Arthropod Endosymbiosis and Evolution

17

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Contents

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17.1 Introduction

The association of "two species that live on or in one another" was first described in the nineteenth century, and the word symbiosis was proposed to denote this biological phenomenon (Sapp 1994). The discovery that lichens are organisms generated by the integration of a fungus and blue-green algae, that is, cyanobacteria, was followed by a number of other studies that have shown how the association of different species is widespread in nature and characterized by different degrees of benefit-sharing. Symbiosis encompasses both antagonistic relationships, in which one organism takes advantage of the other, and mutualistic relationships, where both partners gain advantage from their association. There are also cases where no clear benefit or harm is evident for both interacting species, which are then, in some cases, considered commensals. The term symbiosis applies to all these type of species associations, and not only to mutualism, as is sometimes erroneously done (Sapp 1994).

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The Darwinian model of gradual evolution, based on competitive selection acting on random mutations accumulated over time, was considered for a long time the major evolutionary pattern driving the diversification of all living organisms. However, gradualism does not account for evident leaps revealed from fossil records and molecular data. These evolutionary leaps have stimulated two hypotheses, not mutually exclusive (Ryan 2002). The punctuated equilibrium theory states that evolutionary leaps, denoted as saltations, are generated by drastic changes in the selection scenario, for example, the intense colonization of new ecological niches becoming available after catastrophic mass extinctions. The symbiosis theory provides an alternative interpretation of these evolutionary leaps, which are considered the final result of the fusion of different biological entities, to give rise to a new taxon (Ryan 2002). Therefore, symbiosis can be viewed not only as a peculiar mode of life, but also as a biological phenomenon that has important evolutionary implications, significantly contributing to the evolution of life on Earth (Sagan 1967; Margulis 1993). This possibility has been for a long time underestimated, but an increasing amount of molecular and functional data corroborate this hypothesis (Margulis 2009).

The most species-rich taxon of multicellular organisms is the arthropods, which have successfully colonized virtually every habitat and niche on Earth. Recent surveys reveal an equally rich diversity of symbiotic associations between different types of arthropods and micro-organisms. A comprehensive overview of these interactions is beyond the scope of a single review chapter. Thus, the purpose of this contribution is to summarize current knowledge about the interactions between primarily insects and certain groups of bacteria and viruses, which have been studied in more detail and allow the best appreciation of the considerable impact of endosymbiosis on the evolution of arthropods. In the first section of this chapter, we discuss the range of beneficial endosymbiotic associations that have evolved between insects and bacteria. In the second, we discuss the role of intracellular bacteria in manipulating the reproduction of insects and other arthropods, while our third section discusses the role selected taxa of viruses play as beneficial symbionts of parasitoid wasps and other insects.

17.2 Bacteria as Obligate and Facultative Symbionts of Insects

A comprehensive understanding of the microbial diversity associated with arthropod populations is far from being defined. The information currently available clearly indicates that bacteria, in particular those in the groups of α- and γ-Proteobacteria, are among the major players, as they are more prone to establish tight interactions with arthropod tissues, either as pathogens or as mutualists. The mechanistic bases of these latter associations, and the details of how the interacting symbionts share the emerging benefit, have not always been fully elucidated. In some cases, the symbionts are mutually obligate, due to strong functional ties, such as nutritional complementation of poor diets, which do not allow them to live independently of one another. There are many other forms of symbiosis that are facultative associations, in which the arthropod can survive in the absence of the associated micro-organism. A large variety of evolutionary novelties are generated by these facultative symbioses, which are not always obvious to interpret from a mechanistic point of view and often have more than one function. Currently, our best understanding of both obligate and facultative symbioses is derived from the study of aphids and other plant feeding Hemiptera, which will offer the large majority of the case studies presented hereafter.

17.2.1 Obligate Nutritional Endosymbionts

Obligate microbial symbionts are a common feature among arthropods that have nutritionally poor or imbalanced diets (Buchner 1965; Douglas

1989; Moran et al. 2008). The microbial partners are highly diverse, representing a wide array of bacterial and fungal lineages acquired independently by a variety of ancestral arthropods (Moran et al. 2008; Gibson and Hunter 2010). The vast majority of research on these obligate associations has focused on bacterial partners; hence, our discussion here will also centre upon bacteria.

Like most animals, arthropods including insects are incapable of synthesizing essential amino acids and are generally dependent on gaining these protein building blocks through consumption (Chapman 1998). In contrast, many microbes are competent to synthesize all amino acids. Likewise, many vitamins and cofactors can be synthesized by microbes, but not by arthropods (Chapman 1998). For arthropods that feed upon diets that are deficient in amino acids (plant sap) or vitamins (animal blood), the inadequately available components must be provided by other means. Through light microscopy, early researchers found that arthropods feeding on such poor dietary sources often housed microbes in specialized cells (interchangeably referred to as mycetocytes or bacteriocytes) sometimes grouped together in organ-like structures (mycetomes or bacteriomes) and that these microbes were transmitted vertically from mother to offspring (reviewed in Buchner 1965). Early hypotheses that these mycetocyte-associated microbes play vital nutritional roles have now been validated by empirical, molecular, and genomic analyses (Buchner 1965; Moran et al. 2008; Shigenobu and Wilson 2011).

The pea aphid, *Acyrthosiphon pisum*, and its obligate bacterial symbiont, *Buchnera aphidicola*, present a case study. Aphids feed exclusively on plant phloem, which is carbohydrate rich but very low in essential amino acids (Gündüz and Douglas 2009 and references therein). Analyses of the *Buchnera* genome have found it to be highly reduced compared with free-living bacterial relatives, having lost many critical functions including synthetic pathways for many non-essential amino acids (e.g. Shigenobu et al. 2000). However, despite the overall genome erosion exhibited by the symbiont, the essential amino acid synthetic pathways remain largely intact, suggesting that the essential amino acids are provided to the host by the bacterial symbiont. Analyses of the recently published pea aphid genome, along with expression studies, have confirmed the perfect complementarity of aphid and bacterial metabolisms; the aphid generally provides the non-essential amino acids, and the bacterium synthesizes the essential amino acids (Hansen and Moran 2011; Shigenobu and Wilson 2011). Furthermore, synthesis of some amino acids (e.g. valine, leucine) requires metabolites contributed by both partners: neither host nor bacterium would be capable of synthesizing these amino acids on their own (Hansen and Moran 2011).

These intricate metabolic interdependencies reflect millions of years of coevolution between aphids and bacteria. Almost all aphids contain *Buchnera* symbionts, and phylogenetic analyses have indicated parallel evolution between bacteria and host, with divergences among the bacterial lineages corresponding to divergences among the aphids (Baumann 2005). This pattern of cocladogenesis is consistent with an initial infection of the ancestor of all aphids, estimated to have occurred more than 180 million years ago (mya) (Moran et al. 2008).

Similar stories can be told with other arthropod hosts and their obligate symbiotic lineages, as summarized in Table 17.1. Many of these symbioses are ancient in origin, although some more recent associations have been identified (e.g. Lamelas et al. 2008). Despite the diverse origins of the microbes, some common themes are evident in their evolutionary histories. First, it is very common for the symbiont genome to be extremely reduced, sometimes approaching an order of magnitude smaller than free-living bacterial relatives (Table 17.1; Moran et al. 2008; Toft and Anderson 2010). Factors contributing to this process of genome shrinkage include vertical transmission and insufficient purifying selection of small populations leading to fixation of deleterious alleles, high mutational rates due to loss of DNA repair machinery, and/or mutational bias towards adenine and thymine leading to transcription slippage (Moran et al. 2008; Tamas et al. 2008; Allen et al. 2009;

Table 17.1 Some obligate bacterial nutritional endosymbionts of arthropods

Host taxa[a]	Bacterial symbiont	Bacterial phylum	Est. age of association	Location of bacteriocytes	Bacterial		References
					Genome size	GC content (%)	
Blattodea							
Blattidae + Mastotermes darwiniensis	*Blattabacterium* spp.	Bacteroidetes	>150 my	Fat body	637 kB	27	Moran et al. (2008); Lopez-Sanchez et al. (2009)
Phthiraptera							
Pediculus spp. (human lice)	*Riesia pediculicola*	γ-Proteobacteria	13–25 my	Stomach disc bacteriome	575 kB		Allen et al. (2009); Kirkness et al. (2010)
Pedicinus obtusus (old world monkey louse)	*Puchtella pedicinophila*	γ-Proteobacteria		Midgut epithelium + ovaries			Fukatsu et al. (2009)
Hemiptera							
"Auchenorrhyncha"	*Sulcia* spp.	Bacteroidetes	260–280 my	2 Bacteriomes in haemocoel	246–277 kB	21–22.6	Moran et al. (2005a); McCutcheon and Moran (2010)
Cicadellidae (sharpshooters)	+ *Baumannia cicadellinicola*[b]	γ-Proteobacteria	>100 my	Same bacteriomes as *Sulcia*	686 kB	33	Moran et al. (2008)
Pentastirini (Cixiidae planthoppers)	+ *Purcelliella pentastirinorum*	γ-Proteobacteria	25–120 my	Separate bacteriome from *Sulcia*			Bressan et al. (2009)
Clastoptera arizonana (spittlebug)	+ *Zinderia insecticola*	β-Proteobacteria		Same bacteriomes as *Sulcia*	208 kB	13.5	McCutcheon and Moran (2010)
Cicadidae (cicadas)	+ *Hodgkinia cicadicola*	α-Proteobacteria		Same bacteriocytes as *Sulcia*	144 kB	58.4	McCutcheon et al. (2009)
Sternorrhyncha							
Pseudococcidae (scales)	*Tremblaya* spp.	β-Proteobacteria	100–200 my	1 Bacteriome in haemocoel	139 kB	58.8	Baumann (2005); McCutcheon and von Dohlen (2011)
	+ *Moranella endobia*[c]	γ-Proteobacteria		Within *Tremblaya* cells	538 kB	43.5	McCutcheon and von Dohlen (2011)
Rhizoecini	*Brownia rhizoecola*	Bacteroidetes		1 Bacteriome in haemocoel			Gruwell et al. (2010)
Diaspididae (armoured scales)	*Uzinura diaspidicola*	Bacteroidetes	>100 my	Dispersed throughout haemocoel			Moran et al. (2008)
Psylloidea (psyllids)	*Carsonella ruddii*	γ-Proteobacteria	100–250 my	1 Bacteriome in haemocoel	160 kB	17	Baumann (2005); Nakabachi et al. (2006)

(continued)

Table 17.1 (continued)

Host taxa[a]	Bacterial symbiont	Bacterial phylum	Est. age of association	Location of bacteriocytes	Bacterial		References
					Genome size	GC content (%)	
Aleyrodoidea (whiteflies)	*Portiera aleyrodidarum*	γ-Proteobacteria	100–200 my	2 Bacteriomes in haemocoel			Baumann (2005)
Aphidoidea (aphids)	*Buchnera aphidicola*	γ-Proteobacteria	>180 my	1 Bacteriome in haemocoel	416–641 kB	20–26	Moran et al. (2008)
Cinara cedri	+ *Serratia symbiotica*	γ-Proteobacteria		Same bacteriome as Buchnera	1.76 Mb	29	Lamelas et al. (2011)
Heteroptera							
Cimicidae (bed bugs)	*Wolbachia* sp.	α-Proteobacteria		2 gonad-associated bacteriomes	~1.3 Mb		Hosokawa (2010)
Chilacis typhae (a lygaeid)	*Rohrkolberia cinguli*	γ-Proteobacteria		Midgut epithelium			Kuechler et al. (2011)
Kleidocerys resedae (a lygaeid)	*Kleidoceria schneideri*	γ-Proteobacteria		1 Bacteriome near midgut			Kuechler et al. (2010)
Coleoptera							
Dryophthoridae + Molytinae weevils	*Nardonella* spp.	γ-Proteobacteria	>125 my	1 Larval bacteriome surrounding fore/midgut			Conord et al. (2008)
Sitophilus spp. (grain weevils)	SPE	γ-Proteobacteria	~20 my	Ditto	~3 Mb		(Charles et al. 1997); Conord et al. (2008)
Curculio spp. (seed weevils)	*Curculioniphilus buchneri*	γ-Proteobacteria		1 Bacteriome at larval midgut			Toju et al. (2010)
Hymenoptera							
Camponotini (carpenter ants)	*Blochmannia* spp.	γ-Proteobacteria	>50 my	Midgut epithelium	706–792 kB	27–29	Moran et al. (2008)
Diptera							
Glossina spp. (tsetse flies)	*Wigglesworthia glossinidia*	γ-Proteobacteria	>40 my	1 Bacteriome at gut	698 kB	22	Moran et al. (2008)

[a] Inferred taxonomic distribution. In many cases, some lineages within the taxon have subsequently lost or replaced the symbiont. The absence of a taxonomic group from this list does not necessarily indicate a lack of primary symbionts. Additional taxa (e.g. Membracidae) have primary symbionts (Buchner 1965; Douglas 1989) but have not received modern molecular attention

[b] Symbionts with a plus sign are cosymbionts with the preceding symbiont that lacks a plus sign

[c] This symbiont is contained within the *Tremblaya* cosymbiont

Moran et al. 2009). Genome shrinkage is an ongoing process (Moran et al. 2009), but evidence suggests that the rate of erosion decreases with the age of the symbiotic association (Allen et al. 2009). Second, the genomes of obligate microbial symbionts are very stable, showing no rearrangement over millions of years (Tamas et al. 2002). This stability is partially explained by a lack of mobile genetic elements (Shigenobu et al. 2000; Moran et al. 2008) which, in combination with the isolation of these symbionts, also means there is little opportunity for gene acquisition through horizontal transfer. Finally, there is a correlation between the estimated age of the host/microbe association and symbiont genome size, with microbes in the oldest associations (e.g. *Sulcia*) having the smallest and least functional genomes. In many of these ancient symbioses, additional symbionts (cosymbionts) occur within the same host; however, in some cases (e.g. *Carsonella*), essential symbiont metabolic functions have been lost without compensation by other symbionts (Baumann 2005; Moran et al. 2005b; Nakabachi et al. 2006). Understanding the continued viability of these puzzling minimalistic symbionts will likely await detailed genomic analysis of their hosts.

Concurrent with all these evolutionary changes in symbiotic microbes, host arthropods have also evolved in many ways to accommodate their inhabitants. As previously indicated, many lineages house their symbionts in host-derived membranes, within specialized cells (bacteriocytes), which in turn may be clustered into epithelial-bound bacteriomes (Table 17.1; Buchner 1965; Douglas 1989). Multiple symbionts within the same host may occupy different bacteriomes, the same bacteriome or even the same bacteriocytes (Buchner 1965). Ensuring transmission of these obligate symbionts to subsequent generations is key to the continued existence of their hosts. Because many obligate nutritional symbionts are housed in bacteriomes that are physically separated from the germline, quite complicated and variable pathways of symbiont transmission have evolved among host taxa (Buchner 1965; Douglas 1989). To date, little is known regarding the mechanistic bases for transmission.

Progress is being made in developing an understanding of the general regulation of obligate symbionts by the host, and how an immune response by the host to the symbiont is avoided. Among aphids, *Buchnera* has lost many regulatory genes and has static transcriptional dynamics (Shigenobu and Wilson 2011 and references therein). The pea aphid host, in contrast, has a greater diversity of regulatory genes than any arthropod sequenced thus far and is thought to be largely responsible for regulation of its domesticated microbe (Shigenobu and Wilson 2011). With respect to immunity, aphids have lost many immune genes and pathways that are highly conserved among animals, including other insects (Shigenobu et al. 2000). Whether such loss occurred prior to initial colonization by *Buchnera* (thus facilitating development of a symbiotic interaction) or as an evolutionary consequence of the obligate bacterial association is unclear. Regardless, the absence of a strong immune response by the aphid likely facilitates further symbiotic interactions and probably contributes to the plethora of facultative bacterial symbioses that are also present in these hosts.

In *Sitobion* weevils, a different immune dynamic is evident between host and symbiont. The antibacterial host protein coleoptericin A (ColA) is strongly expressed in symbiont-bearing tissues and apparently contributes to the characteristic bacterial gigantism of the symbionts through inhibition of cytokinesis (Login et al. 2011). Moreover, when transcription of ColA was reduced using RNAi, the bacterial symbiont was subsequently found to have escaped from the bacteriome into other larval tissues, indicating that ColA plays an important role in controlling both symbiont location and number (Login et al. 2011). Interestingly, the genus *Sitobion* has undergone a relatively recent symbiont replacement (Conord et al. 2008), but ColA may have similar effects on the ancestral weevil symbiont *Nardonella* (Login et al. 2011), suggesting that regulatory mechanisms for one symbiosis may indeed facilitate subsequent symbiotic interactions.

These examples make it clear that arthropods and their symbionts have had profound

evolutionary effects upon one another. The critical role these nutritional endosymbionts play in allowing their hosts to use otherwise inadequate diets suggests that initial symbiont acquisition was an evolutionary novelty that allowed expansion into un- or under-occupied ecological niches. Thereafter, however, genetic variation between obligate symbionts likely has not been directly responsible for the radiation and diversification of their host taxa (Clark et al. 2010). The evolutionary processes experienced by these symbionts have usually consisted of stable maintenance of genomic content or genome shrinkage, rather than recombination and innovation. In fact, loss of function in endosymbionts may act as a constraint upon the host. For example, at least some of the variation between pea aphid clones in amino acid requirements can be traced to deleterious mutations in the *Buchnera* genome (Vogel and Moran 2011). Such limitations on the part of nutritional symbionts would act as one (of presumably numerous) constraints on dietary breadth of their hosts.

In general, it is probably fair to conclude that obligate nutritional endosymbionts are not an ongoing source of evolutionary innovation for their hosts. The evolutionary forces that result in genome reduction, combined with a lack of recombination, winnow the genetic complement of obligate endosymbionts down to the bare minimum, or even below (e.g. *Carsonella*; Nakabachi et al. 2006). In such instances, compensation is often provided by other endosymbionts (Moran et al. 2008) that likely originated as facultative "guests" (Buchner 1965) within the host. Facultatively mutualistic endosymbionts have different genomic properties from their obligate counterparts (see below), and it seems most plausible that symbiont-derived evolutionary innovation among hosts is associated with facultative, rather than obligate, endosymbionts.

17.2.2 Facultative Endosymbionts

In contrast to obligate symbionts, "facultative" symbionts are not a requisite from the host's perspective: these bacteria often do not infect every member of a host species and can be experimentally removed (through heat or antibiotic curing) without ill effects on the host. From the perspective of the bacteria, association with an arthropod host is usually obligate. Without considering pathogenic bacteria, we focus on facultative endosymbionts that maintain themselves in host populations through one of two routes: reproductive manipulation or mutualism. Bacteria in the former category are parasites that manipulate host reproduction to promote their own spread and maintenance in the host population, whereas bacteria in the latter category provide their host with fitness benefits, resulting in a selective advantage for infected hosts (Moran et al. 2008). Historically, endosymbiont taxa were considered to fall exclusively into one category or the other, but this distinction has become blurred. A number of examples now have been described, wherein "reproductive manipulators" have been found to provide fitness benefits to their hosts under some circumstances (summarized in White 2011). Nevertheless, many of the evolutionary consequences associated with facultative symbiont infection are tied to the phenotypes elicited by these symbionts, so it is appropriate to consider the broad categories of reproductive manipulators and facultative mutualists separately; it should be recognized, however, that the two categories are not mutually exclusive (Himler et al. 2011) and that any particular bacterial taxon (e.g. *Wolbachia*) might be acting as a reproductive manipulator in some hosts, but as a facultative (Weeks et al. 2007) or obligate mutualist (Hosokawa et al. 2010) in others.

Facultative endosymbionts typically have reduced genomes relative to free-living bacteria, if not as massively reduced as obligate nutritional symbionts (Toft and Anderson 2010). They have usually lost some critical functions and are incapable of resuming a host-independent lifestyle (Degnan et al. 2009; Darby et al. 2010). Additionally, while transmission of facultative endosymbionts is primarily vertical, they lack the pattern of cocladogenesis that characterizes obligate symbionts and their hosts, indicating that horizontal transfer among host taxa has occurred

over evolutionary time (Russell et al. 2003; Werren et al. 2008). Indeed, the genomes of facultative symbionts are typically characterized by evidence of genetic flexibility (e.g. mobile DNA; Newton and Bordenstein 2011), suggesting that these symbionts retain much greater capacity to adapt to new host environments than obligate nutritional symbionts that are irrevocably committed to a particular host lineage.

17.2.2.1 Facultative Symbionts as Mutualists

Facultative mutualists tend to affect their hosts in a manner that is conditionally, rather than universally, beneficial (White 2011). This distinction is partially tautological: symbionts that confer traits that are beneficial under all environmental conditions would likely be categorized as obligate rather than as facultative, because they are probably fixed at 100 % prevalence in host populations and cause a decrease in host fitness if removed. However, evolutionary pressures to ensure vertical transmission of such beneficial associates would presumably render these associations obligate in relatively short order. In contrast, facultative mutualists that provide conditional benefits will be selected for in some environments and selected against in others. These symbionts may, therefore, persist at less than fixed levels due to balancing selection and can be large contributors to the phenotypic variability expressed by the host (White 2011). To date, the host phenotypes that have been shown to be affected by facultative symbionts include (1) defence against natural enemies, (2) interaction with host plants, and (3) environmental tolerances.

Facultative Symbionts and Defence

In aphids, the three most common facultative symbionts are *Hamiltonella defensa*, *Regiella insecticola*, and *Serratia symbiotica*. Each is present in ~15 % of aphid species (Russell et al. 2003; Oliver et al. 2010), and all have been implicated in defence of at least one host aphid species. *Hamiltonella* and *Serratia* have both been shown to protect the pea aphid from parasitism by braconid parasitoids in the genus *Aphidius* (Oliver et al. 2003), and *Regiella* has been shown to have the same effect in the peach-potato aphid, *Myzus persicae* (Vorburger et al. 2010). *Regiella* also defends the pea aphid against the fungus *Pandora* (Scarborough et al. 2005). Outside of aphids, some symbionts previously considered to be solely reproductive manipulators have also been shown to have defensive properties. For example, *Spiroplasma*, which is a male-killer in multiple host taxa (Anbutsu and Fukatsu 2011), protects *Drosophila neotestacea* from *Howardula* nematodes (Jaenike et al. 2010). Similarly, *Wolbachia* in some populations of *D. melanogaster* protects the host against viruses (e.g. Hedges et al. 2008). The relative prevalence of mutualistic versus manipulative strains of these symbionts remains unclear, but given the widespread occurrence of *Wolbachia*, it is certainly possible that many more arthropods benefit from defensive bacterial symbionts than previously realized.

Clearly, defensive symbionts are only beneficial in environments in which the targeted natural enemies are present. For example, in laboratory population studies of the pea aphid, *Hamiltonella*-infected aphids were selectively favoured over uninfected aphids in the presence of the parasitoid *Aphidius ervi*, but were at a disadvantage and decreased in frequency when the parasitoids were absent (Oliver et al. 2008). This suggests that there is a fitness cost associated with maintaining *Hamiltonella* infection. Furthermore, natural enemies are not necessarily just passive victims of symbiotic defensive measures, but can themselves evolve resistance to host defence (Dion et al. 2011). The selective pressures exerted on defensive symbionts in natural populations are likely to depend on the prevalence, identity, and coevolutionary history of natural enemies that are present in a particular locale. It is, therefore, little surprise that the observed frequency of symbiont infection can be highly variable among host populations (e.g. Ferrari et al. 2012).

This dynamic selective environment is matched by symbionts that apparently have a much more dynamic and versatile genetic make-

up than observed in obligate nutritional symbionts. Of the facultative mutualistic symbionts whose genome has received attention thus far, most have ample mobile DNA, often exceeding the amount found in free-living bacteria (Newton and Bordenstein 2011). For *Hamiltonella* in the pea aphid, the mechanism for defence appears to be directly mediated through a bacteriophage, known as APSE phage (Moran et al. 2005a; Oliver et al. 2009). Different variants of this phage are present in different host populations (Degnan and Moran 2008) and encode different toxins (Oliver et al. 2010), which presumably have a range of effectiveness against different natural enemies. Other facultative symbionts also have phages (e.g. Darby et al. 2010), suggesting the possibility of interspecific exchange of genetic material among co-occurring facultative mutualists (Degnan and Moran 2008), which can in turn be horizontally transmitted within and among host taxa (Russell et al. 2003; Moran and Dunbar 2006). While interspecific horizontal gene transfer is rampant in free-living bacteria (Ochman et al. 2000) and may also be common among *Wolbachia* strains (Klasson et al. 2009), evidence for phage exchange among different lineages of facultative mutualists remains elusive (Degnan et al. 2010).

Facultative Symbionts as Mediators of Host Plant Specialization

One of the earliest patterns that became evident with respect to facultative symbionts was that for symbionts of polyphagous herbivores, symbiont prevalence could vary depending upon host plant (Leonardo and Muiru 2003). In the pea aphid, *Regiella* is much more common in aphid clones that are specialized on clover, whereas other symbionts (e.g. *Hamiltonella*, *Serratia*) are less common in aphids on clover (Ferrari et al. 2012). Likewise, a recent study of facultative symbionts of the weevil *Curculio sikkimensis* has also found host-associated differences in symbiont community composition (Toju and Fukatsu 2011).

Such correlative patterns are suggestive that facultative symbionts may play a role in host plant specialization or even the generation of host races or subspecies (Tsuchida et al. 2004). However, subsequent studies that have experimentally manipulated symbiont composition indicate that the relationship between symbiont and host plant utilization may be complex. Tsuchida et al. (2004) found that curing a pea aphid clone of *Regiella* substantially decreased the aphid's performance on clover, whereas Leonardo (2004) found no effect of *Regiella* removal on the performance of multiple aphid clones. Ferrari et al. (2007) found host genotype by symbiont interactions in aphid performance on clover, whereas McLean et al. (2011) found that *Regiella* removal generally decreased aphid fitness, regardless of host plant. The balance of evidence therefore does not support a direct role for *Regiella* in host plant specialization in pea aphid (McLean et al. 2011). It remains to be seen whether facultative symbionts of other polyphagous herbivores are more directly involved in host plant utilization.

Phytophagous arthropods may also benefit from facultative symbionts that influence plant physiology. Larvae of the leafminer *Phyllonorycter blancardella* that develop in senescent apple leaves have a distinctive "green island phenotype" in which the surrounding leaf material remains photosynthetically active long after the rest of the leaf, due to a high concentration of cytokinins within the mine (Giron et al. 2007). It was recently demonstrated that this physiological effect is bacterially mediated, presumably by the endosymbiont *Wolbachia* (Kaiser et al. 2010). Endophagy is a widespread feeding habit among phytophagous insects that encourages intimate and specialized interactions between the insect and the plant. While the *Wolbachia*/*Phyllonorycter*/*Malus* interaction is currently an isolated example, it seems likely that bacterial endosymbionts might play a role in other endophagous insect/plant interactions.

Facultative Symbionts and Environmental Tolerance

Facultative symbionts can also modify the environmental tolerances of their hosts. Once again returning to the well-documented pea aphid system, the facultative symbiont *S. symbiotica*

protects infected hosts from heat shock (Russell and Moran 2006). Survival of *Serratia*-infected pea aphids was greater, following brief exposure to high temperatures than aphids uninfected by *Serratia* (Russell and Moran 2006). Correlative evidence suggests that *Rickettsia* endosymbionts in the whitefly *Bemisia tabaci* may play a similar role (Brumin et al. 2011). Facultative symbionts have also been tested for influence on frost resistance in the aphid *Sitobion avenae*, but not found to have an effect (Lukasik et al. 2011). It is conceivable that facultative symbionts may mediate many other stress responses for their hosts (e.g. toxins such as insecticides, UV, salinity), but to date, no evidence has been presented to support these possibilities.

The Role of Facultative Endosymbionts in Host Evolution

Given the major phenotypes generated by facultative endosymbionts, and the variability in symbiont prevalence among host populations, it is perhaps unsurprising that these bacteria can drive rapid evolutionary shifts in their hosts. Himler et al. (2011) recently demonstrated that the symbiont *Rickettsia* provides major fitness benefits to the whitefly *B. tabaci*. This selective advantage provided to symbiont-bearing whiteflies drove a "symbiont sweep" through whitefly populations in the south-western US: the symbiont was virtually absent from host populations prior to the year 2000, but was near fixation in multiple populations over hundreds of miles by 2006. Similarly, Jaenike et al. (2010) have shown a geographical gradient in *Spiroplasma* infection of *D. neotestacea* across North America, suggesting that the symbiont is spreading because symbiont-bearing flies enjoy protection against invasive nematodes. In aphids, field-cage studies have demonstrated that aphid populations can evolve quickly in response to altered climate conditions, with increased frequency of symbionts that protect against heat shock (Harmon et al. 2009). Latitudinal clines in facultative symbiont prevalence (e.g. Tsuchida et al. 2002) also suggest that symbionts are involved in the climatic adaptation of their hosts. As climate change and invasive species continue to modify prevailing environmental conditions, facultative symbionts may play an important role in the resilience of their hosts.

Facultative symbionts may, on occasion, also provide traits that lead their hosts to a new evolutionary trajectory. For example, it is suggestive that sharpshooters (leafhoppers in the tribes Proconiini and Cicadellini within the family Cicadellidae), one of the very few kinds of insects to feed on xylem, have the necessary vitamins provided to them by the cosymbiont *Baumannia* (McCutcheon and Moran 2007). *Baumannia* likely began its association with an ancestral sharpshooter as a facultative symbiont in conjunction with the more ancient obligate symbiont *Sulcia*. *Sulcia*, which infects the larger Auchenorryncha clade of hemipterans, does not provide vitamins to the hosts in any lineages examined thus far (McCutcheon and Moran 2007, 2010; McCutcheon et al. 2009). It is therefore tempting to conclude that acquisition of *Baumannia* by sharpshooters facilitated a shift in host ecology and evolution. More recent transitions from facultative to obligate symbiosis have been documented in some systems (e.g. *Serratia* in *Cinara cedri*; Lamelas et al. 2011) and are suspected in others (e.g. *Hamiltonella* in a group of *Uroleucon* aphids; Degnan and Moran 2008). In the latter case, the functional basis for the obligate nature of the symbiosis remains unclear. Given that *Hamiltonella* in pea aphid has lost much of its biosynthetic machinery (Degnan et al. 2009), it will be interesting to learn what role *Hamiltonella* might be playing in this clade of aphids. The presence of phage and other mobile DNA in facultative symbionts suggests that acquisition of new traits through horizontal gene transfer remains a possibility for this versatile group of symbionts.

17.2.2.2 Bacteria as Reproductive Parasites of Insects and Other Arthropods

Most known reproductive parasites of arthropods are heritable, maternally transmitted intracellular bacteria that alter the reproduction of their hosts in ways that promote their own

fitness. To ensure their own vertical transmission to the host progeny, reproductive manipulators have evolved mechanisms that favour a female-biased host sex ratio and are detrimental to the non-transmitting sex (the male), including thelytokous parthenogenesis, feminization, and male-killing (MK) (Table 17.2). Alternatively, by inducing cytoplasmic incompatibility, they inhibit the reproduction of uninfected or differently infected individuals and can spread without skewing the sex ratio of the host population. Such manipulations can increase the number of infected hosts within a population even where they reduce the fitness of the host (Werren and O'Neill 1997; Engelstädter and Hurst 2009).

Diversity and Transmission of Reproductive Parasites

The ability to manipulate arthropod reproduction has evolved frequently in phylogenetically diverse bacterial taxa including *Wolbachia* and *Rickettsia* (α-Proteobacteria), *Arsenophonus* (γ-Proteobacteria), *Cardinium*, and *Flavobacterium* (Bacteroidetes), and *Spiroplasma* (Mollicutes) (Duron et al. 2008). *Wolbachia* is the most abundant endosymbiont of insects, with 66 % of species estimated to be infected (Hilgenboeker et al. 2008). Similarly, its prevalence in isopods was estimated at 47 % (Bouchon et al. 2009). In contrast, other bacteria are less pervasive (Duron et al. 2008), with *Cardinium* species, for example, being found in 6–7 % of the arthropod species screened to date (Zchori-Fein and Perlman 2004), but reaching higher prevalence in arachnids (Perlman et al. 2010). In addition, sex ratio distortion phenotypes have also been found in crustacean amphipods infected by representatives of the eukaryotic lineage of the Microsporidia (Terry et al. 2004).

Bacterial reproductive parasites have evolved sophisticated adaptations to move in the cellular environment and infect host reproductive tissues. *Wolbachia*, for example, relies on the host cell cytoskeleton and molecular motors, like dynein and kinesin-1, to move inside and between host cells (Serbus et al. 2008). Reproductive parasites in general are distributed in the host's ovary and infect the developing oocytes. Within the egg, most symbionts localize to the germ pole, a mechanism for increasing the probability that bacteria persist in germ cells and are transmitted to host progeny (Veneti et al. 2004; Giorgini et al. 2010). However, although reproductive tissues of germline origin are the main target tissue, reproductive parasites have also been detected in different somatic tissues in many hosts (Dobson et al. 1999; Ijichi et al. 2002) where they can diversely affect the host biology. For example, a *Wolbachia* strain, *w*MelPop, proliferates massively in adult *Drosophila*'s brain, retina, and muscles, causing tissue degeneration and early death of hosts (Min and Benzer 1997). Further, *Wolbachia* infect haemocytes in isopods and have been implicated in reducing the host immunocompetence and longevity of infected individuals (Chevalier et al. 2012). From an evolutionary perspective, *Wolbachia*'s ability to infect cells of the immune system is very intriguing as regulation of the host immune system can be regarded as a strategy that reproductive parasites use to form long-term symbiotic relationships with their hosts (Siozios et al. 2008). Finally, in some *Drosophila* species, *Wolbachia* are highly abundant in the somatic stem cell niche in the germarium and from there are able to reach the germline, implying that infection of somatic stem cell niche may contribute to efficient vertical transmission (Frydman et al. 2006).

While most bacterial reproductive parasites persist intracellularly, a few exceptions are known. For example, *Arsenophonus nasoniae*, the MK agent of the parasitoid wasp *Nasonia vitripennis*, establishes a persistent intercellular infection that is maternally inherited without infecting the egg cytoplasm. Larval progeny instead acquires *A. nasoniae* by feeding. Because many wasp larvae develop in a single fly pupa, horizontal transmission can also occur between matrilines and different species of *Nasonia* (Duron et al. 2010).

Studies indicate that strictly transovarially transmitted reproductive manipulators must also occasionally be transmitted horizontally as evidenced by the incongruence between reproductive symbionts and host phylogenies. That is,

closely related bacterial strains infect evolutionary distant host species, indicating that horizontal transfer between host species has occurred multiple times (Werren et al. 1995, 2009). Reproductive parasites with strong ability to infect somatic tissues and circulate in the haemolymph have been thought to be more prone to horizontal transfer (Dobson et al. 1999; Caspi-Fluger et al. 2012). At an ecological timescale, possible mechanisms of horizontal transmission in a given ecological community include predation and parasitism (Huigens et al. 2004a; Dedeine et al. 2005; Jaenike et al. 2007), plant-mediated transmission (Caspi-Fluger et al. 2012), and passage of haemolymph between infected and uninfected individuals (Rigaud and Juchault 1995). However, incongruence between symbiont and host phylogenies suggests that the interaction between symbiont and host is rarely permanent and that arthropods often lose an infection over time.

Cytoplasmic Incompatibility

The most common reproductive manipulation is cytoplasmic incompatibility (CI). CI is induced by both *Wolbachia* and *Cardinium* and has been reported to occur in many taxa of insects, mites, and isopods (Bourtzis et al. 2003; Ros and Breeuwer 2009). CI occurs when uninfected female hosts are reproductively incompatible with infected males (unidirectional CI), while all other crosses are compatible (Table 17.2). Because uninfected females do not produce offspring in incompatible crosses, they suffer a fitness cost compared with infected females that produce viable offspring. As a result, the infection will spread in the host population. In addition, for *Wolbachia*, bidirectional CI can occur when males and females are infected by different symbiont strains (O'Neill and Karr 1990). In general, the expression of CI is the mortality of the developing embryo due to the loss of the paternal set of chromosomes, but in haplodiploid Hymenoptera incompatible eggs may also develop as normal males (Breeuwer and Werren 1990; Perrot-Minnot et al. 2002). Sometimes *Wolbachia* and *Cardinium* stably infect a common host that expresses CI, but only one of the two is the reproductive manipulator (Ros and Breeuwer 2009; White et al. 2009).

Although not transmitted through the male germline, *Wolbachia* are present in developing sperm and are eliminated only during the final stages of sperm maturation. CI is due to sperm modification occurring during spermatogenesis (Clark et al. 2008; Serbus et al. 2008), possibly through changes in the expression of genes associated with spermatogenesis (Zheng et al. 2011a, b). However, little is known about the molecular mechanism of symbiont-induced CI. A most striking hypothesis has been postulated for *Wolbachia* and is based on a two-component "modification–rescue" model according to which symbionts induce modifications of sperm during spermatogenesis in infected males and rescue of this modification happens if the egg is infected with the same strain. If the modified sperm do not meet the appropriate symbiont in the egg, embryonic development will be arrested (Werren 1997; Poinsot et al. 2003; Bossan et al. 2011).

Evidence on the cytological mechanism of CI for *Wolbachia* (nothing is yet known about *Cardinium*) suggests that asynchronous development of male and female pronuclei caused by disruption of the cell cycle in early embryonic mitosis prevents karyogamy in incompatible crosses. While the female chromosomes separate normally during anaphase, the paternal chromosomes either fail to segregate or exhibit extensive bridging and fragmentation during segregation. This results in an embryo with a complete maternal chromosome complement but with a reduced or absent paternal chromosome complement. In compatible crosses, *Wolbachia* present in the female reproductive tissues restore coordination between the male and female pronuclei. For extensive description of cytological mechanisms of *Wolbachia*-induced CI, see Tram and Sullivan (2002), Serbus et al. (2008), and Landmann et al. (2009).

Thelytokous Parthenogenesis

Thelytokous parthenogenesis is a form of reproduction where unmated females produce only female offspring through restoration of diploidy in unfertilized eggs (Table 19.2).

Table 17.2 Reproductive manipulations induced by microbial symbionts

Reproductive manipulations	Micro-organisms involved	Type of reproduction	Offspring produced
Cytoplasmic incompatibility	*Wolbachia* *Cardinium*	Compatible crosses:	
		i♀ × u♂[a]	i♀ + i♂
		u♀ × u♂	u♀ + u♂
		i♀ × i♂	i♀ + i♂
		Incompatible crosses:	No offspring or i♂ in haplodiploids
		u♀ × i♂ (unidirectional)	
		i_a♀ × i_b♂ (bidirectional)[b] i_b♀ × i_a♂	
Male-killing	Many bacteria	Biparental reproduction:	
	Microsporidia an RNA virus	i♀ × u♂	i♀
Parthenogenesis in haplodiploid arthropods	*Wolbachia*	Thelytokous reproduction:	
	Rickettsia	i♀ (2n) → infected eggs (n) → i♀ embryos (2n)[c]	i♀ (2n)
	Cardinium		
Feminization in diploid arthropods	*Wolbachia*	Biparental reproduction:	
	Microsporidia	i♀ ZW × u♂ ZZ	i♀ ZW + i♀ ZZ
		i♀ ZZ × u♂ ZZ	i♀ ZZ
Feminization in haplodiploid arthropods	*Cardinium*	Thelytokous reproduction:	
		Brevipalpus phoenicis mites	
		i♀(n) → i♂ eggs (n) → i♀ embryo (n)	i♀ (n)
		Encarsia hispida wasps	
		i♀ (2n) → i♂ eggs (2n) → i♀ embryos (2n)	i♀ (2n)

[a] i♀ and u♀, and i♂ and u♂ are infected and uninfected female, and infected and uninfected male, respectively
[b] i_a and i_b means infected by two different bacterial strains
[c] (n) and (2n) mean haploid and diploid, respectively

In haplodiploid arthropods, thelytokous reproduction is common and has evolved independently in many different lineages. However, in most cases, thelytoky is associated with the occurrence of an endosymbiotic micro-organism (Stouthamer 1997). Feeding females antibiotics restores the production of males in a number of thelytokous species, showing that micro-organisms are the causal agents of the reproductive phenotype. At least three intracellular bacteria, including *Cardinium*, *Rickettsia* and *Wolbachia*, induce thelytoky, especially in the Hymenoptera (Huigens and Stouthamer 2003; Hunter and Zchori-Fein 2006; Giorgini et al. 2010). Parthenogenesis-inducing (PI) *Wolbachia* and *Cardinium* also occur in other haplodiploid arthropods, such as mites, scale insects, and thrips.

In hymenopterans infected by PI-*Wolbachia,* thelytokous parthenogenesis is automictic and occurs by disruption of the cell cycle during early embryogenesis, followed by gamete duplication (Gottlieb et al. 2002; Pannebakker et al. 2004). The haploid nuclei fail to separate and result in a single diploid nucleus containing two identical sets of chromosomes. As this mechanism produces completely homozygous females, it should result in strong inbreeding depression and should not be expected in outbreeding species like most diploid organisms (Stouthamer 1997). Indeed, gamete duplication has only been found in hymenopteran species that tolerate high rates of inbreeding. However, in the parasitoid wasp *Neochrysocharis formosa*, *Rickettsia*-induced parthenogenesis occurs by an

apomictic cloning mechanism with the absence of meiotic recombination and reduction, and final development of heterozygous females (Adachi-Hagimori et al. 2008). A functionally apomictic parthenogenesis is also induced by *Wolbachia* in the mite *Bryobia pretiosa* (Weeks and Breeuwer 2001). Apomixis is the most common form of parthenogenesis within diplodiploid arthropods (Suomalainen et al. 1987) and occurs in some uninfected Hymenoptera (Vavre et al. 2004). Parthenogenesis mechanisms that maintain heterozygosity keep open the chance that endosymbiotic bacteria could be involved in the evolution of thelytokous reproduction in outbreeding species as well (Adachi-Hagimori et al. 2008; Rodriguero et al. 2010).

Feminization

Feminization is the development of genetic males into functional females. A well-known example occurs in isopods with female heterogametic sex determination, where *Wolbachia*-infected ZZ males are morphologically, anatomically, and functionally identical to ZW females (Table 17.2). In ZZ males, *Wolbachia* inhibits androgenic gland differentiation and the synthesis of the androgenic hormone, which promotes the differentiation of male gonads and secondary characters (Bouchon et al. 2009). Similarly, microsporidia induce feminization in amphipods. In insects, however, sexual differentiation is not under hormonal control; consequently, for the full expression of feminization, symbionts have to infect all somatic cells and interact with the genes involved in sex determination. In diploid insects, feminizing *Wolbachia* is known in a ZZ/ZW butterfly species (Narita et al. 2007) and in a leafhopper with XX/X0 sex determination system (Negri et al. 2006). Within haplodiploid arthropods, feminization has been reported only for *Cardinium*. It induces obligate thelytokous reproduction in two host species using different mechanisms (Table 17.2). In the mite *Brevipalpus*, *Cardinium* feminizes unfertilized haploid eggs that develop into functional haploid females (Weeks et al. 2001). In the parasitoid wasp *Encarsia hispida*, diploid males are the by-product of diploidy restoration in unfertilized eggs and *Cardinium* is required to feminize diploid male embryos and guarantee female offspring production (Giorgini et al. 2009).

Because thelytokous females can produce progeny without males, PI and feminizing endosymbionts can reach fixation in haplodiploid species without causing population extinction (Huigens and Stouthamer 2003; Giorgini et al. 2009). In some exceptional cases, infected thelytokous females retain the ability to mate and produce infected daughters from both fertilized and unfertilized eggs. Consequently, infected females can coexist in the field with individuals of uninfected bisexual populations, as in *Wolbachia*-infected populations of *Trichogramma* wasps (Stouthamer et al. 2001). However, most natural parthenogenetic populations have lost the ability to reproduce sexually, and reproduction relies on infection by endosymbiotic bacteria that have now become obligate symbionts (Huigens and Stouthamer 2003; Russell and Stouthamer 2011).

Male-Killing

MK endosymbionts selectively kill male offspring of their arthropod hosts (Table 17.2). A diversity of male-killers, from the bacterial genera *Wolbachia*, *Rickettsia*, *Spiroplasma*, and *Arsenophonus*, and undescribed Flavobacteria and γ-Proteobacteria has been reported in many insect orders and in pseudoscorpions (Hurst et al. 2003; Zeh and Zeh 2006; Majerus and Majerus 2010). Infectious male-killers in insects also include microsporidia and an RNA virus (Hurst et al. 2003; Nakanishi et al. 2008). The MK phenotype, because it favours the transmitting female sex, is not selected against in the bacteria and becomes an advantageous trait for the symbionts if female offspring benefit from the death of their brothers. Infected females gain an advantage over uninfected females through fitness compensation originating from reduced competition between siblings, resource reallocation obtained through the consumption of dead males, or reduced rates of inbreeding. In general, species that lay eggs in clutches, exhibit cannibalism behaviour or aggregated distributions in

breeding sites and use temporary resources may be particularly susceptible to invasion by male-killers (Jaenike et al. 2003; Majerus 2003). Ladybird beetles are a classical example. MK bacteria have to interact with components of the sex determination system of their hosts to express selectively their phenotype in the two sexes, but the mechanism is not fully understood (Bentley et al. 2007). As there is a diversity of MK agents, different mechanisms are expected (Veneti et al. 2005; Ferree et al. 2008; Riparbelli et al. 2012).

All of the aforementioned forms of reproductive manipulation depend on bacterial density within the reproductive tissues of the host. Efficiency of symbiont transmission through the host germline, penetrance of the reproductive phenotype, and infection prevalence in the host population are all strictly correlated with bacterial density (Jaenike 2009). Bacterial density is regulated by genetic factors of the host and the symbiont itself and is strongly influenced by environmental factors, like temperature, antibiotics, and host age (Jaenike 2009; Bordenstein and Bordenstein 2011). The general variation in bacterial density in response to temperatures indicates that there can be large spatial, temporal, and seasonal differences in endosymbiont densities and functions in natural populations.

Interactions Between Reproductive Manipulators and the Host Immune System

To establish successful symbiotic associations with diverse hosts and be able to infect both reproductive and somatic tissues, reproductive parasites must cope with the immune system of their hosts (Siozios et al. 2008), but the mechanisms that endosymbionts use to escape the cellular and humoral host defences are still unclear. Regulation of the host immune system can be regarded as a strategy that reproductive parasites use to form long-term symbiotic relationships with their hosts. *Wolbachia* up-regulation of the host immune genes leading to symbiont-mediated protection against pathogens or predators (Brennan et al. 2008; Moreira et al. 2009; Kambris et al. 2010) may be an effective way by which vertically transmitted symbionts can invade a host population, possibly explaining the high prevalence of weak reproductive parasites in field populations (Brownlie and Johnson 2009). To date, however, the hypothesis that *Wolbachia* interferes with pathogens by preactivating the immune response of its host is based only on studies of immune genes expression in transinfected hosts (naturally uninfected hosts infected by *Wolbachia* in the laboratory). In contrast, no differences in the up-regulation of immune genes have been found between hosts naturally infected by *Wolbachia* and uninfected insects with identical genetic background (Rancés et al. 2012; Wong et al. 2011). It has been found, however, that in the case of viral pathogens, *Wolbachia* reduces virus replication in both naturally infected and transinfected hosts, suggesting that immune priming by *Wolbachia* might not be the only mechanism responsible for viral interference (Rancés et al. 2012).

Wolbachia can also reduce the immunocompetence of hosts by reducing the efficiency of the cellular immune response (for example, preventing the encapsulation of parasitoid wasp eggs; Fytrou et al. 2006) and by down-regulating immune genes (Chevalier et al. 2012), leading to a reduced lifespan of infected individuals (Braquart-Varnier et al. 2008; Sicard et al. 2010). Stable infections of such costly symbionts, like feminizing *Wolbachia* in isopods, can be maintained in natural populations as a by-product of the genomic conflict between symbionts and their hosts. For example, in natural populations of *Armadillidium vulgare*, the frequencies of infected feminized individuals are generally lower than what would be predicted based on feminizing effects alone, possibly due to the lower fitness of immunodepressed feminized individuals (Braquart-Varnier et al. 2008).

Apoptosis of infected cells is an effective immune barrier that intracellular bacteria have to overcome in order to survive and to establish stable associations with host tissues (Batut et al. 2004). To this end, *Wolbachia* shows antiapoptotic pathways which, in the parasitoid wasp *Asobara tabida,* appear to have also a positive impact on host oogenesis by regulating the apoptosis of nurse cells (Dedeine et al. 2001;

Pannebakker et al. 2007). It is reasonable to assume that, at least in *Wolbachia*, the immunomodulating function of antiapoptotic factors may significantly contribute to the regulation of host reproduction, thus driving the evolutionary shift from facultative parasitism towards obligate mutualism (Miller et al. 2010).

Evolution of Host Resistance Genes, Sex Determination Mechanisms, and Genetic Systems

Reproductive parasites, and maternally inherited symbionts in general, have conflicting interests with their hosts. Within infected host populations, microbial genes are selected to favour a female-biased host sex ratio, which increases the prevalence of the symbionts, whereas host genes, generally biparentally inherited, are selected to prevent the action of the symbionts and restore an unbiased sex ratio (Werren and Beukeboom 1998; Caubet et al. 2000). As a consequence of the genetic conflict occurring between microbial genes and host nuclear genes, changes in the host sex determination system may evolve or resistance genes that prevent transmission of the symbiont to host germline or suppress the symbiont activity can be selected.

Occurrence of genetic conflict in response to feminizing *Wolbachia* has been observed in the isopod female heterogametic *A. vulgare* (reviewed by Bouchon et al. 2009). In this species, genetic ZZ males are converted to phenotypic functional females and the female-determining W chromosome is lost in the infected populations. However, a polygenic system of resistance genes involved in reducing the symbiont transmission rate compensates for the absence of males in infected populations. Furthermore, there are uninfected populations with ZZ individuals reversed to females by a feminizing (*f*) element thought to be a mobile genetic element acquired by the host nuclear genome via lateral transfer from *Wolbachia*. The *f* element can also be stabilized on a Z male chromosome, originating a new W-like chromosome. An autosomal dominant masculinizing (*M*) gene, which restores maleness in the presence of the *f* element but is ineffective for feminizing *Wolbachia*, has been found in some populations and interpreted as an effect of the genomic conflict between the selfish *f* element and the host genome. The autosome carrying the *M* gene behaves as a new sex chromosome originating a male heterogametic system of sex determination. These findings have suggested a dynamic evolution of sex determination in *A. vulgare* driven by *Wolbachia* infections and by the occurrence of intragenomic conflicts between different sex ratio distorters and the autosomal genes that promote the selection of new autosomal masculinizing genes. This would explain the low or null morphological differentiation of sex chromosomes in isopods and the occurrence of female and male heterogametic systems in closely related species of isopods.

In a different example, the *Wolbachia* strain *wSca* manipulates the sex of the moth *Ostrinia scapulalis* by interfering with the sex-specific splicing of *Osdsx* gene (Sugimoto and Ishikawa 2012), a homologue of *doublesex* (*dsx*) working at the bottom of the sex determination cascade, which is transcribed into either a male or female isoform by sex-specific splicing and regulates the sex-specific gene expression in somatic cells of insects (Gempe and Beye 2011). *wSca* causes feminization of ZZ genetic males early in development (infected male embryos express the female-type $Osdsx^{FL}$) and subsequently kills the same individuals. However, the male-type $Osdsx^{M}$ is expressed in all individuals cured from infection irrespective of the genetic sex. This indicates that elimination of *wSca* causes the masculinization of ZW females, and consequently, a factor in the female-determining cascade is degraded in *wSca*-infected hosts (Sugimoto and Ishikawa 2012).

Genes that suppress MK have been identified in some insects (Majerus and Majerus 2010), and their spread can occur very quickly in the field, for example, taking only few generations to change the 99 % female sex ratio of some infected populations of the butterfly *Hypolimnas bolina* to a sex ratio near parity (Charlat et al. 2007a).

In the case of CI bacteria, being infected is beneficial for females as their eggs are saved from the deleterious effect of CI and is

detrimental for males as they suffer a fitness cost in mating with uninfected females (Turelli 1994; Snook et al. 2000). Even if selection would favour infection to spread to fixation, it is expected that uninfected individuals are always produced due to inefficiency of endosymbiont transmission. So, nuclear genes reducing levels of CI can be selected for. CI-*Wolbachia* can produce a physiological cost on infected males by significantly reducing the production of sperm (Snook et al. 2000; Lewis et al. 2011); thus, it is expected that endosymbionts can promote evolutionary changes in the functioning of the male germline. Host resistance genes that prevent the entry of *Wolbachia* into testes have been suspected (Poinsot et al. 1998).

In addition to their role in the evolution of sex determination systems, bacterial endosymbionts have been hypothesized to be a driving factor in the evolution of genetic systems (Ross et al. 2010). In particular, haplodiploidy could have originated in diplodiploid arthropods, following the spread of MK endosymbionts that caused the destruction of the paternal chromosome set in diploid males (Normark 2004). Under such circumstances, coevolutionary responses by the host would be predicted and genes that save viability and fertility of haploid males can be selected. If this should be the case, models predict the evolution of a paternal genome elimination-based haplodiploid system if haplodizing endosymbionts become beneficial for female hosts and the infection fixed (Kuijper and Pen 2010).

In the thelytokous parasitoid wasp *E. hispida*, *Cardinium* is required to feminize diploid male embryos and thus must interact with elements of the host sex determination system (Giorgini et al. 2009). Diploid males are produced by antibiotic-fed females. These findings suggest a possible route for the collapse of haplodiploidy into a diplodiploid genetic system. Hosts may contribute to or take over the process of asexual diploidy restoration from symbionts if this reduces mortality of parthenogenetic daughters. Reversion to diplodiploidy from haplodiploidy is quite rare, but in one of two examples of scale insects highlighted by Normark (2004), symbionts appear to play a role. Although relatives are haplodiploid, Buchner (1965) noted that female embryos in the family Stictococcidae are diploid and infected with a bacterium, whereas males are also diploid, but free of bacteria.

Host Population Genetics

Vertically inherited reproductive parasites influence the evolutionary dynamics of host population genetics dramatically. Symbionts and mitochondria are simultaneously inherited through the egg cytoplasm, and because infected individuals have a reproductive advantage over the uninfected ones, the spread of a reproductive parasite will sweep from the infected host populations the mitochondrial haplotypes not associated with infection. The final result will be the reduction in host mtDNA diversity (Johnstone and Hurst 1996). Less frequently, reproductive parasites can also alter the frequency of host nuclear genes. Theoretical models suggest that infections with early MK bacteria impede the spread of beneficial alleles, facilitate the spread of deleterious alleles, and reduce nuclear genetic variation in infected host populations. The reason for this is the strongly reduced fitness of infected females combined with no or very limited gene flow from infected females to uninfected individuals. Most mutations originating in infected individuals are therefore lost, and the effective population size for nuclear genes is reduced almost to the number of uninfected individuals. The impact of reproductive parasites on host population genetics is reviewed by Engelstädter and Hurst (2009).

Reproductive Manipulators as Drivers of Host Reproductive Isolation and Speciation

One effect of CI is the reproductive isolation between differently infected hosts. As a result, CI-inducing endosymbionts could have a role in driving speciation processes in their hosts (Werren 1998; Bordenstein 2003; Telschow et al. 2007). However, because the penetrance of CI is frequently incomplete, vertical transmission of symbionts is not always perfect, and gene flow can occur in compatible cross-directions, it

is unlikely that CI alone drives speciation. Instead, theoretical and empirical works on *Wolbachia*-induced CI suggest a complementary role in species formation along with other genetic and/or geographical mechanisms that restrict gene flow between diverging populations (Telschow et al. 2005). For example, between two closely related parasitoid wasps, *N. vitripennis* and *N. giraulti*, reproductive isolation is maintained both by complete bidirectional CI and by nuclear incompatibilities, leading to hybrid inviability and hybrid sterility (Breeuwer and Werren 1995). However, bidirectional CI was found to be the principal contributor to reproductive isolation between the sibling species *N. giraulti* and *N. longicornis*, each fixed for infection by a specific *Wolbachia* strain; here, *Wolbachia*-induced reproductive isolation has occurred in the early stages of speciation, because other postmating isolating mechanisms, like hybrid inviability and hybrid sterility, are still not present (Bordenstein et al. 2001).

CI causing bacteria can also promote speciation by promoting premating isolation (Telschow et al. 2005). For example, asymmetrical reinforcement has been observed in the field in uninfected *Drosophila. subquinaria* as a consequence of secondary contact with *D. recens*, which is infected near fixation (98 % infection prevalence) with *Wolbachia* causing strong intraspecific and interspecific CI. However, hybrid inviability is not manifested in matings between infected *D. recens* females and uninfected *D. subquinaria* males. Females of *D. subquinaria* from the zone of sympatry exhibit stronger levels of mate discrimination against *D. recens* males than do females from allopatric populations. Furthermore, there was substantial behavioural isolation within *D. subquinaria*, because females sympatric with *D. recens* discriminate against allopatric conspecific males, whereas females allopatric with *D. recens* show no discrimination against any conspecific males. These findings show that interspecific CI may contribute not only to postmating isolation but also to reinforcement, particularly in the uninfected species. The resulting reproductive character displacement not only increases behavioural isolation from the *Wolbachia*-infected species, but may also lead to behavioural isolation between populations of the uninfected species (Jaenike et al. 2006).

Coevolution of reproductive parasites with their host towards a mutualistic association may also play a role in diversifying and separating host populations and eventually driving speciation (Miller et al. 2010). As an example, *Wolbachia* has been implicated in driving sexual isolation between six semispecies of *D. paulistorum* that occur sympatrically in Middle and South America. Each semispecies harbours a specific *Wolbachia* strain that provides a fitness benefit to its host, being essential for oogenesis and development. *Wolbachia* are ancestrally fixed, obligate mutualists of all *D. paulistorum* semispecies, perfectly transmitted by the mother and causing strong bidirectional CI and hybrid male sterility in the laboratory. In nature, however, incompatible matings between semispecies are avoided by female mating choice and courtship behaviour. In their native *D. paulistorum* hosts, *Wolbachia* manipulate sexual behaviour by triggering premating isolation via selective mate avoidance, that is, avoiding mates harbouring another, incompatible symbiont variant. It was assumed that symbiont-directed mate recognition could have evolved in order to prevent strong bidirectional CI and reduced sexual success of potential hybrids, thereby ensuring continuing vertical transmission of the symbiont (Miller et al. 2010).

In asexual populations of haplodiploid arthropods, continuous thelytokous reproduction caused by PI or feminizing symbionts can lead to degradation of genes involved in sexual reproduction, for example, in genes involved in male mating behaviour and fertility or encoding female sexual traits, because these gene are not maintained by selection anymore (Pijls et al. 1996; Arakaki et al. 2000; Gottlieb and Zchori-Fein 2001). Furthermore, such mutations may be selected for if they improve the fitness of asexual females. For example, degradation of costly genes involved in female behaviour or sperm usage could reallocate resources in favour of oogenesis or other fitness traits. As infection by

a PI symbiont spreads, degradation of sexual traits would accumulate, leading to prezygotic isolation between infected asexual populations and uninfected sexual ones (Pannebaker et al. 2005). Sexual degradation will make thelytokous reproduction irreversible in infected populations even if the symbiont is lost, eventually resulting in a speciation event (Bordenstein 2003; Adachi-Hagimori et al. 2011). Consequently, if the symbiont is lost and the host does not come up with an alternative mechanism, this will result in extinction of the infected host.

Gene Acquisition from Reproductive Parasites

Reproductive parasites have also been a source of new genes for hosts via lateral transfer (Werren et al. 2008). It has been found that one-third of sequenced invertebrate genomes contain *Wolbachia* gene insertions and that 70 % of *Wolbachia*-infected arthropod and nematode hosts might have a nuclear insert (Dunning Hotopp 2011). The largest lateral transfer has been found in *D. ananassae* where almost the entire genome of *Wolbachia* (~1.4 Mb) has been integrated into an insect chromosome. However, although some inserted *Wolbachia* genes are transcribed, their biological functions are still unknown. Recently, it was found that the genome of *N. vitripennis* encodes 13 ankyrin repeat proteins with a C-terminal domain (PRANC), and these proteins are found in diverse *Wolbachia* strains (Werren et al. 2010). Phylogenetic analysis of the PRANC domain reveals that *Nasonia* wasps acquired one or more of these proteins from *Wolbachia* with subsequent gene duplication and divergence. Most of the genes are transcribed in both males and females and in different life stages, suggesting that in some cases, lateral gene transfer can be an effective source of new functional genes.

The Role of Reproductive Parasites in Altering Host Behaviour

Biases in the sex ratio of a population are expected to alter which sex competes for mates (Emlen and Oring 1977). Reproductive parasites that skew sex ratio towards females and then decrease the frequency of males in a population are expected to reduce both the intensity of male–male competition and the opportunity for female choice between males. As a consequence, alterations of the mating system and reproductive strategy should occur in favour of female–female competition and male choice (Charlat et al. 2003). As an example, a sex-role reversal has been reported in some populations of the butterfly *Acraea encedon* characterized by high frequency of MK-*Wolbachia* infection and female-biased sex ratio (Jiggins et al. 2000). However, in female-biased populations of the butterfly *H. bolina* infected by a MK-*Wolbachia*, contrary to expectation, female mating frequency increases rather than decreases along with infection prevalence, until male mating capacity becomes limiting (Charlat et al. 2007b). This increasing female promiscuity has been explained as a facultative response to the increasing fatigue and reduced mating resource of males, which produce smaller spermatophores as mating frequency increases. Reduced investment (sperm transfer) by males when paired with infected individuals, potentially leading to variation in host mate preferences, has also been found in crustaceans infected by feminizing *Wolbachia* (Rigaud and Moreau 2004) or microsporidia (Dunn et al. 2006). This is advantageous for males as they are severely sperm limited, and feminized males have lower fecundity than uninfected females.

Reproductive behaviours can also evolve in arthropods infected by reproductive parasites to limit the spread of costly infections. For example, some infections have been found to negatively influence host body size, fecundity, survival, larval competitiveness, male fertility, and sperm production (Snook et al. 2000; Huigens et al. 2004b; Rigaud and Moreau 2004). In the mite *Tetranychus urticae*, *Wolbachia*-associated unidirectional CI can be avoided by females at the premating level through both precopulatory and ovipositional behaviours that increase chances of successful compatible matings; infected females aggregate their offspring, thereby promoting sib mating, while uninfected females preferably mate with uninfected males

and, in doing so, directly reduce opportunities for CI expression (Vala et al. 2004). In *D. melanogaster*, *Wolbachia* plays a role in mate discrimination between infected and uninfected populations with identical genetic background and it has been suggested that *Wolbachia* might have evolved the capacity to modulate host pheromone expression and/or perception (Koukou et al. 2006). In a different study, however, neither male nor female *D. melanogaster* nor *D. simulans* exhibit significant *Wolbachia*-associated precopulatory mate preferences (Champion de Crespigny and Wedell 2007).

CI-*Wolbachia* infection in *D. simulans* negatively affects sperm competition in infected males, suggesting that polyandrous females can utilize differential sperm competitive ability to bias the paternity of the progeny and reduce the penetrance of reproductive manipulators (Champion de Crespigny and Wedell 2006). Reduced success in sperm competition associated with infection of CI-*Wolbachia* in *Drosophila* could play a role in the evolution of host reproductive strategies, like the selection for polyandry in species with CI-inducing endosymbionts, to avoid the fitness cost associated with infections. However, this hypothesis has not been supported by theoretical models (Champion de Crespigny et al. 2008).

PI-bacteria can change the female's host selection behaviour to successfully invade a host population. For example, PI-*Cardinium* manipulates the oviposition choice of its parasitoid host *Encarsia pergandiella*, causing the female wasp to lay unfertilized infected eggs into hosts that are competent for female but not male development (Kenyon and Hunter 2007).

17.3 Viruses as Beneficial Symbionts of Insects

In contrast to bacteria, which are well recognized to form beneficial symbiotic associations, viruses are almost always considered parasites whose life cycles reduce host fitness while benefiting their own (Villarreal 2005; Moreira and Lopez-Garcia 2009). In the case of insects and other arthropods, nearly all studies focus on the role of viruses in causing severe or chronic disease (Bonning 2005). However, recent studies reveal that some viruses have evolved to become obligate beneficial symbionts of parasitoid wasps. The most elegant example of this is for all members of the *Polydnaviridae*, which are exclusively beneficial symbionts of parasitoid wasps. However, selected poxviruses, ascoviruses, and phages that infect bacterial symbionts of insects also exhibit features that suggest they too have evolved into obligate or facultative beneficial symbionts.

17.3.1 Polydnaviruses as Beneficial Symbionts

By far the best example of viruses evolving into beneficial symbionts is the family *Polydnaviridae* (reviewed in Webb and Strand 2005; Strand 2010). These large, double-stranded (ds) DNA viruses are exclusively associated with approximately 40,000 species of parasitoid wasps in the families Braconidae and Ichneumonidae and are divided into two genera called the *Bracovirus* (BV) and Ichnovirus (IV). Most polydnavirus (PDV)-carrying wasps parasitize larval-stage hosts in the order Lepidoptera (moths and butterflies). Each wasp species carries a genetically unique PDV that exists in two forms. The proviral form is integrated into the genome of every cell in wasps of both sexes, and transmission to offspring is strictly vertical through the germline (Fig. 17.1). The encapsidated form of the genome that is packaged into virions consists of multiple, circular dsDNAs, which have aggregate sizes that range from 190 to more than 600 kbp. This makes PDVs the only known dsDNA viruses with multipartite genomes and also underlies the naming of the family. PDVs only replicate in pupal- and adult-stage female wasps in specialized cells that form a region of the ovary called the calyx (Fig. 17.1). Virions accumulate to high density in the lumen of the calyx to form "calyx fluid", and females inject a quantity of calyx fluid together with eggs into each host they parasitize. Virions rapidly infect

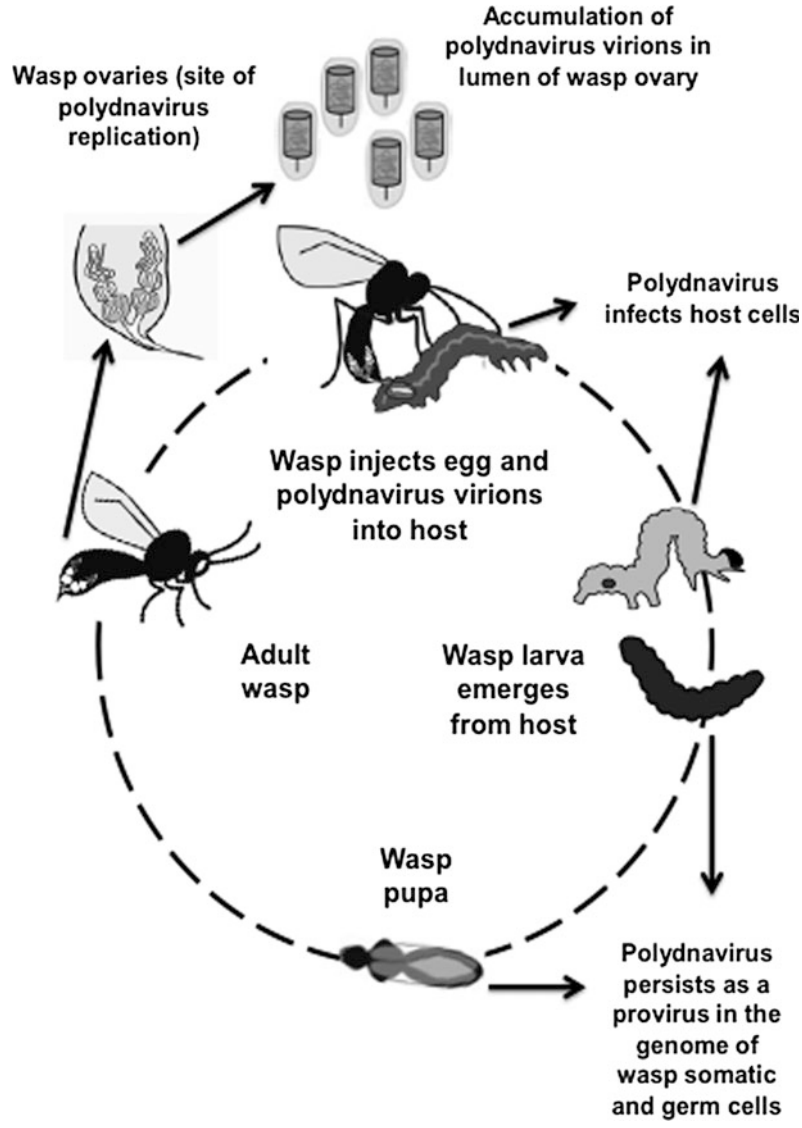

Fig. 17.1 Polydnavirus life cycle. The infection of host tissues and expression of virulence factors largely contribute to the disruption of the immune response and the alteration of development and reproduction in parasitized hosts

host cells, but they never replicate. They do, however, express a number of genes whose products alter host physiology in ways that are essential for survival of wasp offspring (Fig. 17.1). PDVs are thus beneficial symbionts because they fully depend on wasps for persistence, while wasps fully depend on the virulence genes that replication-defective PDV virions deliver to caterpillar hosts.

17.3.1.1 BVs and IVs Have Different Evolutionary Origins

BVs and IVs were originally placed into one family because of their multipartite dsDNA genomes, similar life cycles, and strict association with parasitoid wasps. However, three lines of evidence indicate that BVs and IVs have different evolutionary origins. First, phylogenetic studies indicate that BV-carrying braconids form

a monophyletic assemblage called the microgastroid complex. This group evolved an estimated 100 mya and is relatively distant from the taxon of Ichneumonidae that carry IVs (Whitfield 2002; Murphy et al. 2008). Since no PDVs are known from a common ancestor of these wasp lineages, these data strongly suggest the association of BVs with braconids and IVs with ichneumonids arose independently. Second, partial or complete sequencing of 11 encapsidated genomes (6 BVs, 5 IVs) reveal that BVs from different wasps encode several homologous genes as do IVs. However, BVs share few genes with IVs. Third, morphological studies show that BV virions have barrel-shaped capsids surrounded by one envelope, while IV virions have fusiform capsids and two envelopes. Although each assembles in the nuclei of calyx cells, BV virions are released by cell lysis, while IV virions bud through the plasma membrane. Overall, these findings suggest that BVs evolved from a virus that interacted with the common ancestor of microgastroids. Whether the IV-ichneumonid association also arose from a single virus and wasp ancestor remains unclear, but these progenitors differ from the ancestors of BV-carrying braconids. Finally, the traits that BVs and IVs share most likely arose through convergent evolution and the similar roles each plays in parasitism.

BV and IV virions unquestionably look like viruses, but their encapsidated genomes yielded the surprising finding that no viral genes with predicted roles in genome replication, transcription, or virion formation are present (Burke and Strand 2012; Drezen et al. 2012). The encapsidated genomes of PDVs also have eukaryotic architectural features that include low coding densities and many intron-containing genes. These findings explain why PDVs do not replicate in the hosts that wasps parasitize, but also raise the spectre that PDVs are not of viral origin but products of wasp genes that generate virus-like structures. Recent studies from three braconids, however, provide strong evidence that BVs evolved from a viral ancestor (Bezier et al. 2009; Wetterwald et al. 2010). Specifically, analysis of transcripts expressed in ovaries during BV replication identified homologues in each wasp that are core genes of nudiviruses. Nudiviruses themselves are relatively poorly studied, but they are a sister taxon of the *Baculoviridae*, which are well-known pathogens of insects (Wang and Jehle 2009; Jehle 2010).

Nudiviruses and baculoviruses infect insects or other arthropods, have large circular dsDNA genomes (>100 kbp), and like BVs produce single-enveloped virions. Most baculoviruses are virulent pathogens whose life cycles are distinguished by the coordinated expression of several core genes, high-level replication of the genome in host cell nuclei, and the release of large numbers of virions by cell lysis. Many nudiviruses also establish lytic infections, but some selectively infect the reproductive organs of their insect host (HzNV-2) and establish persistent infections (HzNV-1) that are characterized by a shutdown of most genes expressed during a productive infection and maintenance of the genome as both an episome and an integrated provirus. One route for the evolution of BVs, therefore, is that a nudivirus established a latent infection in the reproductive tract of the ancestral microgastroid. Studies of one ichneumonid also identify features that suggest a viral origin for IVs. However, a lack of homology with genes from other known viruses suggests IVs either evolved from an undiscovered or extinct virus group or have diverged, so greatly that it is not possible to detect sequence similarities with other viruses (Volkoff et al. 2010).

17.3.1.2 Roles of Polydnaviruses in Parasitism of Hosts

PDVs are essential for wasp survival because they deliver virulence genes to hosts that have two broad functions: (1) they prevent the host's immune system from killing wasp offspring and (2) they alter the growth, development, and metabolism of hosts in ways that facilitate wasp development while leading to host death. PDV-carrying wasps are highly specialized organisms with each species parasitizing only one or a few species of host insects. The immunological and

developmental interactions between wasps and hosts also differ, with wasps from distantly related taxa generally exhibiting greater differences than wasps from the same or closely related genera. The encapsidated genomes of PDVs are thus likely to change in response to the selective pressures that act on each wasp in its coevolutionary interactions with hosts. Strict Mendelian inheritance, however, would also suggest that PDV isolates from wasps in distantly related taxa are likely to differ more than isolates from closely related wasps. Although relatively few isolates have been sequenced, current data strongly support these predictions by showing that aggregate genome size, genomic segment numbers, and gene content are most similar among isolates from closely related wasps (Webb and Strand 2005; Pennacchio and Strand 2006; Strand 2010). The encapsidated genomes of BVs from distantly related braconids in the subfamilies Microgastrinae and Cheloninae in contrast share no genes even though their proviral genomes encode the same nudivirus-like core gene set. The encapsidated genomes of BVs and IVs also, as expected, largely encode different genes.

Approximately half of the genes in PDV encapsidated genomes form multimember families that often reside on multiple genomic segments (Strand 2010). Several of these families show signatures of evolving by duplication from a single ancestral gene (Huguet et al. 2012). Several families also show evidence of evolving under diversifying or positive selection in response to alterations in a given host or shifts in the host range of a given wasp. The ankyrin repeat (*ank*) and protein tyrosine phosphatase (*ptp*) families of BVs, and *cys-motif* family of IVs are present in isolates from diverse taxa, suggesting that each have ancient origins and produce products of broad importance in parasitism. Other gene families are only known in isolates from a particular taxon of wasps, which suggests they were acquired more recently and have more specialized roles in parasitism.

Most of the genes in the encapsidated genomes of PDVs are homologues of known genes from eukaryotes, which in turn suggests that many were acquired from wasps or the hosts they parasitize (Burke and Strand 2012; Drezen et al. 2012). This is clearly the case for some genes like a family of predicted sugar transporters present in BVs from the wasp genus *Glyptapanteles.* However, the high divergence rates of more conserved gene families like *anks* and *ptps* make it impossible to discern whether the ancestral gene was acquired from an insect or another eukaryote. Recent studies also suggest that the *ank* gene family present in IVs was acquired by horizontal gene transfer (HGT) from a BV. Lastly, a few genes in the encapsidated genomes of PDVs are not of eukaryotic origin and have also been acquired by HGT through unknown mechanisms.

Understanding of PDV gene function in subversion of host immune defences or growth is restricted to a small number of BV isolates (reviewed in Strand 2010, 2012). The primary immune defence against parasitoids is encapsulation, which occurs when pattern recognition receptors bind to the surface of wasp eggs. This stimulates particular types of immune cells (haemocytes) to adhere to the parasitoid and form a multicellular sheath. Several pattern recognition receptors, cytokines, and adhesion molecules regulate haemocyte adhesion, while antimicrobial peptides (AMPs) and other genes regulated by NF-kB transcription factors of the Toll and/or Imd pathways are induced during the early phases of capsule formation. Capsules also often melanize due to activation of the phenoloxidase (PO) cascade. In turn, parasitoids die in capsules from asphyxiation, cytotoxic molecules generated by the PO cascade, and/or the activity of AMPs and other effector molecules. Members of the conserved members of viral *ank* family function as inhibitor kB (IkB) mimics that negatively regulate host NF-kBs, which are implicated in disabling haemocyte adhesion and phagocytosis. Two unique gene families (*glc, egf*) from *Microplitis demolitor* bracovirus (MdBV) have also been shown to block encapsulation and activation of the PO cascade, while studies of *Chelonus inanitus* bracovirus (CiBV) implicate three genes in altered host

development. Physiological studies clearly show that IVs also produce gene products that disable encapsulation and host growth, but the specific genes involved largely remain uncharacterized.

17.3.2 Entomopoxviruses as Beneficial Symbionts

Whereas all members of the Polydnaviridae are beneficial symbionts, a few isolates from other virus families have also formed similar associations with parasitoid wasps. One such case is a member of the Poxiviridae, which are also large DNA viruses that have linear dsDNA genomes. Members of the family that infect insects in several orders are referred to as entomopoxviruses (Perera et al. 2010). However, one putative entomopox isolate named DlEPV is associated with the braconid wasp *Diachasmimorpha longicaudata*, which parasitizes the larval stage of the Caribbean fruit fly, *Anastrepha suspensa* and related tephritids (Lawrence 2005). Similar to PDV-carrying wasps, *D. longicaudata* injects DlEPV into its host when ovipositing. This wasp also injects another virus named DlRhV, because it morphologically resembles rhabdoviruses, which are negative-sense single-stranded (ss) RNA viruses. DlEPV and DlRhV both appear to replicate in the accessory glands of the wasp, and both entities also appear to replicate in parasitized hosts (Lawrence and Akin 1990; Lawrence and Matos 2005). The function of DlRhV remains unknown, but studies show that DlEPV infects host haemocytes and induces cytopathic effects that disable encapsulation, in order to allow the wasp's offspring survival (Lawrence 2005). Unlike PDVs, however, relatively little is known about the transmission and replication of either DlEPV or DlRhV in the wasp or its host. In turn, it is also unclear whether persistence of either virus totally depends on the wasp or whether these viruses are capable of persisting independently in tephritids or other insects.

17.3.3 Ascoviruses as Parasitoid-Vectored Pathogens and Potential Beneficial Symbionts

The Ascoviridae is a family whose members have large, circular dsDNA genomes and whose hosts are exclusively larval- and pupal-stage Lepidoptera in the family Noctuidae (Federici et al. 1991; Bideshi et al. 2010). These viruses also appear to be exclusively transmitted by parasitoids that acquire ascoviruses when they insert their ovipositor into an infected host. Most ascoviruses are then horizontally transmitted to a new host when the wasp parasitizes another host (Stasiak et al. 2005). Most ascoviruses cause distinct pathology that ultimately results in death of the host larva or pupa and the progeny of the parasitoid (Stasiak et al. 2005). However, one ascovirus isolate associated with the ichneumonid wasp species *Diadromus pulchellus*, DpAV4, has evolved to become an essential immunosuppressive symbiont, which persists without apparent replication as an episome in all cells of wasps and which is vertically transmitted to offspring (Bigot et al. 1997). However, DpAV4 replicates, asymptomatically, in the reproductive tract of female wasps, and DpAV4 virions are injected along with the egg at oviposition. Unlike most ascoviruses, when the pupae of the lepidopteran species *Acrolepiopsis assectella* are parasitized, DpAV4 replication does not immediately occur but is instead synchronized with the development of parasitoid juvenile offspring (Bigot et al. 1997). When this virus is transmitted by other *Diadromus* species, its mutualistic role remains unaltered, but it rapidly replicates and functions as a pathogen when transmitted by other ichneumonids (Bigot et al. 1997; Stasiak et al. 2005). Therefore, it seems that the success of ascovirus/wasp relationship is modulated by unknown factors in wasps from the genus *Diadromus* that control virus replication in the host. Thus, certain ascoviruses can potentially have a pathogenic, mutualistic, or non-pathogenic relationship with

a specific wasp vector, depending upon the species system in which the relationship evolved. In particular, the presence of other interacting viruses may play an important role in determining the final outcome of this viral symbiosis, as explained in detail in the next section.

17.3.4 Cypoviruses as Modulators of Ascovirus Function in Parasitoids

The Reoviridae are segmented dsRNA viruses that infect a diversity of animals. One genus that specifically infects insects is the genus *Cypovirus*. Similar to ascoviruses like DpAV4, several cypoviruses have been identified from *Diodromus* ichneumonids (Renault et al. 2003, 2005). The best studied forms are DpRV1 and DpRV2, from *D. puchellus.* DpRV1 is always found in association with DpAV4 and appears to play a key role in modulating DpAV4 replication, so that the parasitoid's offspring develops before the host dies. This regulatory process appears to be controlled by an additional RNA of wasp origin, which is packaged in the DpRV1 virions (Renault et al. 2005; Stasiak et al. 2005). If true, this represents an alternative way to deliver wasp genetic material using viruses as vectors. Other cypoviruses, as well as other RNA viruses identified from parasitic wasps, have no known role in disabling host immune defences or promoting successful parasitism (Renault 2012).

17.3.5 Viruses that Manipulate Parasitoid Behaviour

The association between viruses and parasitic wasps goes beyond what is described above and may have a considerable influence not only on host physiological regulation and immune suppression, but also on other phenotypic traits, sometimes not very easy to define. For example, the figitid wasp *Leptopilina boulardi*, which parasitizes *Drosophila* spp., carries a virus, named LbFV, which promotes superparasitism (i.e. solitary parasitoids laying more than one egg per host, which results in egg wastage as only one individual will complete development), a phenomenon that is rarely observed in uninfected *L. boulardi* wasps (Varaldi et al. 2005). LbFV is a filamentous virus of unclear taxonomic status that appears to be both maternally and horizontally transmitted (Varaldi et al. 2009). Superparasitism favours horizontal transmission of the virus but whether infection benefits the wasp is not fully clear. A population modelling approach suggests LbFV spread, and prevalence could positively influence parasitism rates of *L. boulardi* (Patot et al. 2010; Varaldi et al. 2012). Empirical studies also indicate that infected wasps have higher egg loads (i.e. fecundity) due possibly to superparasitism creating selection pressure that favours increased investment by wasps in egg production.

17.3.6 Viruses and Aphid Polyphenism

Aphids have complex life cycles, which usually include a sexual generation that consists of winged (alatae) adults, followed by a number of parthenogenetic generations, comprised of wingless (apterae) forms (Zera and Denno 1997). Parthenogenesis promotes rapid colony expansion when host plant resources are abundant and/or environmental conditions are favourable, while alatae formation promotes dispersal when resources become scarce and/or poor as a consequence of crowding. The cues involved in formation of alatae are vary and include a number of environmental factors, such as temperature, population density, nutrition quality of the plants, interactions with natural enemies and ants (Dixon 1998; Muller et al. 2001).

Many factors have been implicated in regulating aphid development (Fereres and Moreno 2009). Very recently, however, studies with rosy apple aphid, *Dysaphis plantaginea*, implicate infection by a densovirus named *D. plantaginea densovirus* (DplDNV) in alate and apterae development. DplDNV infection also often co-occurs with *Rosy apple aphid virus* (RAAV), a taxonomically unassigned RNA virus (Ryabov et al. 2009). Densoviruses have very small (4–6 kb)

ssDNA genomes, and many isolates have been identified that infect insects. DplDNV-infected aphids produce winged aphids in response to crowding or poor plant quality, while uninfected aphids or aphids infected by RAAV fail to produce winged forms. DplDNV infection also reduces aphid reproduction, but by promoting dispersal through formation of winged forms, DplDNV is thought to benefit aphid carriers, on balance.

17.3.7 Aphid Bacterial Symbionts and their Viral Phages

As previously discussed, nearly all aphids depend on primary symbionts like *B. aphidicola* for survival and reproduction (Baumann 2005), while some facultative symbionts (*H. defensa, R. insecticola, S. symbiotica*) provide benefits to aphids through enhanced defence against parasitoids (Oliver et al. 2010). In the case of the pea aphid, *A. pisum*, resistance traits against parasitoids are associated with lysogenic lambdoid bacteriophages that infect the facultative symbiont (Moran et al. 2005a; Oliver et al. 2009). While not fully defined, some of these phage-associated factors disrupt development of parasitoid eggs while others affect the development of wasp larvae by possibly interfering with function of specialized wasp cells called teratocytes (Li et al. 2002; Falabella et al. 2005, 2009). The precise role of phages in the association that exists between aphids and their bacterial symbionts remains poorly understood. However, it is possible that the phages infecting symbionts may function as beneficial symbionts of the bacteria, the aphid, or both.

17.3.8 Plant Viruses and Insect Vectors

Several studies also report interactions between plant viruses and aphids, related homopterans, or other insects that serve as vectors (Stout et al. 2006). The most detailed information is available on aphids, for which alterations attributed to virus infection include increased attraction to infected host plants and increased fecundity when feeding on virus-infected plants and subsequent increased production of alates which promotes virus spread (reviewed in Kluth et al. 2002; Colvin et al. 2006; Stout et al. 2006). The better performance of aphids on virus-infected plants has been poorly investigated at functional and molecular levels. Only recently, it has been demonstrated that *Cucumber mosaic virus* (CMV) encodes a protein that disrupts plant antiviral mechanisms, by enhancing the effect of salicylic acid (SA) on certain defence genes against pathogens and, at the same time, by inhibiting changes in the expression level of 90 % of the genes regulated by jasmonic acid (JA) (Lewsey et al. 2010), notably active against insects. Indeed, this reinforces the plant colonization strategy adopted by stylet feeders, such as aphids and whiteflies, which promote SA-induced gene expression, to down-regulate JA-mediated defence responses against insects, via JA/SA cross-talk (Thomma et al. 2001; Gatehouse 2002; De Vos et al. 2005; Zarate et al. 2007). The study of the salivary secretome of aphids shows the occurrence in the saliva of plant effector molecules similar to those used by different plant pathogens (Carolan et al. 2011), which may partly account for this interaction strategy. Collectively, all this evidence indicates that sucking insects may weaken plant barriers against insects by activating, with the help of plant pathogens, defence responses to which they are not sensitive but that redirect in their favour overall plant defence metabolism.

These complex tripartite interactions also may have a profound impact on biological invasions by alien species or populations, which may displace the local ones with the help of associated plant pathogens they transmit. The best studied case is offered by the biotype B of the whitefly *B. tabaci*, which has replaced the indigenous population (biotype ZHJ1) with the help of two whitefly-transmitted begomoviruses (Jiu et al. 2007). Host plants infected by these viruses enhance the fecundity and longevity of the biotype B individuals while have a negligible effect on the indigenous ZHJ1 population. This is considered one of the mechanisms accounting for the impressive invasive ability of biotype B (Jiu et al. 2007).

17.3.9 Viruses Helping Mosquitoes in Taking Their Blood Meal

Mosquitoes feeding on their hosts must complete their blood meal rapidly, to reduce the risk of being killed by an annoyed host. *Aedes aegypti* seems to do better in locating and using a blood vessel when host hamsters are infected by *Rift Valley fever virus* (RVFV), as this pathogen is supposed to disrupt haemostasis and facilitate mosquito feeding (Rossignol et al. 1985). Therefore, RVFV may provide a benefit to the vector insect, which enhances its own acquisition and transmission. The use of host pathogens by ectoparasitic arthropods to facilitate their feeding seems to be not an uncommon evolutionary pathway. It has been recently observed that *Varroa destructor*, a parasitic mite of honeybees, actively transmits the *Deformed wing virus* (DWV), which seem to disrupt host immune response, with likely negative effects on haemolymph clotting and melanization (Nazzi et al. 2012); this is an important functional requirement for feeding and successful development of mites and other ectoparasitic arthropods (Pennacchio and Strand 2006).

17.3.10 Evolutionary Patterns in Insect–Virus Associations

The examples provided above indicate that similar to bacteria, some viruses have evolved obligate or facultatively beneficial associations with insects. The most common theme is the domestication of viral pathogens by parasitic arthropods for suppressing the immune defences of hosts. The adaptive value of these symbiotic associations inextricably links life cycles and allows the colonization of new ecological niches. This generates fast evolution and speciation rates, both for parasitic organisms and for their viral symbionts, which effectively promote the introduction of new genomic traits favouring a rapid adaptation to new environments (Roossinck 2005). Current data also suggest such symbioses begin as a loose association with a viral pathogen but can culminate, in the case of polydnaviruses, with entities that can no longer persist independently of one another.

The "alliance" of parasitic organisms with viral pathogens of the host seems to be an effective strategy also for insects attacking plants. The tight association between stylet feeding insects and viral plant pathogens provides a good example of how the latter can be used for suppressing plant defence responses. If and how some of the effector molecules present in aphid saliva, resembling those of pathogenic origin, may originate from HGT remains an intriguing question, which is certainly worthy of future research.

17.4 Conclusions

The study of symbiosis in arthropods demonstrates how the microbial diversity associated with these animals is an astonishing source of evolutionary novelty, which goes far beyond the simple complementation of nutritionally poor diets, required for the exploitation of difficult ecological niches. Even though our understanding of the biological bases of many specific symbiotic associations still remains incomplete, it is evident that the many impacts micro-organisms may have on host physiology, reproduction, and development promote the appearance of novel variants exposed to natural selection. Current molecular technologies offer the opportunity to unravel the intimate functional mechanisms underlying the establishment and maintenance of symbiotic associations and to shed light on some basic research issues, such as how chronic mutualistic interactions are discriminated by the host from pathogenic invasions and how these different categories of micro-organisms may be part of complex interactions affecting host immunity. These studies in arthropods offer new tools for investigating important aspects across the related fields of symbiosis and immunity, which have attracted increasing attention in the last few years

(Silverman and Paquette 2008; Gross et al. 2009; Ryu et al. 2010).

The Darwinian evolution model and that of symbiotic evolution are both based on the concept that evolution is driven by positive selection of the more fit variants, even though they propose different theories on how these variants are generated. In the Darwinian model, the gradual changes are the final outcome of random mutation and selection processes. The symbiogenic theory builds upon this model, by stating that genetic novelties and recombination occur among different biological entities (i.e. interacting symbionts) and not exclusively within the same species (Margulis 1993; Sapp 2009; Carrapiço 2010). These changes generated by cross-species association and recombination have the potential to generate much faster evolutionary rates, ultimately driven by natural selection. The unparalleled number and variety of host–symbiont associations in arthropods can offer a unique contribution to the active debate on the model best accounting for the evolution of the biological diversity we observe in nature. Based on the limited number of case studies presented in this chapter, we can reasonably conclude that symbiosis is one of the major drivers of diversification and evolution of natural populations of arthropods; the in-depth molecular and functional analysis of symbiotic interactions will disclose new basic information in evolutionary biology and will offer new tools for the development of innovative technologies for pest control. A cursory inspection of the available literature on arthropod symbiosis convincingly corroborates the model of symbiogenic evolution in multiple arthropod lineages. There is no doubt that selection, acting on horizontal mergers among different symbionts, has caused permanent and irreversible changes that result in new taxonomic entities. This can be interpreted as a consequence of a more general trend in biology: alliance of coexisting forms of life and integration of simpler functions generate new emergent properties.

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