivo has yet to be tested genetically, however, when added to diets in vitro, 4MI3M-Glc is a potent aphid anti-feedant and both 4MI3M-Glc and IMI3M-Glc decrease aphid fecundity more than their precursor I3M-Glc (Kim and Jander 2007). In fact, the reduction of aphid fecundity was similar to the aglycones 13M, 4MI3M, and IMI3M. The role of 4MI3M-Glc and IMI3M-Glc in vivo is further complicated by the apparent instability of indole glucosinolates in the aphid body (Kim and Jander 2007). For example, while intact aliphatic glucosinolates are excreted in aphid honeydew, reduced levels of indole glucosinolates are recovered from the honeydew.

2.5 The Role of Innate Immunity in Non-host Resistance

If a pathogen or insect survives the pre-formed and pre-invasion non-host resistance mechanisms, the induced post-invasion mechanisms of non-host resistance are potent deterrents. Post-invasion non-host resistance is based on the triggering of the host plant's immune system and has substantive overlaps with PTI induced by microbe-associated molecular patterns (MAMPs) and ETI (see Sect. 3). This mechanism of non-host resistance is often associated with localized cell death with similarities to and distinctions from the ETI hypersensitive response. It is clear that in Arabidopsis, indolic glucosinolates have an anti-microbial/anti-feedant role and an additional role as signaling molecules during innate immune responses. Both *PEN2* and *PEN3* are required for MAMP-induced callose deposition (Clay et al. 2009; Luna et al. 2011). Consistent with this finding, aphid whole-body extracts (a source of aphid effectors and elicitors) induce callose deposition and this response is attenuated in the *pen2* mutant (Prince et al. 2014).

3 Pattern-triggered Immunity

As the first line of the innate immune response, host plants detect invaders using plasma membrane-localized pattern recognition receptors (PRRs) (Zipfel 2014). PRRs perceive highly conserved molecules (e.g., MAMPs) to trigger the core of immune responses or PTI. These responses include production of reactive oxygen species (ROS), deposition of callose, and reprogramming of the transcriptome to activate defenses. PRRs are frequently encoded by receptor-like kinases (RLKs) or receptor-like proteins (RLPs). PRR-dependent PTI is also activated by host-derived damage-associated molecular patterns (DAMPs) released by pathogen or pest attack (Heil and Land 2014).

3.1 Well-known Players – FLS2 and EFR and Their Ligands

The best studied microbial MAMPs and their PRR partners are flagellin and FLS2 (FLAGELLIN SENSING 2) and elongation factor Tu (EF-Tu) and EFR (EF-TU RECEPTOR) (Newman et al. 2013). FLS2 is a trans-membrane leucine-rich repeat receptor kinase (LRR-RK) that binds a conserved stretch of 22 amino acids of flagellin (flg22) (Chinchilla et al. 2007). First identified in Arabidopsis, homologs of *FLS2* are ubiquitous in sequenced plant genomes and functional orthologs have been identified in crops such as rice (*Oryza sativa*), tomato (*Solanum lycopersicum*) and grapevine (*Vitis vinifera*) (Robatzek et al. 2007; Takai et al. 2008; Trda et al. 2014).

Unlike flg22, perception of the 18-amino acid EF-Tu epitope (elf18) is limited to members of Brassicaceae and is conferred by EFR, a membrane-localized LRR-RK (Zipfel et al. 2006). This restricted ability to perceive EF-Tu among plants provided an opportunity to test the ability to enhance resistance to bacterial pathogens by transgenic interfamily transfer of EFR. Indeed, transgenic tomato and *Nicotiana benthamiana* expressing *At*EFR exhibit broad-spectrum bacterial resistance indicating that PRRs can be used to engineer effective resistance in crops (Lacombe et al. 2010). Interestingly, recently it has been shown that a distinct 50-amino acid epitope of EF-Tu (EFa50) is recognized in rice by a yet unidentified receptor (Furukawa et al. 2014). Therefore, recognition of EF-Tu has evolved independently in plant species.

3.2 Additional Early Players – PRR Interactors

Upon ligand binding, both *At*FLS2 and *At*EFR form heterodimers with SERK3/BAK1 (SOMATIC EMBRYOGENESIS RECEPTOR KINASE3/BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1). BAK1 is a LRR-RK that is required for flg22- and elf18-induced PTI (Heese et al. 2007).

BAK1 is a co-receptor for flg22 since it directly binds to flg22 (Sun et al. 2013). BAK1 is also required for PTI activation by additional MAMPs/DAMPs (Zipfel 2014); however, it is unclear whether BAK1 binds to these MAMPs/DAMPS in a ligand-dependent manner. The *bak1-5* mutant, which has no pleiotropic effects on brassinosteriod signaling or cell death, displays enhanced susceptibility to both adapted and non-adapted microbial pathogens (Roux et al. 2011). BAK1 cooperates genetically with BKK1 (BAK1-like/SERK4) in non-host and PTI responses. Similarly, *bak1-5* plants exhibit enhanced susceptibility to the non-adapted pea aphid pest *Acyrthosiphon pisum* as reflected in increased aphid longevity on *bak1-5* plants (Prince et al. 2014). While *M. persicae* longevity on *bak1-5* plants was not tested, this generalist aphid had similar fecundity on *bak1-5* and wild-type plants (Prince et al. 2014). These data suggest that the adapted *M. persicae* is able to circumvent the *BAK1*-dependent immune responses (Fig. 2).

In addition to BAK1, several PRRs require the cytoplasmic RLK BIK1 (BOTRYTIS-INDUCED KINASE 1) to activate PTI by microbial pathogens. In the FLS2 and EFR recognition systems, BIK1 directly interacts with these two recep-

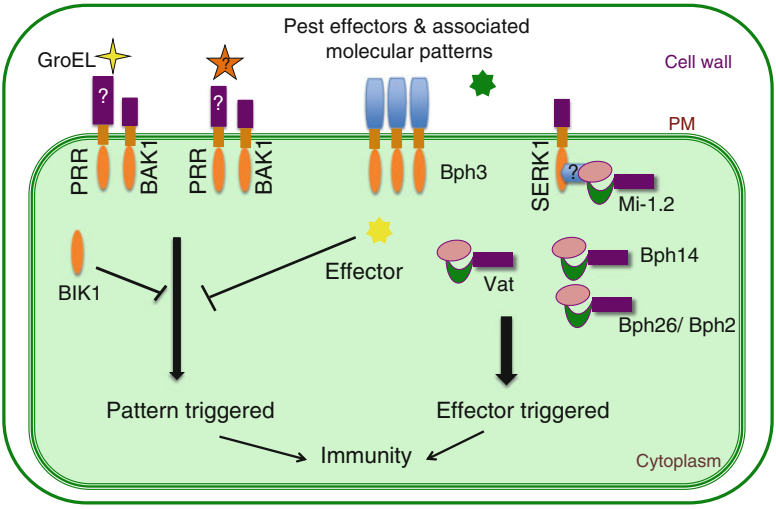

Fig. 2 Model for plant resistance to hemipteran insects. Unknown, presumed plasma membrane (PM)-localized Pattern Recognition Receptor (PRR) recognize the aphid endosymbiont *Buchnera* GroEL and at least one other aphid-associated molecular pattern. Both recognition events require the PM-localized receptor-like kinase (RLK) BAK1. The cytoplasmic RLK BIK1 acts as negative regulator of aphid defense. The tomato coiled-coil nucleotide-binding leucine-rich repeat (CC-NLR) protein Mi-1.2 confers broad spectrum resistance to potato aphids, root-knot nematodes, whiteflies, and psyllids. Mi-1.2 requires the PM-localized RLK SERK1 for the aphid resistance. The melon CC-NLR Vat confers resistance to cotton aphid, while the rice CC-NLR Bph14 and Bph26/Bph2 confer resistance to brown planthopper. The rice *Bph3* locus consists of three PM-localized lectin receptor kinases (*Os*LecRK1-*Os*LecRK3) and confers broad-spectrum resistance to brown and white-backed planthoppers

tors and with BAK1 (Lu et al. 2010; Zhang et al. 2010). Upon MAMP perception, BIK1 is phosphorylated by BAK1 and released from the recognition complex to presumably activate PTI (Lu et al. 2010; Zhang et al. 2010; Laluk et al. 2011). Therefore, BIK positively regulates PTI and immunity to the fungal pathogen *Botrytis cinerea* (Veronese et al. 2006). In contrast to its positive role against microbial pathogens, BIK1 has a negative role in aphid resistance as *bik1* mutants exhibited reduced *M. persicae* fecundity (Fig. 2) (Lei et al. 2014). Collectively, these data indicate that while the same players may participate in aphid- and microbial-induced PTI, the mechanisms that regulate the early events in aphid and possibly other hemiptera perception and signal transduction are likely to be distinct.

3.3 Aphid-derived Elicitors

While the exact aphid-derived trigger(s) of the BAK-1-dependent immune response is not known, clues exist as to its nature. Using whole body extracts of *M. persicae*, an aphid elicitor(s) that stimulate the hallmarks of PTI (e.g., callose deposition, ROS production, and induction of PTI sentinel genes) was identified (Prince et al. 2014). The elicitor(s) is proteinaceous as boiling or proteinase K treatment abolishes the extract-induced PTI responses. In addition, elicitor activity is found in both 3- to 10-kDa and >10-kDa fractions of the *M. persicae* extracts. While only the 3–10 kDa fraction induces a ROS burst, both fractions reduce susceptibility to aphids when infiltrated into Arabidopsis leaves. This aphid extract-induced reduced susceptibility is BAK1-dependent. These data suggest that the two fractions represent a single >10-kDa elicitor and its degradation products or two distinct elicitors of different sizes. Finally, whole body extracts from an *Arabidopsis*-adapted aphid (*B. brassicae*) and two non-adapted aphids (*A. pisum* and *Sitobion avenae*) were able to induce PTI marker genes suggesting that the PTI trigger(s) is evolutionary conserved among aphids (Prince et al. 2014).

The data from the aphid extracts are consistent with an earlier study that identified a 3- to 10-kDa fraction of *M. persicae* saliva to contain a proteinaceous elicitor that slowed aphid population growth (De Vos and Jander 2009). It is unknown if the whole-body elicitor(s) from the four aphid species and the salivary elicitor(s) from *M. persicae* are the same or distinct molecules (De Vos and Jander 2009; Prince et al. 2014). In addition, while it is unknown if the salivary elicitor action is BAK1-dependent, defense mutant analyses indicated that the salivary elicitor-induced resistance to *M. persicae* was independent of the defense hormones jasmonic acid (JA)-, salicylic acid (SA)-, ethylene-dependent signaling, as well as the lipase-like protein PAD4 (PHYTOALEXIN DEFICIENT4). To date, the identity of the PRR that perceives the *M. persicae* elicitor(s) is unknown but aphid elicitor-induced ROS does not involve the FLS2-, ERF- or CERK1 (CHITIN ELICITOR RECEPTOR KINASE1) PRRs (Prince et al. 2014). Moreover, it is not known whether the elicitor activity of the aphid whole body or salivary extracts is of aphid origin or of aphid-associated bacterial endosymbiont origin. Considering that aphid saliva contains

proteins of endosymbiont origin, the PTI could be activated by patterns associated with the endosymbionts (Chaudhary et al. 2014; Vandermoten et al. 2014). In fact, recent information indicates that an endosymbiont-derived protein activates host PTI responses (see Sect. 3.5 below).

3.4 Chitin – A Possible Hemipteran-relevant MAMP and Its PRRs

A well-known MAMP that is most relevant to perception of insects is chitin. Chitin is composed of linear homopolymer of β-(1,4)-linked N-acetyl-D glucosamine (GlcNac) monomers and is part of the exoskeleton and mouthparts of arthropods, including hemipterans (Merzendorfer and Zimoch 2003). It is also the major component of fungal cell walls and chitin fragments were first discovered as a fungal MAMP (Shibuya and Minami 2001). Plasma membrane-localized receptor proteins (RP) with lysin motif (LysM)-containing ectodomains that bind chitin, have been identified from both rice and Arabidopsis. While similar proteins are used to perceive chitin, the mechanisms in rice and *Arabidopsis* are distinct.

The rice LysM-proteins *Os*CEBiP (CHITIN OLIGOSACCHARIDE ELICITOR-BINDING PROTEIN1) and *Os*CERK1 are partners in chitin perception and both are critical for fungal immunity (Kaku et al. 2006). *Os*CEBiP binds chitin but it lacks a kinase domain. *Os*CERK1 has a low affinity for chitin and is an active kinase (Shimizu et al. 2010). Upon chitin binding, *Os*CEBiP forms a homo-dimer and this complex binds *Os*CERK1 to form a hetero-oligomeric receptor complex, which activates downstream immune responses (Kaku et al. 2006; Hayafune et al. 2014). Additional rice LysM-containing plasma membrane-localized proteins *Os*LYP4 and *Os*LYP6 are also involved in chitin signaling (Liu et al. 2012a).

Unlike rice, the CERK1 and CEBiP homologues of Arabidopsis function in two independent chitin perception and signaling pathways that confer fungal immunity (Miya et al. 2007; Faulkner et al. 2013). The plasma membrane-localized *At*CERK1 binds chitin and induces immunity to fungal pathogens (Miya et al. 2007). Ligand binding induces *At*CERK1 homo-dimerization and activation of downstream signaling (Liu et al. 2012b). This chitin-induced PTI is BAK1 independent (Albrecht et al. 2012). The Arabidopsis *Os*CEBiP homologue, *At*LYM2 (LYSIN MOTIF DOMAIN-CONTAINING GLY-COSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN 2) also binds chitin and is thought to be associated with membranes via a GPI anchor (Faulkner et al. 2013). Unlike *At*CERK1, *At*LYM2 is enriched in plasmodesmata membranes and reduces the flux of molecules through plasmodesmata upon chitin perception (Faulkner et al. 2013). The role of *At*LYM2 in plasmodesmata suggests that restricting cell-to-cell communication is an important part of activating the suite of defense genes associated with fungal immunity.

It is intriguing to speculate that chitin-induced PTI forms a layer of defense against hemiptera. Chitin-induced PTI in both rice and Arabidopsis require chitin fragments of specific sizes (e.g., heptamers and octamers) to trigger receptor

dimerization and activation of defense responses (Liu al. 2012b; Hayafune et al. 2014). While it is unknown whether such chitin units are released by hemiptera during exoskeleton shedding, it is rather surprising that the role of chitin-induced PTI against hempiteran insects has not been investigated and hemiptera performance on Arabidopsis *cerk1* or *lym2* mutants or rice *Os*CERK1 and *Os*CBiP RNAi lines have not been evaluated. To date, we are aware of only one study that has evaluated a chitin-perception mutant in hemiptera interaction. Prince et al. (2014) showed that the ROS burst induced by whole-body aphid extracts was not impaired in the Arabidopsis triple mutant *fls2 efr cerk1*. These data lead to one of two conclusions. First, chitin perception may not be essential for the aphid-induced ROS burst. Alternatively, chitin perception is important but the quantity or sizes of the chitin oligomers present in the aphid whole-body extracts are not equivalent to the natural chitin oligomers released during molting of the aphid exoskeleton or mouthparts or during egg hatching. The roles of chitin and its receptors in plant immunity against aphids and other hemipteran remain to be discovered.

3.5 The Role of Hemipteran-associated Microbes in PTI

A growing body of evidence indicates that hemiptera-associated microbes are directly involved in modulating host defenses. Aphid saliva and honeydew contain aphid-derived proteins as well as proteins from its primary bacterial endosymbiont, *Buchnera aphidicola* (Sabri et al. 2013; Chaudhary et al. 2014; Vandermoten et al. 2014). *Buchnera* is an obligate mutualist endosymbiotic γ-Protobacterium that has co-evolved with aphids and is essential for their reproduction and survival (Wilson et al. 2010). In addition, aphid honeydew contains proteins from the aphid gut microflora expanding the repertoire of possible aphid-associated signals that activate plant immune responses (Sabri et al. 2013).

The most abundant primary aphid endosymbiont protein is the chaperonin GroEL, which constitutes 10 % of the total *Buchnera* proteins (Baumann et al. 1996). *Buchnera* GroEL has been identified in aphid saliva and honeydew (Sabri et al. 2013; Chaudhary et al. 2014; Vandermoten et al. 2014). *Buchnera* GroEL from the potato aphid *Macrosiphum euphorbiae* induces canonical PTI defense responses including ROS, induction of defense marker genes and callose deposition suggesting that GroEL is a MAMP (Chaudhary et al. 2014). Consistent with its role as a molecular pattern, heat treatment of GroEL did not affect its PTI elicitor activity. Perception of GroEL in Arabidopsis requires BAK1 suggesting the presence of a yet unidentified cell surface receptor for GroEL that likely functions with BAK1 (Fig. 2). Expressing GroEL in transgenic Arabidopsis or delivery of GroEL into tomato plants using bacterial type three secretion system, reduces aphid fecundity indicating that GroEL induces defense responses against aphids (Chaudhary et al. 2014; Elzinga et al. 2014). Since the aphid-*Buchnera* mutualism is obligate, it is intriguing to speculate that the plant immune system is exploiting this strict mutual dependency to recognize the aphid intruder.

Contrary to the role of the *Buchnera* GroEL in activating effective defenses against the aphid, a secondary endosymbiont of whiteflies *Hamiltonella defensa* exploits the antagonistic relationship between SA and JA defense hormones to suppress effective plant defenses to benefit its insect host (Su et al. 2015). Unlike *Buchnera*-aphid interactions, *H. defensa* is a facultative endosymbiont of aphids and *B. tabaci* and strains of these herbivores lacking *H. defensa* exist. The presence of *H. defensa* in *B. tabaci* confers greater longevity and fecundity to whiteflies. While both *B. tabaci* with and without *H. defensa* induce expression of SA- and JA-regulated marker genes in the first 24 h of feeding on tomato, at later time points whiteflies harboring *H. defensa* preferentially induce non-effective SA-dependent responses to suppress effective JA-dependent responses (Su et al. 2015). Consistent with these findings is the fact that long-term interactions of whitefly nymphs with host plants Arabidopsis and Lima beans also appear to use SA-JA crosstalk to suppress the most effective defenses against whiteflies (Zarate et al. 2007; Walling 2009; Zhang et al. 2009; 2013). While the nature of the *H. defensa*-associated signal is unknown, it appears to be a non-proteinaceous salivary molecule less than 3 kDa in size (Su et al. 2015).

4 Effectors

Hemipteran insects form an intimate relationship with their hosts. With the exception of the tissue-damaging hemiptera, such as leafhoppers (Backus 1988), there are relatively few visible symptoms of hemipteran feeding on plants when infestation levels are low. In addition, while feeding on susceptible hosts, hemipteran mouthparts or stylets are in continuous contact with the host tissues for prolonged periods. This intimate and symptomless relationship is thought to have evolved because of the ability to a hemipteran insect disguise itself to evade recognition or suppress immune response triggered by the plant surveillance system (Fig. 2) (Walling 2008). It has been postulated that insect saliva is the key player in this process.

Pioneering work by Peter Miles has shown that aphid saliva is a complex mixture of biomolecules with potential roles in overcoming plant immune responses (Miles 1999). Consensus has built that similar to microbial pathogens (Feng and Zhou 2012; Pais et al. 2013), hemipterans are able to manipulate host responses to their advantage (Zarate et al. 2007; Hogenhout and Bos 2011; Elzinga et al. 2014). Until recently, this conclusion has been achieved mainly from studying gene expression changes in the plant host after exposure to the insects (Zhu-Salzman et al. 2004; Zarate et al. 2007; Casteel et al. 2012; VanDoorn et al. 2015) or studying biochemical activity of aphid saliva (Miles 1999; Will et al. 2013). Recent advances in genomics technology and molecular approaches have allowed identification and direct investigation of the role of insect effectors in interactions with their hosts (Coleman et al. 2015; Walling and Kaloshian 2015 – this book).

To date, most of this work has been performed with aphid pests. Transcriptome profiling and proteomics analysis of whole insects, insect salivary glands or

proteome of salivary glands or saliva combined with bioinformatics tools have built a collection of candidate effectors that could be involved in the insect-host interactions (Walling and Kaloshian 2015). By expressing candidate effectors *in planta* either by stable transgenic approaches, transient expression or delivery by bacterial type-three secretion system, the roles for some salivary proteins in enhancing or reducing aphid survival and/or fecundity have been identified (Bos et al. 2010; Pitino and Hogenhout 2013; Chaudhary et al. 2014; Elzinga et al. 2014).

In spite of this recent progress in identifying aphid effectors that alter plant responses to aphids, hemiptera effectors that initiate ETI (see below), also known as avirulence (*Avr*) proteins, remain elusive. While several brown planthopper *Avr* loci have been mapped (Jing et al. 2014; Kobayashi et al. 2014), lack of good genetic systems and efficient gene-silencing approaches in most hempiterans, including aphids, is limiting the progress in this area of research (Coleman et al. 2015). Recently the first arthropod *Avr* gene from the gall midge pest *Mayetiola destructor* was cloned. This *Avr* gene, *vH13*, is recognized by the wheat *H13* resistance (*R*) gene and encodes a small 116-amino acid protein with no sequence similarity to known proteins (Aggarwal et al. 2014).

5 Effector-triggered Immunity

ETI is a second pathogen-sensing mechanism and is mediated by plant R proteins activating strong immune responses against pathogens and pests. Identifying plant resistance to hempiteran insects is useful for developing pest-resistant crops; when they are available, resistant genotypes are at the foundation of most integrated pest management strategies (Naranjo and Ellsworth 2009; Smith and Clement 2012). In the past half-decade, a number of loci conferring resistance to aphids and planthoppers have been identified and mapped (Smith and Clement 2012; Fujita et al. 2013; Kamphuis et al. 2013). Quite surprisingly, only five *R* loci to hempiteran insects have been cloned to date.

5.1 Cloned Resistance Loci

The five cloned *R* loci confer resistance to aphids or planthoppers. These loci include the tomato *Mi-1.2* gene and the melon (*Cucumis melo*) *Vat* gene conferring resistance to the aphids *M. euphorbiae* and *Aphis gossypii*, respectively (Rossi et al. 1998; Vos et al. 1998; Dogimont et al. 2014). The remaining three *R* loci are the rice loci *Bph3*, *Bph14*, and *Bph26* (also known as *Bph2*), which confer resistance to brown planthopper, *Nilaparvata lugens* (Du et al. 2009; Liu et al. 2014; Tamura et al. 2014). Four of the five *R* genes (*Mi1.2*, *Vat*, *Bph14* and *Bph26*) belong to the largest class of disease *R* genes encoding coiled-coil (CC) nucleotide-binding and leucine-rich repeat receptor (NLR) proteins (Fig. 2) (Rossi et al. 1998; Vos et al.

1998; Du et al. 2009; Rafiqi et al. 2009; Dogimont et al. 2014; Tamura et al. 2014). The four CC-NLRs lack organellar localization signals and therefore are thought to be confined to the cytoplasm. The genes at the *Bph3* locus have a distinct structure from the CC-NLRs.

Bph3 locus was initially identified as a major quantitative trait locus (QTL) with additional minor QTL loci influencing this broad-spectrum planthopper resistance (Lakshminarayana and Khush 1977). The *Bph3* locus contains three genes encoding plasma membrane-localized lectin receptor kinases (*OsLecRK1*, *OsLecRK2* and *OsLecRK3*) (Fig. 2) (Liu et al. 2014). In addition to the extracellular lectin-binding domain, these *Os*LecRKs contain an extracellular PAN/APPLE-like domain, which is predicted to bind protein or carbohydrate, as well as an intracellular serine/threonine kinase domain, all characteristic of G-type LecRKs. *Bph3* locus was initially identified as a major quantitative trait locus (QTL) with additional minor QTL loci influencing this broad-spectrum planthopper resistance (Lakshminarayana and Khush 1977). The cloning and detailed analysis of these loci showed that the three *OsLecRK* genes work additively to confer planthopper resistance (Liu et al. 2014). However, transgenic rice plants expressing all three *OsLecRK* genes were not as resistant as the resistant parent suggesting the requirement for additional minor QTL loci (Liu et al. 2014). Alternatively, this could reflect position effects on the expression of the *OsLecRK1-OsLecRK3* transgenes.

While *Mi-1.2* and *Bph3* encode different class of R proteins, they both confer broad–spectrum pest resistance. Besides conferring resistance to potato aphids, *Mi-1.2* confers resistance to whiteflies [*B. tabaci* B (MEAM1) and Q (MED)], psyllids (*Bactericerca cockerelli*), and three species of root-knot nematodes (*Meloidogyne arenaria*, *M. incognita* and *M. javanica*) (Roberts and Thomason 1986; Rossi et al. 1998; Vos et al. 1998; Nombela et al. 2000, 2001, 2003; Casteel et al. 2006). The *Bph3* locus confers resistance to four brown planthopper biotypes, as well as to white-backed planthopper (*Sogatella furcifera*) (Pathak and Heinrichs 1982; Shen et al. 2003; Cheng et al. 2013; Liu et al. 2014). While *Bph3* resistance is durable and has been effective in the field for over 30 years (Fujita et al. 2013), isolates of root knot nematode and potato aphid populations overcoming *Mi-1.2*-mediated resistance are well documented (Kaloshian et al. 1996; Goggin et al. 2001). The putative structure and extracellular nature of the three OsLecRKs comprising the *Bph3* locus, combined with the broad-spectrum durable resistance against planthoppers, suggest that Bph3 is functioning as a PRR and may recognize a conserved molecular pattern among planthoppers or the DAMPs released because of their feeding (Liu et al. 2014).

5.2 Forming Pairs

Consensus is emerging that R proteins function in pairs either as homodimers and/or heterodimers. For example, while the Arabidopsis RPS4 forms homodimers, RPS4 also forms heterodimers with RRS1 (Williams et al. 2014). RPS4 encodes a Toll-interleukin1 receptor (TIR)-domain NLR, while RRS1 encodes a TIR-NLR

with a WRKY domain at its C-terminus. The RPS4 and RRS1 pair confers broad-spectrum resistance to microbial pathogens including two bacterial pathogens, *Pseudomonas syringae* pv. *tomato* DC3000 expressing the effector AvrRps4 and *Ralstonia solanacearum* expressing the PopP2 effector, and to the fungal pathogen, *Colletotrichum higginsianum* with a yet uncharacterized effector (Birker et al. 2009; Narusaka et al. 2009). Interestingly, transferring this pair of genes to several plant species including those to taxonomically different families, such as tomato and cucumber (*Cucumis sativus*), also conferred broad-spectrum resistance (Narusaka et al. 2013).

The pair-of-genes requirement is not limited to the TIR-NLR proteins, as several monocot and dicot CC-NLR protein pairs also confer resistance (Cesari et al. 2014). For example, the melon *Fom-1* and *Prv* gene-pair confers resistance to the fungal pathogen *Fusarium oxysporum* and *Papaya ring-spot virus*. In addition, there are three sets of rice *R* gene pairs that mediate resistance to the rice blast fungus *Magnaporthe oryzae* and form heterodimers including: *RGA4*/*RGA5*, *Pik-1*/*Pink-2* and *Pi5-1*/*Pi5-2* (Ashikawa et al. 2008; Okuyama et al. 2011; Yuan et al. 2011; Brotman et al. 2013; Zhai et al. 2014). All of these NLR gene pairs are located next to each other on the chromosome with short intergenic regions; this close proximity of the genes avoids separation due to recombination and could potentially mediate co-regulation (Cesari et al. 2014).

In addition to sharing NLR domains, another feature of the R protein pairs is that one partner contains an atypical non-conserved domain that is often the target of the pathogen effector [reviewed in Cesari et al. (2014)]. Given the number of examples of R protein pairs that confer resistance to microbial pathogens, it seems likely that a similar strategy may be used for resistance to hemipteran insects. For example, while neither *Bph26* nor *Bph25* confer resistance to a virulent biotype of *N. lugens*, pyramiding of these rice planthopper resistance genes provides resistance suggesting that plant *R* gene pairs may also work together for insect resistance (Myint et al. 2012).

In addition to the hetero-dimer R protein pairs, several CNL and TNL R proteins form homodimers or homo-oligomers either before or after effector recognition (Bernoux et al. 2011; Maekawa et al. 2011). The CC domain and the TIR domains of the CNL and TNL R proteins, respectively, appear to facilitate multimer formation, as truncated R proteins with only the CC or TIR domains form dimers. While dimerization and elicitor-dependent activation seem to be key events for downstream signaling for microbial pathogen resistance (Cesari et al. 2014), it is not clear whether homo-dimerization is also required for the function of the R proteins against hempiteran insects.

5.3 Structure Function and Activation of Immune Receptors

Although large gaps remain in our understanding how NLR proteins are activated and transduce downstream signals for microbes and insect pests, consensus models for these steps are emerging. These models have largely been derived from

knowledge of the tomato Mi-1.2 protein, other plant NLR proteins, and structurally related proteins from animal systems (Takken and Goverse 2012). These models propose conformational resting and activated states for NLR proteins based on known protein domains and motifs (Riedl et al. 2005; Yan et al. 2005; van Ooijen et al. 2008; Maekawa et al. 2011; Takken and Goverse 2012; Qi and Innes 2013). In its resting state (prior to pest perception), NLRs are in a signaling competent but auto-inhibited conformation. In the "off" state, the NLR protein is folded allowing its NB domain to bind ADP. In the presence of a pest or pathogen effector, there is a conformational change in the NLR that releases the protein from auto-inhibition with a concomitant exchange of ADP for ATP. In its ATP-bound conformation, the NLR is active (the "on" state) allowing interaction with other proteins to initiate downstream signaling. Subsequent, ATP hydrolysis to returns the NLR to its resting state.

5.4 Direct or Indirect Recognition

NLR proteins either directly interact with their cognate effector or act indirectly by guarding an effector target or a decoy protein. Although most R proteins are thought to recognize their effectors indirectly (Rafiqi et al. 2009), several examples exist for direct interaction between NLR and pathogen effectors (Jia et al. 2000; Deslandes et al. 2003; Dodds et al. 2006). Since no hemipteran insect effector has been identified, it is unknown whether NLRs conferring resistance to insect pests interact directly or indirectly with their cognate pest effectors. Furthermore the domains that are critical for insect-effector recognition have not been investigated for most R proteins that confer resistance to insects. The exception is Mi-1.2. Using chimeric Mi-1.2 constructs, the importance of Mi-1.2's LRR domain in resistance and possibly effector recognition was shown (Hwang and Williamson 2003). However, since Mi-1.2's cognate effector protein(s) have not been identified, it is not possible to determine if the LRR domain directly binds the pest effectors.

More recently, the CC domain of a rice NLR (Pikh-1) was shown to directly interact with its cognate effector (Zhai et al. 2014). These data are consistent with the model that in NLR's resting state, the CC domain is folded and is in close proximity to the C-terminal end of the LRR. This conformational status may allow the LRR domain to indirectly sense the effector bound to the CC domain. This could be the case for Mi-1.2. Alternatively, LRR domain may bind (or guard) the CC domain. In this scenario, upon effector binding to the CC domain, the LRR detects a perturbation of CC domain to initiate defense signaling. A similar scenario was recently shown for the Arabidopsis RPS5 NLR protein; it guards the receptor-like cytoplasmic kinase PBS1. RPS5 senses PBS1 cleavage by the bacterial effector AvrPphB (Qi et al. 2012).

5.5 *R Complexes and Downstream Signaling*

The signaling components acting downstream of hemipteran R proteins are largely unexplored. NLR proteins conferring resistance to microbial pathogens frequently require the chaperone HSP90 (Heat Shock Protein 90) and the co-chaperones Rar1 (Required for MLA12 resistance 1) and SGT1 (Suppressor of the G2 allele of SKP1). To date, this has only been investigated for tomato's *Mi-1.2*-mediated resistance. Consistent with microbial pathogens, silencing of tomato's *Hsp90* or *Sgt1*, but not *Rar1*, attenuates *Mi-1.2*-mediated resistance to nematodes and the potato aphid indicating the requirement for both *Hsp90* and *Sgt1* in *Mi-1.2* resistance (Bhattarai et al. 2007). Hsp90 and Sgt1 may ensure the proper folding of Mi-1.2 and stabilize the resting and/or active conformational states of this NLR. Alternatively, Hsp90 and Sgt1 may influence the steady-state levels of Mi-1.2. This is consistent with the roles of these chaperones in the regulation of other NLR proteins (Kadota et al. 2010). In addition to its role as positive regulator, Sgt1 is also a negative regulator of NRL function. Sgt1 binds to the COP9 signalosome and the ubiquitin E3 ligase SCF (Skp1-Cullin-F box) complex that targets proteins for degradation by the proteasome (Liu et al. 2002). Therefore, these chaperones maybe universally needed for folding, stability and competence of the NLR immune complexes, including those with roles in hemipteran resistance.

Additional components identified in Mi-1.2-signaling cascade for aphids and nematodes include the receptor-like kinase *Sl*SERK1, mitogen-activated protein kinase cascades, and transcription factors WRKY70 and WRKY72 (Bhattarai et al. 2010; Mantelin et al. 2011; Atamian et al. 2012). The roles for these components in *Mi-1.2* resistance were identified using virus-induced gene silencing. Interestingly, while *Sl*SERK1 is required for aphid resistance, it is not required for nematode resistance. Considering that *Sl*SERK1 is plasma membrane localized, *Sl*SERK1 could act early in the Mi-1.2-signaling pathway with distinct signaling or recognition complexes for aphids and nematodes. It is not yet clear if *Sl*SERK1 is required for whitefly or psyllid resistance (Nombela et al. 2003; Casteel et al. 2006). The branching of the Mi-1.2-triggered immune responses to aphids, nematodes, whiteflies, and psyllids is anticipated because the mechanisms of resistance appear to be distinct. While *Mi-1.2* controls a phloem-mediated resistance to potato aphids, *Mi-1.2*-mediated resistance appears to effect resistance factors in the leaf apoplast impacting the ability of whiteflies to reach the phloem and psyllid response to Mi-1.2 volatile blends.

Finally, the *Rme1* (*Required for resistance to Meloidogyne*) locus is also required for *Mi-1.2* resistance against aphids, nematodes and whiteflies (Martinez de Ilarduya et al. 2001, 2004). *Rme1* was identified after screening a mutagenized population of resistant (*Mi-1.2*) tomato for compromised resistance to nematodes. Since the mutation in *Rme1* affects three pest resistance mechanisms (e.g., resistance to aphids, nematodes and whiteflies), it is possible that it is the target for different effectors from both nematode and hempiteran pests. It is therefore important to unravel the identity of *Rme1* to identify this potentially important virulence target.

6 Conclusions

In the past decade, major strides have been made in characterizing resistance signaling to hemipteran insects. The existence of PTI against hemiptera and early events in basal defense are being investigated and identifying extracellular immune receptors now are within reach. Similarly, the role of hemiptera-associated endosymbionts in plant immune responses is being unraveled. While these advances have been rapid and informative, limited progress has been made in cloning hemiptera *R* gene loci and limited information exists on their early signaling events. Since the majority of these *R* genes were cloned in the past few years, structure-function information for these R proteins, with the exception of Mi-1.2, is absent from the literature. With the recent technological advances and reduction in costs of genome sequencing, a large numbers of plant genomes and crop cultivars are scheduled for sequencing (Matasci et al. 2014). The availability of sequenced crop genomes, combined with development of genetic maps and marker-assisted selection, will accelerate the cloning of additional *R* genes against hemipteran pests. Similarly, the development of pest genetics and rapidly expanding genomics resources will also hasten the identification of hemipteran effectors that are recognized by R proteins and their mechanisms of recognition. The next decade promises major new discoveries in plant-hemipteran interactions.

References

Aggarwal, R., Subramanyam, S., Zhao, C. Y., Chen, M. S., Harris, M. O., & Stuart, J. J. (2014). Avirulence effector discovery in a plant galling and plant parasitic arthropod, the Hessian Fly (*Mayetiola destructor*). *PLoS One, 9*, e100958.

Albrecht, C., Boutrot, F., Segonzac, C., Schwessinger, B., Gimenez-Ibanez, S., Chinchilla, D., Rathjen, J. P., de Vries, S. C., & Zipfel, C. (2012). Brassinosteroids inhibit pathogen-associated molecular pattern-triggered immune signaling independent of the receptor kinase BAK1. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 303–308.

Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J., Matsumoto, T., Ono, K., & Yano, M. (2008). Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. *Genetics, 180*, 2267–2276.

Atamian, H. S., Eulgem, T., & Kaloshian, I. (2012). *SlWRKY70* is required for *Mi-1*-mediated resistance to aphids and nematodes in tomato. *Planta, 235*, 299–309.

Backus, E. A. (1988). Sensory systems and behaviors which mediate Hemipteran plant-feeding – a taxonomic overview. *Journal of Insect Physiology, 34*, 151–165.

Barth, C., & Jander, G. (2006). Arabidopsis myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. *The Plant Journal, 46*, 549–562.

Batista, R., & Oliveira, M. M. (2009). Facts and fiction of genetically engineered food. *Trends in Biotechnology, 27*, 277–286.

Baumann, P., Baumann, L., & Clark, M. A. (1996). Levels of *Buchnera aphidicola* chaperonin GroEL during growth of the aphid *Schizaphis graminum. Current Microbiology, 32*, 279–285.

Bednarek, P., Pislewska-Bednarek, M., Svatos, A., Schneider, B., Doubsky, J., Mansurova, M., Humphry, M., Consonni, C., Panstruga, R., Sanchez-Vallet, A., Molina, A., & Schulze-Lefert,

P. (2009). A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science, 323*, 101–106.

Bellotti, A. C., & Arias, B. (2001). Host plant resistance to whiteflies with emphasis on cassava as a case study. *Crop Protection, 20*, 813–823.

Bernoux, M., Ellis, J. G., & Dodds, P. N. (2011). New insights in plant immunity signaling activation. *Current Opinion in Plant Biology, 14*, 512–518.

Bhattarai, K. K., Li, Q., Liu, Y., Dinesh-Kumar, S. P., & Kaloshian, I. (2007). The *Mi-1*-mediated pest resistance requires *Hsp90* and *Sgt1*. *Plant Physiology, 144*, 312–323.

Bhattarai, K. K., Hagop, S. A., Kaloshian, I., & Eulgem, T. (2010). WRKY72-type transcription factors contribute to basal immunity in tomato and Arabidopsis as well as gene-for-gene resistance mediated by the tomato *R* gene *Mi-1*. *The Plant Journal, 63*, 229–240.

Birker, D., Heidrich, K., Takahara, H., Narusaka, M., Deslandes, L., Narusaka, Y., Reymond, M., Parker, J. E., & O'Connell, R. (2009). A locus conferring resistance to *Colletotrichum higginsianum* is shared by four geographically distinct Arabidopsis accessions. *The Plant Journal, 60*, 602–613.

Borgen, B. H., Ahuja, I., Thangstad, O. P., Honne, B. I., Rohloff, J., Rossiter, J. T., & Bones, A. M. (2012). 'Myrosin cells' are not a prerequisite for aphid feeding on oilseed rape (*Brassica napus*) but affect host plant preferences. *Plant Biology, 14*, 894–904.

Bos, J. I. B., Prince, D., Pitino, M., Maffei, M. E., Win, J., & Hogenhout, S. A. (2010). A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS Genetics, 6*, e1001216.

Brookes, G., & Barfoot, P. (2006). Global impact of biotech crops: Socio-economic and environmental effects in the first ten years or commercial use. *AgBioforum, 9*, 131–151.

Brotman, Y., Normantovich, M., Goldenberg, Z., Zvirin, Z., Kovalski, I., Stovbun, N., Doniger, T., Bolger, A. M., Troadec, C., Bendahmane, A., Cohen, R., Katzir, N., Pitrat, M., Dogimont, C., & Perl-Treves, R. (2013). Dual resistance of melon to *Fusarium oxysporum* races 0 and 2 and to Papaya ring-spot virus is controlled by a pair of head-to-head-oriented NB-LRR genes of unusual architecture. *Molecular Plant, 6*, 235–238.

Cao, D., Liu, Y., Walker, W. B., Li, J., & Wang, G. (2014). Molecular characterization of the *Aphis gossypii* olfactory receptor gene families. *PLoS One, 9*, e101187.

Casteel, C. L., Walling, L. L., & Paine, T. D. (2006). Behavior and biology of the tomato psyllid, *Bactericerca cockerelli*, in response to the *Mi-1.2* gene. *Experimental and Applied Acarology, 121*, 67–72.

Casteel, C. L., Hansen, A. K., Walling, L. L., & Paine, T. D. (2012). Manipulation of plant defense responses by the tomato psyllid (*Bactericerca cockerelli*) and its associated endosymbiont *Candidatus* Liberibacter Psyllaurous. *PLoS One, 7*, e35191.

Cesari, S., Bernoux, M., Moncuquet, P., Kroj, T., & Dodds, P. N. (2014). A novel conserved mechanism for plant NLR protein pairs: The "integrated decoy" hypothesis. *Front Plant Sci, 5*, 606.

Chaudhary, R., Atamian, H. S., Shen, Z., Briggs, S. P., & Kaloshian, I. (2014). GroEL from the endosymbiont *Buchnera aphidicola* betrays the aphid by triggering plant defense. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 8919–8924.

Cheng, X. Y., Zhu, L. L., & He, G. C. (2013). Towards understanding of molecular interactions between rice and the brown planthopper. *Molecular Plant, 6*, 621–634.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J. D. G., Felix, G., & Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature, 448*, 497–501.

Clay, N. K., Adio, A. M., Denoux, C., Jander, G., & Ausubel, F. M. (2009). Glucosinolate metabolites required for an Arabidopsis innate immune response. *Science, 323*, 95–101.

Coleman, A. D., Mugford, S. T., & Hogenhout, S. A. (2015). Silencing of aphid genes by dsRNA feeding from plants. In H. Czosnek & M. Ghanim (Eds.), *Management of insect pests to agriculture: Lessons learned from deciphering their genome, transcriptome and proteome* (pp. YYYY–ZZZZ). Cham: Springer.

Collins, N. C., Thordal-Christensen, H., Lipka, V., Bau, S., Kombrink, E., Qiu, J. L., Huckelhoven, R., Stein, M., Freialdenhoven, A., Somerville, S. C., & Schulze-Lefert, P. (2003). SNARE-protein-mediated disease resistance at the plant cell wall. *Nature, 425*, 973–977.

De Vos, M., & Jander, G. (2009). *Myzus persicae* (green peach aphid) salivary components induce defence responses in *Arabidopsis thaliana. Plant, Cell and Environment, 32*, 1548–1560.

De Vos, M., Van Oosten, V. R., Van Poecke, R. M. P., Van Pelt, J. A., Pozo, M. J., Mueller, M. J., Buchala, A. J., Metraux, J. P., Van Loon, L. C., Dicke, M., & Pieterse, C. M. J. (2005). Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. *Molecular Plant-Microbe Interactions, 18*, 923–937.

Deslandes, L., Olivier, J., Peeters, N., Feng, D. X., Khounlotham, M., Boucher, C., Somssich, L., Genin, S., & Marco, Y. (2003). Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 8024–8029.

Dicke, M., & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: Beyond the 'cry for help'. *Trends in Plant Science, 15*, 167–175.

Dodds, P. N., Lawrence, G. J., Catanzariti, A. M., Teh, T., Wang, C. I. A., Ayliffe, M. A., Kobe, B., & Ellis, J. G. (2006). Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 8888–8893.

Doering, T. F. (2014). How aphids find their host plants, and how they don't. *The Annals of Applied Biology, 165*, 3–26.

Dogimont, C., Chovelon, V., Pauquet, J., Boualem, A., & Bendahmane, A. (2014). The Vat locus encodes for a CC-NBS-LRR protein that confers resistance to *Aphis gossypii* infestation and *A. gossypii*-mediated virus resistance. *The Plant Journal, 80*, 993–1004.

Du, B., Zhang, W. L., Liu, B. F., Hu, J., Wei, Z., Shi, Z. Y., He, R. F., Zhu, L. L., Chen, R. Z., Han, B., & He, G. C. (2009). Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 22163–22168.

Elbaz, M., Halon, E., Malka, O., Malitsky, S., Blum, E., Aharoni, A., & Morin, S. (2012). Asymmetric adaptation to indolic and aliphatic glucosinolates in the B and Q sibling species of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Molecular Ecology, 21*, 4533–4546.

Elzinga, D. A., De Vos, M., & Jander, G. (2014). Suppression of plant defenses by a *Myzus persicae* (green peach aphid) salivary effector protein. *Molecular Plant-Microbe Interactions, 27*, 747–756.

FAO. (2009). *Food and Agriculture Organization article: 1.02 billion people hungry. One sixth of humanity undernourished – more than ever before*. http://www.fao.org/news/story/en/item/20568/icode/

Faulkner, C., Petutschnig, E., Benitez-Alfonso, Y., Beck, M., Robatzek, S., Lipka, V., & Maule, A. J. (2013). LYM2-dependent chitin perception limits molecular flux via plasmodesmata. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 9166–9170.

Feng, F., & Zhou, J.-M. (2012). Plant-bacterial pathogen interactions mediated by type III effectors. *Current Opinion in Plant Biology, 15*, 469–476.

Fujita, D., Kohli, A., & Horgan, F. G. (2013). Rice resistance to planthoppers and leafhoppers. *Critical Reviews in Plant Sciences, 32*, 162–191.

Furukawa, T., Inagaki, H., Takai, R., Hirai, H., & Che, F.-S. (2014). Two distinct EF-Tu epitopes induce immune responses in rice and Arabidopsis. *Molecular Plant-Microbe Interactions, 27*, 113–124.

Gatehouse, J. A. (2008). Biotechnological prospects for engineering insect-resistant plants. *Plant Physiology, 146*, 881–887.

Gerling, D. (1990). *Whiteflies: Their bionomics, pest status and management*. Andover: Intercept Ltd.

Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R. C., & Kant, M. R. (2012). Plant glandula trichomes as targets for breeding or engineering of resistance to herbivores. *International Journal of Molecular Sciences, 13*, 17077–17103.

Goggin, F. L., Williamson, V. M., & Ullman, D. E. (2001). Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the tomato resistance gene *Mi*. *Environmental Entomology, 30*, 101–106.

Hayafune, M., Berisio, R., Marchetti, R., Silipo, A., Kayama, M., Desaki, Y., Arima, S., Squeglia, F., Ruggiero, A., Tokuyasu, K., Molinaro, A., Kaku, H., & Shibuya, N. (2014). Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. *Proceedings of the National Academy of Sciences of the United States of America, 111*, E404–E413.

He, M., & He, P. (2014). Molecular characterization, expression profiling, and binding properties of odorant binding protein genes in the whitebacked planthopper, *Sogatella furcifera*. *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology, 174*, 1–8.

Heath, M. C. (2000). Nonhost resistance and nonspecific plant defenses. *Current Opinion in Plant Biology, 3*, 315–319.

Heese, A., Hann, D. R., Gimenez-Ibanez, S., Jones, A. M. E., He, K., Li, J., Schroeder, J. I., Peck, S. C., & Rathjen, J. P. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 12217–12222.

Heil, M., & Land, W. G. (2014). Danger signals – damaged-self recognition across the tree of life. *Frontiers in Plant Science, 5*, 578.

Hogenhout, S. A., & Bos, J. I. B. (2011). Effector proteins that modulate plant-insect interactions. *Current Opinion in Plant Biology, 14*, 422–428.

Hwang, C.-F., & Williamson, V. M. (2003). Leucine-rich repeat-mediated intramolecular interactions in nematode recognition and cell death signaling by the tomato resistance protein Mi. *The Plant Journal, 34*, 585–593.

Jander, G. (2014). Revisiting plant-herbivore co-evolution in the molecular biology era. In *Annual plant reviews* (pp. 361–384). Wiley.

Jia, Y., McAdams, S. A., Bryan, G. T., Hershey, H. P., & Valent, B. (2000). Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *The EMBO Journal, 19*, 4004–4014.

Jing, S., Zhang, L., Ma, Y., Liu, B., Zhao, Y., Yu, H., Zhou, X., Qin, R., Zhu, L., & He, G. (2014). Genome-wide mapping of virulence in brown planthopper identifies loci that break down host plant resistance. *PLoS One, 9*, e98911.

Kadota, Y., Shirasu, K., & Guerois, R. (2010). NLR sensors meet at the SGT1-HSP90 crossroad. *Trends in Biochemical Sciences, 35*, 199–207.

Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiyama, C., Dohmae, N., Takio, K., Minami, E., & Shibuya, N. (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 11086–11091.

Kaloshian, I., Williamson, V. M., Miyao, G., Lawn, D. A., & Westerdahl, B. B. (1996). Identification of "resistance breaking" field populations of root-knot nematodes on tomato in California. *California Agriculture, 50*, 18–19.

Kamphuis, L. G., Zulak, K., Gao, L.-L., Anderson, J., & Singh, K. B. (2013). Plant–aphid interactions with a focus on legumes. *Functional Plant Biology, 40*, 1271–1284.

Kazana, E., Pope, T. W., Tibbles, L., Bridges, M., Pickett, J. A., Bones, A. M., Powell, G., & Rossiter, J. T. (2007). The cabbage aphid: A walking mustard oil bomb. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 274*, 2271–2277.

Kempema, L. A., Cui, X., Holzer, F. M., & Walling, L. L. (2007). Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiology, 143*, 849–865.

Kim, J. H., & Jander, G. (2007). *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. *The Plant Journal, 49*, 1008–1019.

Kishaba, A. N., Castle, S. J., Coudriet, D. L., McCreight, J. D., & Bohn, G. W. (1992). Virus transmission by *Aphis gossypii* Glover to aphid-resistant and susceptible muskmelons. *Journal of the American Society for Horticultural Science, 117*, 248–254.

Kliebenstein, D. J. (2014). Quantitative genetics and genomics of plant resistance to insects. In *Annual plant reviews* (pp. 235–262). Wiley, Chichester, UK.

Kliebenstein, D. J., Kroymann, J., & Mitchell-Olds, T. (2005). The glucosinolate-myrosinase system in an ecological and evolutionary context. *Current Opinion in Plant Biology, 8*, 264–271.

Kobayashi, T., Yamamoto, K., Suetsugu, Y., Kuwazaki, S., Hattori, M., Jairin, J., Sanada-Morimura, S., & Matsumura, M. (2014). Genetic mapping of the rice resistance-breaking gene of the brown planthopper *Nilaparvata lugens. Proceedings of the Royal Society of London. Series B: Biological Sciences, 281*, 20140726.

Kwon, C., Neu, C., Pajonk, S., Yun, H. S., Lipka, U., Humphry, M., Bau, S., Straus, M., Kwaaitaal, M., Rampelt, H., El Kasmi, F., Jurgens, G., Parker, J., Panstruga, R., Lipka, V., & Schulze-Lefert, P. (2008). Co-option of a default secretory pathway for plant immune responses. *Nature, 451*, 835–841.

Lacombe, S., Rougon-Cardoso, A., Sherwood, E., Peeters, N., Dahlbeck, D., van Esse, H. P., Smoker, M., Rallapalli, G., Thomma, B., Staskawicz, B., Jones, J. D. G., & Zipfel, C. (2010). Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nature Biotechnology, 28*, 365–394.

Lakshminarayana, A., & Khush, G. S. (1977). New genes for resistance to brown planthopper in rice. *Crop Science, 17*, 96–100.

Laluk, K., Luo, H. L., Chai, M. F., Dhawan, R., Lai, Z. B., & Mengiste, T. (2011). Biochemical and genetic requirements for function of the immune response regulator BOTRYTIS-INDUCED KINASE1 in plant growth, ethylene signaling, and PAMP-triggered immunity in Arabidopsis. *Plant Cell, 23*, 2831–2849.

Last, R. L., Jones, A. D., Pichersky, E., Barry, C. S., Kim, J., Schilmiller, A., Kang, K. Y., & Gonzales-Vigil, E. (2012). Hairy genomics: Studies of secretory glandular trichomes in tomato and relatives. *Pharmaceutical Biology 50*, 619–619.

Lei, J. X., Finlayson, S. A., Salzman, R. A., Shan, L. B., & Zhu-Salzman, K. (2014). BOTRYTIS-INDUCED KINASE1 modulates Arabidopsis resistance to green peach aphids via PHYTOALEXIN DEFICIENT4. *Plant Physiology, 165*, 1657–1670.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S., & Schulze-Lefert, P. (2005). Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. *Science, 310*, 1180–1183.

Liu, Y., Schiff, M., Serino, G., Deng, X. W., & Dinesh-Kumar, S. P. (2002). Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene-mediated resistance response to Tobacco mosaic virus. *Plant Cell, 14*, 1483–1496.

Liu, B., Li, J. F., Ao, Y., Qu, J., Li, Z., Su, J., Zhang, Y., Liu, J., Feng, D., Qi, K., He, Y., Wang, J., & Wang, H. B. (2012a). Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. *Plant Cell, 24*, 3406–3419.

Liu, T., Liu, Z., Song, C., Hu, Y., Han, Z., She, J., Fan, F., Wang, J., Jin, C., Chang, J., Zhou, J. M., & Chai, J. (2012b). Chitin-induced dimerization activates a plant immune receptor. *Science, 336*, 1160–1164.

Liu, Y., Wu, H., Chen, H., Liu, Y., He, J., Kang, H., Sun, Z., Pan, G., Wang, Q., Hu, J., Zhou, F., Zhou, K., Zheng, X., Ren, Y., Chen, L., Wang, Y., Zhao, Z., Lin, Q., Wu, F., Zhang, X., Guo, X., Cheng, X., Jiang, L., Wu, C., Wang, H., & Wan, J. (2014). A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. *Nature Biotechnology, 33*(3), 301–5.

Lu, D. P., Wu, S. J., Gao, X. Q., Zhang, Y. L., Shan, L. B., & He, P. (2010). A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate

immunity. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 496–501.

Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., & Ton, J. (2011). Callose deposition: A multifaceted plant defense response. *Molecular Plant-Microbe Interactions, 24*, 183–193.

Maekawa, T., Cheng, W., Spiridon, L. N., Toeller, A., Lukasik, E., Saijo, Y., Liu, P., Shen, Q.-H., Micluta, M. A., Somssich, I. E., Takken, F. L. W., Petrescu, A.-J., Chai, J., & Schulze-Lefert, P. (2011). Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. *Cell Host & Microbe, 9*, 187–199.

Mantelin, S., Peng, H. C., Li, B., Atamian, H., Takken, F. L. W., & Kaloshian, I. (2011). The receptor-like kinase SISERK1 is required for *Mi-1*-mediated resistance to potato aphids in tomato. *The Plant Journal, 67*, 459–471.

Martinez de Ilarduya, O., Moore, A. E., & Kaloshian, I. (2001). The tomato *Rme1* locus is required for *Mi-1*-mediated resistance to root-knot nematodes and the potato aphid. *The Plant Journal, 27*, 417–425.

Martinez de Ilarduya, O., Nombela, G., Hwang, C. F., Williamson, V. M., Muniz, M., & Kaloshian, I. (2004). *Rme1* is necessary for *Mi-1*-mediated resistance and acts early in the resistance pathway. *Molecular Plant-Microbe Interactions, 17*, 55–61.

Matasci, N., Hung, L.-H., Yan, Z., Carpenter, E., Wickett, N., Mirarab, S., Nguyen, N., Warnow, T., Ayyampalayam, S., Barker, M., Burleigh, J., Gitzendanner, M., Wafula, E., Der, J., dePamphilis, C., Roure, B., Philippe, H., Ruhfel, B., Miles, N., Graham, S., Mathews, S., Surek, B., Melkonian, M., Soltis, D., Soltis, P., Rothfels, C., Pokorny, L., Shaw, J., DeGironimo, L., & Stevenson, D. (2014). Data access for the 1,000 Plants (1KP) project. *GigaScience, 3*, 17.

Merzendorfer, H., & Zimoch, L. (2003). Chitin metabolism in insects: Structure, function and regulation of chitin synthases and chitinases. *Journal of Experimental Biology, 206*, 4393–4412.

Miles, P. W. (1999). Aphid saliva. *Biological Reviews, 74*, 41–85.

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., & Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 104*, 19613–19618.

Muller, C., & Riederer, M. (2005). Plant surface properties in chemical ecology. *Journal of Chemical Ecology, 31*, 2621–2651.

Myint, K. K. M., Fujita, D., Matsumura, M., Sonoda, T., Yoshimura, A., & Yasui, H. (2012). Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvata lugens* Stal) in the rice cultivar ADR52. *Theoretical and Applied Genetics, 124*, 495–504.

Naranjo, S. E., & Ellsworth, P. C. (2009). Fifty years of the integrated control concept: Moving the model and implementation forward in Arizona. *Pest Management Science, 65*, 1267–1286.

Narusaka, M., Shirasu, K., Noutoshi, Y., Kubo, Y., Shiraishi, T., Iwabuchi, M., & Narusaka, Y. (2009). RRS1 and RPS4 provide a dual resistance-gene system against fungal and bacterial pathogens. *The Plant Journal, 60*, 218–226.

Narusaka, M., Kubo, Y., Hatakeyama, K., Imamura, J., Ezura, H., Nanasato, Y., Tabei, Y., Takano, Y., Shirasu, K., & Narusaka, Y. (2013). Interfamily transfer of dual NB-LRR genes confers resistance to multiple pathogens. *PLoS One, 8*, e55954.

Newman, M. A., Sundelin, T., Nielsen, J. T., & Erbs, G. (2013). MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Frontiers in Plant Science, 4*, 139.

Nombela, G., Beitia, F., & Muniz, M. (2000). Variation in tomato host response to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to acyl sugar content and presence of the nematode and potato aphid resistance gene *Mi. Bulletin of Entomological Research, 90*, 161–167.

Nombela, G., Beitia, F., & Muniz, M. (2001). A differential interaction study of *Bemisia tabac*i Q-biotype on commercial tomato varieties with or without the *Mi* resistance gene, and comparative host responses with the B-biotype. *Entomologia Experimentalis et Applicata, 98*, 339–344.

Nombela, G., Williamson, V. M., & Muniz, M. (2003). The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci. Molecular Plant-Microbe Interactions, 16*, 645–649.

Nurnberger, T., & Lipka, V. (2005). Non-host resistance in plants: New insights into an old phenomenon. *Molecular Plant Pathology, 6*, 335–345.

Oerke, E. C. (2006). Crop losses due to pests. *The Journal of Agricultural Science, 144*, 31–43.

Okuyama, Y., Kanzaki, H., Abe, A., Yoshida, K., Tamiru, M., Saitoh, H., Fujibe, T., Matsumura, H., Shenton, M., Galam, D. C., Undan, J., Ito, A., Sone, T., & Terauchi, R. (2011). A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *The Plant Journal, 66*, 467–479.

Pais, M., Win, J., Yoshida, K., Etherington, G. J., Cano, L. M., Raffaele, S., Banfield, M. J., Jones, A., Kamoun, S., & Saunders, D. G. O. (2013). From pathogen genomes to host plant processes: The power of plant parasitic oomycetes. *Genome Biology, 14*, 211.

Panda, N., & Khush, G. S. (1995). *Host plant resistance to insects* (p. 431). Wallingford: CAB International.

Pathak, P. K., & Heinrichs, E. A. (1982). Selection of biotype population-2 and population-3 of *Nilaparvata lugens* (Homoptera, Delphacidae) by exposure to resistant rice varities. *Environmental Entomology, 11*, 85–90.

Pitino, M., & Hogenhout, S. A. (2013). Aphid protein effectors promote aphid colonization in a plant species-specific manner. *Molecular Plant-Microbe Interactions, 26*, 130–139.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology, 51*, 309–330.

Prince, D. C., Drurey, C., Zipfel, C., & Hogenhout, S. A. (2014). The leucine-rich repeat receptor-like kinase BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1 and the cytochrome P450 PHYTOALEXIN DEFICIENT3 contribute to innate immunity to aphids in Arabidopsis. *Plant Physiology, 164*, 2207–2219.

Qi, D., & Innes, R. W. (2013). Recent advances in plant NLR structure, function, localization, and signaling. *Frontiers in Immunology, 4*, 348–348.

Qi, D., DeYoung, B. J., & Innes, R. W. (2012). Structure-function analysis of the coiled-coil and leucine-rich repeat domains of the RPS5 disease resistance protein. *Plant Physiology, 158*, 1819–1832.

Rafiqi, M., Bernoux, M., Ellis, J. G., & Dodds, P. N. (2009). In the trenches of plant pathogen recognition: Role of NB-LRR proteins. *Seminars in Cell and Developmental Biology, 20*, 1017–1024.

Reina-Pinto, J. J., & Yephremov, A. (2009). Surface lipids and plant defenses. *Plant Physiology and Biochemistry, 47*, 540–549.

Ribaut, J.-M., de Vicente, M. C., & Delannay, X. (2010). Molecular breeding in developing countries: Challenges and perspectives. *Current Opinion in Plant Biology, 13*, 213–218.

Riedl, S. J., Li, W., Chao, Y., Schwarzenbacher, R., & Shi, Y. (2005). Structure of the apoptotic protease-activating factor 1 bound to ADP. *Nature, 434*, 926–933.

Robatzek, S., Bittel, P., Chinchilla, D., Kochner, P., Felix, G., Shiu, S. H., & Boller, T. (2007). Molecular identification and characterization of the tomato flagellin receptor LeFLS2, an orthologue of *Arabidopsis* FLS2 exhibiting characteristically different perception specificities. *Plant Molecular Biology, 64*, 539–547.

Roberts, P. A., & Thomason, I. J. (1986). Variabilty in reproduction of isolates of *Meloidogyne incognita* and *Meloidogyne javanica* on resistant tomato genotypes. *Plant Disease, 70*, 547–551.

Rossi, M., Goggin, F. L., Milligan, S. B., Kaloshian, I., Ullman, D. E., & Williamson, V. M. (1998). The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 9750–9754.

Roux, M., Schwessinger, B., Albrecht, C., Chinchilla, D., Jones, A., Holton, N., Malinovsky, F. G., Tör, M., de Vries, S., & Zipfel, C. (2011). The Arabidopsis leucine-rich repeat receptor–like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell, 23*, 2440–2455.

Sabri, A., Vandermoten, S., Leroy, P. D., Haubruge, E., Hance, T., Thonart, P., De Pauw, E., & Francis, F. (2013). Proteomic investigation of aphid honeydew reveals an unexpected diversity of proteins. *PLoS One, 8*, e74656.

Shen, J., Wang, Y., Sogawa, K., Hattori, M., & Liu, G. J. (2003). Virulence of the populations of the whitebacked planthopper, *Sogatella furcifera* reared on different resistant rice varieties. *Chinese Journal of Rice Science, 11*, 57–61.

Shibuya, N., & Minami, E. (2001). Oligosaccharide signalling for defence responses in plant. *Molecular Plant-Microbe Interactions, 59*, 223–233.

Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., Minami, E., Okada, K., Yamane, H., Kaku, H., & Shibuya, N. (2010). Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. *The Plant Journal, 64*, 204–214.

Smadja, C., Shi, P., Butlin, R. K., & Robertson, H. M. (2009). Large gene family expansions and adaptive evolution for odorant and gustatory receptors in the pea aphid, *Acyrthosiphon pisum. Molecular Biology and Evolution, 26*, 2073–2086.

Smadja, C. M., Canback, B., Vitalis, R., Gautier, M., Ferrari, J., Zhou, J. J., & Butlin, R. K. (2012). Large-scale candidate gene scan reveals the role of chemoreceptor genes in host plant specialization and speciation in the pea aphid. *Evolution, 66*, 2723–2738.

Smith, C. M., & Chuang, W.-P. (2014). Plant resistance to aphid feeding: Behavioral, physiological, genetic and molecular cues regulate aphid host selection and feeding. *Pest Management Science, 70*, 528–540.

Smith, C. M., & Clement, S. L. (2012). Molecular bases of plant resistance to arthropods. *Annual Review of Entomology, 57*, 309–328.

Stam, R., Mantelin, S., McLellan, H., & Thilliez, G. (2014). The role of effectors in nonhost resistance to filamentous plant pathogens. *Front Plant Sci, 5*, 582.

Stein, M., Dittgen, J., Sanchez-Rodriguez, C., Hou, B. H., Molina, A., Schulze-Lefert, P., Lipka, V., & Somerville, S. (2006). Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. *Plant Cell, 18*, 731–746.

Su, Q., Oliver, K. M., Xie, W., Wu, Q., Wang, S., & Zhang, Y. (2015). The whitefly-associated facultative symbiont *Hamiltonella defensa* suppresses induced plant defences in tomato. *Functional Ecology*, 29, 1007–1018.

Sun, Y. D., Li, L., Macho, A. P., Han, Z. F., Hu, Z. H., Zipfel, C., Zhou, J. M., & Chai, J. J. (2013). Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. *Science, 342*, 624–628.

Takai, R., Isogai, A., Takayama, S., & Che, F. S. (2008). Analysis of flagellin perception mediated by flg22 receptor OsFLS2 in rice. *Molecular Plant-Microbe Interactions, 21*, 1635–1642.

Takken, F. L. W., & Goverse, A. (2012). How to build a pathogen detector: Structural basis of NB-LRR function. *Current Opinion in Plant Biology, 15*, 375–384.

Tamura, Y., Hattori, M., Yoshioka, H., Yoshioka, M., Takahashi, A., Wu, J., Sentoku, N., & Yasui, H. (2014). Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. indica cultivar ADR52. *Scientific Reports, 4*, 5872.

Tanguy, S., & Dedryver, C. A. (2009). Reduced BYDV-PAV transmission by the grain aphid in a *Triticum monococcum* line. *European Journal of Plant Pathology, 123*, 281–289.

Trda, L., Fernandez, O., Boutrot, F., Heloir, M.-C., Kelloniemi, J., Daire, X., Adrian, M., Clement, C., Zipfel, C., Dorey, S., & Poinssot, B. (2014). The grapevine flagellin receptor VvFLS2 differentially recognizes flagellin-derived epitopes from the endophytic growth-promoting bacterium *Burkholderia phytofirmans* and plant pathogenic bacteria. *The New Phytologist, 201*, 1371–1384.

van Ooijen, G., Mayr, G., Kasiem, M. M. A., Albrecht, M., Cornelissen, B. J. C., Takken, F. L. W., van Ooijen, G., Mayr, G., Kasiem, M. M. A., Albrecht, M., Cornelissen, B. J. C., & Takken, F. L. W. (2008). Structure-function analysis of the NB-ARC domain of plant disease resistance proteins. *Journal of Experimental Botany, 59*, 1383–1397.

Vandermoten, S., Harmel, N., Mazzucchelli, G., De Pauw, E., Haubruge, E., & Francis, F. (2014). Comparative analyses of salivary proteins from three aphid species. *Insect Molecular Biology, 23*, 67–77.

VanDoorn, A., de Vries, M., Kant, M. R., & Schuurink, R. C. (2015). Whiteflies glycosylate salicylic acid and secrete the conjugate via their honeydew. *Journal of Chemical Ecology, 41*, 52–58.

Veronese, P., Nakagami, H., Bluhm, B., AbuQamar, S., Chen, X., Salmeron, J., Dietrich, R. A., Hirt, H., & Mengiste, T. (2006). The membrane-anchored BOTRYTIS-INDUCED KINASE1 plays distinct roles in Arabidopsis resistance to necrotrophic and biotrophic pathogens. *Plant Cell, 18*, 257–273.

Vos, P., Simons, G., Jesse, T., Wijbrandi, J., Heinen, L., Hogers, R., Frijters, A., Groenendijk, J., Diergaarde, P., Reijans, M., FierensOnstenk, J., deBoth, M., Peleman, J., Liharska, T., Hontelez, J., & Zabeau, M. (1998). The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. *Nature Biotechnology, 16*, 1365–1369.

Wagner, G. J., Wang, E., & Shepherd, R. W. (2004). New approaches for studying and exploiting an old protuberance, the plant trichome. *Annals of Botany, 93*, 3–11.

Walker, G. P. (1987). Probing and oviposition behavior of the bayberry whitefly (Homoptera, Aleyrodidae) on young and mature lemon leaves. *Annals of the Entomological Society of America, 80*, 524–529.

Walling, L. L. (2008). Avoiding effective defenses: Strategies employed by phloem-feeding insects. *Plant Physiology, 146*, 859–866.

Walling, L. L. (2009). Adaptive defense responses to pathogens and pests. *Advances in Botanical Research, 51*, 551–612.

Walling, L. L., & Kaloshian, I. (2015). Plant-herbivore interactions in the era of big data. In M. Ghanim & H. Csoznek (Eds.), *Management of insect pests to agriculture: Lessons learned from deciphering their genome, transcriptome and proteome* (pp. YYYY–ZZZZ). Cham: Springer.

Walling, L. L., & Thompson, G. A. (2013). Behavioral and molecular-genetic basis of resistance against phloem feeding insects. In A. van Bel & G. A. Thompson (Eds.), *Phloem: Molecular cell biology, systemic communication, biotic interactions*. Oxford: Wiley-Blackwell.

Will, T., Furch, A. C. U., & Zimmermann, M. R. (2013). How phloem-feeding insects face the challenge of phloem-located defenses. *Frontiers in Plant Science, 4*, 336.

Williams, S. J., Sohn, K. H., Wan, L., Bernoux, M., Sarris, P. F., Segonzac, C., Ve, T., Ma, Y., Saucet, S. B., Ericsson, D. J., Casey, L. W., Lonhienne, T., Winzor, D. J., Zhang, X., Coerdt, A., Parker, J. E., Dodds, P. N., Kobe, B., & Jones, J. D. G. (2014). Structural basis for assembly and function of a heterodimeric plant immune receptor. *Science, 344*, 299–303.

Wilson, A. C., Ashton, P. D., Calevro, F., Charles, H., Colella, S., Febvay, G., Jander, G., Kushlan, P. F., Macdonald, S. J., Schwartz, J. F., Thomas, G. H., & Douglas, A. E. (2010). Genomic insight into the amino acid relations of the pea aphid, *Acyrthosiphon pisum*, with its symbiotic bacterium *Buchnera aphidicola. Insect Molecular Biology, 19*(Suppl 2), 249–258.

Yan, N., Chai, J., Lee, E. S., Gu, L., Liu, Q., He, J., Wu, J. W., Kokel, D., Li, H., Hao, Q., Xue, D., & Shi, Y. (2005). Structure of the CED-4-CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans. Nature, 437*, 831–837.

Yencho, G. C., Cohen, M. B., & Byrne, P. F. (2000). Applications of tagging and mapping insect resistance loci in plants. *Annual Review of Entomology, 45*, 393–422.

Yuan, B., Zhai, C., Wang, W., Zeng, X., Xu, X., Hu, H., Lin, F., Wang, L., & Pan, Q. (2011). The *Pik-p* resistance to *Magnaporthe oryzae* in rice is mediated by a pair of closely linked CC-NBS-LRR genes. *Theoretical and Applied Genetics, 122*, 1017–1028.

Zarate, S. I., Kempema, L. A., & Walling, L. L. (2007). Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology, 143*, 866–875.

Zhai, C., Zhang, Y., Yao, N., Lin, F., Liu, Z., Dong, Z., Wang, L., & Pan, Q. (2014). Function and interaction of the coupled genes responsible for *Pik-h* encoded rice blast resistance. *PLoS One, 9*, e98067.

Zhang, Z., Feechan, A., Pedersen, C., Newman, M.-A., Qiu, J. L., Olesen, K. L., & Thordal-Christensen, H. (2007). A SNARE-protein has opposing functions in penetration resistance and defence signalling pathways. *The Plant Journal, 49*, 302–312.

Zhang, P. J., Zheng, S. J., van Loon, J. J. A., Boland, W., David, A., Mumm, R., & Dicke, M. (2009). Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 21202–21207.

Zhang, J., Li, W., Xiang, T. T., Liu, Z. X., Laluk, K., Ding, X. J., Zou, Y., Gao, M. H., Zhang, X. J., Chen, S., Mengiste, T., Zhang, Y. L., & Zhou, J. M. (2010). Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host & Microbe, 7*, 290–301.

Zhang, P. J., Li, W. D., Huang, F., Zhang, J. M., Xu, F. C., & Lu, Y. B. (2013). Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. *Journal of Chemical Ecology, 39*, 612–619.

Zhu-Salzman, K., Salzman, R. A., Ahn, J.-E., & Koiwa, H. (2004). Transcriptional regulation of Sorghum defense determinants against a phloem-feeding aphid. *Plant Physiology, 134*, 420–431.

Zipfel, C. (2014). Plant pattern-recognition receptors. *Trends in Immunology, 35*, 345–351.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T., & Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. *Cell, 125*, 749–760.

Silencing of Aphid Genes by dsRNA Feeding from Plants

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Abstract RNA interference (RNAi) has been used to knock down gene expression and study gene function in many insect species, including economically important plant pests such as aphids. RNAi can be achieved by dsRNA injection into insect bodies, insect feeding on artificial medium containing dsRNAs and by exposure of insects to transgenic plants that transiently or stably produce dsRNAs corresponding to the insect genes (plant-mediated RNAi). The latter approach could be used to control insect pests in crops.

1 Overview

Over the past 15 years RNA interference (RNAi) has been successfully exploited as a reverse-genetics tool to study gene function in various organisms. Some of the earliest RNAi studies in insects include work on the fruit fly, *Drosophila melanogaster* (Elbashir et al. 2001). Since then RNAi has been successfully utilized in multiple insect systems using a variety of means, including direct injection of dsRNA/siRNA into larvae or adults, exogenous application of double-stranded RNA (dsRNA)/small-interfering RNA (siRNA), transfection using bacterial or viral expression systems, and feeding of dsRNA/siRNA on artificial diets or via transgenic plant expression (Mao et al. 2007; Yu et al. 2013). RNAi of insect genes achieved via insect feeding on transgenic plants, or 'plant-mediated RNAi' has been established to study gene function in aphids. Aphids are of considerable economic concern due to feeding on important crop plants and as vectors of numerous plant viruses. Plant-mediated RNAi can be utilized to determine the function of aphid genes involved in diverse processes such as plant-insect interactions, virus transmission, development and metabolism. Plant-mediated RNAi may further be used as a tool to control aphid pests in the field via transgenic crop plants.

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2 RNA Interference

RNA interference (RNAi) is a means of post-transcriptional gene regulation conserved across eukaryotes, including both plants and insects, and has many essential roles in normal growth and development, and in defense responses against viruses or transposable elements (Hannon 2002). Endogenous gene-regulation via RNAi occurs via the production of microRNAs (miRNA) from precursor transcripts, which facilitate the recognition and degradation of target transcripts in a sequence-specific manner. RNAi also operates in the context of defence against viruses through the processing of viral double-stranded RNA (dsRNA) molecules into small-interfering RNAs (siRNA) derivatives. These siRNAs target viral RNA molecules in a sequence-specific manner leading to reduced virus RNA abundance (Fire et al. 1998). Thus, it is possible to introduce a dsRNA molecule exogenously, designed to suppress endogenous target gene expression via highly-specific depletion of target transcripts. This has been used as a method to study gene function in many organisms. Here we discuss a method to suppress gene expression in aphids through the expression of dsRNAs corresponding to aphid genes in the host plants on which the aphids are feeding.

3 RNAi in Aphid Systems

RNAi-mediated gene knockdown was first achieved in aphids through direct injection of dsRNA or siRNA into aphid hemolymph. This approach was used to silence *C002*, a gene strongly expressed in the salivary glands of pea aphids (Mutti et al. 2006). Silencing this gene resulted in lethality of the aphids on plants, but not on an artificial diet, indicating that C002 functions in aphid interaction with the plant host (Mutti et al. 2006, 2008). Microinjection of long dsRNA into pea aphids also leads to silencing of genes encoding a calreticulin and a cathepsin by 30–40 % (Jaubert-Possamai et al. 2007). The calreticulin is a calcium-binding protein that is produced in most aphid tissues, while at least some cathepsins (cysteine proteases) are specifically expressed in the pea aphid gut. Thus, RNAi is achievable for genes that are expressed across a range of different tissues (Jaubert-Possamai et al. 2007).

Feeding of dsRNA produced *in-vitro* from an artificial diet can also suppress expression of the corresponding aphid gene. Pea aphids fed on an artificial diet containing dsRNA corresponding to the aquaporin transcript lead to down-regulation by more than twofold within 24 h (Shakesby et al. 2009). Consistent with the role of aquaporins in osmoregulation, this resulted in elevated osmotic pressure in the hemolymph (Shakesby et al. 2009). In a similar study, feeding of dsRNA targeting *vATPase* transcripts from an artificial diet achieved a 30 % decrease in transcript levels in pea aphids and a significant increase in aphid mortality (Whyard et al. 2009).

4 Plant-Mediated RNA Interference

As aphid RNAi was shown to be feasible through the above methods, more recent studies have achieved RNA in aphids by feeding the aphids on transgenic plants expressing dsRNAs (Pitino et al. 2011; Bhatia et al. 2012; Pitino and Hogenhout 2013; Guo et al. 2014; Coleman et al. 2015). Briefly, cDNA fragments corresponding to known aphid transcripts can be cloned into a hairpin vector for expression of dsRNAs under control of a suitable promoter e.g. 35S promoter for constitutive expression. Upon feeding from host tissues aphids may acquire the long dsRNAs or *in planta* processed shorter versions of the dsRNAs, including siRNAs.

The green peach aphid (*Myzus persicae*) has been the main aphid species used for plant-mediated RNAi (PMRi) approaches, chiefly because it is capable of feeding on the model plants *Nicotiana benthamiana* and *Arabidopsis thaliana*, whereas the model aphid species, the pea aphid (*Acyrthosiphon pisum*) is restricted to legumes. PMRi has also been shown to be effective in other aphid species (Xu et al. 2014), and also across other insect taxa: in Coleoptera (Baum et al. 2007), and Lepidoptera (Mao et al. 2007). DsRNA corresponding to aphid genes can be produced transiently in *N. benthamiana* leaves by infiltration with *Agrobacterium tumeficiens* bearing the dsRNA-encoding tDNA. As well, stable transgenic *A. thaliana* plants can be produced by *Agrobacterium*-mediated transformation (Pitino et al. 2011; Coleman et al. 2015). The former is quicker and less labor intensive and hence is highly suitable for candidate screening, whereas the latter is suited to more detailed analyses and longer-term studies of aphid populations (Coleman et al. 2015).

Plant-mediated RNAi holds certain advantages over other methods. For example: the process allows for experiments in a more biologically relevant context; less manipulation of target insects is required; it avoids damaging of the insect; and it is applicable to multiple insects at once. Plant-mediated RNAi is amenable for functional genomic studies and may prove suitable for direct control of insect pests in the field.

5 Application of Plant-Mediated RNAi in Aphid Functional Genomics

Genomics information is currently being gathered for a multitude of insect species, for example the i5k initiative is a 5-year project aiming to sequence the genomes of 5000 insect and related arthropod species by 2016 (i5k Consortium 2013). This opens up RNAi-based tools to an increasing number of insect systems. The genome of the pea aphid (*A. pisum*) is published (The International Aphid Genomics Consortium 2010), and the *M. persicae* draft genome sequence is available ahead of publication (http://www.aphidbase.com/aphidbase/). PMRi may therefore be a valuable tool to use alongside the growing wealth of sequence data.

Plant-mediated RNAi of three genes, *Rack1*, *MpC002* and *MpPIntO2* (also known as *Mp2*), was shown to reduce fecundity of *M. persicae* (Pitino et al. 2011; Pitino and Hogenhout 2013; Coleman et al. 2015). These studies have highlighted the important roles of these genes for the aphid. Plant-mediated RNAi of another target gene, *MpPIntO1* (also known as *Mp1*), also successfully depleted the transcript abundance but did not result in reduced fecundity of the aphids (Pitino and Hogenhout 2013). It has also been shown that RNAi is equally effective for genes expressed predominantly in different tissues, i.e. *Rack1* in the gut and *MpC002* in the salivary glands (Pitino et al. 2011).

Transgenic plants producing dsRNA corresponding to *Myzus persicae* serine protease (*MySP*) resulted in significant attenuation of the expression of *MySP* in green peach aphids and a corresponding decline in gut protease activity (Bhatia et al. 2012). Down-regulation of *MySP* resulted in a significant reduction in aphid fecundity. In another study, several genes from *M. persicae* were targeted for silencing by PMRi, including the acetylcholinesterase 2 coding gene (*MpAChE2*), V-type proton ATPase subunit E-like (*V-ATPaseE*), and the tubulin folding cofactor D (*TBCD*); all of which resulted in successful reduction of expression and increased aphid resistance in transgenic plants (Guo et al. 2014). Similarly, plant-mediated RNAi was used to target the carboxylesterase (*CbE E4*) gene in *Sitobion avenae* using transgenic wheat (Xu et al. 2014). This severely impaired *S. avenae* tolerance towards Phoxim insecticides.

PMRi has multiple applications in diverse areas of aphid research including development, metabolism, insecticide resistance, as well as interactions with hosts, viruses and endosymbionts. PMRi could be used to investigate aphid genes involved in insecticide resistance e.g. detoxifying enzymes such as cytochrome P450s (Ramsey et al. 2010), to understand how insects quickly develop pesticide resistance, potentially providing a means to suppress pesticide resistance in the field, and also assisting in the development of novel pesticides.

As aphids subjected to PMRi are reared on host plants, this makes it particularly amenable to study plant-insect interactions. It could therefore contribute towards understanding how insects overcome plant defenses and adapt to their hosts. Aphid species differ in their life strategies and host range. The pea aphid, *A. pisum*, for example, is a specialist legume feeder whereas the green peach aphid, *M. persicae*, can feed on over 40 plant families, and is able to overcome the diverse defence mechanisms of all of these. This is achieved, at least in part, through the action of effectors, which are secreted proteins in the aphid saliva that modulate host plant defence mechanisms. RNAi has been used to understand the function of aphid salivary proteins involved in colonization of host plants (Bos et al. 2010; Pitino and Hogenhout 2013). Finally, it is not fully understood how aphids mediate the transmission of plant viruses. PMRi can be used to identify aphid proteins involved in the non-persistent and persistent transmission of plant viruses, and reduce the harm to crop plants.

6 Potential of Plant-Mediated RNAi to Control Aphid Pests

Aphids are among the most important insect pests of arable crops in temperate regions worldwide (Blackman and Eastop 2000). As well as causing direct feeding damage to crops, aphids are capable of transmitting about 30 % of the plant virus species discovered to date (Ng and Perry 2004; Hogenhout et al. 2008).

As well as use as a reverse-genetics tool to study gene function, there is also potential to use RNAi as a direct means of pest control. A breakthrough study by Baum et al. (2007) demonstrated the potential of RNAi to control coleopteran insect pests. Transgenic maize plants that were engineered to produce dsRNAs corresponding to the western corn rootworm resulted in significantly reduced feeding damage as a result of rootworm attack. Silenced insects displayed larval stunting and increased mortality (Baum et al. 2007). In another study, the model plants *Nicotiana tabacum* and *A. thaliana* were modified to produce dsRNA corresponding to a cytochrome P450 gene of the cotton bollworm (Mao et al. 2007). When larvae were fed transgenic leaves, levels of the cytochrome P450 mRNA were reduced and larval growth retarded (Mao et al. 2007).

Down-regulation of various aphid genes via PMRi has been shown to reduce aphid fecundity, indicating that this technique may have useful applications in agriculture to manage aphid infestation (Pitino et al. 2011; Bhatia et al. 2012; Pitino and Hogenhout 2013; Xu et al. 2014; Coleman et al. 2015). Aphid populations reproduce mainly via parthenogenic reproduction, facilitating rapid exponential population growth. Even a relatively small effect on population growth rate may have substantial agricultural impact (Coleman et al. 2015). Furthermore, there is an enormous library of genes that could be targeted to provide a wide-range of useful phenotypic effects other than direct mortality or reduced fecundity. This could include, for example, increased susceptibility to pesticides to integrate current pest-control strategies (Xu et al. 2014), or to block virus transmission.

One of the main advantages of plant-mediated RNAi is the selectivity of RNAi based on the sequence identity of the targeted transcript. It is feasible to design constructs for highly specific targeting at the level of the single species or even biotype, or alternatively a more broad-range construct based on conserved target regions may be designed to target multiple insects. The number of RNAi targets could also be stacked in transgenic plants, allowing the plant to produce a strong cocktail of dsRNAs which the target insect would struggle to overcome. The potential uses of plant-mediated RNAi are therefore multifarious and could be a powerful tool in future aphid control strategies.

References

Baum, J. A., Bogaert, T., Clinton, W., Heck, G. R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., Vaughn, T., & Roberts, J. (2007). Control of coleopteran insect pests through RNA interference. *Nature Biotechnology, 25*(11), 1322–1326.

Bhatia, V., Bhattacharya, R., Uniyal, P. L., Singh, R., & Niranjan, R. S. (2012). Host generated siRNAs attenuate expression of serine protease gene in *Myzus persicae. PLoS One, 7*(10), e46343.

Blackman, R. L., & Eastop, V. F. (2000). *Aphids on the world's crops: an identification and information guide*. Chichester, UK: Wiley-Blackwell.

Bos, J. I. B., Prince, D., Pitino, M., Maffei, M. E., Win, J., & Hogenhout, S. A. (2010). A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS Genetics, 6*(11), e1001216.

Coleman, A. D., Wouters, R. H. M., Mugford, S. T., & Hogenhout, S. A. (2015). Persistence and transgenerational effect of plant-mediated RNAi in aphids. *Journal of Experimental Botany, 66*(2), 541–548.

Elbashir, S. M., Martinez, J., Patkaniowska, A., Lendeckel, W., & Tuschl, T. (2001). Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO Journal, 20*(23), 6877–6888.

Fire, A., Xu, S. Q., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans. Nature, 391*(6669), 806–811.

Guo, H., Song, X., Wang, G., Yang, K., Wang, Y., Niu, L., Chen, X., & Fang, R. (2014). Plant-generated artificial small RNAs mediated aphid resistance. *PLoS One, 9*(5), e97410.

Hannon, G. J. (2002). RNA interference. *Nature, 418*(6894), 244–251.

Hogenhout, S. A., Ammar, E. D., Whitfield, A. E., & Redinbaugh, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology, 46*, 327–359.

i5K Consortium. (2013). The i5K initiative: advancing arthropod genomics for knowledge, human health, agriculture, and the environment. *Journal of Heredity, 104*(5), 595–600.

Jaubert-Possamai, S., Le Trionnaire, G., Bonhomme, J., Christophides, G. K., Rispe, C., & Tagu, D. (2007). Gene knockdown by RNAi in the pea aphid *Acyrthosiphon pisum. BMC Biotechnology, 7*, 8.

Mao, Y. B., Cai, W. J., Wang, J. W., Hong, G. J., Tao, X. Y., Wang, L. J., Huang, Y. P., & Chen, X. Y. (2007). Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature Biotechnology, 25*, 1307–1313.

Mutti, N. S., Park, Y., Reese, J. C., & Reeck, G. R. (2006). RNAi knockdown of a salivary transcript leading to lethality in the pea aphid, *Acyrthosiphon pisum. Journal of Insect Science, 6*.

Mutti, N. S., Louis, J., Pappan, L. K., Pappan, K., Begum, K., Chen, M. S., Park, Y., Dittmer, N., Marshall, J., Reese, J. C., & Reeck, G. R. (2008). A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences of the United States of America, 105*(29), 9965–9969.

Ng, J. C. K., & Perry, K. L. (2004). Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology, 5*(5), 505–511.

Pitino, M., & Hogenhout, S. A. (2013). Aphid protein effectors promote aphid colonization in a plant species-specific manner. *Molecular Plant-Microbe Interactions, 26*(1), 130–139.

Pitino, M., Coleman, A. D., Maffei, M. E., Ridout, C. J., & Hogenhout, S. A. (2011). Silencing of aphid genes by dsRNA feeding from plants. *PLoS One, 6*(10), e25709.

Ramsey, J. S., Rider, D. S., Walsh, T. K., De Vos, M., Gordon, K. H. J., Ponnala, L., Macmil, S. L., Roe, B. A., & Jander, G. (2010). Comparative analysis of detoxification enzymes in *Acyrthosiphon pisum* and *Myzus persicae. Insect Molecular Biology, 19*, 155–164.

Shakesby, A. J., Wallace, I. S., Isaacs, H. V., Pritchard, J., Roberts, D. M., & Douglas, A. E. (2009). A water-specific aquaporin involved in aphid osmoregulation. *Insect Biochemistry and Molecular Biology, 39*(1), 1–10.

The International Aphid Genomics Consortium. (2010). Genome sequence of the pea aphid *Acyrthosiphon pisum. PLoS Biology, 8*(2), e1000313.

Whyard, S., Singh, A. D., & Wong, S. (2009). Ingested double-stranded RNAs can act as species-specific insecticides. *Insect Biochemistry and Molecular Biology, 39*(11), 824–832.

Xu, L., Duan, X., Lv, Y., Zhang, X., Nie, Z., Xie, C., Ni, Z., & Liang, R. (2014). Silencing of an aphid carboxylesterase gene by use of plant-mediated RNAi impairs *Sitobion avenae* tolerance of Phoxim insecticides. *Transgenic Research, 23*(2), 389–396.

Yu, N., Christiaens, O., Liu, J., Niu, J., Cappelle, K., Caccia, S., Huvenne, H., & Smagghe, G. (2013). Delivery of dsRNA for RNAi in insects: an overview and future directions. *Insect Science, 20*(1), 4–14.

Management of Rice Planthoppers Through Recent Advanced Research

Hiroaki Noda

Abstract Rice planthoppers are the most serious insect pest in rice production, and improvements in the management of rice planthoppers is of the highest-priority. Three major rice planthopper species, *Nilaparvata lugens*, *Sogatella furcifera*, and *Laodelphax striatellus*, show characteristics different from each other. In this chapter, various aspects of the three planthopper species, especially most important species *N. lugens*, are described from the viewpoint of planthopper management. Much attention is paid to recent molecular studies of planthoppers and related organisms.

1 Overview

Knowledge of planthopper management has been accumulated from field evidence and ecological, behavioral, and physiological studies of planthoppers and rice plants. There have been remarkable recent advances in molecular studies of rice planthoppers, and genomic and genetic studies are also showing steady progress. Gene functional studies of rice planthoppers have started to use RNAi techniques and other recent technologies. One negative aspect, however, is the gap that exists between the accumulated knowledge of planthopper management strategies and the recent growing knowledge of molecular studies. Even though molecular studies of planthoppers still lack depth in comparison with some model insects, it is worth introducing recent achievements of molecular and genomics studies into planthopper management, which this chapter aims to do. A number of important aspects of planthopper management are illustrated and explained using recent knowledge, including molecular and genomics studies, together with data and conclusions from older rice planthopper studies.

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There are three major rice planthoppers: the brown (rice) planthopper *Nilaparvata lugens* (BPH), the white-backed (rice) planthopper *Sogatella furcifera* (WBPH), and the small brown (rice) planthopper *Laodelphax striatellus* (SBPH). Since the most serious pest in rice production is the BPH among many pest species of rice plants (Wilson and Claridge 1985), the BPH is the central figure in this chapter. The other two planthopper species are also dealt with in some aspects, and these species are not far behind the BPH in their economic importance according to circumstances in some regions. The scientific reports for molecular aspects of rice planthoppers have been greatly increased in number in these a few years and will be expected to rise further. This chapter may not include all of the striking reports published, but the author has tried to include the majority of literature that is important in regards to planthopper management.

2 Basic Concepts and Current Status of Planthopper Control

The three major rice planthopper species are all virus vectors. However, the BPH and the WBPH cause major damage by sucking the phloem sap of the rice plant. In contrast, the SBPH cause less sucking damage, but it is an important pest in the transmission of viruses. The BPH and the WBPH became much more serious pests following the Green Evolution of rice in the 1960s, during which time large quantities of fertilizer were used for new rice varieties (Dyck et al. 1979). Paddy fields, where rice plants with rich nutrients are cultivated, have become ideal environments for planthopper growth and development. Asian countries have been dealing with rice planthopper problems for several decades (Cheng 2009; Watanabe et al. 2009; Catindig et al. 2009; Bottrell and Schoenly 2012).

Applied entomologists have investigated various aspects of planthopper management, including meteorological conditions for planthopper flight, planthopper population change following immigration into rice fields, rice plant resistance to planthoppers, virulence of planthoppers against resistant rice varieties, insecticide application to planthoppers, insecticide-resistant strains of planthoppers, and virus transmission by planthoppers. Currently, major control methods include cultivation of planthopper-resistant rice varieties and the use of insecticides to keep planthopper populations low. Biological control by natural enemies is also an important method of planthopper management, but biological control is seldom performed in a responsible manner and is typically restricted to trials of conservation by natural enemies in the rice fields of practical farmers. In order to effectively control planthopper populations, particularly by chemical means in subtropical and temperate regions, it is important to understand the temporal developmental stages of planthoppers and the temporal population density of planthoppers within rice fields.

Additional information, such as the timing of the main planthopper immigration, immigrant population size, biological features of the immigrants (virulence level of breaking resistant rice varieties or insecticide-resistance level), and temporal growing stages of rice plants, is also helpful in controlling planthopper populations in the field.

The BPH and the WBPH live throughout the whole year, mainly in tropical regions of Southeast Asia (Catindig et al. 2009; Hu et al. 2012), and they do not undergo diapause, nor can they overwinter in northern subtropical and temperate regions. In contrast, the SBPH is distributed over a wide geographical area, including Europe (Achon et al. 2013), and undergoes diapause during the nymphal stage in cool regions (Noda 1992; Wang et al. 2013). Thus, the three planthopper species occur during different seasons, and the timings of population increases and the critical period for chemical control differ among the three species. In temperate regions, the SBPH appears in rice fields in May or June, just after transplanting. Viruliferous SBPHs transmit rice stripe virus to rice plants in spring and early summer, thus, it is important to control populations of the SBPH during these seasons to prevent plant viral disease. The WBPH and the BPH do not overwinter in temperate regions and immigrate rice fields after long-distance flights each year (Kisimoto and Rosenberg 1994). The flights are supported by strong winds, such as the westerly jet, that occur in the rainy season in east Asia (June and July). These two species emigrate from tropical and southern subtropical regions into northern subtropical and temperate regions from May to July. The initial population size of the WBPH is larger than that of the BPH. The WBPH lays large numbers of eggs and its population peaks in the following generation or the generation after that in mid-summer. Populations of the BPH gradually increase in the field and cause damage two or three generations following the arrival of the immigrants (early or mid-autumn). Therefore, the timing of chemical control against the WBPH and the BPH differs and careful surveying of planthoppers in the field is necessary when making decisions regarding the use of chemical controls. In tropical regions, the cropping season and the system appear to affect the population density in the respective rice fields as well as the initial population size. Obtaining temporal population growth patterns of planthoppers in each area is a key issue for effective planthopper control.

Many insecticides have been used to control planthoppers, including organophosphorus, carbamate, and pyrethroid compounds (Heinrichs 1994). Neonicotinoid insecticides have been used since the 1990s, and have successfully suppressed outbreaks of rice planthoppers, especially those of the BPH. Remarkably, the residual and potent activity of the neonicotinoid insecticides enabled farmers to use just a single application during the nursery stage, removing the need to consider specific timing for insecticide application throughout the rice growing season. Once farmers apply the insecticide during the nursery stage of the rice plants, the insecticides remain effective for long periods, typically until the flight season of the WBPH and the BPH. In many rice fields in Japan, nursery rice plants are planted in May–

June, following the application of persistent systemic insecticides to them (nursery box application). This labor-saving control method provides effective control of the immigrants, which appear in June–July, providing farmers with a significant benefit. However, excessive use of neonicotinoid insecticides in Asian countries has produced insecticide-resistant strains of planthoppers (Matsumura et al. 2009), thus, labor-saving insecticide application on nursery plants has been less effective. Since 2005, insecticide-resistant strains have resulted in outbreaks of planthoppers (Catindig et al. 2009), and the establishment of effective planthopper control methods is urgently required.

In these circumstances, recent progress in the study of planthoppers, especially in molecular and genomic fields, may provide useful information and methods against the threat of planthoppers. Enserink et al. (2013) discussed insecticide use and its problems, stating that "pest control can become much smarter, and science has a major role to play." As regards the planthopper problem, molecular and genomic studies can have an additional key role to play in more sophisticated management of planthoppers.

3 Rice Damage by Planthopper Attack

The three major rice planthoppers, *Nilaparvata lugens* (BPH), *Sogatella furcifera* (WBPH), and *Laodelphax striatellus* (SBPH), differ in the symptoms of damage they cause to rice plants based on their behavioral, ecological, and physiological features. Damage caused by these planthoppers can be largely divided into two categories: (1) damage caused by rice plant viruses transmitted by the planthoppers, and (2) damage caused by the planthoppers sucking sap from the rice plants.

More than 15 viruses are known to occur in rice plants (Nault 1994; Hibino 1996; Zhou et al. 2008) and 5 viruses are transmitted by rice planthoppers (Table 1).

Table 1 Rice plant viruses transmitted by three major rice planthoppers

Virus name	Abbreviation	Genus	Main vector	Transmission manner
Rice grassy stunt virus	RGSV	Tenuivirus	*Nilaparvata lugens*	Persistent
Rice ragged stunt virus	RRSV	Oryzavirus	*Nilaparvata lugens*	Persistent
Southern rice black-streaked dwarf virus	SRBSDV	Fijivirus	*Sogatella furcifera*	Persistent?
Rice stripe virus	RSV	Tenuivirus	*Laodelphax striatellus*	Persistent
Rice black-streaked dwarf virus	RBSDV	Fijivirus	*Laodelphax striatellus*	Persistent

Southern rice black-streaked dwarf virus (SRBSDV), transmitted by the WBPH, was discovered relatively recently (Zhou et al. 2008). Rice stripe virus (RSV) is transmitted by the SBPH, and rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) are transmitted by the BPH. Serious outbreaks of RSV, RGSV, and RRSV have also been observed. Symptoms of virus diseases are described in many studies (Hibino 1996; Cabauatan et al. 2009; Zhou et al. 2013), and can be seen here: RSV at http://www.dpvweb.net/dpv/showdpv.php?dpvno=375, RGSV at http://www.dpvweb.net/dpv/showdpv.php?dpvno=320, and RRSV at http://www.dpvweb.net/dpv/showdpv.php?dpvno=248. Planthopper viruses are not discussed in depth, and are dealt with under the section of associated organisms of rice planthoppers.

Sucking damage to rice plants is mainly caused by the BPH and the WBPH. The SBPH rarely causes sucking damage to rice plants because population levels of this species in rice fields are typically low. The BPH and the WBPH often attack en masse and infestations of these two planthoppers are sometimes intolerably heavy for rice plants. However, the two planthoppers inflict damage to different stages of rice plants, and the damage symptoms of these two planthoppers are also quite different.

Sucking damage caused by the BPH (Fig. 1a, b) is often typified by "hopperburn" (resulting in the complete death of plants by desiccation), which is caused by heavy infestations of the planthopper. Hopperburn usually occurs in the middle to

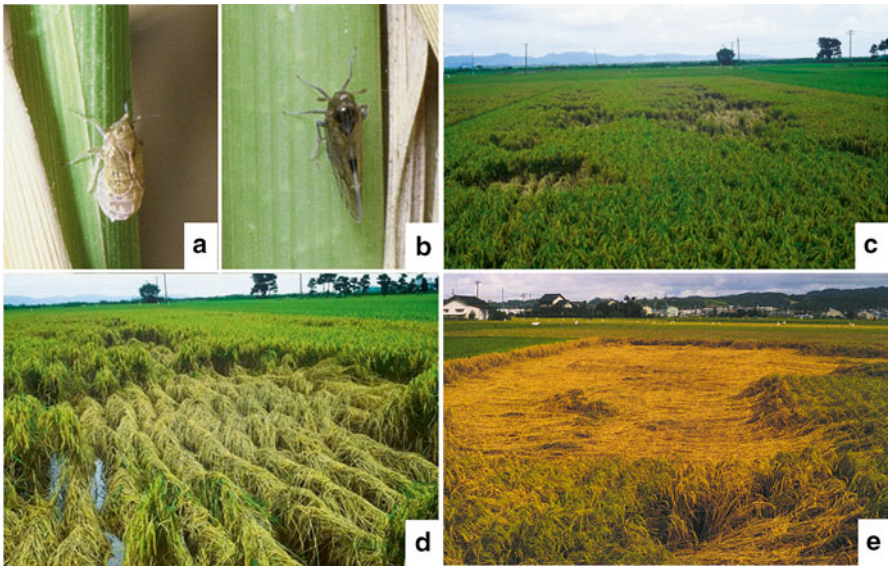

Fig. 1 The brown planthopper *Nilaparvata lugens* and the damage it causes to rice plants. (**a**) pregnant brachypterous adult female; (**b**) macropterous adult male; (**c**) hopperburn during the middle of the ripening stage of rice plants; (**d**) hopperburn during the late ripening stage of rice plants; (**e**) field-wide death of rice plants following heavy infestation

late stages of rice plant development; whereas an infestation of the BPH during the reproductive growth stages (early to middle stages) of rice plants sometimes reduces the number of tillers and panicles, and limits the total weight gain of the plants (Bae and Pathak 1970). Since the BPH prefers the lower stems of rice plants, they migrate down the plants after arriving as winged adults and then increase in numbers on the lower stems. Once they settle at the base of a rice plant, they seldom move to another rice plant unless conditions deteriorate. The BPH, therefore, shows patchy distribution across rice fields. Hopperburn occurs at the point when the BPH propagates in the rice fields (Fig. 1c). Heavy sucking by many planthoppers from the phloem deprives the rice plant of fluids and destroys the physiological homeostasis of the rice plant. Field-wide hopperburn occasionally occurs during the late stage of rice plant development due to the widespread dispersal of the BPH across the field (Fig. 1d, e). Furthermore, rice plants may be less tolerant of rapid water extraction by the BPH sucking during the late stage of development. A large time lag exists between the immigration of the BPH and the occurrence of hopperburn because hopperburn occurs following an increase in the population size of the BPH as they transition through multiple generations in the rice field (Kuno 1979). This lag time is an important period for predicting possible damage to rice plants and controlling the BPH population to prevent damage. It is, therefore, important to know how many planthoppers are locally distributed in the field and the development stage of the majority of planthoppers. When the population size of planthoppers is predicted to exceed acceptable levels, chemical control should be applied during the early to early-middle nymphal stages, because mature nymphs suck the plant heavily and hopperburn usually begins to occur when the majority of the BPH population is at the late nymphal stage.

Damage caused by the WBPH differs from that caused by the BPH, and three types of WBPH damage are typically recognized in the field. First, the immigrant generation of the WBPH has a larger population size than that of the BPH, and the population is relatively uniformly distributed across the rice field. WBPH often damages young stage of rice plants. The WBPH lays eggs by vertically rupturing the stem, resulting in yellow discoloration and brown scars on the stems and leaves (Fig. 2a). The young rice plants also sustain damage from sucking. This damage appears to delay plant growth and reduce the number of tillers. Second, if the mature nymphs of the next generation of immigrants meet the ear-heading stage of the rice plant, they attack the emerging glumes, of which sucked-parts turn brown (Fig. 2b, c) (Noda 1986a), and heavily attacked panicles do not ripen (Fig. 2d). Third, rice plants sometimes die following heavy infestation by the WBPH (Fig. 2e), though this damage occurs less frequently. Hopperburn caused by the WBPH usually occurs in young stages of the rice plant and is often inflicted by the next generation of WBPH immigrants, sometimes accompanying attacks by other pests or diseases. The boundary of hopperburn in the rice field is less clear than that caused by the BPH.

Fig. 2 Damage caused by the white-backed planthopper *Sogatella furcifera*. (**a**) damage in young rice plants from oviposition and heavy sucking by immigrant adults; (**b**) browning on a young panicle of a rice plant caused by sucking; (**c**) nymphs attacking emerging rice plant ear, (**d**) sterile panicles on the ear, which were attacked by planthoppers in the field; (**e**) firing of young rice plants following heavy planthopper infestation

4 Mouthparts and Feeding

Planthoppers are vascular feeders, and rice planthoppers extract nutrients from the phloem sap of rice plants. The vascular bundle of plants consists of two main transport tissues (phloem and xylem) that effect the long distance translocation of heterogeneous constituents in higher-level plants (Will et al. 2013). The stem of rice plants is composed of leaf sheaths, and the BPH prefers to probe into the thick segment of the outer leaf sheath (Wang et al. 2008a), where thick vascular bundles are located. Planthoppers have a pointed mouthpart for vascular feeding, which consists of a pointed part, the labium, situated on the labrum, where the stylets are located (Fig. 3a). The stylets penetrate the distal part of the labium and appear from the tip of the labium (Fig. 3b). Four stylets (Fig. 3c), which are derived from pairs of mandibles and maxillae, form a stylet fascicle (Backus 1985). The mandibular stylets encompass the maxillary stylets, and the maxillary stylets form two canals: a food canal and a smaller salivary canal (Sogawa 1982; Backus 1985). The plant sap is taken in through the food canal. When planthoppers feed, the tip of the labium is positioned on the surface of the rice plant, and the stylets are inserted into the plant. Salivary fluid is discharged from the tip of the stylet fascicle, and the salivary flange is created on the plant (Fig. 4) and the stylet sheath (salivary sheath) is created inside the plant. The stylet flange and the stylet sheath are usually

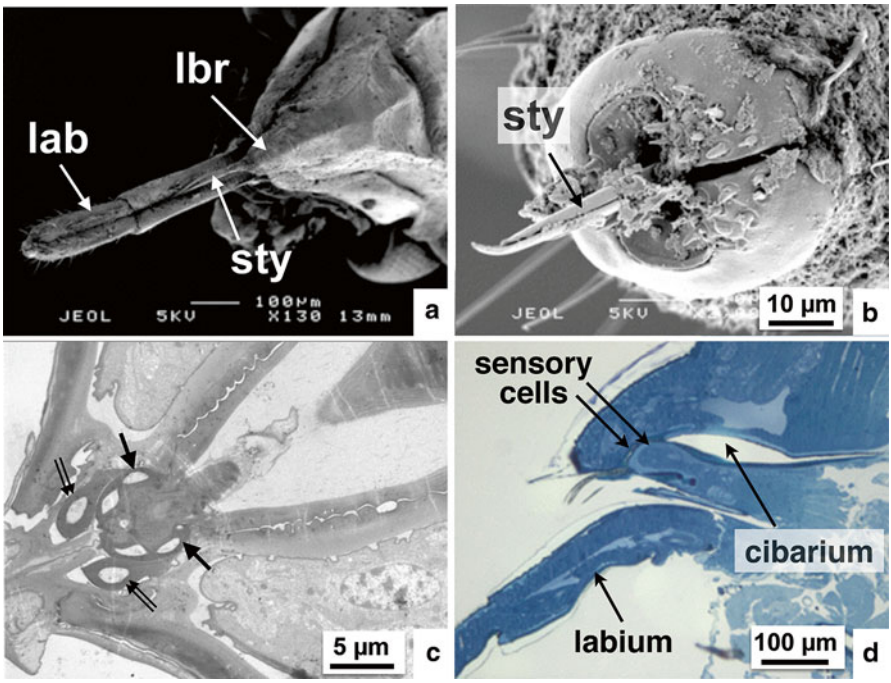

Fig. 3 Electron micrographs of the mouthparts of *Nilaparvata lugens*. (**a**) Scanning electron micrograph of the labium (*lab*), stylets (*sty*), and labrum (*lbr*); (**b**) Scanning electron micrograph of tip of the labium and projecting stylets; (**c**) Transmission electron micrograph of transverse section of stylets near the basal part in the labrum, showing pairs of maxillary (*arrow*) and mandibular (*double arrows*) stylets; (**d**) Transverse section of the head. Food taken through the stylets is transferred into the cibarium that connects to the alimentary canal. Sensory cells are observed in the anterior part of the cibarium

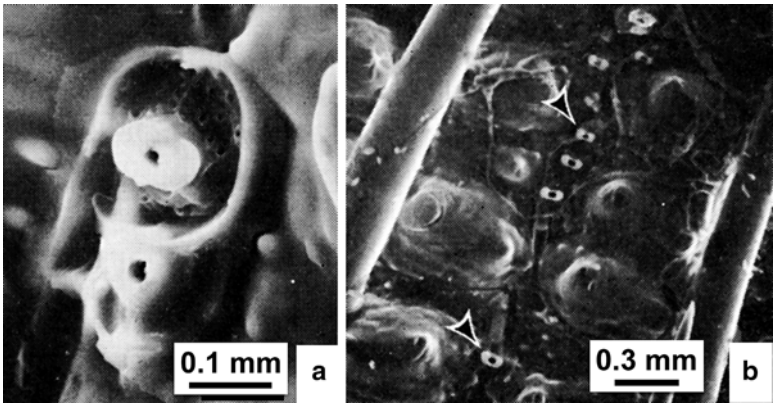

Fig. 4 Scanning electron micrographs of salivary flanges of *Sogatella furcifera*. (**a**) salivary flanges; (**b**) salivary flanges on the hollow region of the rice plant panicle (From Noda 1986a)

connected. The stylet sheath envelops the entire stylet fascicle in the plant and ensures smooth sucking from the phloem sieve elements. Planthoppers explore for phloem by stylet probing, which usually creates side branches on the stylet sheath within the rice plant. The salivary flange is considered useful for ensuring that the labium remains vertically mounted on the plants. The stylet sheath is typically formed in many species of phytophagous hemipteran insects (Morgan et al. 2013).

The salivary glands of the BPH are composed of a group of principal glands that are made of eight different types of secretory follicles (lobes) and an accessary gland (Sogawa 1965). Each follicle contains different salivary materials, including enzymes and stylet sheath materials. The saliva of plant-sap sucking insects is usually divided into two categories: watery and gelling (gel, coagulable) saliva (Miles 1968, 1972, 1999). The watery saliva contains various enzymes that are involved in digestion and detoxification, and is also considered to be used to trigger plant defenses and for escaping from plant defenses. In contrast, gelling saliva solidifies, forming a stylet sheath within the plant and a salivary flange on the outside of the plant.

A number of enzymes that function during feeding, digestion, and detoxification are reported to exist in the salivary glands of leafhoppers and planthoppers: for example, diphenol oxidase (or laccase) (Sogawa 1968a; Hattori et al. 2005), glucosidases (Sogawa 1968b; Nakamura and Hattori 2013), and endoglucanase (Backus et al. 2012). Enzymes for carbohydrate digestion, such as amylase or sucrose, observed in aphids (Miles 1972, 1999), may also be present in the saliva of planthoppers. Proteomic studies of aphids have revealed that the saliva includes digestive and detoxifying enzymes as well as other proteins plausibly involved in signaling cascades and stylet sheath formation (Carolan et al. 2009, 2011; Rao et al. 2013). Comprehensive transcriptome analyses have also been conducted for the BPH (Ji et al. 2013). The BPH secretes a catalase-like protein, which is considered to work against hydrogen peroxide created by rice plants as an endogenous defense substance (Petrova and Smith 2014). Expressed sequence tag (EST) studies, however, have revealed that only four enzymes are found in the top 30 highly expressed genes in the salivary glands of the BPH: enolase (phosphopyruvate hydratase), aldolase (probably fructose-1,6-bisphosphate aldolase), PNPase (purine nucleoside phosphorylase, inosine phosphorylase), and ADP/ATP translocase (Noda et al. 2008; Hasegawa et al. 2015). Moreover, in planthoppers, these enzymes do not seem to actively participate in food digestion. Enolase and aldolase are enzymes involved in glycolysis, which occurs in the cells rather than during the digestive process of ingesting food (Murray et al. 2006). PNPase and ADP/ATP translocase are involved in purine metabolism and the energy acquisition process, respectively. These enzymes work within cells, especially ADP/ATP translocase, which is a transporter protein involved in the inner mitochondrial membrane. This demonstrates that the genes which encode enzymes that are involved in food digestion are not actively expressed in the salivary glands. Proteomic analysis of the BPH supports this phenomenon. Enzymes involved in cell metabolism were observed together with housekeeping proteins, but glycosidase and phenoloxidases were not detected in the salivary glands (Konishi et al. 2009), demonstrating that the

expression level of these enzymes is relatively low. Salivary enzymes that are involved in food digestion or detoxification may be effective even in small amounts.

EST and proteomic analyses have revealed that important proteins are involved in interactions between insects and host plants, rather than as food digestive enzymes. Proteins with an EF-hand domain that are thought to be found in watery saliva (Konishi et al. 2009; Hasegawa et al. 2015) are found in the salivary glands. The EF-hand domain is the most common calcium-binding motif in proteins, and the most abundant protein in the green rice leafhopper *Nephotettix cincticeps* is a protein with EF-hand motifs (Hattori et al. 2012). The *Nephotettix* protein has been shown to possess calcium-binding ability. The two genes, both of which encodes proteins with EF-hand motifs, were the 9th and 10th most highly expressed during EST analysis of the salivary glands of the BPH. Calcium-binding proteins have also been reported in the salivary glands of aphids (Will et al. 2007; Carolan et al. 2009) and are considered to be a common component in the saliva of phloem (or vascular) feeders. Damage to sieve tubes disturbs the existing pressure conditions, which affects intracellular calcium (Ca^{2+}) levels (Will et al. 2013). Calcium is known to be a mediator for plugging sieve tubes of plants. The EF-hand motif is thought to work as a Ca^{2+} chelating agent to prevent sieve tube occlusion (King and Zeevaart 1974; Knoblauch et al. 2001; Hattori et al. 2012). Ca^{2+} is also required for callose synthase activity (Kauss et al. 1983; Hayashi et al. 1987), which is thought to be involved in the wound response in the sieve tubes (Kauss 1987). EF-hand motif proteins or calcium-binding proteins are considered to facilitate continuous feeding by inhibiting sieve-element plugging, which is a plant defense response against damage to the sieve tube elements.

The major structural proteins that form the stylet sheath should be highly expressed in the salivary glands. Large quantities of the components of gelling saliva appear to be required to produce the stylet sheath, which is quite a contrast to the small amount of enzymes found in watery saliva; small amounts of enzymes may be sufficient for their functioning. A stylet sheath protein, which is produced in the principal gland cell of the salivary glands, is reported in BPH (Huang et al. 2015). The gene of this protein shows alternative splicing, and knockdown of the both splicing variants by RNAi resulted in a lethal phenotype, indicating this protein is essential for feeding. Some salivary proteins are rich in tyrosine, which appears to contribute to the formation of tight cross-linking, enabling the synthesis of the tough stylet sheath to allow stable and continuous feeding by the BPH (Hasegawa et al. 2015). These tyrosine-rich proteins were not observed during a typical proteomic study of the salivary glands (Konishi et al. 2009) because of the difficulty in dissociating the gelling salivary proteins.

An important mechanism within planthopper mouthparts is a sensory mechanism used for feeding. Though it is still unclear how planthoppers detect the phloem and evaluate the suitability of the plant sap for feeding, the sensory receptors in the mouthpart are likely to play an important role. Chemical compounds in plant tissues influence BPH stylet probing (Sogawa 1974), which is considered to be important for locating the phloem. Food intake by the BPH is markedly affected by various nutrients and their concentration (Sakai and Sogawa 1976). The BPH presumably feeds through a complicated process that is controlled by mechanical and chemical senses. Sensory receptors are known to exist in three regions of the mouthparts of

BPH; the labial tip, the stylets, and the cibarium (posteriorly-located canal of the stylets, providing a connecting link between the food canal of the maxillary stylets and the esophagus) (Fig. 3d). The BPH possesses many sensory receptors on the labium and a mechanoreceptive dendrite and chemoreceptive dendrite are located in a peg receptor on the sensory field (Foster et al. 1983a). Basically, planthoppers have two apical pairs of sensory fields (dorsal and ventral) (Brozek and Bourgoin 2013), and these receptors may influence initiation of feeding on rice plants. Sensory neurons are also located in the mandibular and maxillary stylets of the BPH; these stylets have cavities containing the dendrites (Foster et al. 1983b). The stylet sensilla of planthoppers appear to be used for stylet probing, and the mechanosensory function of these sensilla is thought to allow the planthoppers to discriminate the vascular bundle from other tissues. The stylet sensory organs have been found in all hemipterans examined (Backus 1985). Cibarial (precibarial) sensilla are also found in aphids, leafhoppers, and planthoppers. The stylet food duct opens to a narrow canal, which leads to the wide cibarium and then to the esophagus. Three groups of receptors are found in the narrow canal of the BPH (Foster et al. 1983b). These receptors appear to be involved in gustatory responses during the initial stages of stylet probing and continuous feeding of phloem sap. Proteins related to chemoreception are reported in BPH; 11 genes coding for odorant binding proteins (OBPs), 17 for chemosensory proteins (CSPs), 50 for odorant receptors (ORs), and 10 for gustatory receptors (GRs) are found from genome analyses (Xue et al. 2014). Expression profiles of CSPs are also studied (Yang et al. 2014). Little is known about the receptors and their related molecules, and these areas need to be elucidated in future studies.

5 Molecular Studies and Tools

Molecular studies of rice planthoppers were limited during the 2000s, except for studies related to certain metabolic enzymes and insecticide resistance. Meanwhile, EST data was provided for the BPH (Noda et al. 2008) and RNAi was proven to be effective for functional studies. Technical progress in RNA sequencing (RNA-Seq) has boosted molecular studies of rice planthoppers. Transcriptome analyses are actively performed in rice planthoppers, especially in the BPH, and differentially expressed genes (DEGs) have been compared among different samples (Xue et al. 2010; Peng et al. 2011; Bao et al. 2012; Zhai et al. 2013; Yu et al. 2014). Additionally, there has been an increasing number of reports on BPH genes that are thought to be important for food intake, metabolism, detoxification, and reproduction, etc. Proteomic studies have also been conducted (Zhai et al. 2013), and studies of noncoding RNA (Chen et al. 2012; Xiao et al. 2015) and the function of microRNA are beginning to be undertaken (Chen et al. 2013; Xu et al. 2013). A draft genome sequence of 1.14 gigabase was reported in BPH, showing there are many insertion sequences present in this rather large-sized genome (Xue et al. 2014). The publication of the BPH genome sequence facilitates the molecular studies of rice planthoppers.

For gene functional studies, RNAi is a powerful technique, which is very effective in planthoppers. A number of methods of dsRNA uptake have been examined in various insects (Yang et al. 2011) and these methods strongly influence the efficiency of gene silencing. Microinjection is often used in insects, and soaking can be used for embryos. Using microinjection, Liu et al. (2010b) reported RNAi in the *calreticulin* gene, the *cathepsin B-like protease* gene, and the *nicotinic acetylcholine receptor subunit* gene *NlnAChR_β2* of the BPH. The use of an artificial diet is a convenient and more natural method of introducing dsRNA into insects. Chen et al. (2010) performed dsRNA ingestion of the *trehalose phosphate synthase* gene in the BPH, and reported that mRNA levels decrease by approximately 70 % and enzyme activity decreased by 50 %. In the case of two *trahalase* genes in the SBPH, mRNA expression level decreased by 40–50 % and enzyme activity by approximately 25 % (Zhang et al. 2012a). However, oral delivery of dsRNA is not so efficient in comparison with injection of dsRNA and this has been confirmed in the corn planthopper *Peregrinus maidis* by introducing dsRNA in a liquid diet (Yao et al. 2013). Many reports deal with nymphal and adult RNAi, but probable parental RNAi was shown by Xu et al. (2013) using the *Distal-less* gene, a homeodomain transcription factor, by observing distal limb structures in the offspring of the injected female 5th instar nymph. Parental RNAi was also observed when dsRNA of the *chitin synthase* gene was injected into female adults, resulting in incomplete embryonic development (Kawai and Noda 2010).

There is a favorable outlook for *in vitro* studies of gene function, metabolism, and virus studies, etc. Establishing planthopper cell lines was considered to be a difficult task; however, cell lines are now available for the BPH (Xu et al. 2014; Chen et al. 2014) and the WBPH (Jia et al. 2012; Mao et al. 2013). These studies successfully took over from the long-lasting efforts of Kimura and Omura using Kimira's medium (Kimura and Omura 1988; Omura and Kimura 1994). Approaches are also under way to establish rice planthopper cell lines using modified Grace's medium (K. Tateishi 2015, personal communication; J. Kobayashi 2015, personal communication).

6 Organisms Associated with Rice Planthoppers

Rice planthoppers have associations with various organisms inside and outside the body (Fig. 5). The most economically important associates are rice plant viruses; five rice plant viruses are known to be transmitted by rice planthoppers. RGSV and RRSV are transmitted by the BPH, SRBSDV is transmitted by the WBPH, and RSV and rice black-streaked dwarf virus (RBSDV) are transmitted by the SBPH (Table 1) (Hibino 1996; Zhou et al. 2008). RGSV, RRSV, RSV, and RBSDV propagate in the host planthoppers and are transmitted in a persistent manner; SRBSDV is also considered to be transmitted in a persistent manner because the Fijivirus can multiply in the body of vector hoppers (Zhou et al. 2008). Transovarial passage is known in RSV; RSV is transmitted from female adults to their progeny through eggs (Hibino 1996). Rice stripe disease, caused by RSV, spread throughout the Jiangsu province in China in the middle of the 2000s and high viruliferous rates were

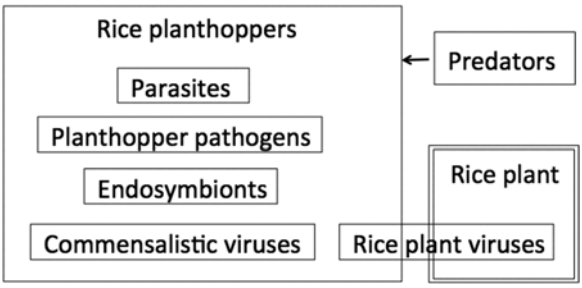

Fig. 5 Schematic view of organisms associated with rice planthoppers. Rice plant viruses transmitted by planthoppers propagate in both planthoppers and rice plants. Environmental microorganisms with which planthoppers may come into contact with are excluded

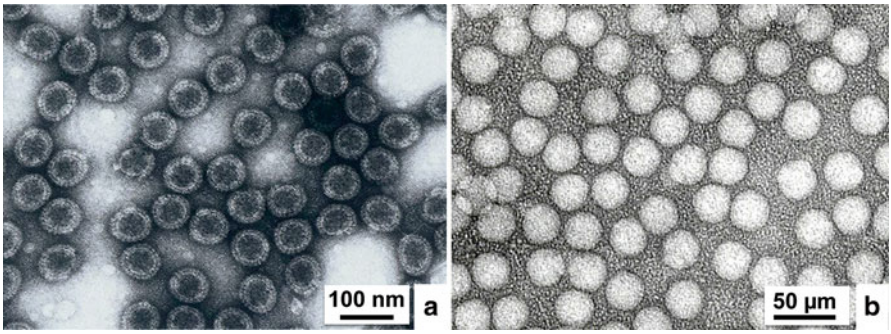

Fig. 6 Organisms associated with rice planthoppers I: commensalistic viruses. (**a**) Electron micrograph of Nilaparvata lugens reovirus; (**b**) Electron micrograph of Himetobi P virus isolated from *Nilaparvata lugens*

recorded in the SBPH (Otuka et al. 2012b). It has been reported that introduction of an RSV-resistant plants suppressed the viruliferous rate (Xiao et al. 2010). RGSV and RRSV spread through southern Vietnam in the middle of the 2000s together with an outbreak of the BPH, resulting in significant damage to rice products (Cabauatan et al. 2009). SRBSDV has been recorded in both rice and maize and has recently been characterized (Zhou et al. 2008). SRBSDV has spread through northern Vietnam and southern China, and is expanding its distribution (Zhou et al. 2013). Since outbreaks of virus diseases occur periodically, it is important to elucidate the underlying problems associated with these diseases and establish control methods.

Commensalistic viruses that propagate in planthoppers but are not plant pathogens are known to exist. These viruses may have been referred to as latent, persistent, hereditary, symbiotic, or asymptomatic viruses (Noda and Nakashima 1995). Nilaparvata lugens reovirus (NLRV) (Fig. 6a) was first reported as a commensalistic virus in the BPH (Noda et al. 1991) and Himetobi P virus was reported in the SBPH (Toriyama et al. 1992) (Fig. 6b). In addition to hosting NLRV and Himetobi P virus, some BPH strains also harbor Nilaparvata lugens commensal X virus (NLCXV) (Nakashima et al. 2006). Because of their relative abundance in BPH, virus particles

of these three viruses have been isolated. However, transcriptome analyses have revealed new viruses, with three types of iflavirus found in the BPH (Murakami et al. 2013, 2014).

To date, a few planthopper pathogens have been reported. Li (1985) reported eight fungi and one bacterial pathogen from rice planthoppers and leafhoppers, and field prevalence of the fungus *Entomophthora delphacis* has been reported in the BPH (Shimazu 1976). However, germination of this fungus requires water or quite high humidity (Shimazu 1977). *Beauveria bassiana* and *Metarhizium anisopliae* are considered to be useful entomopathogenic fungi for the control of planthopper populations (Toledo et al. 2010). The bacterial pathogen *Serratia marcescens*, which frequently produces a red pigment, is sometimes observed in the laboratory colonies. However, little is known about the destructive force of these entomopathogens against planthopper population growth in the field.

Planthoppers harbor endosymbionts; one species of fungi and several species of bacteria have been confirmed to live within the cells of planthoppers. Yeast-like symbiont (YLS), a prerequisite microorganism for host planthoppers (Fig. 7a, b), is a member of Clavicipitaceae in the ascomycetes (Suh et al. 2001). YLS is transmitted to the next generation via the ovary through infection of the posterior region of the ovariole (Yukuhiro et al. 2014). Genome sequence of YLS in BPH is available (Fan et al. 2015). However, YLS has not been successfully cultured in a cell-free or a cell-mediated *in vitro* cultivation. YLS is the only mutualistic microorganism to associate with the three species of host planthoppers, whereas all other bacterial symbionts are facultative in rice planthoppers. *Wolbachia* (Fig. 7c) has been reported in the SBPH, the WBPH, and the BPH, and is known to cause reproductive cytoplasmic incompatibility in the SBPH (Noda et al. 2001). *Cardinium* (Fig. 7d) infects the WBPH, causing cytoplasmic incompatibility (Nakamura et al. 2012; Zhang et al. 2012b). Rickettsia are sometimes observed in rice planthoppers when using PCR analysis but these bacteria are yet to be characterized in rice planthoppers. *Arsenophonus* is also a facultative symbiont in the BPH (Tang et al. 2010). Facultative symbionts often cause sexual or reproductive alteration, as described above for *Wolbachia* and *Cardinium*. In the SBPH, *Spiroplasma* is a causative agent of male-killing (Sanada-Morimura et al. 2013).

Rice planthoppers are attacked by many parasites and egg parasitoids may be important natural enemies of planthoppers. Parasitoids belonging to the genus *Anagrus* (Mymaridae) are major egg parasitoids in tropical and temperate regions (Otake 1970; Chiu 1979). Members of the Trichogrammatidae are also important parasitoids in rice planthoppers, and the major nymphal and adult parasites include drynid wasps, strepsipterans, and a nematode. Several species of drynid wasps, including *Haplogonatopus*, often parasitize rice planthoppers (Gurr et al. 2011; Mita et al. 2012), and the strepsipteran parasitoid *Elenchus japonicus*, which consists of at least three genotypes (Matsumoto et al. 2011), has also been observed in rice planthoppers (Chiu 1979). Drynid wasps and the strepsipteran parasitoid are found in the planthopper migrants on the East China Sea, indicating long distance co-migration of these parasites with host planthoppers (Noda 1986b; Kifune and Maeta 1986). Another important natural enemy of planthoppers is the parasitic nematode *Agamermis unka* (Chiu 1979; Choo and Kaya 1990) (Fig. 7e). This large-

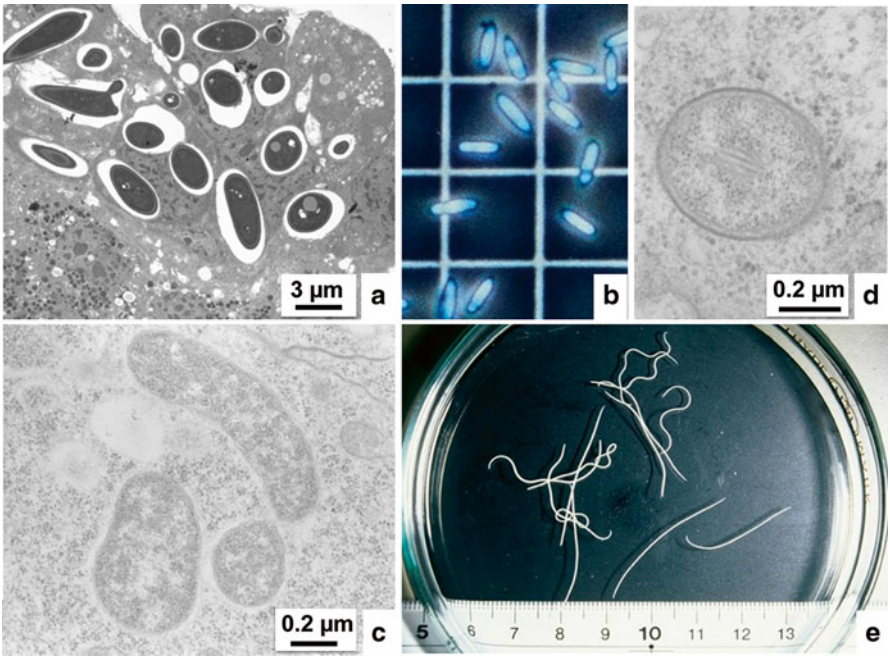

Fig. 7 Organisms associated with rice planthoppers II: symbionts and nematodes. (**a**) Electron micrograph of yeast-like symbiont of *Nilaparvata lugens*, which infects the epithelial plug cells of the ovary; (**b**) Light micrograph of isolated yeast-like symbionts (the tick marks represent 25 µm squares); (**c**) Electron micrograph of *Wolbachia* in *Laodelphax striatellus*; (**d**) Electron micrograph of *Cardinium* in *Sogatella furcifera*; (**e**) parasitic nematode *Agamermis unka* isolated from *N. lugens*; the *scale* shows cm

sized nematode parasitized planthoppers at high rate around 20 years ago but its parasitic rate has recently decreased in Japan for reason(s) that have yet to be identified (K. Hidaka 2015, personal communication).

Predators of planthoppers, mainly spiders, are also considered to actively work in the suppression of planthopper population growth (Preap et al. 2001). Though insecticide use affects the survival of this important group of natural enemies (Tanaka et al. 2000), tracking data on the effects of insecticide use on populations of spiders and planthoppers have not been sufficiently analyzed.

7 Control Using Resistant Rice Varieties

Utilization of resistant rice varieties is probably the most important factor in planthopper management to ensure stable rice production in tropical regions. The mechanisms of resistance of rice varieties against planthoppers are exhibited in the behavioral traits of the planthoppers, which were classified by Painter as

"non-preference (antixenosis)" and "antibiosis" (Sogawa 1982). The BPH is not considered to show an orientational response, induced by olfactory or visual cues, between susceptible and resistant rice varieties. However, there are significant differences in the behavior displayed for stylet insertion and exploratory probing on susceptible and resistant rice varieties, including differences in probing frequency and honeydew excretion. More feeding marks (salivary flanges) are observed on resistant rice varieties than on susceptible ones (Sogawa and Pathak 1970; Sogawa 1982). Therefore, host plant selection is thought to be achieved after stylet probing, suggesting rice plants use a feeding deterrent as a resistance mechanism (Sogawa and Pathak 1970; Sogawa 1982; Ghaffar et al. 2011) or they may mechanically inhibit sucking plant sap (Hao et al. 2008). Electronic monitoring of feeding behavior of the BPH on a resistant rice variety suggests that a resistance mechanism prevents sustained phloem ingestion after the phloem ingestion is initiated (Velusamy and Heinrichs 1986; Kimmins 1989). This behavior is different from that observed on non-host plants, where the BPH is rarely successful in achieving primary ingestion, indicating that probing is interrupted before the stylet reaches the sieve elements (Hattori 2001). Virtually all varietal resistance observed in the field can be explained by feeding inhibition of planthoppers on the resistant varieties of rice, which eventually leads to non-preference of the resistant varieties. Planthoppers readily settle, and proceed to sustained sucking, on susceptible varieties of rice. In rice fields, immigrant macropterous planthoppers leave areas of resistant rice varieties because they cannot ingest plant sap from the phloem, resulting in low population numbers of planthoppers in areas of resistant rice varieties. Consequently, planthoppers settle on susceptible rice varieties in the field.

In order to clarify the resistance mechanisms of rice plants, low molecular weight compounds from resistant rice varieties have been explored using organic chemistry techniques. However, promising molecules that may confer resistance have not been observed to date. Trans-aconitic acid, which has been observed to significantly suppress BPH feeding, was isolated from a non-host plant, the barnyard grass (Kim et al. 1975, 1976). However, the concentration of trans-aconitic acid in the phloem sap of rice plants was much lower than the levels observed to inhibit feeding of planthoppers in the feeding tests (Nagata and Hayakawa 1998). As mentioned above, the stylet of BPH can reach the phloem in both resistant and susceptible rice plants (Sogawa 1982); nevertheless BPH does not ingest sap from the phloem in the resistant rice plants (Hattori 2001). Therefore, the resistant factor is generally thought to be present in the phloem, where sustained sucking of the BPH is blocked (Sogawa and Pathak 1970).

Planthoppers show high nymphal mortality and strongly reduced fecundity, when the BPH is added to resistant rice varieties under caged condition (Sogawa and Pathak 1970; Sogawa 1982). Unsuccessful ingestion of nutrients or poor sap feeding result in developmental delays and low fecundity in the planthoppers. A lack of sustained sucking is simply measured as a low honeydew excretion level. This antibiosis does not appear to be caused by hazardous materials in the food, but rather by feeding inhibition. Therefore, important areas to consider in future studies

are the mechanisms used by planthoppers to taste their food, the factors that result in interruption of feeding, and the mechanisms involved in feeding interruption.

Recent molecular techniques have facilitated studies to compare resistant and susceptible rice plants at the transcriptomic, proteomic, and metabolomic levels. A number of rice plant genes whose expressions are influenced by planthopper feeding have been reported (Zhang et al. 2004; Yuan et al. 2005; Hu et al. 2011), and differentially expressed genes between resistant and susceptible rice varieties against planthopper attack have also been studied (Zhang et al. 2004; Wang et al. 2008c). These studies have revealed that planthopper attacks reduce photosynthetic rates of the leaves and up- or down-regulate a number of genes related to various metabolic pathways. Differential expression of transcription factor genes was studied between BPH resistant (Rathu Heenati) and susceptible (Taichun Native 1) rice varieties using microarray analysis (Wang et al. 2012). Expression levels of the transcription factors, which are up-stream *cis* regulators, may affect expression of rice resistance-related genes against the BPH. Although many differentially expressed genes were reported, it is not yet clear which genes are specifically involved in resistance against the BPH.

In addition to transcriptomic analyses, proteomic analyses have been performed on rice plants infested by planthoppers, revealing expression of jasmonic acid synthesis proteins, oxidative stress response proteins, and others (Wei et al. 2009). Protein content was also compared among wild type IR64 rice, a susceptible rice mutant, and a resistant rice mutant (Sangha et al. 2013). This study suggests that S-like RNase is involved in delivering enhanced levels of resistance against the BPH. A metabolomics study revealed that a BPH attack causes complex metabolic changes involving transamination, the GABA shunt, the TCA cycle, and gluconeogenesis/glycolysis, etc. (Liu et al. 2010a). Many candidate rice genes or proteins that are up- or down-regulated by planthopper infestations are listed in the transcriptomic, proteomic, and metabolomic analyses. BPH infestations were found to induce complex biological changes in gene expressions and protein regulations in rice plants. Further progress in this area is required before key responsible genes or proteins can be identified.

Of the mechanisms used by rice plants to regulate and inhibit planthopper sucking, one of the important ones is callose deposition in the phloem sieve element, which results in mechanical disturbance of planthopper feeding. The sieve elements are sensitive to biotic and abiotic disturbance, and have sealing mechanisms, such as protein plugging and callose formation. Callose deposition is considered to be a defense response of plants (Shinoda 1993; Luna et al. 2011). Callose is a linear β-1,3-glucan polymer with a number of 1,6 branches, and it is found in large quantities in the sieve plates of the phloem (McNairn and Currier 1967). Callose is produced enzymatically by callose synthases in the presence of Ca^{2+}, it is located in the plasma membrane, and is deposited extracellularly around sieve pores (Will et al. 2013). BPH feeding has been observed to up-regulate expression of the *callose synthase* genes and induce callose deposition in the sieve tube, where the stylets penetrate (Hao et al. 2008). In susceptible rice plants, β-1,3-glucanases avoid callose deposition. Thus, callose deposition is considered to be an important resistance

mechanism of resistant rice plants (Hao et al. 2008; Du et al. 2009), although a proteomic study has shown that proteins related to callose metabolism remain unchanged, even after BPH feeding (Wei et al. 2009).

Following experiences and reflections from previous rice breeding strategies, developing rice cultivars, referred to as Green Super Rice, has been proposed based on recent molecular studies (Zhang 2007). As far as developing resistant rice varieties against planthoppers are concerned, a number of candidate genes have been reported that could be introduced into rice to control planthopper populations. Transgenic rice, created by the introduction of toxic genes into susceptible rice varieties, is considered to control planthoppers. This approach is similar to Bt-genetically modified crops that are successfully cultivated to control lepidopteran pests. Introduction of a *lectin* gene, *Allium sativum* leaf agglutinin, resulted in potent entomotoxic effects on the BPH, the WBPH, and the green rice leafhopper *Nephotettix virescens* (Yarasi et al. 2008). This transgenic strategy is also useful for controlling viral diseases of rice plants. Expression of virus genes in rice plants is a well-established method to confer resistance to rice plants against viruses (Sasaya et al. 2014).

Transgenic plants that express dsRNA are also an attractive pest control method (Gordon and Waterhouse 2007; Price and Gatehouse 2008), and this method is based on RNA interference (RNAi, RNA silencing) (Lundgren and Duan 2013). dsRNA transgenic plants were created using three genes expressed in the midgut of the BPH, the *hexose transporter* gene *NlHT1*, the *carboxypeptidase* gene *Nlcar*, and the *trypsin-like serine protease* gene *Nltry* (Zha et al. 2011). The transcript levels of the planthopper genes were reduced by 40–70 % in the 3rd instar nymphs by day four of feeding. However, a lethal phenotypic effect was not observed. Appropriate target genes for RNAi need to be selected to create dsRNA transgenic rice plants. Little is known about the RNAi pathway in insects, other than for *Drosophila* (Burand and Hunter 2013); the planthopper RNAi pathway needs to be clarified in order to effectively use RNAi technology in pest control.

To date, at least 28 major BPH resistance genes have been identified in both *indica* varieties and wild rice species (Brar et al. 2009; Cheng et al. 2013; Jing et al. 2014). The resistant gene *BPH14* originating in *Oryza officinalis* was first cloned as a resistance gene against the BPH (Du et al. 2009). This gene has a coiled-coil (C-C), a nucleotide binding site (NBS), and a leucine-rich repeat (LRR) domains, which may activate defense responses in the plant when the BPH attacks. Expression of this gene activates the salicylic acid signaling pathway, induces callose deposition in phloem cells, and induces trypsin inhibitor production following planthopper infestation. *BPH26*, which is carried by the *indica* cultivar ADR52 together with *BPH25*, also encodes CC-NBS-LRR protein (Tamura et al. 2014). *BPH26* is identical to *BPH2*, which is found in the *indica* cultivar ASD7. In addition to *BPH14* and *BPH26*, *BPH17* is reported to possess lectin receptor kinase genes as a resistant factor from *indica* cultivar Rathu Heenati (Liu et al. 2015). Though the resistant gene of this lectin receptor kinase is reported to be *BPH3* in the original paper, it corresponds to *BPH17* (Y. Tamura 2015, personal communication). *BPH29* originating in *Oryza rufipogon* has been also cloned (Wang et al. 2015). This gene pos-

sesses B3 DNA-binding domain, which is highly conserved in vascular plants. *BPH18* is also reported to be cloned, though the details remain vague (Jena and Kim 2010). Precise resistance mechanisms are expected to be elucidated using these resistant genes or using transgenic rice with these genes.

One of the main issues in rice planthopper management is the appearance of virulent planthopper strains capable of attacking resistant rice varieties. This resistance-breaking ability of planthoppers was first recognized in the resistance rice variety Mudgo in 1969 (Pathak et al. 1969). Wild BPH in rice fields already had resistance-breaking abilities against some rice varieties (Seo et al. 2009). The virulent strains have been called "biotypes" of the BPH. The virulent BPH population that adapted to the resistance conferred by the *BPH1* gene, which was derived from the Mudgo variety, was termed Biotype 2. In contrast, planthopper populations that can only survive on rice varieties that do not carry any BPH resistance genes are designated as Biotype 1. Biotype 3 planthoppers possess a virulent gene to the BPH resistance gene *BPH2* (*BPH2*-virulent biotype) and have the ability to attack the resistance rice variety ASD7. The term "biotypes" is used by applied biologists to distinguish populations of insects that differ from one another in the traits they possess. The term "biotypes" is generally applied to a wide range of biologically distinct entities, but is highlighted as having little descriptive power (Diehl and Bush 1984; Downie 2010). In planthoppers, Claridge and Den Hollander (1980) recommend avoiding the use of the term biotype when referring to the BPH because a colony of BPH biotypes composed of varying degrees of biotype traits have been considered to be polygenic in nature (Claridge and Den Hollander 1980; Den Hollander and Pathak 1981). Nevertheless, the term "biotype" has been used to describe planthopper populations that have the ability to overcome rice plants resistant to planthoppers (Cheng et al. 2013; Kobayashi et al. 2014; Jing et al. 2014). Progress in the study of rice plant and planthopper interactions will facilitate reaching a consensus on the pros and cons of using the term "biotype" when referring to resistance-breaking traits in the BPH.

To elucidate the gene(s) involved in the resistance-breaking ability of planthoppers, a genetic linkage map is currently under construction (Jairin et al. 2013). Both male (XY type) and female (XX type) planthoppers have 30 chromosomes (Noda and Tatewaki 1990). The linkage map has revealed that a single recessive gene controls virulence to *BPH1*, which is on linkage group 10 (Kobayashi et al. 2014). The locus of genes responsible for virulence of Biotype 2 (*BPH1*) is independently reported on the basis of a different linkage map (Jing et al. 2014). The results of the two reports differ despite studying the same virulence factor; a single recessive gene is reported in the former study (Kobayashi et al. 2014) and three major genes are reported in the latter study (Jing et al. 2014). The virulence of the BPH is considered to be controlled by polygenic determinants (Den Hollander and Pathak 1981) and a gene-for-gene correspondence between resistance and virulence that is widely accepted in pathogen-plant interactions (Cheng et al. 2013) has been refuted in the BPH (Denno and Roderick 1990). However, the former study may be paralleled with gene-for-gene theory, suggesting careful re-evaluation of previous virulence factor analyses. The latter study assumes that the mechanisms of non-preference

(antixenosis) and antibiosis are different. The main difference between the two studies is in the methods used to evaluate virulence; Kobayashi et al. (2014) use the volume and pH of the excreted honeydew as indicators of virulence and Jing et al. (2014) use the conventional preference test (similar to the seedbox screening test (Horgan 2009; Brar et al. 2009)) and survival rate. The release of the BPH whole genome (Xue et al. 2014) will facilitate elucidating the virulence factors in BPH.

8 Pesticide Resistance

Organic synthetic insecticides have been used for more than 60 years to control rice planthoppers (Heinrichs 1994), and insecticide resistance has been recorded in rice planthoppers since the 1960s. Insecticides are classified based on the mode of action (MOA) by the Insecticide Resistance Action Committee (IRAC). Insecticides for rice planthoppers include eight MOA groups, cyclodiene organochlorines (Group 2A), organophosphates (1B), carbamates (1A), pyrethroids (3A), neonicotinoids (4A), phenylpyrazoles (fipronil, 2B), chitin biosynthesis inhibitors type 1 (buprofezin, 16), and selective feeding blockers (pymetrozine, 9B). Resistance in rice planthoppers has been recorded for one or more compounds in all of the MOA groups, except the group for selective feeding blockers (http://www.irac-online.org/pests/nilaparvata-lugens/). Resistance to benzen hexachloride (BHC) was reported in rice planthoppers in the 1960s, and resistance to organophosphates and carbamates was first noticed in rice fields in the late 1960s and was prominent during the 1970s (Ozaki and Kassai 1970, 1982; Nagata et al. 1979; Heinrichs 1979; Hama and Hosoda 1983). An insecticide belonging to the chitin biosynthesis inhibitors was used in East Asia in the 1980s together with carbamates and pyrethroids. At present, neonicotinoid and phenylpyrazole compounds are mainly used to control planthopper populations.

Mechanisms of insecticide resistance in insects can typically be classified into two major groups: increased detoxification capabilities and alteration of the insecticide target sites (target insensitivity) (Matsumura 2009). Resistance as a result of high activity of detoxification enzymes was first reported in rice planthoppers when high esterase activity was detected in marathion resistant SBPH strains (Ozaki and Kassai 1970). Detoxification as a result of high esterase activity was the main factor contributing to resistance of the BPH to organophosphorus and carbamate insecticides. High activity of detoxification enzymes is a result of amplification of the *carboxylesterase* gene (Small and Hemingway 2000), resulting in elevated mRNA levels of this gene (Vontas et al. 2000). Elevated glutathione S-transferases have been shown to confer resistance to pyrethroids in a laboratory-selected colony of BPH, which appears to be due to gene amplification (Vontas et al. 2001, 2002). In contrast, target insensitivity against carbamate insecticides has been reported in a laboratory-selected BPH strain due to low acetylcholinesterase (AChE) sensitivity (Hama and Hosoda 1983; Yoo et al. 2002). However, the mutation site on the *ace-*

tylcholinesterase gene, which seems to confer carbamate insecticide resistance, has not been elucidated in resistant BPH strains to date. A point mutation of the *AChE* gene, G185S, has been reported in methamidophos (organophosphorus insecticide) resistance strains, which may be responsible for the insensitivity of AChE (Yang et al. 2010).

The decreased efficacy of organophosphates, carbamates, and pyrethroids in controlling populations of the BPH became obvious in the field during the 1990s (Nagata 1984; Matsumura et al. 2008). At that time, neonicotinoid insecticides were introduced to control planthopper populations. Soon after, a phenylpyrazole insecticide, fipronil, was released on the market. These new insecticides successfully suppressed the planthopper populations that had acquired multiple-insecticide resistance. Damage to rice plants, particularly that inflicted by BPH, was almost repressed by the use of these insecticides by the year 2000. Neonicotinoid insecticides, mainly imidacloprids, were heavily used in Asian countries, resulting in a strong retort from planthoppers on rice production in Asia in 2005. In 2005, Asian countries encountered heavy outbreaks of the BPH, which had not been previously observed. Neonicotinoid resistant strains of the BPH were recorded (Gorman et al. 2008; Matsumura et al. 2008; Wang et al. 2008b) and fipronil resistant strains of the WBPH and the SBPH were detected (Matsumura et al. 2008, 2014). Though resistance levels to insecticides vary, the susceptibility of local planthopper populations to these insecticides apparently decreases with repeated use of the insecticides (Sanada-Morimura et al. 2011; He et al. 2012; Matsumura et al. 2014).

The BPH shows a high level of resistance against neonicotinoid insecticides, especially imidacloprid, the most widely used insecticide for planthopper control (Gorman et al. 2008; Matsumura et al. 2008, 2014; Wang et al. 2008b; Wen et al. 2009; He et al. 2012). Crossing experiments of imidacloprid resistant and susceptible strains of the BPH suggest that multiple autosomal genes are involved in resistance in a field population of the BPH (Wang et al. 2009). The main resistance factor is considered to be detoxification microsomal enzymes called P450 monooxygenases. Imidacloprid resistant strains show high P450 activity, and piperonyl butoxide (PBO), which is an inhibitor of P450 and a synergist used in insecticidal experiments of a wide variety of pesticides, exhibited synergism on imidacloprid (Liu et al. 2003; Wen et al. 2009). Therefore, increased cytochrome P450 monooxygenase activity, which catalyzes imidacloprid in the planthoppers, was the major mechanism of imidacloprid resistance in the strains studied (Puinean et al. 2010). In resistant strains, overexpression of P450 enzymes has been shown, for example, in *CYP6ER1* (Bass et al. 2011) and *CYP6AY1* genes (Ding et al. 2013). RNA interference (RNAi) of the *CYP6AY1* gene recovered imidacloprid susceptibility in the resistant strain (Ding et al. 2013). In the field populations examined so far, high P450 monooxygenase activity caused by the selective pressure of repeated neonicotinoid insecticide application is the main causative factor of resistance. The members of P450 gene family of BPH are reported by Lao et al. (2015). Some populations of the SBPH show resistance against imidacloprid, but precise studies have not been conducted to date.

As for imidacloprid resistance in the BPH, an alternative resistance mechanism, site mutation of the target gene, has been reported in a laboratory-selected strain. Neonicotinoids bind nicotinic acetylcholine receptors (nAChRs), causing continuous nervous excitation. Around ten different *nAChR* subunit genes are present in insects and these subunit genes are grouped into two types (alpha and beta) (Tomizawa and Casida 2001; Jones and Sattelle 2010). Five subunits from both types usually form one functional receptor. A single point mutation at a conserved position (Y151S) was found in two nAChR subunits, Nlα1 and Nlα3 (Liu et al. 2005). This mutation was further analyzed for the resistance mechanism and function of the nicotinic acetylcholine receptor of the planthopper (Li et al. 2010). However, mutation in *nAChR* genes has not been observed in field populations of planthoppers to date.

The BPH has highly developed resistance against imidacloprid, whereas the WBPH and the SBPH show fipronil resistance (Matsumura et al. 2008, 2014) at present. Fipronil is an antagonist for the γ-aminobutyric acid (GABA) receptor and inhibits the flux of chloride ions into the nerve cells, causing hyper-excitation (Buckingham et al. 2005). A point mutation in the 2nd transmembrane domain (Narusuye et al. 2007) confers resistance against fipronil in the WBPH (Nakao et al. 2010) and SBPH (Nakao et al. 2011). This mutation has been found in many field populations of the SBPH and is supposed to be the major cause of resistance to fipronil. In the WBPH, another mutation has been observed in the cytoplasmic loop between the 3rd and 4th membrane-spanning region and this is also thought to be involved in resistance (Nakao et al. 2012).

Insecticide resistance has become conspicuous. In order to manage planthopper outbreaks effectively, a number of important issues related to resistance problems need to be considered. First, planthopper resistance levels in the field need to be monitored. Methods that determine the frequency of resistance mutation of the target genes and the activity of detoxification enzymes are useful for predicting levels of insecticide resistance in planthopper populations. Periodic monitoring of insecticide resistance levels in the laboratory, by application of frequently used insecticides, is also useful for identifying effective compounds, though it is an exacting task. Second, based on the monitoring results and the actual conditions of field application of insecticides by farmers, efficient insecticides should substitute those for which resistance is developing. Third, as the application window for planthopper control, i.e., the optimum period for spraying insecticides, is narrow, predictions of the main temporal planthopper stages and population densities in rice fields are important for decision making regarding insecticide use. In addition to these management tasks in the field, new approaches in the laboratory are also required to explore new targets in planthoppers, develop new insecticides, and create new insecticide concepts, such as RNAi insecticides.

9 Occurrence Forecast

In many rice fields in temperate regions, rice plants are planted following application of persistent systemic insecticides to seedlings in nursery-boxes. This early insecticide treatment provides effective control against the first planthopper immigrants and against subsequent propagation of offspring. However, recent insecticide resistance in planthoppers means that later foliar application of insecticides is now required. Gaining perspective on annual trends of planthopper occurrence is important for decision making regarding additional chemical controls. The BPH and the WBPH live in tropical and southern subtropical regions all year round and immigrate into wide areas of rice fields across Asia, indicating the importance of predicting the source, flight path, immigration time, immigration density, and biological traits of the immigrants.

Following a long dispute about the life cycle and annual source of the BPH and the WBPH in temperate regions, long distance flight in the planthoppers was proven in 1967 by accident. A large number of planthoppers were found on an ocean weather ship in the Pacific Ocean more than 500 km from land (Asahina and Tsuruoka 1968). Thus, over water flight of more than 500 km was apparent, and this evidence for long distance flight of planthoppers readily explains the unexpected emergence of rice planthoppers in the rice fields of Japan and Korea (Kisimoto 1976, 1987). A prediction model for planthopper migration was proposed by Seino et al. (1987), and the model was developed by other scientists enabling the migration source and migration route of planthoppers to be predicted by backward trajectory analysis (Otuka et al. 2005; Otuka 2009). Real-time analysis of planthopper migration in East Asia can be seen at http://web1.jppn.ne.jp/docs_cgi/umnkyoso/in planthopper migration seasons.

Long distance mass migration of the BPH and the WBPH has been well analyzed. The density of rice planthopper immigrants in regions of Japan, and probably in Korea and northern China, was influenced by (i) the average temperature during the winter season in North Vietnam, where planthoppers successfully overwinter; (ii) the strong upper wind from North Vietnam to South China in April and May, when the first stage of migration occurs; and (iii) the strong upper wind from South China to the northern and eastern regions in June and July, when the second stage of migration occurs (Syobu et al. 2012). Typhoons have also been shown to influence the migration of planthoppers (Otuka et al. 2012a; Hu et al. 2013). The planthopper migration in spring and early summer occurs northward or northeastward, whereas southward migration of planthoppers is reported in autumn (Cheng et al. 1979; Kisimoto 1987). This return migration in autumn appears to mix the planthopper populations in Asia, resulting in mixing of the genetic material of planthoppers. In addition to long distance migration, short distance migration (Otuka et al. 2014) also helps to mix populations. The SBPH has also been shown to undertake long distance mass migration, and the emigration period and migration route have been recently analyzed (Otuka et al. 2012b; Otuka 2013). These analyses explain the

spread of rice stripe disease, whose causative agent, RSV, is transmitted by the SBPH.

Research has shown that immature females undertake long distance migration and sexual maturation occurs after their immigration into rice fields. Zheng et al. (2014) reported that female BPH and WBPH caught in light traps during the peak emigration periods showed negligible or no ovarian development. Furthermore, no signs of ovarian development were apparent in three species of planthopper migrants caught on the East China Sea (31° N, 126° E) (Noda 1986b). Fifty-three females of WBPH, 26 females of BPH, and 14 females of SBPH were dissected on board the ship immediately after being caught, revealing no ovarian development. The spermatheca of individuals was also dissected to observe sperm using light microscopy, resulting in no copulation in all female planthoppers tested. Thus, premating flight was apparent in the planthoppers.

To distinguish regional populations and predict the migrant source of planthoppers, molecular markers have been studied. Sequence variation among populations of the BPH and the WBPH was first reported by Mun et al. (1999) in the mitochondrial cytochrome oxidase subunit I gene (COI, *cox1*). The nucleotide sequences of the *cox* genes (*cox1* and *cox2*) were later determined for 579 BPH (1928 bp) and 464 WBPH (1927 bp) individuals collected from 31 and 25 locations, respectively, resulting in 30 and 20 mitochondrial haplotypes for the BPH and the WBPH, respectively (Matsumoto et al. 2013). However, very little differentiation was observed among local populations for both planthopper species. This may be due to mingling of populations through long distance migration, resulting in genetically intermixed Asian populations. Though whole mitochondrial sequences of the three species of planthopper are available (Zhang et al. 2013, 2014; Lv et al. 2015), it may be difficult to obtain mitochondrial genes whose sequences could be used as molecular markers to distinguish geographical variation. Internal transcribed spacer (ITS) regions 1 and 2 of the rRNA gene were also compared among many local populations in Asia, but the usefulness of the ITS region for geographical discrimination of planthoppers was not indicated because of sequence variation unrelated to planthopper locality (Fu et al. 2012).

Microsatellite markers have been reported for the BPH and significant genetic differentiation was detected using these markers in China field populations (Sun et al. 2011; Jing et al. 2012). Analyses using chemical elements to identify different geographic populations of planthoppers have also been undertaken (Miao et al. 2012). Although these methods successfully distinguish populations, they are, as yet, unrecognized in their usefulness in distinguishing regional populations across Asia and in estimating migration routes. The use of internal fauna, e.g. symbionts or intestinal bacteria, for distinguishing regional populations has been proposed. However, since planthopper populations are genetically mixed as a result of migration in Asia, careful evaluation using large scale populations will be necessary for marker based population discrimination.

10 Perspective

The BPH has been a threat to rice production in Asia for several decades (Dyck and Thomas 1979; Heong and Hardy 2009). The WBPH and the SBPH are also economically important and some common problems exist for the three species, such as countermeasures for insecticide resistance, establishment of occurrence forecasting, and breeding of resistant rice varieties. Fortunately, molecular studies of rice planthoppers have been steadily progressing in recent years following the previous overview of this topic (Noda 2009). The entire genome of the BPH has been sequenced (Xue et al. 2014), rice resistance genes against the BPH, have been elucidated (Du et al. 2009; Tamura et al. 2014; Liu et al. 2015; Wang et al. 2015), and planthopper genes thought to be responsible for breaking resistance have been mapped on to the BPH chromosome (Kobayashi et al. 2014; Jing et al. 2014). Many important genes and molecules have been reported and prominent target genes for chemical control will be revealed within due course.

Nevertheless, a clear blueprint for planthopper control does not exist. Since cultural control methods have quite limited effects and cannot be applied for the majority of rice production in Asia, three main control methods are available: insecticides, resistant rice varieties, including transgenic rice, and natural enemies. Essentially, as a consequence of sequencing the planthopper genome, and recent transcriptomic, proteomic, and metabolomic studies, much smarter planthopper management systems need to be developed and utilized. Planthopper management should be advanced using a well-balanced combination of accumulated knowledge for management strategies and recent advanced techniques.

There are a number of important aspects of rice planthopper studies and these contribute to basic and applied entomology in a variety of ways. First, studies of host resistance against planthoppers and resistance-breaking planthoppers are good models for plant host-insect interactions. In particular, the rice genome is one of the properly maintained important crop genomes, and planthopper genomic information is also available. The study of planthoppers complements the work being conducted with aphids in relation to the feeding behavior of sucking insects, which is an important trait in analyzing crop damage. Second, planthoppers' phase variation in wings, macropterous and brachypterous forms, is not only an important topic for flight ability enabling long distance migration but also an attractive theme in insect adaptation and evolution. The BPH and the WBPH do not undergo typical diapause and are not influenced by seasonal environmental changes. Their wing dimorphism is simply determined by food quality and density in the nymphal stage of the corresponding generation. Recently, insulin receptors have been reported to be involved in planthopper wing dimorphism (Xu et al. 2015). Third, planthoppers provide a rare opportunity for studying the development of insecticide resistance on a global scale because of the planthoppers' long distance migration and the use of similar insecticides across wide areas. Insecticide resistance is an acute and serious problem, and the sharing of information is becoming vastly more important in the management of planthopper infestations.

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References

Achon, M. A., Subira, J., & Sin, E. (2013). Seasonal occurrence of *Laodelphax striatellus* in Spain: Effect on the incidence of maize rough dwarf virus. *Crop Protection, 47*, 1–5.

Asahina, S., & Tsuruoka, Y. (1968). Records of the insects visited a weather ship located at the Ocean Weather Station "Tango" on the Pacific, II. *Kontyu, 36*, 190–202.

Backus, E. A. (1985). Anatomical and sensory mechanisms of leafhopper and planthopper feeding behavior. In L. R. Nault & J. G. Rodriguez (Eds.), *The leafhoppers and planthoppers* (pp. 163–194). New York: Wiley.

Backus, E. A., Andrews, K. B., Shugart, H. J., Greve, L. C., Labavitch, J. M., & Alhaddad, H. (2012). Salivary enzymes are injected into xylem by the glassy-winged sharpshooter, a vector of *Xylella fastidiosa. Journal of Insect Physiology, 58*, 949–959.

Bae, S. H., & Pathak, M. D. (1970). Life history of *Nilaparvata lugens* (Homoptera: Delphacidae) and susceptibility of rice varieties to its attacks. *Annals of the Entomological Society of America, 63*, 149–155.

Bao, Y. Y., Wang, Y., Wu, W. J., Zhao, D., Xue, J., Zhang, B. Q., Shen, Z. C., & Zhang, C. X. (2012). De novo intestine-specific transcriptome of the brown planthopper *Nilaparvata lugens* revealed potential functions in digestion, detoxification and immune response. *Genomics, 99*, 256–264.

Bass, C., Carvalho, R. A., Oliphant, L., Puinean, A. M., Field, L. M., Nauen, R., Williamson, M. S., Moores, G., & Gorman, K. (2011). Overexpression of a cytochrome P450 monooxygenase, CYP6ER1, is associated with resistance to imidacloprid in the brown planthopper, *Nilaparvata lugens. Insect Molecular Biology, 20*, 763–773.

Bottrell, D. G., & Schoenly, K. G. (2012). Resurrecting the ghost of green revolutions past: The brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. *Journal of Asia-Pacific Entomology, 15*, 122–140.

Brar, D. S., Virk, P. S., Jena, K. K., & Khush, G. S. (2009). Breeding for resistance to planthoppers in rice. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 401–427). Los Baños: International Rice Research Institute.

Brozek, J., & Bourgoin, T. (2013). Morphology and distribution of the external labial sensilla in Fulgoromorpha (Insecta: Hemiptera). *Zoomorphology, 132*, 33–65.

Buckingham, S. D., Biggin, P. C., Sattelle, B. M., Brown, L. A., & Sattelle, D. B. (2005). Insect GABA receptors: Splicing, editing, and targeting by antiparasitics and insecticides. *Molecular Pharmacology, 68*, 942–951.

Burand, J. P., & Hunter, W. B. (2013). RNAi: Future in insect management. *Journal of Invertebrate Pathology, 112*, S68–S74.

Cabauatan, P. Q., Cabunagan, R. C., & Choi, I.-R. (2009). Rice viruses transmitted by the brown planthopper *Nilaparvata lugens* Stål. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 357–368). Los Baños: International Rice Research Institute.

Carolan, J. C., Fitzroy, C. I. J., Ashton, P. D., Douglas, A. E., & Wilkinson, T. L. (2009). The secreted salivary proteome of the pea aphid *Acyrthosiphon pisum* characterised by mass spectrometry. *Proteomics, 9*, 2457–2467.

Carolan, J. C., Caragea, D., Reardon, K. T., Mutti, N. S., Dittmer, N., Pappan, K., Cui, F., Castaneto, M., Poulain, J., Dossat, C., Tagu, D., Reese, J. C., Reeck, G. R., Wilkinson, T. L., & Edwards, O. R. (2011). Predicted effector molecules in the salivary secretome of the pea aphid (*Acyrthosiphon pisum*): A dual transcriptomic/proteomic approach. *Journal of Proteome Research, 10*, 1505–1518.

Catindig, J. L. A., Arida, G. S., Baehaki, S. E., Bentur, J. S., Cuong, L. Q., Norowi, M., Rattanakarn, W., Sriratanasak, W., Xia, J., & Lu, Z. (2009). Situation of planthoppers in Asia. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 191–220). Los Baños: International Rice Research Institute.

Chen, J., Zhang, D., Yao, Q., Zhang, J., Dong, X., Tian, H., Chen, J., & Zhang, W. (2010). Feeding-based RNA interference of a trehalose phosphate synthase gene in the brown planthopper, *Nilaparvata lugens. Insect Molecular Biology, 19*, 777–786.

Chen, Q. H., Lu, L., Hua, H. X., Zhou, F., Lu, L. X., & Lin, Y. J. (2012). Characterization and comparative analysis of small RNAs in three small RNA libraries of the brown planthopper (*Nilaparvata lugens*). *PLoS One, 7*, e32860.

Chen, J., Liang, Z. K., Liang, Y. K., Pang, R., & Zhang, W. Q. (2013). Conserved microRNAs miR-8-5p and miR-2a-3p modulate chitin biosynthesis in response to 20-hydroxyecdysone signaling in the brown planthopper, *Nilaparvata lugens. Insect Biochemistry and Molecular Biology, 43*, 839–848.

Chen, H. Y., Zheng, L. M., Mao, Q. Z., Liu, Q. F., Jia, D. S., & Wei, T. Y. (2014). Development of continuous cell culture of brown planthopper to trace the early infection process of oryzaviruses in insect vector cells. *Journal of Virology, 88*, 4265–4274.

Cheng, J. A. (2009). Rice planthopper problems and relevant caused in China. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 157–177). Los Baños: International Rice Research Institute.

Cheng, S., Chen, J., Si, H., Yan, L., Chu, T., Wu, C., Chien, J., & Yen, G. (1979). Studies on the migrations of brown planthopper *Nilaparvata lugens* Stål. *Acta Entomologica Sinica, 22*, 1–21.

Cheng, X. Y., Zhu, L. L., & He, G. C. (2013). Towards understanding of molecular interactions between rice and the brown planthopper. *Molecular Plant, 6*, 621–634.

Chiu, S. (1979). Biologidal control of the brown planthopper. In I. R. R. Institute (Ed.), *Brown planthopper: Threat to rice production in Asia* (pp. 335–355). Los Baños: International Rice Research Institute.

Choo, H. Y., & Kaya, H. K. (1990). Parasitism of brown planthopper and whitebacked planthopper by *Agamermis unka* in Korea. *Journal of Nematology, 22*, 513–517.

Claridge, M. F., & Den Hollander, J. (1980). The biotypes of the rice brown planthopper, *Nilaparvata lugens. Entomologia Experimentalis et Applicata, 27*, 23–30.

Den Hollander, J., & Pathak, P. K. (1981). The genetics of the biotypes of the rice brown planthopper, *Nilaparvata lugens. Entomologia Experimentalis et Applicata, 29*, 76–86.

Denno, R. F., & Roderick, G. K. (1990). Population biology of planthoppers. *Annual Review of Entomology, 35*, 489–520.

Diehl, S. R., & Bush, G. L. (1984). An evolutionary and applied perspective of insect biotypes. *Annual Review of Entomology, 29*, 471–504.

Ding, Z. P., Wen, Y. C., Yang, B. J., Zhang, Y. X., Liu, S. H., Liu, Z. W., & Han, Z. J. (2013). Biochemical mechanisms of imidacloprid resistance in *Nilaparvata lugens*: Over-expression of cytochrome P450 CYP6AY1. *Insect Biochemistry and Molecular Biology, 43*, 1021–1027.

Downie, D. A. (2010). Baubles, bangles, and biotypes: A critical review of the use and abuse of the biotype concept. *Journal of Insect Science, 10*, 176.

Du, B., Zhang, W. L., Liu, B. F., Hu, J., Wei, Z., Shi, Z. Y., He, R. F., Zhu, L. L., Chen, R. Z., Han, B., & He, G. C. (2009). Identification and characterization of *Bph14*, a gene conferring resis-

tance to brown planthopper in rice. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 22163–22168.

Dyck, V. A., & Thomas, B. (1979). The brown planthopper problem. In I. R. R. Institute (Ed.), *Brown planthopper: Threat to rice production in Asia* (pp. 3–17). Los Baños: International Rice Research Institute.

Dyck, V. A., Misra, B. C., Alam, S., Chen, C. N., Hsieh, C. Y., & Rejesus, R. S. (1979). Ecology of the brown planthopper in the tropics. In I. R. R. Institute (Ed.), *Brown planthopper: Threat to rice production in Asia* (pp. 61–98). Los Baños: International Rice Research Institute.

Enserink, M., Hines, P. J., Vignieri, S. N., Wigginton, N. S., & Yeston, J. S. (2013). The pesticide paradox. *Science, 341*, 728–729.

Fan, H. W., Noda, H., Xie, H. Q., Suetsugu, Y., Zhu, Q. H., & Zhang, C. X. (2015). Genomic analysis of an ascomycete fungus from the rice planthopper reveals how it adapts to an endosymbiotic lifestyle. *Genome Biology and Evolution, 7*, 2623–2634.

Foster, S., Goodman, L. J., & Duckett, J. G. (1983a). Ultrastructure of sensory receptors on the labium of the rice brown planthopper. *Cell and Tissue Research, 230*, 353–366.

Foster, S., Goodman, L. J., & Duckett, J. G. (1983b). Sensory receptors associated with the stylets and cibarium of the rice brown planthopper, *Nilapavarta lugens. Cell and Tissue Research, 232*, 111–119.

Fu, Q., Matsumoto, Y., Matsumura, M., Hirai, Y., Sato, Y., & Noda, H. (2012). Presence of a short repeat sequence in internal transcribed spacer (ITS) 1 of the rRNA gene of *Sogatella furcifera* (Hemiptera: Delphacidae) from geographically different populations in Asia. *Applied Entomology and Zoology, 47*, 95–101.

Ghaffar, M. B. A. B., Pritchard, J., & Ford-Lloyd, B. (2011). Brown planthopper (*N. lugens* Stål) feeding behaviour on rice germplasm as an indicator of resistance. *PLoS One, 6*, e22137.

Gordon, K. H. J., & Waterhouse, P. M. (2007). RNAi for insect-proof plants. *Nature Biotechnology, 25*, 1231–1232.

Gorman, K., Liu, Z., Denholm, I., Bruggen, K. U., & Nauen, R. (2008). Neonicotinoid resistance in rice brown planthopper, *Nilaparvata lugens. Pest Management Science, 64*, 1122–1125.

Gurr, G. M., Liu, J., Read, D. M. Y., Catindig, J. L. A., Cheng, J. A., Lan, L. P., & Heong, K. L. (2011). Parasitoids of Asian rice planthopper (Hemiptera: Delphacidae) pests and prospects for enhancing biological control by ecological engineering. *Annals of Applied Biology, 158*, 149–176.

Hama, H., & Hosoda, A. (1983). High aliesterase activity and low acetylcholinesterase sensitivity involved in organo-phosphorus and carbamate resistance of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera, Delphacidae). *Applied Entomology and Zoology, 18*, 475–485.

Hao, P. Y., Liu, C. X., Wang, Y. Y., Chen, R. Z., Tang, M., Du, B., Zhu, L. L., & He, G. (2008). Herbivore-induced callose deposition on the sieve plates of rice: An important mechanism for host resistance. *Plant Physiology, 146*, 1810–1820.

Hattori, M. (2001). Probing behavior of the brown planthoppe, *Nilaparvata lugens* Stål (Homoptera: Delphacidae) on a non-host barnyard grass, and resistant and susceptible varieties of rice. *Applied Entomology and Zoology, 36*, 83–89.

Hattori, M., Konishi, H., Tamura, Y., Konno, K., & Sogawa, K. (2005). Laccase-type phenoloxidase in salivary glands and watery saliva of the green rice leafhopper, *Nephotettix cincticeps. Journal of Insect Physiology, 51*, 1359–1365.

Hattori, M., Nakamura, M., Komatsu, S., Tsuchihara, K., Tamura, Y., & Hasegawa, T. (2012). Molecular cloning of a novel calcium-binding protein in the secreted saliva of the green rice leafhopper *Nephotettix cincticeps. Insect Biochemistry and Molecular Biology, 42*, 1–9.

Hayashi, T., Read, S. M., Bussell, J., Thelen, M., Lin, F. C., Brown, R. M., & Delmer, D. P. (1987). Udp-glucose: (1->3)-β-glucan synthases from mung bean and cotton – Differential-effects of Ca^{2+} and Mg^{2+} on enzyme properties and on macromolecular structure of the glucan product. *Plant Physiology, 83*, 1054–1062.

He, Y., Zhang, J., Xiao, P., Chen, L., & Chen, J. (2012). Susceptibility of three rice planthoppers to insecticides in Zhejiang province. *Acta Agriculturae Zhejiangensis, 24*, 642–646.

Heinrichs, E. A. (1979). Chemical control of the brown planthopper. In I. R. R. Institute (Ed.), *Brown planthopper: Threat to rice production in Asia* (pp. 145–167). Los Baños: International Rice Research Institute.

Heinrichs, E. A. (1994). Impact of insecticides on the resistance and resurgence of rice planthoppers. In R. F. Denno & T. J. Perfect (Eds.), *Planthoppers: Their ecology and management* (pp. 571–598). New York: Chapman & Hall, Inc.

Heong, K. L., & Hardy, B. (2009). *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia*. Los Baños: International Rice Research Institute.

Hibino, H. (1996). Biology and epidemiology of rice viruses. *Annual Review of Phytopathology, 34*, 249–274.

Horgan, F. (2009). Mechanisms of resistance: A major gap in understanding planthopper-rice interactions. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 281–302). Los Baños: International Rice Research Institute.

Hu, J., Zhou, J. B., Peng, X. X., Xu, H. H., Liu, C. X., Du, B., Yuan, H. Y., Zhu, L. L., & He, G. C. (2011). The *Bphi008a* gene interacts with the ethylene pathway and transcriptionally regulates MAPK genes in the response of rice to brown planthopper feeding. *Plant Physiology, 156*, 856–872.

Hu, S. J., Fu, D. Y., Liu, X. J., Zhao, T., Han, Z. L., Lu, J. P., Wan, H. L., & Ye, H. (2012). Diversity of planthoppers associated with the winter rice agroecosystems in southern Yunnan, China. *Journal of Insect Science, 12*, 29.

Hu, G., Lu, F., Lu, M. H., Liu, W. C., Xu, W. G., Jiang, X. H., & Zhai, B. P. (2013). The influence of typhoon Khanun on the return migration of *Nilaparvata lugens* (Stål) in eastern China. *PLoS One, 8*, e57277.

Huang, H. J., Liu, C. W., Cai, Y. F., Zhang, M. Z., Bao, Y. Y., & Zhang, C. X. (2015). A salivary sheath protein essential for the interaction of the brown planthopper with rice plants. *Insect Biochemistry and Molecular Biology, 66*, 77–87.

Jairin, J., Kobayashi, T., Yamagata, Y., Sanada-Morimura, S., Mori, K., Tashiro, K., Kuhara, S., Kuwazaki, S., Urio, M., Suetsugu, Y., Yamamoto, K., Matsumura, M., & Yasui, H. (2013). A simple sequence repeat- and single-nucleotide polymorphism-based genetic linkage map of the brown planthopper, *Nilaparvata lugens. DNA Research, 20*, 17–30.

Jena, K. K., & Kim, S. M. (2010). Current status of brown planthopper (BPH) resistance and genetics. *Rice, 3*, 161–171.

Ji, R., Yu, H. X., Fu, Q., Chen, H. D., Ye, W. F., Li, S. H., & Lou, Y. G. (2013). Comparative transcriptome analysis of salivary glands of two populations of rice brown planthopper, *Nilaparvata lugens*, that differ in virulence. *PLoS One, 8*, e79612.

Jia, D. S., Chen, H. Y., Zheng, A. L., Chen, Q., Liu, Q. F., Xie, L. H., Wu, Z. J., & Wei, T. Y. (2012). Development of an insect vector cell culture and RNA interference system to investigate the functional role of Fijivirus replication protein. *Journal of Virology, 86*, 5800–5807.

Jing, S. L., Zhou, X., Yu, H. J., Liu, B. F., Zhang, C. X., Wang, S. Z., Peng, X. X., Zhu, L. L., Ding, Y., & He, G. C. (2012). Isolation and characterization of microsatellite markers in brown planthopper (*Nilaparvata lugens* Stål). *International Journal of Molecular Sciences, 13*, 9527–9533.

Jing, S., Zhang, L., Ma, Y., Liu, B., Zhao, Y., Yu, H., Zhou, X., Qin, R., Zhu, L., & He, G. (2014). Genome-wide mapping of virulence in brown planthopper identifies loci that break down host plant resistance. *PLoS One, 9*, e98911.

Jones, A. K., & Sattelle, D. B. (2010). Diversity of insect nicotinic acetylcholine receptor subunits. In S. H. Thany (Ed.), *Insect nicotinic acetylcholine receptors* (Vol. 683, pp. 25–43). New York: Springer.

Kauss, H. (1987). Some aspects of calcium-dependent regulation in plant-metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology, 38*, 47–72.

Kauss, H., Kohle, H., & Jeblick, W. (1983). Proteolytic activation and stimulation by Ca^{2+} of glucan synthase from soybean cells. *FEBS Letters, 158*, 84–88.

Kifune, T., & Maeta, Y. (1986). New host records of *Elenchus japonicus* (Esaki et Matsumoto, 1931) (Strepsiptera, Elenchidae) from Japan and the East China Sea. *Kontyu, 54*, 359–360.

Kim, M. K., Koh, H.-S., Ichikawa, T., Fukami, H., & Ishii, S. (1975). Antifeedant of barnyard grass against the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Applied Entomology and Zoology, 10*, 116–122.

Kim, M. K., Koh, H.-S., Obata, T., Fukami, H., & Ishii, S. (1976). Isolation and identification of trans-aconitic acid as the antifeedant in barnyard grass against the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Applied Entomology and Zoology, 11*, 53–57.

Kimmins, F. M. (1989). Electrical penetration graphs from *Nilaparvata lugens* on resistant and susceptible rice varieties. *Entomologia Experimentalis et Applicata, 50*, 69–79.

Kimura, I., & Omura, T. (1988). Leafhopper cell cultures as a means for phyoreovirus research. *Advances in Disease Vector Research, 5*, 111–135.

King, R. W., & Zeevaart, J. A. (1974). Enhancement of phloem exudation from cut petioles by chelating agents. *Plant Physiology, 53*, 96–103.

Kisimoto, R. (1976). Synoptic weather conditions inducing long-distance immigration of planthoppers, *Sogatella furcifera* Horváth and *Nilaparvata lugens* Stål. *Ecological Entomology, 1*, 95–109.

Kisimoto, R. (1987). Ecology of planthopper migration. In M. R. Wilson & L. R. Nault (Eds.), *Proceedings of 2nd international workshop on leafhopper and planthopper of economic importance* (pp. 41–54). London: CAB International Institute of Entomology.

Kisimoto, R., & Rosenberg, L. J. (1994). Long-distance migration in delphacid planthoppers. In R. F. Denno & T. J. Perfect (Eds.), *Planthoppers: Their ecology and management* (pp. 302–322). New York: Chapman & Hall, Inc.

Knoblauch, M., Peters, W. S., Ehlers, K., & van Bel, A. J. E. (2001). Reversible calcium-regulated stopcocks in legume sieve tubes. *Plant Cell, 13*, 1221–1230.

Kobayashi, T., Yamamoto, K., Suetsugu, Y., Kuwazaki, S., Hattori, M., Jairin, J., Sanada-Morimura, S., & Matsumura, M. (2014). Genetic mapping of the rice resistance-breaking gene of the brown planthopper *Nilaparvata lugens. Proceedings of the Royal Society B, 281*, 20140726.

Konishi, H., Noda, H., Tamura, Y., & Hattori, M. (2009). Proteomic analysis of the salivary glands of the rice brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Applied Entomology and Zoology, 44*, 525–534.

Kuno, E. (1979). Ecology of the brown planthopper in temperate regions. In I. R. R. Institute (Ed.), *Brown planthopper: Threat to rice production in Asia* (pp. 45–60). Los Baños: International Rice Research Institute.

Lao, S. H., Huang, X. H., Huang, H. J., Liu, C. W., Zhang, C. X., & Bao, Y. Y. (2015). Genomic and transcriptomic insights into the cytochrome P450 monooxygenase gene repertoire in the rice pest brown planthopper, *Nilaparvata lugens. Genomics, 106*, 301–309.

Li, H. K. (1985). Entomopathogenic microorganisms of rice planthoppers and leafhoppers in China. *International Rice Research Newsletter, 10*, 13–14.

Li, J., Shao, Y., Ding, Z. P., Bao, H. B., Liu, Z. W., Han, Z. J., & Millar, N. S. (2010). Native subunit composition of two insect nicotinic receptor subtypes with differing affinities for the insecticide imidacloprid. *Insect Biochemistry and Molecular Biology, 40*, 17–22.

Liu, Z. W., Han, Z. J., Wang, Y. C., Zhang, L. C., Zhang, H. W., & Liu, C. J. (2003). Selection for imidacloprid resistance in *Nilaparvata lugens*: Cross-resistance patterns and possible mechanisms. *Pest Management Science, 59*, 1355–1359.

Liu, Z. W., Williamson, M. S., Lansdell, S. J., Denholm, I., Han, Z. J., & Millar, N. S. (2005). A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper). *Proceedings of the National Academy of Sciences of the United States of America, 102*, 8420–8425.

Liu, C. X., Hao, F. H., Hu, J., Zhang, W. L., Wan, L. L., Zhu, L. L., Tang, H. R., & He, G. C. (2010a). Revealing different systems responses to brown planthopper infestation for pest sus-

ceptible and resistant rice plants with the combined metabonomic and gene-expression analysis. *Journal of Proteome Research, 9*, 6774–6785.

Liu, S. H., Ding, Z. P., Zhang, C. W., Yang, B. J., & Liu, Z. W. (2010b). Gene knockdown by intrathoracic injection of double-stranded RNA in the brown planthopper, *Nilaparvata lugens*. *Insect Biochemistry and Molecular Biology, 40*, 666–671.

Liu, Y. Q., Wu, H., Chen, H., Liu, Y. L., He, J., Kang, H. Y., Sun, Z. G., Pan, G., Wang, Q., Hu, J. L., Zhou, F., Zhou, K. N., Zheng, X. M., Ren, Y. L., Chen, L. M., Wang, Y. H., Zhao, Z. G., Lin, Q. B., Wu, F. Q., Zhang, X., Guo, X. P., Cheng, X. I., Jiang, L., Wu, C. Y., Wang, H. Y., & Wan, J. M. (2015). A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. *Nature Biotechnology, 33*, 301–305.

Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., & Ton, J. (2011). Callose deposition: A multifaceted plant defense response. *Molecular Plant-Microbe Interactions, 24*, 183–193.

Lundgren, J. G., & Duan, J. J. (2013). RNAi-based insecticidal crops: Potential effects on nontarget species. *Bioscience, 63*, 657–665.

Lv, L., Peng, X., Jing, S., Liu, B., Zhu, L., & He, G. (2015). Intraspecific and interspecific variations in the mitochondrial genomes of *Nilaparvata* (Hemiptera: Delphacidae). *Journal of Economic Entomology, 108*, 2021–2029.

Mao, Q. Z., Zheng, S. L., Han, Q. M., Chen, H. Y., Ma, Y. Y., Jia, D. S., Chen, Q., & Wei, T. Y. (2013). New model for the genesis and maturation of viroplasms induced by Fijiviruses in insect vector cells. *Journal of Virology, 87*, 6819–6828.

Matsumoto, Y., Matsumura, M., Hoshizaki, S., Sato, Y., & Noda, H. (2011). The strepsipteran parasite *Elenchus japonicus* (Strepsiptera, Elenchidae) of planthoppers consists of three genotypes. *Applied Entomology and Zoology, 46*, 435–442.

Matsumoto, Y., Matsumura, M., Sanada-Morimura, S., Hirai, Y., Sato, Y., & Noda, H. (2013). Mitochondrial cox sequences of *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera, Delphacidae): Low specificity among Asian planthopper populations. *Bulletin of Entomological Research, 103*, 382–392.

Matsumura, F. (2009). Insecticides. In V. H. Resh & R. T. Carde (Eds.), *Encyclopedia of insects* (2nd ed., pp. 502–505). San Diego: Academic Press (Elsevier, Inc).

Matsumura, M., Takeuchi, H., Satoh, M., Sanada-Morimura, S., Otuka, A., Watanabe, T., & Van Thanh, D. (2008). Species-specific insecticide resistance to imidacloprid and fipronil in the rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera* in East and South-east Asia. *Pest Management Science, 64*, 1115–1121.

Matsumura, M., Takeuchi, H., Satoh, M., Sanada-Morimura, S., Otuka, A., Watanabe, T., & Thanh, D. V. (2009). Current status of insecticide resistance in rice planthoppers in Asia. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 233–243). Los Baños: International Rice Research Institute.

Matsumura, M., Sanada-Morimura, S., Otuka, A., Ohtsu, R., Sakumoto, S., Takeuchi, H., & Satoh, M. (2014). Insecticide susceptibilities in populations of two rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera*, immigrating into Japan in the period 2005–2012. *Pest Management Science, 70*, 615–622.

Mcnairn, R. B., & Currier, H. B. (1967). Sieve plate callose. A factor in blockage of axial phloem transport. *Naturwissenschaften, 54*, 591.

Miao, Q., Wu, J., Tang, Q., Cheng, J., & Fu, Q. (2012). Feasibility of recognizing different geographic populations of the brown planthopper, *Nilaparvata lugens* (Hemiptera:Delphacidae), using chemical elements. *Acta Entomologica Sinica, 55*, 535–544.

Miles, P. W. (1968). Insect secretions in plants. *Annual Review of Phytopathology, 6*, 137–164.

Miles, P. W. (1972). The saliva of Hemiptera. *Advances in Insect Physiology, 9*, 183–255.

Miles, P. W. (1999). Aphid saliva. *Biological Reviews, 74*, 41–85.

Mita, T., Matsumoto, Y., Sanada-Morimura, S., & Matsumura, M. (2012). Passive long-distance migration of apterous dryinid wasps parasitizing rice planthoppers. In L. Stebens (Ed.), *Global advances in biogeography* (pp. 49–60). Rijeka: Intech.

Morgan, J. K., Luzio, G. A., Ammar, E. D., Hunter, W. B., Hall, D. G., & Shatters, R. G. (2013). Formation of stylet sheaths in aere (in air) from eight species of phytophagous hemipterans from six families (Suborders: Auchenorrhyncha and Sternorrhyncha). *PLoS One, 8*, e62444.

Mun, J. H., Song, Y. H., Heong, K. L., & Roderick, G. K. (1999). Genetic variation among Asian populations of rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae): Mitochondrial DNA sequences. *Bulletin of Entomological Research, 89*, 245–253.

Murakami, R., Suetsugu, Y., Kobayashi, T., & Nakashima, N. (2013). The genome sequence and transmission of an iflavirus from the brown planthopper, *Nilaparvata lugens. Virus Research, 176*, 179–187.

Murakami, R., Suetsugu, Y., & Nakashima, N. (2014). Complete genome sequences of two iflaviruses from the brown planthopper, *Nilaparvata lugens. Archives of Virology, 159*, 585–588.

Murray, R. K., Granner, D. K., & Rodwell, V. W. (2006). *Harper's illustrated biochemistry* (27th ed.). New York: McGraw-Hill Companies, Inc.

Nagata, T. (1984). Insecticide resistance in the brown planthopper. *Chinese Journal of Entomology, 4*, 117–124.

Nagata, T., & Hayakawa, T. (1998). Antifeeding activity of aconitic acids and oxalic acid on brown planthopper, *Nilaparvata lugens* (Stål) and green rice leafhopper, *Nephotettix cincticeps* (Uhler). *Japanese Journal of Applied Entomology and Zoology, 42*, 115–121.

Nagata, T., Masuda, T., & Moriya, S. (1979). Development of insecticide resistance in the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera, Delphacidae). *Applied Entomology and Zoology, 14*, 264–269.

Nakamura, M., & Hattori, M. (2013). Purification of beta-glucosidase from the salivary glands of the green rice leafhopper, *Nephotettix cincticeps* (Uhler) (Hemiptera: Cicadellidae), and its detection in the salivary sheath. *Applied Entomology and Zoology, 48*, 489–497.

Nakamura, Y., Yukuhiro, F., Matsumura, M., & Noda, H. (2012). Cytoplasmic incompatibility involving *Cardinium* and *Wolbachia* in the white-backed planthopper *Sogatella furcifera* (Hemiptera: Delphacidae). *Applied Entomology and Zoology, 47*, 273–283.

Nakao, T., Naoi, A., Kawahara, N., & Hirase, K. (2010). Mutation of the GABA receptor associated with fipronil resistance in the whitebacked planthopper, *Sogatella furcifera. Pesticide Biochemistry and Physiology, 97*, 262–266.

Nakao, T., Kawase, A., Kinoshita, A., Abe, R., Hama, M., Kawahara, N., & Hirase, K. (2011). The A2′N mutation of the RDL gamma-aminobutyric acid receptor conferring fipronil resistance in *Laodelphax striatellus* (Hemiptera: Delphacidae). *Journal of Economic Entomology, 104*, 646–652.

Nakao, T., Hama, M., Kawahara, N., & Hirase, K. (2012). Fipronil resistance in *Sogatella furcifera*: Molecular cloning and functional expression of wild-type and mutant RDL GABA receptor subunits. *Journal of Pesticide Science, 37*, 37–44.

Nakashima, N., Kawahara, N., Omura, T., & Noda, H. (2006). Characterization of a novel satellite virus and a strain of Himetobi P virus (Dicistroviridae) from the brown planthopper, *Nilaparvata lugens. Journal of Invertebrate Pathology, 91*, 53–56.

Narusuye, K., Nakao, T., Abe, R., Nagatomi, Y., Hirase, K., & Ozoe, Y. (2007). Molecular cloning of a GABA receptor subunit from *Laodelphax striatella* (Fallén) and patch clamp analysis of the homo-oligomeric receptors expressed in a D*rosophila* cell line. *Insect Molecular Biology, 16*, 723–733.

Nault, L. R. (1994). Transmission biology, vector specificity and evolution of planthopper-transmitted plant viruses. In R. F. Denno & T. J. Perfect (Eds.), *Planthoppers: Their ecology and management* (pp. 429–448). New York: Chapman & Hall, Inc.

Noda, H. (1986a). Damage to ears of rice plants caused by the white-backed planthopper, *Sogatella furcifera* (Homoptera: Delphacidae). *Applied Entomology and Zoology, 21*, 474–476.

Noda, H. (1986b). Pre-mating flight of rice planthopper migrants (Homoptera, Delphacidae) collected on the East-China-Sea. *Applied Entomology and Zoology, 21*, 175–176.

Noda, H. (1992). Geographic variation of nymphal diapause in the small brown planthopper in Japan. *Jarq-Japan Agricultural Research Quarterly, 26*, 124–129.

Noda, H. (2009). How can planthopper genomics be useful for planthopper management? In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 429–445). Los Baños: International Rice Research Institute.

Noda, H., & Nakashima, N. (1995). Non-pathogenic reoviruses of leafhoppers and planthoppers. *Seminars in Virology, 6*, 109–116.

Noda, H., & Tatewaki, R. (1990). Reexamination of chromosomes of three species of rice planthoppers (Homoptera, Delphacidae). *Applied Entomology and Zoology, 25*, 538–540.

Noda, H., Ishikawa, K., Hibino, H., & Omura, T. (1991). A reovirus in the brown planthopper, *Nilaparvata lugens. Journal of General Virology, 72*, 2425–2430.

Noda, H., Koizumi, Y., Zhang, Q., & Deng, K. J. (2001). Infection density of Wolbachia and incompatibility level in two planthopper species, *Laodelphax striatellus and Sogatella furcifera. Insect Biochemistry and Molecular Biology, 31*, 727–737.

Noda, H., Kawai, S., Koizumi, Y., Matsui, K., Zhang, Q., Furukawa, S., Shimomura, M., & Mita, K. (2008). Annotated ESTs from various tissues of the brown planthopper *Nilaparvata lugens*: A genomic resource for studying agricultural pests. *BMC Genomics, 9*, 117.

Omura, T., & Kimura, I. (1994). Leafhopper cell culture for virus research. In K. Maramorosch & A. H. McIntosh (Eds.), *Arthropod cell culture systems* (pp. 91–107). Philadelphia: CRC Press.

Otake, A. (1970). Studies on the egg parasites of the smaller brown planthopper, *Laodelphax striatellus* (Fallén): Hemiptera: Delphacidae: IV. Seasonal trends in parasitic and dispersal activities, with special reference to *Anagrus* nr. *flaveolus* Waterhouse (Hymenoptera: Mymaridae). *Applied Entomology and Zoology, 5*, 95–104.

Otuka, A. (2009). Migration of rice planthoppers and simulation techniques. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 343–356). Los Baños: International Rice Research Institute.

Otuka, A. (2013). Migration of rice planthoppers and their vectored re-emerging and novel rice viruses in East Asia. *Frontiers in Microbiology, 4*, 309.

Otuka, A., Dudhia, J., Watanabe, T., & Furuno, A. (2005). A new trajectory analysis method for migratory planthoppers, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) and *Nilaparvata lugens* (Stål), using an advanced weather forecast model. *Agricultural and Forest Entomology, 7*, 1–9.

Otuka, A., Huang, S. H., Sanada-Morimura, S., & Matsumura, M. (2012a). Migration analysis of *Nilaparvata lugens* (Hemiptera: Delphacidae) from the Philippines to Taiwan under typhoon-induced windy conditions. *Applied Entomology and Zoology, 47*, 263–271.

Otuka, A., Zhou, Y. J., Lee, G. S., Matsumura, M., Zhu, Y. Q., Park, H. H., Liu, Z. W., & Sanada-Morimura, S. (2012b). Prediction of overseas migration of the small brown planthopper, *Laodelphax striatellus* (Hemiptera: Delphacidae) in East Asia. *Applied Entomology and Zoology, 47*, 379–388.

Otuka, A., Sakamoto, T., Chien, H. V., Matsumura, M., & Sanada-Morimura, S. (2014). Occurrence and short-distance migration of *Nilaparvata lugens* (Hemiptera: Delphacidae) in the Vietnamese Mekong Delta. *Applied Entomology and Zoology, 49*, 97–107.

Ozaki, K., & Kassai, T. (1970). Biochemical genetics of malathion resistance in smaller brown planthopper, *Laodelphax striatellus. Entomologia Experimentalis et Applicata, 13*, 162–172.

Ozaki, K., & Kassai, T. (1982). Development of insecticid resistance by the brown planthopper, *Nilaparvata lagens* Stål (Hemiptera: Delphacidae) and resistance pattern of field populations. *Japanese Journal of Applied Entomology and Zoology, 26*, 249–255.

Pathak, M. D., Cheng, C. H., & Fortuno, M. E. (1969). Resistance to *Nephotettix impicticeps* and *Nilaparvata lugens* in varieties of rice. *Nature, 223*, 502–504.

Peng, X., Zha, W., He, R., Lu, T., Zhu, L., Han, B., & He, G. (2011). Pyrosequencing the midgut transcriptome of the brown planthopper, *Nilaparvata lugens. Insect Molecular Biology, 20*, 745–762.

Petrova, A., & Smith, C. M. (2014). Immunodetection of a brown planthopper (*Nilaparvata lugens* Stål) salivary catalase-like protein into tissues of rice, *Oryza sativa. Insect Molecular Biology, 23*, 13–25.

Preap, V., Zalucki, M. P., Jahn, G. C., & Nesbitt, H. J. (2001). Effectiveness of brown planthopper predators: Population suppression by two species of spider, *Pardosa pseudoannulata* (Araneae, Lycosidae) and *Araneus inustus* (Araneae, Araneidae). *Journal of Asia-Pacific Entomology, 4*, 187–193.

Price, D. R. G., & Gatehouse, J. A. (2008). RNAi-mediated crop protection against insects. *Trends in Biotechnology, 26*, 393–400.

Puinean, A. M., Denholm, I., Millar, N. S., Nauen, R., & Williamson, M. S. (2010). Characterisation of imidacloprid resistance mechanisms in the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Pesticide Biochemistry and Physiology, 97*, 129–132.

Rao, S. A. K., Carolan, J. C., & Wilkinson, T. L. (2013). Proteomic profiling of cereal aphid saliva reveals both ubiquitous and adaptive secreted proteins. *PLoS One, 8*, e57413.

Sakai, T., & Sogawa, K. (1976). Effects of nutrient compounds on sucking response of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). *Applied Entomology and Zoology, 11*, 82–88.

Sanada-Morimura, S., Sakumoto, S., Ohtsu, R., Otuka, A., Huang, S. H., Thanh, D. V., & Matsumura, M. (2011). Current status of insecticide resistance in the small brown planthopper, *Laodelphax striatellus*, in Japan, Taiwan, and Vietnam. *Applied Entomology and Zoology, 46*, 65–73.

Sanada-Morimura, S., Matsumura, M., & Noda, H. (2013). Male killing caused by a spiroplasma symbiont in the small brown planthopper, *Laodelphax striatellus. Journal of Heredity, 104*, 821–829.

Sangha, J. S., Yolanda, H. C., Kaur, J., Khan, W., Abduljaleel, Z., Alanazi, M. S., Mills, A., Adalla, C. B., Bennett, J., Prithiviraj, B., Jahn, G. C., & Leung, H. (2013). Proteome analysis of rice (Oryza sativa L.) mutants reveals differentially induced proteins during brown planthopper (*Nilaparvata lugens*) infestation. *International Journal of Molecular Sciences, 14*, 3921–3945.

Sasaya, T., Nakazono-Nagaoka, E., Saika, H., Aoki, H., Hiraguri, A., Netsu, O., Uehara-Ichiki, T., Onuki, M., Toki, S., Saito, K., & Yatou, O. (2014). Transgenic strategies to confer resistance against viruses in rice plants. *Frontiers in Microbiology, 4*, 409.

Seino, H., Shiotsuki, Y., Oya, S., & Hirai, Y. (1987). Prediction of long distance migration of rice planthoppers to northern Kyushu considering low-level jet stream. *Jounal of Agricultural Meteorology, 43*, 203–208.

Seo, B. Y., Jung, J. K., Choi, B.-R., Park, H. M., & Lee, B. H. (2009). Resistance-breaking ability and feeding behavior of the brown planthopper, *Nilaparvata lugens*, recently collected in Korea. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 303–314). Los Baños: International Rice Research Institute.

Shimazu, M. (1976). *Entomophthora delphacis* isolated from the brown planthopper, *Nilaparvata lugens* (Stål). *Japanese Journal of Applied Entomology and Zoology, 20*, 144–150.

Shimazu, M. (1977). Factors affecting conidial germination of *Entomophthora delphacis* Hori (Entomophthorales: Entomophthoraceae). *Applied Entomology and Zoology, 12*, 260–264.

Shinoda, T. (1993). Callose reaction induced in melon leaves by feeding of melon aphid, *Aphis gossypii* Glover as possible aphid-resistant factor. *Japanese Journal of Applied Entomology and Zoology, 37*, 145–152.

Small, G. J., & Hemingway, J. (2000). Molecular characterization of the amplified carboxylesterase gene associated with organophosphorus insecticide resistance in the brown planthopper, *Nilaparvata lugens. Insect Molecular Biology, 9*, 647–653.

Sogawa, K. (1965). Studies on the salivary glands of rice plant leafhoppers: I. Morphology and histology. *Japanese Journal of Applied Entomology and Zoology, 9*, 275–290.

Sogawa, K. (1968a). Studies on the salivary glands of rice plant leafhoppers: III. Salivary phenolase. *Applied Entomology and Zoology, 3*, 13–25.

Sogawa, K. (1968b). Studies on the salivary glands of rice plant leafhoppers: IV. Carbohydrase activities. *Applied Entomology and Zoology, 3*, 67–73.

Sogawa, K. (1974). Studies on the feeding habits of the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) IV. Probing stimulant. *Applied Entomology and Zoology, 9*, 204–213.

Sogawa, K. (1982). The rice brown planthopper – feeding physiology and host plant interactions. *Annual Review of Entomology, 27*, 49–73.

Sogawa, K., & Pathak, M. D. (1970). Mechanism of brown planthopper resistance in Mudgo variety of rice (Hemiptera: Delphacidae). *Applied Entomology and Zoology, 5*, 145–158.

Suh, S. O., Noda, H., & Blackwell, M. (2001). Insect symbiosis: Derivation of yeast-like endosymbionts within an entomopathogenic filamentous lineage. *Molecular Biology and Evolution, 18*, 995–1000.

Sun, J. T., Zhang, Y. K., Ge, C., & Hong, X. Y. (2011). Mining and characterization of sequence tagged microsatellites from the brown planthopper *Nilaparvata lugens. Journal of Insect Science, 11*, 134.

Syobu, S., Otuka, A., & Matsumura, M. (2012). Annual fluctuations in the immigrant density of rice planthoppers, *Sogatella furcifera* and *Nilaparvata lugens* (Hemiptera: Delphacidae), in the Kyushu district of Japan, and associated meteorological conditions. *Applied Entomology and Zoology, 47*, 399–412.

Tamura, Y., Hattori, M., Yoshioka, H., Yoshioka, M., Takahashi, A., Wu, J., Sentoku, N., & Yasui, H. (2014). Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. *indica* cultivar ADR52. *Science Reporter, 4*, 5872.

Tanaka, K., Endo, S., & Kazano, H. (2000). Toxicity of insecticides to predators of rice planthoppers: Spiders, the mirid bug and the dryinid wasp. *Applied Entomology and Zoology, 35*, 177–187.

Tang, M., Lv, L., Jing, S. L., Zhu, L. L., & He, G. C. (2010). Bacterial symbionts of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). *Applied and Environmental Microbiology, 76*, 1740–1745.

Toledo, A. V., Lenicov, A. M. M. D., & Lastra, C. C. L. (2010). Histopathology caused by the entomopathic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, in the adult plant hopper, *Peregrinus maidis*, a maize virus vector. *Journal of Insect Science, 10*, 35.

Tomizawa, M., & Casida, J. E. (2001). Structure and diversity of insect nicotinic acetylcholine receptors. *Pest Management Science, 57*, 914–922.

Toriyama, S., Guy, P. L., Fuji, S., & Takahashi, M. (1992). Characterization of a new picorna-like virus, Himetobi P-Virus, in planthoppers. *Journal of General Virology, 73*, 1021–1023.

Velusamy, R., & Heinrichs, E. A. (1986). Electronic monitoring of feeding-behavior of *Nilaparvata lugens* (Homoptera, Delphacidae) on resistant and susceptible rice cultivars. *Environmental Entomology, 15*, 678–682.

Vontas, J. G., Small, G. J., & Hemingway, J. (2000). Comparison of esterase gene amplification, gene expression and esterase activity in insecticide susceptible and resistant strains of the brown planthopper, *Nilaparvata lugens* (Stål). *Insect Molecular Biology, 9*, 655–660.

Vontas, J. G., Small, G. J., & Hemingway, J. (2001). Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens. Biochemical Journal, 357*, 65–72.

Vontas, J. G., Small, G. J., Nikou, D. C., Ranson, H., & Hemingway, J. (2002). Purification, molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the rice brown planthopper, *Nilaparvata lugens. Biochemical Journal, 362*, 329–337.

Wang, Y. C., Tang, M., Hao, P. Y., Yang, Z. F., Zhu, L. L., & He, G. C. (2008a). Penetration into rice tissues by brown planthopper and fine structure of the salivary sheaths. *Entomologia Experimentalis et Applicata, 129*, 295–307.

Wang, Y. H., Chen, J., Zhu, Y. C., Ma, C. Y., Huang, Y., & Shen, J. L. (2008b). Susceptibility to neonicotinoids and risk of resistance development in the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Pest Management Science, 64*, 1278–1284.

Wang, Y. Y., Wang, X. L., Yuan, H., Chen, R. Z., Zhu, L. L., He, R. F., & He, G. C. (2008c). Responses of two contrasting genotypes of rice to brown planthopper. *Molecular Plant-Microbe Interactions, 21*, 122–132.

Wang, Y. H., Liu, X. G., Zhu, Y. C., Wu, S. G., Li, S. Y., Chen, W. M., & Shen, J. L. (2009). Inheritance mode and realized heritability of resistance to imidacloprid in the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Pest Management Science, 65*, 629–634.

Wang, Y. B., Guo, H. M., Li, H. C., Zhang, H., & Miao, X. X. (2012). Identification of transcription factors potential related to brown planthopper resistance in rice via microarray expression profiling. *BMC Genomics, 13*, 687.

Wang, L.-F., Fu, S., Xiao, L., Vhen, C., & Xue, F.-S. (2013). Life history, reproduction and overwintering biology of the small brown planthopper, *Laodelphax striatellus* (Hemiptera: Delphacidae), in Nanchang, Jiangxi, East China. *Acta Entomologica Sinica, 56*, 1430–1439.

Wang, Y., Cao, L. M., Zhang, Y. X., Cao, C. X., Liu, F., Huang, F. K., Qiu, Y. F., Li, R. B., & Lou, X. J. (2015). Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. *Journal of Experimental Botany, 66*, 6035–6045.

Watanabe, T., Matsumura, M., & Otuka, A. (2009). Recent occurrences of long-distance migratory planthoppers and factors causing outbreaks in Japan. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 179–189). Los Baños: International Rice Research Institute.

Wei, Z., Hu, W., Lin, Q. S., Cheng, X. Y., Tong, M. J., Zhu, L. L., Chen, R. Z., & He, G. C. (2009). Understanding rice plant resistance to the brown planthopper (*Nilaparvata lugens*): A proteomic approach. *Proteomics, 9*, 2798–2808.

Wen, Y. C., Liu, Z. W., Bao, H. B., & Han, Z. J. (2009). Imidacloprid resistance and its mechanisms in field populations of brown planthopper, *Nilaparvata lugens* Stål in China. *Pesticide Biochemistry and Physiology, 94*, 36–42.

Will, T., Tjallingii, W. F., Thonnessen, A., & van Bel, A. J. E. (2007). Molecular sabotage of plant defense by aphid saliva. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 10536–10541.

Will, T., Furch, A. C. U., & Zimmermann, M. R. (2013). How phloem-feeding insects face the challenge of phloem-located defenses. *Frontiers in Plant Science, 4*, 336.

Wilson, M. R., & Claridge, M. F. (1985). The leafhopper and planthopper faunas of rice fields. In L. R. Nault & J. G. Rodriguez (Eds.), *The leafhoppers and planthoppers* (pp. 381–404). New York: Wiley.

Xiao, D., Li, W., Wei, T., Wu, Z., & Xie, L. (2010). Advances in the studies of rice stripe virus. *Frontiers of Agriculture in China, 4*, 287–292.

Xiao, H., Yuan, Z., Guo, D., Hou, B., Yin, C., Zhang, W., & Li, F. (2015). Genome-wide identification of long noncoding RNA genes and their potential association with fecundity and virulence in rice brown planthopper, *Nilaparvata lugens. BMC Genomics, 16*, 749.

Xu, H. J., Chen, T., Ma, X. F., Xue, J., Pan, P. L., Zhang, X. C., Cheng, J. A., & Zhang, C. X. (2013). Genome-wide screening for components of small interfering RNA (siRNA) and microRNA (miRNA) pathways in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). *Insect Molecular Biology, 22*, 635–647.

Xu, Y. P., Chen, Y. H., & Yu, X. P. (2014). Cell culture of the rice brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *In Vitro Cellular and Developmental Biology-Animal, 50*, 384–388.

Xu, H. J., Xue, J., Lu, B., Zhang, X. C., Zhuo, J. C., He, S. F., Ma, X. F., Jiang, Y. Q., Fan, H. W., Xu, J. Y., Ye, Y. X., Pan, P. L., Li, Q., Bao, Y. Y., Nijhout, H. F., & Zhang, C. X. (2015). Two insulin receptors determine alternative wing morphs in planthoppers. *Nature, 519*, 464–467.

Xue, J. A., Bao, Y. Y., Li, B. L., Cheng, Y. B., Peng, Z. Y., Liu, H., Xu, H. J., Zhu, Z. R., Lou, Y. G., Cheng, J. A., & Zhang, C. X. (2010). Transcriptome analysis of the brown planthopper *Nilaparvata lugens. PLoS One, 5*, e14233.

Xue, J., Zhou, X., Zhang, C. X., Yu, L. L., Fan, H. W., Wang, Z., Xu, H. J., Xi, Y., Zhu, Z. R., Zhou, W. W., Pan, P. L., Li, B. L., Colbourne, J. K., Noda, H., Suetsugu, Y., Kobayashi, T., Zheng, Y., Liu, S., Zhang, R., Liu, Y., Luo, Y. D., Fang, D. M., Chen, Y., Zhan, D. L., Lv, X. D., Cai, Y., Wang, Z. B., Huang, H. J., Cheng, R. L., Zhang, X. C., Lou, Y. H., Yu, B., Zhuo, J. C., Ye, Y. X., Zhang, W. Q., Shen, Z. C., Yang, H. M., Wang, J., Wang, J., Bao, Y. Y., & Cheng, J. A. (2014). Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. *Genome Biology, 15*, 521.

Yang, Z. F., Chen, J., Chen, Y. Q., & Jiang, S. J. (2010). Molecular cloning and characterization of an acetylcholinesterase cDNA in the brown planthopper, *Nilaparvata lugens. Journal of Insect Science, 10*, 102.

Yang, K., He, P., & Dong, S. L. (2014). Different expression profiles suggest functional differentiation among chemosensory proteins in *Nilaparvata lugens* (Hemiptera: Delphacidae). *Journal of Insect Science, 14*, 132.

Yang, G., You, M., Vasseur, L., Zhao, Y., & Liu, C. (2011). Development of RNAi in insects and RNAi-based pest control. In M. Stoytcheva (Ed.), *Pesticides in the modern world – pests control and pesticides exposure and toxicity assessment* (pp. 27–38). Rijeka: InTech.

Yao, J. X., Rotenberg, D., Afsharifar, A., Barandoc-Alviar, K., & Whitfield, A. E. (2013). Development of RNAi methods for *Peregrinus maidis*, the corn planthopper. *PLoS One, 8*, e70243.

Yarasi, B., Sadumpati, V., Immanni, C. P., Vudem, D. R., & Khareedu, V. R. (2008). Transgenic rice expressing *Allium sativum* leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. *BMC Plant Biology, 8*, 102.

Yoo, J. K., Lee, S. W., Ahn, Y. J., Nagata, T., & Shono, T. (2002). Altered acetylcholinesterase as a resistance mechanism in the brown planthopper (Homoptera: Delphacidae), *Nilaparvata lugens* Stål. *Applied Entomology and Zoology, 37*, 37–41.

Yu, H. X., Ji, R., Ye, W. F., Chen, H. D., Lai, W. X., Fu, Q., & Lou, Y. G. (2014). Transcriptome analysis of fat bodies from two brown planthopper (*Nilaparvata lugens*) populations with different virulence levels in rice. *PLoS One, 9*, e88528.

Yuan, H. Y., Chen, X. P., Zhu, L. L., & He, G. C. (2005). Identification of genes responsive to brown planthopper *Nilaparvata lugens* Stål (Homoptera: Delphacidae) feeding in rice. *Planta, 221*, 105–112.

Yukuhiro, F., Miyoshi, T., & Noda, H. (2014). Actin-mediated transovarial transmission of a yeast-like symbiont in the brown planthopper. *Journal of Insect Physiology, 60*, 111–117.

Zha, W. J., Peng, X. X., Chen, R. Z., Du, B., Zhu, L. L., & He, G. C. (2011). Knockdown of midgut genes by dsRNA-transgenic plant-mediated RNA interference in the hemipteran insect *Nilaparvata lugens. PLoS One, 6*, e20504.

Zhai, Y. F., Zhang, J. Q., Sun, Z. X., Dong, X. L., He, Y., Kang, K., Liu, Z. C., & Zhang, W. Q. (2013). Proteomic and transcriptomic analyses of fecundity in the brown planthopper *Nilaparvata lugens* (Stål). *Journal of Proteome Research, 12*, 5199–5212.

Zhang, Q. F. (2007). Strategies for developing green super rice. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 16402–16409.

Zhang, F., Zhu, L., & He, G. C. (2004). Differential gene expression in response to brown planthopper feeding in rice. *Journal of Plant Physiology, 161*, 53–62.

Zhang, Q., Lu, D., Pu, J., Wu, M., & Han, Z. (2012a). Cloning and RNA interference effects of trehalase genes in *Laodelphax striatellus* (Homoptera: Delphacidae). *Acta Entomologica Sinica, 55*, 911–920.

Zhang, X. F., Zhao, D. X., & Hong, X. Y. (2012b). Cardinium-the leading factor of cytoplasmic incompatibility in the planthopper *Sogatella furcifera* doubly infected with *Wolbachia* and *Cardinium. Environmental Entomology, 41*, 833–840.

Zhang, K. J., Zhu, W. C., Rong, X., Zhang, Y. K., Ding, X. L., Liu, J., Chen, D. S., Du, Y., & Hong, X. Y. (2013). The complete mitochondrial genomes of two rice planthoppers, *Nilaparvata lugens* and *Laodelphax striatellus*: conserved genome rearrangement in Delphacidae and discovery of new characteristics of atp8 and tRNA genes. *BMC Genomics, 14*, 417.

Zhang, K. J., Zhu, W. C., Rong, X., Liu, J., Ding, X. L., & Hong, X. Y. (2014). The complete mitochondrial genome sequence of *Sogatella furcifera* (Horváth) and a comparative mitogenomic analysis of three predominant rice planthoppers. *Gene, 533*, 100–109.

Zheng, D. B., Hu, G., Yang, F., Du, X. D., Yang, H. B., Zhang, G., Qi, G. J., Liang, Z. L., Zhang, X. X., Cheng, X. N., & Zhai, B. P. (2014). Ovarian development status and population characteristics of *Sogatella furcifera* (Horváth) and *Nilaparvata lugens* (Stål):implications for pest forecasting. *Journal of Applied Entomology, 138*, 67–77.

Zhou, G. H., Wen, J. J., Cai, D. J., Li, P., Xu, D. L., & Zhang, S. G. (2008). Southern rice black-streaked dwarf virus: A new proposed Fijivirus species in the family Reoviridae. *Chinese Science Bulletin, 53*, 3677–3685.

Zhou, G., Xu, D., Xu, D., & Zhang, M. (2013). Southern rice black-streaked dwarf virus: A white-backed planthopper-transmitted fijivirus threatening rice production in Asia. *Frontiers in Microbiology, 4*, 270.