Chapter 4
Molecular Adaptations of Aphid Biotypes in Overcoming Host-Plant Resistance

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Abstract Host-plant resistance (HPR) is a valuable tactic to control pests of agronomic and horticultural crops. Insects are often the most frequent targets of HPR, especially aphids. However, aphids are prone to adapt and overcome this natural pest resistance, which threatens the efficacy, durability, and sustainability of this strategy. In this short review, we focus on recent genetic and molecular biology research that has advanced our mechanistic understanding of aphid biotype evolution with respect to HPR. We highlight studies that have utilized new population genomic, transcriptomic, and metabolomic techniques. We also draw inferences from studies on the evolution of aphid biotype adaptation on different host plants and discuss how these studies can provide a framework to study aphid biotypes. While research shows the existence of multiple, possible routes for overcoming HPR defenses, the exact mechanism(s) remains unclear. An interdisciplinary approach involving multiple fields, including omics research (population and functional genomics, transcriptomics, metabolomics, proteomics, etc.), endosymbiont biology, as well as the ecological interactions between HPR crops and the aphid pests that they target, is needed.

Abbreviations

AFLP	Amplified fragment length polymorphism
Ca^{2+}	Calcium ion
EST	Esterase
GST	Glutathione *S*-transferase
HPR	Host-plant resistance
Hx	Hydroxamic acid
IDE	Inhibitor of digestive enzyme
JA	Jasmonic acid
LRRs	Leucine-rich repeats

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C. Raman et al. (eds.), *Short Views on Insect Genomics and Proteomics*,
Entomology in Focus 3, DOI 10.1007/978-3-319-24235-4_4

MAPK	Mitogen-associated protein kinase
NBS	Nucleotide-binding site
P450	Cytochrome P450 monooxygenase
PSM	Plant secondary metabolite
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RWA	Russian wheat aphid
SA	Salicylic acid
SNP	Single-nucleotide polymorphism

4.1 Host-Plant Resistance and Biotype Evolution

Host-plant resistance (HPR) is a pest management technique that exploits naturally evolved plant defenses for improved and sustainable crop production [1]. Development of crop varieties with resistance to insect and arthropod pests has a long history, starting as early as crop domestication [2–4]. Not only has HPR been implemented as a management tactic for many crops (see [4] for a recent list of 22 crops), it serves as a model to expand our understanding of insect-plant interactions.

Hemipterans are a frequent target for HPR, and perhaps no group is more targeted than aphids—at least 16 crops are bred for aphid resistance [5, 6]. Most of these HPR crops are highly successful, for example, maize and wheat lines developed by traditional plant breeding techniques can limit aphid damage [6–8]. Alfalfa and sorghum with aphid and leafhopper resistance have an annual economic value of over $400 million [6]. In addition to economic savings, HPR promotes the ecological service of biological control [7] and can lead to a decrease in potentially hazardous chemical applications. In some cases, however, the use of HPR in pest management has been challenging and limited due to many factors including efficacy, economic viability, and lack of availability of resistant varieties [8].

Perhaps the most serious challenge to full implementation of HPR crops is their durability in the face of insect biotype evolution [3, 5, 9]. There are several definitions of the term "biotype," but, in the context of agricultural insect pests and HPR interactions, biotypes are specifically defined by their differential survival or fitness on, or adaptation to, host-plant defenses [3, 5]. Insect populations capable of overcoming resistance are considered *virulent* to the HPR plant, whereas those unable to survive and reproduce are referred to as *avirulent* [3]. Furthermore, *compatible interactions* occur when insects can feed and colonize on a plant, as opposed to *incompatible interactions* which result in insect deterrence and/or death.

Insect pests across a variety of taxa have developed biotypes in response to HPR, such as the Hessian fly (*Mayetiola destructor* [10]), the black currant leaf midge (*Dasineura tetensi* [11]), rice brown plant hopper (*Nilaparvata lugens* [12]), and black pine-leaf scale (*Nuculaspis californica* [13]). However, a large portion of documented biotypes are clustered in the order Hemiptera, specifically within the

family Aphididae [2, 3, 5, 14]. Smith and Chuang [6] listed 17 aphid species that have adapted to HPR, and all of these have multiple biotypes, i.e., differential survival with different HPR genes. In some cases it can take years for virulence to evolve—aphid-resistant strawberries were effective for *c.a.* 50 years [6]. Alternatively, biotypes can occur even before the large-scale deployment of HPR, as was the case with the soybean aphid, *Aphis glycines* [9, 15, 16]. The evolution of biotypes in the soybean aphid was a particularly notable example of rapid biotype evolution because, despite the genetic bottleneck during its North American invasion, virulence was observed within 5 years after invasion.

HPR can be a valuable strategy for insect management, but its use as an alternative to insecticides is limited by the evolution of virulent biotypes. Moreover, very few mechanisms of virulent biotype evolution have been described. Understanding the genetic and molecular factors that explain virulence and biotype adaptation is important to develop strategies that limit increases in its frequency, to extend HPR crops' durability and to improve the sustainability of this management tactic. This short review will highlight important advances in our understanding of how virulent biotype adaptation occurs and also show where additional studies and important tools are needed in order to fully use HPR to its potential.

4.2 Population Genomics in Characterizing Biotype Differentiation

Diehl and Bush [17] developed an evolutionarily based framework for the characterization of insect biotypes, largely based on how genetic variation was partitioned among populations. They hypothesized that if biotypes were truly distinct and evolutionarily defined, then greater genetic similarity should exist among individuals of the same biotype rather than between biotypes, i.e., genetic variation would be better explained by biotype designation and not other factors such as geography. However, this framework had not been fully tested until the wide-scale use and practicality of molecular markers enabled such comparisons. Still, most of these early studies focused on population genetics, migration and structure, or comparisons of intraspecific aphid populations on different species of host plants, and, in many of these cases, genetic differentiation supporting host-associated populations were found [18–21]. Yet, there are only a few studies that used these tools to directly compare aphid biotypes on susceptible and resistant cultivars of the same species.

4.2.1 Biotypes of Raspberry Aphids

Raspberry aphids (*Amphorophora idaei* and *A. agathonica*) have several biotypes that are virulent to aphid-resistance genes in raspberry [22–24]. An earlier study using a restriction fragment length polymorphism (RFLP)-based approach to

analyze ribosomal spacer length variability showed discrete patterns among biotype clones. However, when comparing field populations, a greater extent of variability was observed, complicating attempts to associate genetics with specific biotypes [24].

4.2.2 Greenbug Biotypes

The greenbug, *Schizaphis graminum*, is a commonly found aphid of wheat and sorghum in North America which has 8–13 known biotypes [25, 26]. The genetics of greenbug biotypes has been compared using several marker types, including mtDNA sequencing, RFLPs, random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites [26–29]. All markers largely suggested the presence of three clades, although divergence was relatively recent (0.3–0.6 Ma), and also included polyphyletic assemblages of biotypes; indeed, it appeared that clade representation was better explained by different host-plant species (which included weedy hosts near crop fields) and not by different resistant crop cultivars [28]. The use of 31 microsatellite markers appeared to increase the resolution and ability in defining biotypes, but a population-wide perspective was difficult to interpret as within-biotype variation was not included [26]. Nonetheless, these studies did find substantial genetic variation within aphids from a multitude of wild and cultivated hosts that likely predated the development of resistant cultivars and served as a possible genetic reservoir for adaptation to resistant cultivars [26, 28, 30].

4.2.3 Russian Wheat Aphid Biotypes

The Russian wheat aphid (RWA) (*Diuraphis noxia*) is another significant and worldwide pest of wheat with eight known virulent biotypes in North America [25, 31, 32]. An AFLP comparison of these eight biotypes with other populations from South America, Europe, Africa, and the Middle East showed that at least two invasions occurred in North America, one from Middle East-Africa, and one from Europe [33]. Therefore, North American biotypes did not share recent common ancestors and instead likely emerged after these separate introductions. Two biotypes (RWA1 and 2) were placed in the Middle Eastern-African clade, and biotypes RWA3, 4, and 5 were European in origin. The independent evolution of these biotypes, combined with the high-resolution power of AFLPs, allowed for a clear delineation in genetic differences among biotypes in different clades. However, a comparison of within-biotype variation was not included, as AFLP profiles resulted from pools of 20 individuals. Cui et al. [34] compared sequence polymorphisms of 17 putative salivary transcripts of RWA. A total of 13 of these transcripts contained variation, and some single-nucleotide polymorphisms (SNPs) and indels were

specific to one biotype, albeit at low frequency. Much of the variation was shared among biotypes, and because laboratory strains were used, it is unclear if frequencies in natural populations would have been similar. Interestingly, these authors did find evidence of positive selection and rapid adaptation among these transcripts suggesting that salivary transcripts may play an important role in HPR interactions.

4.2.4 Soybean Aphid Biotypes

A. glycines is a significant agricultural pest of soybean in Asia and is invasive in North America. There are four biotypes, three of which are virulent to various *Rag* genes (**R**esistance to ***A**phis **g**lycines*) [15, 16, 35]. Using microsatellites, Michel et al. [36] were able to find diagnostic markers among laboratory colonies of the avirulent biotype 1 and the virulent biotype 2. However, when SNPs were used to compare avirulent (biotype 1) and virulent (biotype 2) *A. glycines* collected from resistant and susceptible plants in the field, no diagnostic markers were found, and genetic differentiation was not apparent among biotypes [37]. These data mirrored the raspberry aphid study [23] in that substantial genetic diversity was found in field populations but did not cluster by biotype. For the soybean aphid, there was a stronger relationship with genetic isolation by geographic distance, aided by large-scale dispersal late in the growing season [38].

While these traditional molecular marker studies have expanded our understanding of biotypic genetic variation, they have not been able to identify aphid genes that may be under natural selection for virulence nor develop reliable diagnostic markers among biotypes. The reasons for this challenge are varied and complicated. From a population-genetic perspective, a molecular marker-based approach for identifying virulence adaptation will be feasible if selection is stronger than gene flow in aphids undergoing full or partial sexual reproduction. In many aphids, this may not be the case. Selection placed on the aphid population by resistant cultivars may lead to the evolution of virulence, but the use of HPR often occurs in a patchwork mosaic, i.e., different cultivars in different areas or in limited quantities (e.g., ~40 % for *Aphis gossypii*-resistant melon, ~50 % for US sorghum, and limited acreage in soybean; see recent reviews [6, 8]). In some cases, like the greenbug, movement may also be to and from wild grasses and other plants [28, 30]. This heterogeneity would result in more balanced polymorphisms instead of the fixed (or nearly fixed) differences needed for frequency-based molecular marker analyses. Sexual reproduction in many of these aphid species allows for recombination, potentially removing any linkage between molecular markers and the virulence gene(s). An additional complication is the often contentious phenotypic definition of biotype [17, 37, 39], which is based on an insect's response to a resistant plant. Insects showing very similar responses can result from very different genetic backgrounds, as was seen with RWA2 and RWA4 [33]. Furthermore, individuals within a biotype designation may not share the same mechanism of virulence and could instead result from

convergent evolution [33] or coadapted gene complexes that provide a more qualitative aspect of virulence [37].

Newer and high-throughput sequencing technologies utilizing whole-genomic approaches have been widely used for characterizing aphid biotypes on different host-plant associations. For example, host-race evolution in the pea aphid, *Acyrthosiphon pisum*, has long been a research focus for understanding insect adaptation and speciation [21, 40, 41]. A recent combination of quantitative trait loci (QTLs) and a genome scan with AFLPs and 137 microsatellites revealed correlated genomic areas under divergent selection among populations on red clover and alfalfa [42]. A different study using 390 microsatellites also found markers under selection among pea aphids from alfalfa, clover, pea [43], and other host plants [44]. When compared to the pea aphid genome [45], 5 of the 11 outlier markers were linked to important genes (two markers related to olfactory and three markers with salivary transcripts). A targeted resequencing array (e.g., exon capture) of known pea aphid genes analyzed sequence variation of 172 loci among pea aphid from alfalfa, clover, and trefoil [46]. Significant genetic differentiation was found among host races, and much of it was focused on receptors for host odor and assessing plant quality (i.e., gustatory). These recent studies in polyphagous host-plant adaptation will certainly serve as foundations for future investigations on virulent biotype evolution to HPR, especially when additional whole-genome and molecular sequence data from multiple aphid species are obtained.

4.3 Molecular Interactions of Aphid Resistance—The Plant Perspective

The molecular interactions between aphids and their host plants have long been a research focus [3, 5, 6, 47]. Despite this research, only two aphid-resistance (R) genes, *Mi-1.2* and *Vat*, have been cloned. *Mi-1.2* in wild tomato confers resistance to the potato aphid, *Macrosiphum euphoribae* [48], and *Vat* in melon provides resistance to *A. gossypii* [49]. Similar to pathogen-resistance genes in plants, both *Mi-1.2* and *Vat* encode proteins containing a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) [48–52]. *Mi-1.2* guards the aphid-effector target *RME1* in plant cells [53] and activates defense signal transduction as soon as it detects a modification in *RME1*. These plant "R" genes mediate the resistance to aphids through microRNAs [54]. Additional genes in other aphid-resistant plants have been mapped to genic regions known to encode NBS-LRR-like proteins (see [6] for a list of genes).

Molecular studies have shown that the defenses in resistant plants including those possessing NBS-LRR "R" genes are induced after attack by aphids rather than being constitutive [4, 6, 55–59]. The induced defense is advantageous to host plants as it incurs less metabolic cost and is more pest specific [60, 61]. Upon induction, the defense signal is transduced through downstream cascades involving phytohor-

mone pathways which ultimately leads to the synthesis of a variety of defense chemicals (detailed in sections below) [4, 61]. Signaling through the jasmonic acid (JA) pathway seems to be vital for resistance to aphids, although the salicylic acid (SA) pathway can play a role [62]. Interestingly, SA induction by some aphid species makes the plant susceptible, which is seen as a ploy (sometimes called a decoy response) to suppress the more effective JA signaling (detailed below).

4.4 Molecular Interactions of Aphid Resistance—The Aphid Perspective and Virulence Evolution

Upon attack by an avirulent biotype, resistant plants induce defenses, which generally occur in three steps (Fig. 4.1). (1) *Recognition of pest attack*: A plant's surveillance system detects the attack through recognition of the pest's specific signals including molecular patterns and effectors. (2) *Signal transduction*: Detected signals are then carried through a network of signal transduction pathways like mitogen-associated protein kinases (MAPKs) and phytohormones (JA, SA, etc.). (3) *Defensive chemical production*: Signaling pathways eventually lead to the production of plant defense chemicals such as plant secondary metabolites (PSMs), proteases, protease inhibitors, and lectins so as to deter or kill the pest. Virulent biotype adaptation can occur by impeding any of these three steps, such as (1) evading recognition by the plant's surveillance system and preventing defense induction, (2) distorting or manipulating the signal transduction to their own advantage, and (3) developing resistance to plant defense chemicals (Fig. 4.1). Based on the body of knowledge available on aphid biology and aphid-plant interactions, all these scenarios for the defeat of plant resistance by aphid biotypes seem plausible.

4.4.1 Avoidance of Recognition by Plant Surveillance

There is growing evidence which suggests that plants recognize aphid attack through the latter's effector molecules injected into host cells using needle-like stylets [63–66]. Effectors are proteins or other small molecules present in aphid salivary glands which can modify the structure and function of a plant cell [63]. Upon recognition by "R"-gene-mediated surveillance, an aphid effector can trigger the plant defense response. For example, Mp10, an effector from green peach aphid, *Myzus persicae*, induces plant defenses as revealed through its *in planta* transient overexpression in *Nicotiana benthamiana* and activation of both JA and SA signaling pathways [67], which ultimately resulted in reduced aphid fecundity [68]. Similarly, Mp42, Mp56, Mp57, Mp58, and other effectors from the green peach aphid are thought to induce plant defenses, as their transient overexpression in *N. tabacum* and *Arabidopsis thaliana* caused a reduction in aphid fecundity [68, 69].

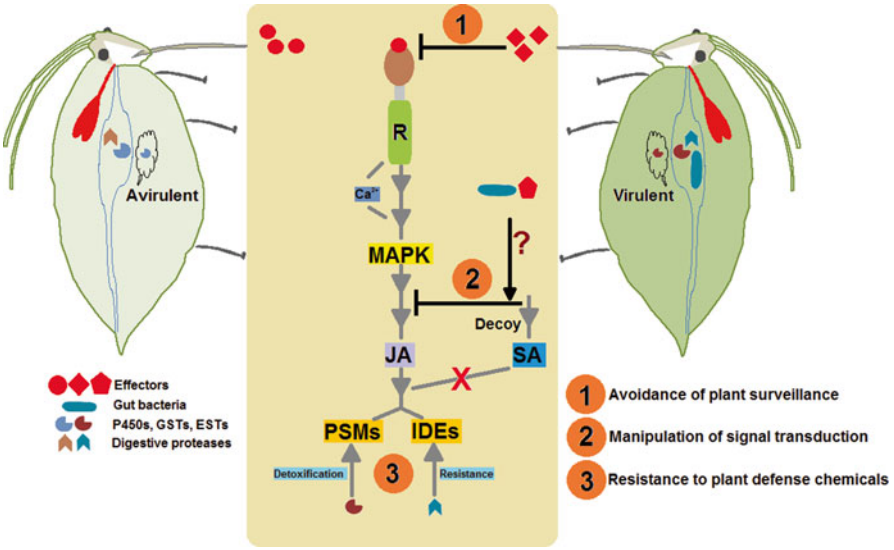

Fig. 4.1 A model summarizing putative strategies adopted by virulent aphid biotypes to overcome HPR. *1. Avoidance of plant surveillance*: Virulent biotypes can avoid recognition by a plant R (resistance)-gene-mediated guard system through diversified/novel salivary effectors. *2. Manipulation of signal transduction*: Virulent biotypes can distort and/or hinder signal transduction pathways to either block the signal transduction or manipulate and divert the transduction in such a way so that the signal is not transduced properly. The latter scenario can lead to an ineffective SA pathway in place of a biologically potent JA pathway, referred to as a decoy response. Aphid gut bacteria or salivary effectors could be involved in inducing the decoy response. *3. Resistance to plant defense chemicals*: Virulent biotypes may evolve resistance to plant secondary metabolites (PSMs) through a detoxification system comprised of cytochrome P450s (P450s), glutathione-s-transferases (GSTs), and esterases (ESTs). Usually, gut and fat body are sites for the occurrence of detoxification events within insects. Virulent biotypes may evolve resistance to inhibitors of digestive enzymes (IDEs) produced by plants through various strategies as described in the text. Ca^{2+} are also involved in other cellular activities during stress such as production of reactive oxygen species (not indicated here). Plant signaling events shown here are based on those described in Wu and Baldwin [61]. *MAPK* mitogen-associated protein kinases, *JA* jasmonic acid, *SA* salicylic acid, *Ca^{2+}* calcium ions

However, from the aphid's perspective, effectors are produced and secreted into plant cells not to induce plant defenses, but to promote their own virulence and colonization [63]. Indeed, effectors like C002 (from the pea aphid and the green peach aphid) [70, 71], Mp1, Mp2, Mp55 (from *M. persicae*) [69, 71], and Me10 and Me23 (from the potato aphid) [72] increase aphid fecundity and virulence on their respective host plants. Thus, to successfully colonize and adapt on resistant plants, virulent aphids may keep the plant defenses in an un-induced state by evading the plant's surveillance through employing a diversified effector or altogether abandoning a particular effector [63] (Fig. 4.1). Population genomics and proteomics research suggests the adoption of diversified effectors as a possible strategy as evidenced by a strong positive selection in many effector genes [34, 43, 71, 73]. For example,

higher non-synonymous variations exist among salivary effector transcripts of biotypes in the RWA and the pea aphid [34, 43]. Future research on the comparative functional analysis of diversified effectors from disparate biotypes within and among aphid species would improve our ability to understand the role of effectors in virulent biotype adaptation.

4.4.2 Manipulation of Signal Transduction Pathways

Once an aphid attack is recognized, the signal is transduced through various cellular compartments to meet the end goal of producing defense chemicals. Signal transduction occurs through multiple layers of intracellular transduction pathways involving Ca^{2+}, reactive oxygen species, MAPKs, phytohormones, and transcription factors [61]. Theoretically, virulent biotypes may either distort and/or hinder any of these pathways to either block the signal transduction or manipulate and divert the transduction in such a way so that the signal is not transduced properly (a "decoy" response; see below) (Fig. 4.1). Both these scenarios will prevent the synthesis of desired defense toxins.

In most cases, JA signaling is the key phytohormone pathway that suppresses aphid colonization [74]. This was made evident through research on the model plant *Arabidopsis* when mutants deficient in JA signaling lost resistance to aphids [75, 76], whereas mutants that were compromised for SA signaling retained resistance to aphids [55, 77]. Furthermore, in compatible interactions where aphids could successfully colonize the plants, higher SA-pathway transcripts were observed; on the other hand, reduced or slightly increased JA-pathway transcripts were detected in susceptible plants [55, 75, 78–80]. These observations have led to the "decoy" hypothesis where aphids are believed to hijack plant defense signaling by manipulating the signal transduction away from the biologically potent JA pathway and toward the ineffective SA pathway. The often negative cross talk of SA signaling with JA signaling can also hinder the effective deployment of plant resistance to aphids. Though the mechanism of manipulation of phytohormone signaling by aphids is not well understood, the infection-promoting effectors or insect gut bacteria could be involved [81] (discussed below). Future studies on the comparative transcriptomic and biochemical analysis of phytohormone and other signaling constituents in cultivars infested with virulent and avirulent biotypes will help to shed light on aphid biotype evolution through manipulation of plant defense signaling.

4.4.3 Development of Resistance to Plant Defense Chemicals

In plants, the successful transduction of induced signal leads to production of a multifaceted defense that can be broadly categorized as toxic and anti-nutritious [60]. Both toxicity and anti-nutrition are manifested through a variety of plant defense chemicals such as PSMs, proteases, and protease inhibitors.

4.4.3.1 Resistance to PSMs

PSMs are metabolic by-products which are not required for normal plant growth and development but possess direct toxicity to pests including aphids [82]. To counter PSMs, insects have evolved their own defense strategies, typically involving detoxification enzymes such as cytochrome P450 monooxygenases (P450s), glutathione *S*-transferases (GSTs), and esterases (ESTs). These detoxification enzymes can readily metabolize PSMs to limit their effectiveness (Fig. 4.1) and lead to virulent biotype adaptation. PSM resistance has been reported in numerous insects [83–86]. Employing detoxification enzymes incurs a low energy and fitness costs to aphids, thus makes it a favorable strategy to overcome HPR [87]. The role of P450s in mediating biotypic host-plant adaptation is supported by the fact that generalist aphids carry a significantly larger repertoire of P450 enzymes than specialists. For example, the generalist green peach aphid, which feeds on more than 100 species in 40 different plant families, has at least 40 % more P450 genes compared to the pea aphid, a specialist which feeds only on a few species within a single plant family (Fabaceae) [88].

There are at least two possible ways by which detoxification can lead to PSM resistance and biotype adaptation: (1) The detoxification enzyme can be produced in higher amounts, most likely through overexpression. For example, the green peach aphid adapts to glucosinolates (a family of PSMs) in *Sinapis alba* by producing more GSTs compared to when feeding on glucosinolate-free *Vicia faba* [83]. (2) Mutation(s) can occur in the catalytic site of detoxification enzymes enabling a much more efficient and effective neutralization of the PSM. In fact, detoxification genes are induced in avirulent aphid biotypes when fed with resistant plants or are exposed to PSMs. For example, GSTs have higher expression in the avirulent biotype 1 of RWA fed with wheat plants containing the *Dn4* resistance gene [89]. Higher activity of GSTs and ESTs has been found in the cereal aphid, *Sitobion avenae*, after feeding on resistant wheat with high concentrations of phenolics (PSMs) or when exposed to gramine (an alkaloid PSM) [90, 91]. Similarly, higher enzymatic activities for P450s, GSTs, and ESTs were found in cereal aphid fed with hydroxamic acid (Hx)-containing wheat compared to those fed with Hx-free oats [92]. Higher EST activity occurred in the corn aphid, *Rhopalosiphum padi*, when feeding on resistant wheat compared to those fed with susceptible plants [93, 94]. Similarly, certain P450s, GSTs, and ESTs are induced when the avirulent biotype 1 of the soybean aphid feeds on a soybean plant possessing the *Rag1* resistance gene [95].

4.4.3.2 Resistance to Inhibitors of Digestive Enzymes

As a part of their defense, plants induce the production of inhibitors that target insect digestive enzymes, the majority of which are proteases and amylases [96]. Protease inhibitors targeting various aphid species have been characterized in different plants [97–99]. However, insects are known to adapt to plant protease

inhibitors in a number of ways (Fig. 4.1): (1) inactivation of protease inhibitors by direct proteolysis by insect gut proteinases [100, 101], (2) overproduction of existing digestive proteases [102], (3) expression of inhibitor-insensitive proteases [101, 103, 104], and (4) inducing isoforms of inhibitor-sensitive proteases [105]. The latter three strategies essentially result in redeployment of the insect digestive arsenal which is regulated by the alteration in gene expression of different digestive enzymes. Indeed, like in other insects, aphids show differential expression of gut digestive enzymes when feeding on HPR crops. For example, there is significant differential regulation of gut proteases among the avirulent biotype 1 and the virulent biotype 2 of RWA fed with *Dn4* wheat [89]. Similarly, there is significantly differential regulation of protease and protease inhibitors in the virulent biotype 3 and avirulent biotype 1 of the soybean aphid feeding on resistant (*Rag1*) soybean [95]. Moreover, aphids exhibit a massive expansion in their repertoire of cathepsin B genes, the major digestive proteinases of hemipterans which can overcome plant protease inhibitors [106].

4.4.4 Role of Bacterial Symbionts in Aphid Biotype Evolution

Aphids are well known for their symbiotic relationships with bacteria. The pea aphid is known to harbor three kinds of bacteria: (1) the obligate endosymbiont, *Buchnera*; (2) several facultative endosymbionts (*Hamiltonella*, *Regiella*, *Serratia*, *Rickettsia*, and *Spiroplasma*); and (3) extracellular gut microbiota which reside in the lumen of the digestive tract (e.g., *Pantoea*, *Bacillus*). However, the contribution of endosymbionts for virulent biotype adaptation may be limited because their intracellular lifestyle hampers the release of factors or gene products directly into the salivary secretions or the gut lumen, where they might assist in overcoming host-plant recognition/defenses, detoxifying plant defense chemicals, or improving digestion [107].

The phenomenon of biotype evolution is characterized by a perpetual arms race requiring defense against novel challenges posed by host plants. Therefore, *Buchnera* is highly unlikely to be involved in aphid biotype evolution as it possesses a dramatically reduced genome and does not acquire novel genes in its symbiotic relationship with aphids [107, 108]. In fact, recent studies suggest *Buchnera* might actually be an antagonist, though inadvertently, to its host aphid. *Buchnera*'s chaperonin, GroEL, can act as a molecular pattern to trigger a plant's defense response, which can negatively affect aphid growth and fecundity [69, 109]. It is speculated that aphids have evolved effectors to suppress *Buchnera* GroEL-triggered immunity in host plants [109]. Some caution is required in this interpretation, however, because these results are based on *in planta* overexpression or exogenous application of GroEL. Alternatively, *Buchnera* may be involved in greenbug virulence [110]. Proteomic variation linked with unique sequence polymorphisms in the EF-Tu protein from *Buchnera* was found within the highly virulent biotype H when compared to avirulent biotypes, although the exact mechanism or role of this

protein is unclear [110]. Future improvements on in vivo studies involving *Buchnera* and research on the localization of *Buchnera* proteins in cells of aphid-infested plants will better discern *Buchnera*'s direct role in virulent biotype evolution.

There has been some evidence to suggest that facultative endosymbionts drive aphid biotype specialization. For example, *Regiella insecticola* improved the fitness of the pea aphid on white clover but not on vetch plants [111]; however, subsequent studies did not support these results [112–114]. In another study, particular facultative endosymbiont species were found to be associated with a particular host-specialized biotype of *A. pisum* [115]. Nonetheless, inferences drawn from such surveys on the role of facultative endosymbionts in governing aphid biotype evolution could be misleading, and, to date, there are no studies to suggest that these bacteria play a role for virulent biotype evolution in relation to HPR. The association of a facultative endosymbiont with a particular aphid biotype may occur due to many other factors, as discussed in [107].

Due to their location, gut bacteria are perhaps best situated to play a direct role in aphid biotype evolution. Bacteria such as *Staphylococcus*, *Pseudomonas*, *Acinetobacter*, *Pantoea*, *Bacillus*, and *Brevundimonas* have been detected from aphid gut; however, functions for most of these are not well known [116–119]. In general, insect gut bacteria are known to perform three major activities which may be significant in virulent biotype adaptation: (1) They aid in digestion by producing inhibitor-resistant proteases [120]; (2) insect gut bacteria can detoxify PSMs, the major plant defense chemicals inside insect gut [121]; and (3) insect gut bacteria can induce decoy responses to suppress effective host-plant defense [81]. Conversely, there is a lack of concrete evidence on any role of gut bacteria in virulent biotype adaptation in aphids, with a few studies reporting only the diversity and abundance of an insect's gut and their transient presence occurring due to inconsistent infections [119]. Nonetheless, there is data to support a role for symbionts in virulent biotype adaptation to HPR in plant hoppers and leafhoppers (suborder Auchenorrhyncha), which are close relatives of the Aphididae (suborder Sternorrhyncha) [122]. More research is certainly necessary regarding the possibility of endosymbionts contributing to virulent biotype adaptation in aphids.

4.5 Conclusion

As much investment is made toward the development of HPR crops, it is imperative to understand virulent biotype adaptation and improve management strategies that extend their durability. There has been an increase in population genomics and transcriptomics research on biotype adaptation to diverse host plants which can offer clues for virulent biotype evolution. Although similar, these comparisons may also be functionally different, as the mechanism(s) of virulent biotype adaptation to HPR (sources within a single plant species) may involve greater specificity than adapting to different plant species. Transitioning among host plants in different genera may involve more complicated and diverse adaptations, stronger selection pressures, and

reproductive isolation. These factors may not all exist to the same degree during adaptation to HPR plants or varieties which may differ by one or a few genes. Since the success of large-scale population genomics and transcriptomics depends on the strength of the selective footprint [123–125], these approaches alone may not be enough to reveal mechanisms of virulent biotype adaptation, i.e., the genomic islands of divergence are too few and too small to be detected from the sea of neutral variation [126, 127]. A combined approach will be necessary which not only includes proteomics and metabolomics but also the interactions with the incredible microbial diversity that aphids house as well. Research using natural populations is also needed to capture the extent of genetic variation and diversity in both aphids and bacteria, as studies have shown dramatic differences in laboratory and natural populations in both taxa [36, 119]. Virulent biotype adaptation to HPR will also need to be investigated in the context of complex agroecosystems that include natural enemies, insecticides, and a patchwork mosaic of crop varieties that differ in maturity, nutrition (e.g., oil, protein, sugars), and many other phenotypic traits. Expanding our understanding of virulent biotype adaptation in aphids will help maintain the efficacy of current HPR crops and will also provide a solid foundation to study virulence or resistance development when the next generation of RNA interference-based insect control is implemented [128].

Acknowledgments We would like to thank members of the Michel Laboratory including L. Wallace and J. Wenger, the M.A.R Mian laboratory at USDA-ARS, and the SoyRes Team from the Center of Applied Plant Sciences at The Ohio State University. Support for this work was provided by the Ohio Agricultural Research and Development Center, The Ohio State University, as well as various soybean checkoff organizations, including the Ohio Soybean Council, North Central Soybean Research Program, and the United Soybean Board.

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