PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2008) **275**, 353–361
doi:10.1098/rspb.2007.1211
Published online 5 December 2007

Review

Symbiont-mediated protection

Eleanor R. Haine*

Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK

Despite the fact that all vertically transmitted symbionts sequester resources from their hosts and are therefore costly to maintain, there is an extraordinary diversity of them in invertebrates. Some spread through host populations by providing their hosts with fitness benefits or by manipulating host sex ratio, but some do not: their maintenance in host lineages remains an enigma. In this review, I explore the evolutionary ecology of vertically transmitted symbionts and their impact on host resistance, and provide an overview of the evidence for the three-way interactions between these symbionts, natural enemies and invertebrate hosts. A number of recent empirical and theoretical studies suggest that vertically transmitted symbionts may protect their hosts from pathogens. If this 'symbiont-mediated protection' is widespread, it is likely that vertically transmitted symbionts contribute significantly to variation in measures of invertebrate resistance to natural enemies.

Keywords: symbiont; vertical transmission; resistance; evolutionary ecology; invertebrate immunity; protection

1. INTRODUCTION

Symbiosis describes a close relationship between members of different species, such as the associations between large multicellular organisms and microbes. The microbial partners are called symbionts (reviewed by Moran 2006). When the interaction benefits both partners, it is a mutualistic symbiosis, but when the symbiont benefits at the expense of its host, the association is one of parasitic symbiosis. The potential durable interactions between hosts and symbionts may lie anywhere on a continuum between parasitism and mutualism (Combes 2001). Generally speaking, microbes at the parasitic end of the symbiosis continuum tend to transmit horizontally (horizontal transmission, HT) from one host to another, while those at the mutualistic end of the symbiosis continuum tend to transmit vertically (vertical transmission, VT) from parent to offspring.

Invertebrates possess an extraordinary diversity of VT symbionts (e.g. *Wolbachia*, *Rickettsia*, *Spiroplasma*, *Arsenophonus*, *Flavobacteria*, *Bacteroidetes* and microsporidia Yen & Barr 1971; Werren *et al.* 1995; Hurst *et al.* 1999; Stouthamer *et al.* 1999; Dunn & Smith 2001; Schulenburg *et al.* 2001; Weeks *et al.* 2001, 2003; Tsuchida *et al.* 2002; Hunter *et al.* 2003; Zchori-Fein & Perlman 2004; Perlman *et al.* 2006), which can broadly be categorized into two groups based on their biology and evolutionary history (summarized by Dale & Moran (2006)). First, there are primary symbionts that are obligate for host survival and reproduction, are strictly vertically transmitted and have been associated with their host lineages for a long evolutionary time. An example is *Buchnera aphidicola*, a Gammaproteobacteria that provides essential nutrients to aphids (Buchner 1965). Second, there are secondary symbionts that are facultative and can undergo low levels of HT by which they can colonize new hosts. However, as they are predominantly vertically transmitted, in order to spread and persist in host populations they often confer some fitness advantage to infected over uninfected individuals, for example, by increasing host survival or reproductive output. An example of a secondary symbiont is the Gammaproteobacteria *Arsenophonus* (e.g. Dale *et al.* 2006). Other secondary symbionts spread because they manipulate host reproduction to increase their transmission through the maternal line; these are the reproductive parasites, such as *Wolbachia pipientis* (Hurst *et al.* 2005), which can spread even if it is deleterious to host fitness. *Wolbachia* is generally not necessary for host survival, but in some hosts it is an obligate symbiont: for example, a strain infecting the *Drosophila* parasitoid, *Asobara tabida* is necessary for host oogenesis (Dedeine *et al.* 2001). The impact of secondary symbionts on host fitness might depend on the environment such that they might be beneficial in one environment and deleterious in another. This is likely to determine why some populations are fixed for secondary symbiont infections while others are not (e.g. Montllor *et al.* 2002).

Infections by VT symbionts are often asymptomatic—they have very few pathogenic effects on their hosts—and as a result they are often undetected unless specifically screened for by molecular methods. The diversity and presence of VT symbionts require an explanation since they exploit their host for resources and are therefore likely to be costly to maintain. In some cases, their persistence can be explained because they undergo low levels of HT, but in general symbionts that are strictly vertically transmitted must either increase the fitness of their host or manipulate host reproduction in ways that benefit their own transmission in order to be maintained in host populations (Werren & O'Neill 1997; Hurst *et al.* 2005). However, there are examples where symbionts do not interfere with

*e.haine@sheffield.ac.uk

Received 5 September 2007
Accepted 14 November 2007

Box 1. Protection by vertically transmitted symbionts

A vertically transmitted parasite (VTP) infection can affect the population dynamics of a system containing a vertically transmitted symbiont, a host and a horizontally transmitted parasite (HTP). The host can be infected with either HTP or VTP, with the HTP superinfecting the VTP-infected host. Figure 1 describes the interactions.

β is the transmission efficiency of the HTP to infect the susceptible host and δ is the amount of protection the VTP affords the host that it is infecting. Susceptible hosts are therefore susceptible to horizontal infection at rate β, and vertically infected hosts are susceptible to horizontal infection at rate $(1-\delta)\beta$ (dashed arrows). a is the birth rate (open arrows). The VTP has a vertical transmission efficiency of p and therefore increases in numbers by $p \times a$, and contributes $(1-p)a$ to the uninfected hosts (solid arrow). Increasing protection allows the VTP to persist in the host–HTP system, and also increases total host population numbers. The host population growth rate is density dependent.

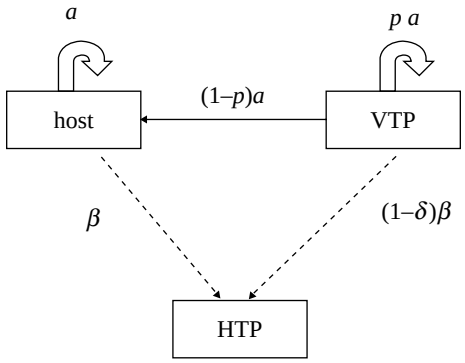

Figure 1. Schematic showing the interactions in a system containing a vertically transmitted symbiont (VTP), a host and a HTP (Jones *et al.* 2007).

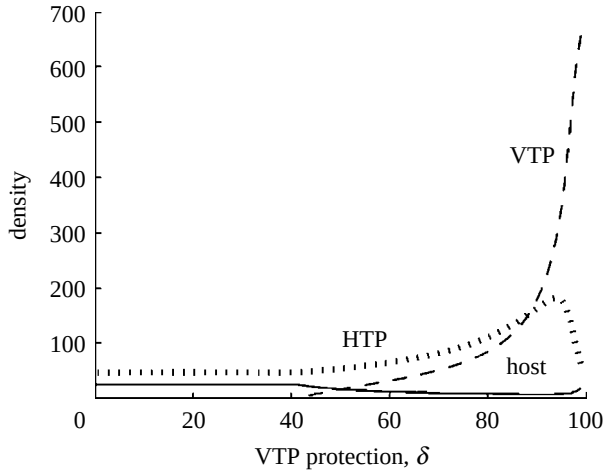

Figure 2. Densities at equilibrium of susceptible hosts, HTP- and VTP-infected hosts, with increasing protection provided by the VTP against the HTP (Jones *et al.* 2007).

Population Dynamics

Protection by the VTP leads to an increase in the HTP numbers until nearly complete protection occurs at which point there is a clear reduction in HTP numbers and an increase in the densities of both the VTP and uninfected hosts (figure 2). In a system where the HTP transmits with free-living infectious particles, the introduction of the protecting VTP stabilizes oscillating interactions, reducing amplitudes and the tendency to cycle (Jones *et al.* 2007).

host reproduction, provide no direct fitness benefit to their host and the mechanism underpinning their maintenance is not understood (e.g. Hoffmann *et al.* 1996).

To complicate matters further, an additional conflict arises when HT parasites (HTPs) and VT symbionts find themselves infecting the same host, since the former reduce host fecundity and often kill the host, while the latter's fitness is intrinsically linked to that of its host owing to its transmission mode (Rigaud & Haine 2005), and it requires that the host survives to reproduce—I propose that the conflict between VT symbionts and HTPs is resolved when VT symbionts provide their host with resistance to pathogens and parasites. The term 'symbiont-mediated resistance' was first coined by Oliver *et al.* (2005) in relation to the empirical evidence that secondary symbionts confer protection against parasitoid attack to their aphid hosts. Such phenomena have been described as 'interference' in theoretical studies (Jones *et al.* 2007) and have been suggested to be an overlooked component of invertebrate immune defence (Loker *et al.* 2004). The consequence of this line of reasoning is that any direct fitness effects of VT symbionts on their hosts (that explain their maintenance) may therefore be realized (and so observed) only when symbiont-bearing hosts are superinfected by horizontally transmitted pathogens.

In this review, I summarize the theoretical studies that have examined the interactions between VT symbionts and HTPs. I then present empirical evidence for symbiont protection of their hosts from natural enemies, discuss the mechanisms by which they do this and consider the interactions between VT symbionts and host immune defences. While this review just deals with invertebrate symbionts, symbiont-mediated protection is also relevant to vertebrates: a recent study found that mice infected by latent herpesviruses are resistant to infection by the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis* (Barton *et al.* 2007).

2. THEORETICAL CONSIDERATIONS ON INTERFERENCE BETWEEN VT SYMBIONTS AND PATHOGENS

In order to be maintained in a host population, the probability of a VT symbiont being successfully transmitted to the next generation must be greater than 1 (Lipsitch *et al.* 1995; Lively *et al.* 2005). This may be achieved if the VT symbiont increases host fitness (e.g. by increasing host fecundity and/or decreasing host mortality). It may also be greater than 1 if it can also transmit horizontally (Lipsitch *et al.* 1995) and, in some cases, a VT symbiont can increase in frequency in a host population even if it reduces host fitness because the symbiont manipulates host reproduction to gain increased transmission (Werren & O'Neill 1997). The dynamics of a host population containing a VT symbiont are complicated when the host population is also infected by an HTP because an HTP will increase the host population death rate, and eventually reduce population density

(Lively *et al.* 2005). Lipsitch *et al.* (1996) examined competition between two parasite strains where both parasites can transmit vertically and horizontally. In most cases, VT symbionts are predominantly vertically transmitted, but Lipsitch *et al.* (1996) also considered the situation in which one of the strains was strictly vertically transmitted and predicted that it could increase in a population that was infected by a HTP but it could not eliminate the HTP. More recently, four papers have specifically examined the theoretical interactions between VT symbionts and HTPs. Three of these models assume that there is complete protection, i.e. a HTP cannot infect hosts that are already infected by a VT symbiont (Lively *et al.* 2005; Faeth *et al.* 2007; Dhirasakdanon & Thieme submitted). However, the assumption that a host cannot be simultaneously infected by a VT symbiont and an HTP is not always biologically realistic because empirical studies have demonstrated that hosts can be simultaneously infected by parasites with both forms of transmission (e.g. Oliver *et al.* 2003, 2005; Haine *et al.* 2005). Owing to the nature of their transmission mode, VT symbionts will inevitably infect a host individual first, after which HTPs may superinfect the same hosts (e.g. Oliver *et al.* 2003, 2005; Haine *et al.* 2005). In their model, Jones *et al.* (2007; box 1) allow the HTP to superinfect hosts that are already infected by a VT symbiont.

When a VT symbiont is introduced to a population at equilibrium for a HTP, Lively *et al.* (2005) predicted that the VT symbiont can reduce the frequency and number of hosts infected by an HTP. It can also increase the number of hosts in the population at equilibrium relative to a population infected only by the HTP: the number of uninfected hosts increases as the VT symbiont increases in frequency, and the frequency of both uninfected and VT symbiont-infected hosts increases with the transmission probability of the VT symbiont. Although the VT symbiont can only invade an uninfected population if its transmission probability is greater than 1, which is biologically impossible in this theoretical system, it can also invade a population that is already infected by an HTP because the HTP drastically reduces host population density, and the effect of host density on individuals' birth and death rates is reduced (Lively *et al.* 2005). Interestingly, the model predicted that density-dependent virulence of the HTP increases as the transmission of the VT parasite increases because the total host density increases with VT parasite transmission (Lively *et al.* 2005). A study with similar assumptions predicted that coexistence is possible provided the VT strain causes less mortality than the HT strain (Dhirasakdanon & Thieme submitted), and Faeth *et al.* (2007) predicted that a VT strain, which would die out on its own, was able to persist by protecting its host against a more harmful HT strain. In all three of these studies the interesting prediction is that, even when the probability of a VT symbiont being successfully transmitted to the next generation is less than or equal to 1 (therefore preventing it from invading an uninfected population), it can invade a host population if it protects the host population from an HTP.

By contrast, both VT symbionts and HTPs can simultaneously infect the same host. Jones *et al.* (2007) made predictions about the population dynamics of a population in which a VT symbiont can protect its host by reducing the transmission efficiency of an HTP. In a population of susceptible hosts, hosts infected by VT symbionts and HTPs, the proportion of hosts infected by VT symbionts and HTPs increases as the protection conferred by the VT symbiont increases, until a threshold is reached at which the protection is so large that there are very few individuals infected by the VT symbiont that are becoming infected by the HTP. As protection reaches 100%, the density of hosts infected by the HTP decreases (Jones *et al.* 2007; box 1), and there is an overall increase in host population density (as also predicted by Lively *et al.* (2005), Faeth *et al.* (2007) and Dhirasakdanon & Thieme (submitted)). Notably, where the HTP is a functional predator (i.e. infected individuals neither recover nor reproduce, Boots (2004)) there is always a greater opportunity for the VT symbiont persist compared with when the HTP acts like a true parasite (i.e. infected individuals can recover and can reproduce; Jones *et al.* 2007). This is because in a 'predatory' system, HTP-infected hosts do not contribute to the population of susceptible hosts, and the susceptible hosts are therefore less able to compete for resources with the population of VT symbiont-infected hosts. The VT symbiont-infected hosts can therefore provide a lower level of protection to the host from the HTP and still persist in the system (Jones *et al.* 2007). Overall, therefore, theory predicts that interference between VT symbionts and HTPs is possible. There is mounting empirical evidence that this possibility is realized in nature.

3. EMPIRICAL EVIDENCE FOR VT SYMBIONT INVOLVEMENT IN HOST RESISTANCE TO NATURAL ENEMIES

As a result of increased awareness of the incidence of symbionts and hence more general screening for their presence in host study organisms, there has been a dramatic increase in the evidence for symbiont-mediated protection over the past 5 years. This evidence can broadly be divided into evidence for symbionts (a) protecting their host from microbial diseases, (b) protecting their host from parasites and (c) protecting their host from predators (summarized in table 1).

(a) *Provision of protection from pathogens*

Symbiotic microbes have the capacity to protect their hosts from pathogenic micro-organisms, and there is evidence for symbiont protection in diverse arthropods including shrimp, lobster, aphids and mosquitoes. For example, symbionts produce antifungal metabolites that protect their crustacean hosts *Palaemon macrodactylus* and *Homarus americanus* from the pathogenic fungus *Lagenidium callinectes* (Gil-Turnes *et al.* 1989; Gil-Turnes & Fenical 1992). Similarly, possession of the facultative symbiont *Regiella insecticola* is associated with host resistance to a fungal pathogen *Pandora neoaphidis* in aphids (Ferrari *et al.* 2004; Scarborough *et al.* 2005). Another interesting phenomenon is that the cardia of the *Anopheles gambiae* gut expresses several anti-*Plasmodium* factors (Warr *et al.* 2007). Research is currently underway that suggests that the presence of the natural bacterial flora in the mosquito 'primes' or activates the immune response against *Plasmodium* in addition to having a direct effect on parasite development in the mosquito gut (G. Dimopoulos 2007, personal communication). The mosquito's capacity

Table 1. Summary of empirical studies finding direct evidence for symbiont-mediated protection against natural enemies. (The symbiont, host and natural enemy in each interaction are listed, along with the protection mechanism where known.)

symbiont	host	natural enemy	protection mechanism	references
Alteromonas sp.	*Palaemon macrodactylus*	fungus	antifungal compound, isatin	Gil-Turnes *et al.* (1989)
unknown bacterium	*Homarus americanus*	fungus	antifungal compound, tyrosol	Gil-Turnes & Fenical (1992)
Regiella insecticola	*Acyrthosiphon pisum*	fungus	?	Ferrari *et al.* (2004) and Scarborough *et al.* (2005)
Serratia symbiotica	*Acyrthosiphon pisum*	parasitoids	?	Oliver *et al.* (2003)
Hamiltonella defensa	*Acyrthosiphon pisum*	parasitoids	toxin produced by bacteriophage	Oliver *et al.* (2003, 2005), Ferrari *et al.* (2004) and Moran *et al.* (2005)
Dictyocoela sp.	*Gammarus roeseli*	parasitic helminth	?	Haine *et al.* (2005)
Pseudomonas sp.	*Paederus* spp.	predatory wolf spider	secretion of pederin toxin	Kellner & Dettner (1996), Kellner (1999, 2001), Piel (2002) and Piel *et al.* (2004*a*)

to transmit malaria is therefore likely to be significantly influenced by its microbial exposure in nature.

(b) *Provision of protection from parasites*

In addition to protecting their hosts from other micro-organisms, symbionts may also protect their hosts from parasitic invertebrates, and there are two examples that support this. First, in the pea aphid, *Acyrthosiphon pisum*, two facultative symbionts, *Serratia symbiotica* and *Hamiltonella defensa*, confer protection to their host from the parasitoids *Aphidius ervi* and *Aphidius eadyi* (Oliver *et al.* 2003, 2005; Ferrari *et al.* 2004). Second, gammarid crustaceans can be infected by Acanthocephala (parasitic helminths). Gammarids are the intermediate hosts in which the helminths develop until a point when they manipulate their host's behaviour to make it more likely to be predated by the helminth's definitive host (Bauer *et al.* 2005). However, when the gammarid is also infected by microsporidian symbionts, the microsporidia reduce the behavioural manipulation by the helminth parasite, effectively reducing the predation risk to their host (Haine *et al.* 2005; Rigaud & Haine 2005). This helminth parasite only partially castrates its gammarid host, therefore by 'sabotaging' the behavioural change, the microsporidia must gain enough advantage in terms of increased host survival to offset the costs of interference. Interestingly, the examples in this section are where the parasite is a functional predator and these are predicted to be more likely to allow the persistence of VT symbionts than true parasites (Jones *et al.* 2007).

(c) *Provision of protection from predators*

Finally, there is evidence that symbionts can produce toxic compounds that protect their hosts from predators. Kellner (1999) first proposed that endosymbionts in *Paederus* beetles produce the polyketide toxin pederin, rather than the beetles themselves. This toxin confers protection to *Paederus* beetle larvae from wolf spiders (Kellner & Dettner 1996). Pederin was subsequently confirmed to originate from a bacterial symbiont of the beetles, rather than the beetles themselves (Kellner 2001, 2002; Piel 2002; Piel *et al.* 2004*a*). Polyketide toxins like pederin are also found in sponges and although at least one of these toxins is of bacterial origin (Faulkner *et al.* 1999; Piel *et al.* 2004*b*), as yet there is no link between polyketides and sponge defences against predators. However, another polyketide produced by the bacterial symbiont *Endobugula sertula* deters fishes from predating its bryozoan host (Davidson *et al.* 2001; Lopanik *et al.* 2004*a*,*b*), and a similar symbiont-mediated mechanism is thought to protect marine isopods from fish predation (Lindquist *et al.* 2005).

4. MECHANISMS OF SYMBIONT-MEDIATED PROTECTION

The mechanisms by which symbionts protect their hosts from natural enemies are diverse. As described above, some produce substances with antimicrobial properties such as isatin (Gil-Turnes *et al.* 1989) and tyrosol (Gil-Turnes & Fenical 1992). Others deter potential predators from their hosts (Kellner & Dettner 1996; Lopanik *et al.* 2004*b*) through the production of toxic compounds called polyketides, such as bryostatin (Lopanik *et al.* 2004*a*), pederin (Piel 2002), and onnamides and theopederins (Piel *et al.* 2004*b*), which can inhibit eukaryotic protein biosynthesis (e.g. Tiboni *et al.* 1968). In addition, it has recently been proposed that a toxin produced by the bacteriophage APSE in the symbiont *H. defensa* may be the mechanism by which this symbiont protects its aphid host from parasitoids (Moran *et al.* 2005). This toxin is similar to Shiga toxin, which disrupts eukaryotic cell processes. It would be interesting to determine whether *S. symbiotica*, the other symbiont that protects aphids from parasitoid attack, and *R. insecticola*, which protects aphids from fungal pathogens, also possess bacteriophages with similar toxin-producing capabilities. By contrast, the mechanism by which microsporidia sabotage the behavioural change induced in gammarids by Acanthocephala remains unclear. Acanthocephala manipulate host behaviour in some gammarid species by altering serotonergic activity (Tain *et al.* 2006), and the microsporidia possibly interferes with this process rather than by directly killing the other parasite, since the Acanthocephala infecting gammarids that are also infected by microsporidia are viable (Haine *et al.* 2005).

It is likely that there are other (hypothetical) mechanisms by which VT symbionts protect their hosts.

Table 2. Symbiont avoidance of host defences. (In each case, the symbiont and host are listed, along with the defence component that has been investigated with regard to the interactions between symbionts and host immunity. 'Yes' and 'no' indicate studies that have investigated whether the symbiont can and cannot, respectively, induce or suppress host defences.)

symbiont	host	host defence	induce	suppress	references
Wolbachia	*Drosophila simulans*	antimicrobial peptides	no	no	Bourtzis *et al.* (2000)
Wolbachia	*Drosophila simulans*	all, encapsulation	no	yes	Fytrou *et al.* (2005)
Wolbachia	*Aedes albopictus*	antimicrobial peptides	no	no	Bourtzis *et al.* (2000)
Rickettsia peacockii	*Dermacentor andersoni*	antimicrobial peptides	no	no	Mattila *et al.* (2007)
Rickettsia peacockii	*Dermacentor andersoni*	phagocytosis		no	Mattila *et al.* (in press)
Spiroplasma poulsonii	*Drosophila melanogaster*	antimicrobial peptides	no	no	Hurst *et al.* (2003)
γ3-proteobacteria S clade	*Sitophilus zeamais*	peptidoglycan recognition proteins	yes		Heddi *et al.* (2005)

For example, in addition to producing growth-inhibiting toxins, symbionts might also directly protect their hosts by outcompeting HTPs for host resources. Alternatively, they may indirectly protect their host by preventing superinfection of the host by HTPs, perhaps by altering host behaviour or augmenting host defences to prevent disease establishment. In all these cases, however, it is essential that symbionts are able to avoid host immune defences. There are three reasons why this is necessary: first, VT symbionts must avoid recognition by the host immune system in order to establish and be maintained in the host; second, where the host is superinfected by a parasite, they must survive the parasite-induced immune response; and third, if the VT symbiont manipulates the host immune defences to overcome a parasite it must also survive the augmented immune response. A tantalizing possibility is that VT symbionts may have the capacity to commandeer host defences to produce a swifter response to a HTP than the host on its own.

5. VT SYMBIONT INTERACTIONS WITH HOST IMMUNE DEFENCES

Hosts are selected to identify invading micro-organisms and swiftly mount immune defences in order to eliminate pathogens. Where the maintenance of a VT symbiont provides the host with a defence advantage, the host must exercise balance between recognizing and defending itself against HTPs, and maintaining beneficial VT symbionts. A VT symbiont may therefore be under selection to avoid being recognized by its host immune system, but at the same time it must not suppress its host's immune defences against pathogens. A VT symbiont's capacity to protect its host from natural enemies and its capacity to avoid its host's immune defences are therefore intrinsically linked. It is therefore essential to explore the interactions between symbionts and their host immune defences in order to understand symbiont-mediated protection.

Despite only possessing innate immune defences (Siva-Jothy *et al.* 2005), invertebrates are able to defend themselves against, and survive, a wide variety of parasites (Poulin 2007). They do this through the sophisticated management of the cellular and humoral components of their immune systems (reviewed in Loker *et al.* 2004; Little *et al.* 2005; Siva-Jothy *et al.* 2005). Their first line of defence comprises barrier defences—cuticle and gut. If these barrier defences are breached, perhaps via a wound, then cellular processes such as phagocytosis, nodulation and encapsulation quickly surround invading pathogens, physically separate them from the haemocoel and kill them (e.g. Cherry & Silverman 2006). Meanwhile a suite of humoral defences are expressed—antimicrobial peptides (AMPs; Bulet *et al.* 2004), phenoloxidase (Suguraman 2002) and coagulation (Theopold *et al.* 2004). Together these defences serve to eliminate any invading parasites or pathogens and physically separate them from the haemocoel. All of these components are relevant to the entry, establishment and persistence of VT symbionts in their hosts, and it is possible that they may also be manipulated by symbionts in order for the VT symbiont to evade, suppress or even upregulate host immune responses.

A straightforward strategy to avoid being recognized by the host immune response is for a symbiont to locate itself inside host cells, where AMPs and other components of haemolymph-based invertebrate immune responses cannot penetrate. Indeed, most VT symbionts are obligately intracellular, having lost the capacity to exist in a free-living state during the evolutionary history of their association with their hosts (e.g. McGraw & O'Neill 2004). A small number of studies have examined the interactions between VT symbionts and invertebrate immunity (table 2). Studies on *Wolbachia* suggest either that there are particular host-genotype/symbiont-genotype interactions that determine whether or not the symbionts interfere with host defences, or that the symbiont only interacts with some, but not all, components of the host immune system (Bourtzis *et al.* 2000; Fytrou *et al.* 2005; Pankewitz *et al.* 2007). For example, *Wolbachia* reduced the ability of *Drosophila simulans* to encapsulate eggs of the parasitoid *Leptopilina heterotoma*, but had no impact on host resistance to an entomopathogenic fungus (Fytrou *et al.* 2005). Other VT symbionts fail to elicit several different components of their host immune responses (*Rickettsia*: Mattila *et al.* 2007, in press; *Spiroplasma*: Hurst *et al.* 2003), but while VT symbionts such as *Spiroplasma* can evade recognition by the host immune system, activation of host defences results in suppression of its titre (Hurst *et al.* 2003). By contrast, the tsetse fly (*Glossina morsitans morsitans*) is infected by three VT symbionts, *Wigglesworthia*, *Sodalis* and *Wolbachia*, but their densities did not change when the host immune system was activated by a challenge with *Escherichia coli* (Rio *et al.* 2006). However, some VT symbionts elicit host immune defences (Heddi *et al.* 2005) and this may be a strategy the host uses to control symbiont numbers and location (Anselme *et al.* 2006). Others are refractory to AMPs compared to their free-living relatives (Hao *et al.* 2001; Baldridge *et al.* 2005; Hu & Aksoy 2005), but the mechanism of this resistance is unknown. The general mode of action of AMPs is to be bactericidal: they are attracted to negatively charged phospholipids in cell

membranes where they form pores or channels that disrupt membrane potential and result in cell death (Zasloff 2002). It is tempting to speculate that VT symbionts can alter their membrane charge, so they are less susceptible to binding by positively charged AMPs.

VT symbionts may evade recognition by the receptors that trigger synthesis of AMPs by losing the surface molecules that elicit immune gene expression or by hiding behind host-derived surface molecules. For example, symbiotic Mollicutes such as spiroplasmas completely lack cell walls (Trachtenberg 1998), and it is the recognition of the constituents of microbial cell wall components such as peptidoglycan that results in the production of AMPs (Leulier *et al.* 2003). The lack of a cell wall may therefore explain their inability to activate the production of AMPs in *Drosophila* (Hurst *et al.* 2003) and could provide a reason why, compared with many VT symbionts, *Spiroplasma* are able to survive freely in the haemolymph and not just inside host cells. By contrast, *Wolbachia* are present in their hosts enclosed in a vacuole enveloped by three layers of membrane, the outer of which is derived from their host (Louis & Nigro 1989). They may therefore evade recognition as non-self by effectively hiding behind a host-derived cloak. Alternatively, VT symbionts may continually vary their surface antigens to avoid recognition. Some VT symbiont surface molecules are under positive selection and may even have been lost in some certain lineages (Schulenberg *et al.* 2000; Blanc *et al.* 2005; Jiggins 2006). However, Jiggins *et al.* (2002) found that there is positive selection on an outer membrane protein in parasitic, but not mutualistic, Rickettsiaceae, and this suggests that host evasion strategies may vary with the position of the VT symbiont on the parasitism–mutualism continuum. A recent review by Finlay & McFadden (2006) gives a comprehensive overview of how pathogens might evade host immune systems and many of the processes described in their review are relevant to VT symbionts.

Considering that VT symbionts can evade invertebrate immune defences and have the potential to manipulate them, invertebrate immune effector systems are likely to be an important line of defence at which the conflict between VT symbionts and HT natural enemies is fought. The capacity of an invertebrate to mount an immune response to pathogens, with and without infections by VT symbionts, should therefore be added to the 'traditional' measures of fecundity, size and longevity as a measure of the fitness consequences of VT symbiont infections.

6. CONCLUSIONS

Given the ubiquity of VT symbionts in invertebrates (Stouthamer *et al.* 1999; Terry *et al.* 2004; Zchori-Fein & Perlman 2004), it is likely that VT symbiont infections contribute significantly to variation in invertebrate resistance to HTP infections (Schmid-Hempel 2003). Theory predicts a three-way interaction between VT symbionts, natural enemies and their hosts, which is mediated by interference by VT symbionts on the impact that natural enemies have on their hosts. There is mounting empirical evidence that these three-way interactions exist and the routes by which they are mediated are diverse. Symbiont-mediated protection is moving from beyond the realm of obscure one-off occurrences, into a phenomenon that is likely to be widespread, and it goes some way towards explaining why VT symbionts are so common in invertebrate lineages. Interference by VT symbionts may well account for variation in behavioural modifications, pathogen prevalence, immune competence, pathogen virulence and host susceptibility.

Fruitful avenues of research will investigate whether the degree of protection depends on the length of time the host and VT symbiont have been associated, or on particular host genotype—VT symbiont genotype combinations. It would be particularly interesting to investigate whether the protection conferred by VT symbionts against microbial pathogens has arisen as a result of coevolution with a particular pathogen that infects that host or whether the protection is a generalized and systemic response to any pathogen whether it is a specialist on its host or an opportunist. Further detailed investigations of the immune- and predator-defence capabilities of VT symbiont-infected and uninfected invertebrates are likely to yield more examples of interference.

It would be particularly valuable to test the theory behind our understanding of these interactions by exploring how transmission rates of both types of parasite affect the level of protection through interference and its impact on host population dynamics (Jones *et al.* 2007), and explore the consequences of such interference on disease epidemiology. In addition, it would be interesting to perform general surveys of VT symbiont prevalences and, using natural history data, test the prediction that VT symbionts are more likely to persist in a system containing a functional predator as opposed to a true parasite (Jones *et al.* 2007). This aspect has potential ecological importance since the interference by VT symbionts (such as those in aphids) with functional predators (e.g. parasitoids) can have implications for food web complexity (Lafferty *et al.* 2006). Symbiont-mediated protection should also be given special attention in any system being developed for biological control purposes, since a control method developed for a VT symbiont-free population of a pest species might not work as efficiently for a population infected by a VT symbiont. Finally, lateral gene transfer between *Wolbachia* and its invertebrate hosts has been demonstrated (Kondo *et al.* 2002; Dunning Hotopp *et al.* 2007) and this opens up the intriguing possibility that invertebrates can potentially acquire VT symbiont genes, such as those that encode toxins such as pederin, to defend themselves against their natural enemies. It is therefore crucial to consider the presence of VT symbionts (and VT symbiont genes) in any studies that measure the immune competence, predator avoidance or other fitness aspects of invertebrates as they are likely to account for much of the variance measured in many different fitness traits.

I am indebted to Jens Rolff, Mike Siva-Jothy and three anonymous referees for useful comments on the manuscript, Thierry Rigaud for inspiration, and I would particularly like to thank Ed Jones and Mike Boots for their help on the theoretical considerations. Many thanks are also due to George Dimopoulos, Josh Mattila and Horst Thieme for access to pre-publication manuscripts and data.

REFERENCES

Anselme, C., Vallier, A., Balmand, S., Fauvarque, M.-O. & Heddi, A. 2006 Host *PGRP* gene expression and bacterial release in endosymbiosis of the weevil *Sitophilus zeamais*. *Appl. Environ. Microbiol.* **72**, 6766–6772. (doi:10.1128/AEM.00942-06)

Baldridge, G. D., Kurtti, T. J. & Munderloh, U. G. 2005 Susceptibility of *Rickettsia monacensis* and *Rickettsia peacockii* to Cecropin A, Ceratotoxin A and Lysozyme. *Curr. Microbiol.* **51**, 233–238. (doi:10.1007/s00284-005-4532-7)

Barton, E. S., White, D. W., Cathelyn, J. S., Brett-McClellan, K. A., Engle, M., Diamond, M. S., Miller, V. L. & Virgin, H. W. 2007 Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* **447**, 326–330. (doi:10.1038/nature05762)

Bauer, A., Haine, E. R., Perrot-Minnot, M.-J. & Rigaud, T. 2005 The acanthocephalan parasite *Polymorphus minutus* alters the geotactic and clinging behaviours of the invasive amphipod *Gammarus roeseli*. *J. Zool.* **267**, 39–43. (doi:10.1017/S0952836905007223)

Blanc, G., Ngwamidiba, M., Ogata, H., Fournier, P.-E., Claverie, J.-M. & Raoult, D. 2005 Molecular evolution of *Rickettsia* surface antigens, evidence of positive selection. *Mol. Biol. Evol.* **22**, 2073–2083. (doi:10.1093/molbev/msi199)

Boots, M. 2004 Modelling insect diseases as functional predators. *Physiol. Entomol.* **29**, 237–239. (doi:10.1111/j.0307-6962.2004.00403.x)

Bourtzis, K., Pettigrew, M. M. & O'Neill, S. L. 2000 *Wolbachia* neither induces nor suppresses transcripts encoding antimicrobial peptides. *Insect Mol. Biol.* **9**, 635–639. (doi:10.1046/j.1365-2583.2000.00224.x)

Buchner, P. 1965 *Endosymbiosis of animals with plant microorganisms*. New York, NY: Wiley.

Bulet, P., Stocklin, R. & Menin, L. 2004 Antimicrobial pepetides from invertebrates to vertebrates. *Immunol. Rev.* **198**, 169–184. (doi:10.1111/j.0105-2896.2004.0124.x)

Cherry, S. & Silverman, N. 2006 Host–pathogen interactions in *Drosophila*, new tricks from an old friend. *Nat. Immunol.* **7**, 911–917. (doi:10.1038/ni1388)

Combes, C. 2001 Parasitism, the ecology and evolution of intimate interactions, pp. 555–578. Chicago, IL: The University of Chicago Press.

Dale, C. & Moran, N. A. 2006 Molecular interactions between bacterial symbionts and their hosts. *Cell* **126**, 453–465. (doi:10.1016/j.cell.2006.07.014)

Dale, C., Beeton, M., Harbison, C., Jones, T. & Pontes, M. 2006 Isolation, pure culture and characterization of "Candidatus Atsenophonus arthropodicus", and intracellular secondary endosymbiont from the hippoboscid louse fly *Pseudolymchia canariensis*. *Appl. Environ. Microbiol.* **72**, 2997–3004. (doi:10.1128/AEM.72.4.2997-3004.2006)

Davidson, S. K., Allen, S. W., Lim, G. E., Anderson, C. M. & Haygood, M. G. 2001 Evidence for the biosynthesis of bryostatins by the bacterial symbiont *Candidatus Endobugula sertula* of the bryozoan *Bugula neritina*. *Appl. Environ. Microbiol.* **67**, 4531–4537. (doi:10.1128/AEM.67.10.4531-4537.2001)

Dedeine, F., Vavre, F., Fleury, F., Loppin, B., Hochberg, M. E. & Bouletreau, M. 2001 Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc. Natl Acad. Sci. USA* **98**, 6247–6252. (doi:10.1073/pnas.101304298)

Dhirasakdanon, T. & Thieme, H. R. Submitted. Persistence of vertically transmitted parasite strains which protect against more virulent horizontally transmitted strains.

Dunn, A. M. & Smith, J. E. 2001 Microsporidian life cycles and diversity, the relationship between virulence and transmission. *Microbes Infect.* **3**, 381–388. (doi:10.1016/S1286-4579(01)01394-6)

Dunning Hotopp, J. C. *et al.* 2007 Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* **317**, 1753–1756. (doi:10.1126/science.1142490)

Faeth, S. H., Hadeler, K. P. & Thieme, H. R. 2007 An apparent paradox of horizontal and vertical disease transmission. *J. Biol. Dyn.* **1**, 45–62. (doi:10.1080/17513750601040367)

Faulkner, D. J., Harper, M. K., Salomon, C. E. & Schmidt, E. W. 1999 Localisation of bioactive metabolites in marine sponges. *Mem. Queensl. Mus.* **44**, 167–173.

Ferrari, J., Darby, A. C., Daniell, T. J., Godfray, H. C. J. & Douglas, A. E. 2004 Linking the bacterial community in pea aphids with host–plant use and natural enemy resistance. *Ecol. Entomol.* **29**, 60–65. (doi:10.1111/j.1365-2311.2004.00574.x)

Finlay, B. B. & McFadden, G. 2006 Anti-immunology, evasion of the host immune system by bacterial and viral pathogens. *Cell* **124**, 767–782. (doi:10.1016/j.cell.2006.01.034)

Fytrou, A., Schofield, P. G., Kraaijeveld, A. R. & Hubbard, S. F. 2005 *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. *Proc. R. Soc. B* **273**, 791–796. (doi:10.1098/rspb.2005.3383)

Gil-Turnes, M. S. & Fenical, W. 1992 Embryos of *Homarus americanus* are protected by epibiotic bacteria. *Biol. Bull.* **182**, 105–108. (doi:10.2307/1542184)

Gil-Turnes, M. S., Hay, M. E. & Fenical, W. 1989 Symbiotic mariner bacteria chemically defend Crustancean embryos from a pathogenic fungus. *Science* **246**, 116–118. (doi:10.1126/science.2781297)

Haine, E. R., Boucansaud, K. & Rigaud, T. 2005 Conflict between parasites with different transmission strategies infecting an amphipod host. *Proc. R. Soc. B* **272**, 2505–2510. (doi:10.1098/rspb.2005.3244)

Hao, Z., Kasumba, I., Lehane, M. J., Gibson, W. C., Kwon, J. & Aksoy, S. 2001 Tsetse immune responses and trypanosome transmission, implications for the development of tsetse-based strategies to reduce trypanosomiasis. *Proc. Natl Acad. Sci. USA* **98**, 12 649–12 653.

Heddi, A., Vallier, A., Anselme, C., Xin, H., Rahbe, Y. & Wackers, F. 2005 Molecular and cellular profiles of insect bacteriocytes, mutualism and harm at the initial evolutionary step of symbiogenesis. *Cell. Microbiol.* **7**, 293–305.

Hoffmann, A. A., Clancy, D. & Duncan, J. 1996 Naturally-occurring *Wolbachia* infection in *Drosophila simulans* that does not cause cytoplasmic incompatibility. *Heredity* **76**, 1–8.

Hu, Y. & Aksoy, S. 2005 An antimicrobial peptide with trypanocidal activity characterized from *Glossina morsitans morsitans*. *Insect Biochem. Mol. Biol.* **35**, 105–115. (doi:10.1016/j.ibmb.2004.10.007)

Hunter, M. S., Perlman, S. J. & Kelly, S. E. 2003 A bacterial symbiont in the *Bacteroidetes* induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella*. *Proc. R. Soc. B* **270**, 2185–2190. (doi:10.1098/rspb.2003.2475)

Hurst, G. D. D., Bandi, C., Sacchi, L., Cochrane, A. G., Bertrand, D., Karaca, I. & Majerus, M. E. N. 1999 *Adonia variegata* (Coleoptera, Coccinellidae) bears maternally inherited flavobacteria that kill males only. *Parasitology* **118**, 125–134. (doi:10.1017/S0031182098003655)

Hurst, G. D. D., Anbutsu, H., Kutsukake, M. & Fukatsu, T. 2003 Hidden from the host, *Spiroplasma* bacteria infecting *Drosophila* do not cause an immune responses, but are suppressed by ectopic immune activation. *Insect Mol. Biol.* **12**, 93–97. (doi:10.1046/j.1365-2583.2003.00380.x)

Hurst, G. D. D., Webberley, M. & Knell, R. 2005 The role of parasites of insect reproduction in the diversification of insect reproductive processes. In *Insect evolutionary ecology* (eds M. D. E. Fellowes, G. J. Holloway & J. Rolff), pp. 205–229. Wallingford, UK: CABI Publishing.

Jiggins, F. M. 2006 Adaptive evolution and recombination of *Rickettsia* antigens. *J. Mol. Evol.* **62**, 99–110. (doi:10.1007/s00239-005-0080-9)

Jiggins, F. M., Hurst, G. D. D. & Yang, Z. 2002 Host–symbiont conflicts, positive selection on an outer membrane protein of parasitic but not mutualistic Rickettsiaceae. *Mol. Biol. Evol.* **19**, 1341–1349.

Jones, E. O., White, A. & Boots, M. 2007 Interference and persistence of vertically transmitted parasites. *J. Theor. Biol.* **246**, 10–17. (doi:10.1016/j.jtbi.2006.12.007)

Kellner, R. L. L. 1999 What is the basis of pederin polymorphism in *Paederus riparius* rove beetles? The endosymbiotic hypothesis. *Entomol. Exp. Appl.* **93**, 41–49. (doi:10.1023/A:1003842927497)

Kellner, R. L. L. 2001 Suppression of pederin biosynthesis through antibiotic elimination of endosymbionts in *Paederus sabaeus*. *J. Insect Physiol.* **47**, 475–483. (doi:10.1016/S0022-1910(00)00140-2)

Kellner, R. L. L. 2002 Molecular identification of an endosymbiotic bacterium associated with pederin biosynthesis in *Paederus sabaeus* (Coleoptera, Staphylinidae). *Insect Biochem. Mol. Biol.* **32**, 389–395. (doi:10.1016/S0965-1748(01)00115-1)

Kellner, R. L. L. & Dettner, K. 1996 Differential efficacy of toxin pederin in deterring potential arthropod predators of *Paederus* (Coloptera, Staphylinidae) offspring. *Oecologia* **107**, 293–300. (doi:10.1007/BF00328445)

Kondo, N., Nikoh, N., Ijichi, N., Shimada, M. & Fukatsu, T. 2002 Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. *Proc. Natl Acad. Sci. USA* **99**, 14 280–14 285. (doi:10.1073/pnas.222228199)

Lafferty, K. D., Dobson, A. P. & Kuris, A. M. 2006 Parasites dominate food web links. *Proc. Natl Acad. Sci. USA* **103**, 11 211–11 216. (doi:10.1073/pnas.0604755103)

Leulier, F., Parquet, C., Pili-Floury, S., Ryu, J.-H., Carolff, M., Lee, W.-J., Mengin-Lecreulx, D. & Lemaitre, B. 2003 The *Drosophila* immune system detects bacteria through speciic peptidoglycan recognition. *Nat. Immunol.* **4**, 478–484. (doi:10.1038/ni922)

Lindquist, N., Barber, P. H. & Weisz, J. B. 2005 Episymbiotic microbes as food and defence for mariner isopods, unique symbioses in a hostile environment. *Proc. R. Soc. B* **272**, 1209–1216. (doi:10.1098/rspb.2005.3082)

Lipsitch, M., Nowak, M. A., Ebert, D. & May, R. M. 1995 The population dynamics of vertically and horizontally transmitted parasites. *Proc. R. Soc. B* **260**, 321–327. (doi:10.1098/rspb.1995.0099)

Lipsitch, M., Siller, S. & Nowak, M. A. 1996 The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* **50**, 1729–1741. (doi:10.2307/2410731)

Little, T. J., Hultmark, D. & Read, A. F. 2005 Invertebrate immunity and the limits of mechanistic immunology. *Nat. Immunol.* **6**, 651–654. (doi:10.1038/ni1219)

Lively, C. M., Clay, K., Wade, M. J. & Fuqua, C. 2005 Competitive co-existence of vertically and horizontally transmitted parasites. *Evol. Ecol. Res.* **7**, 1183–1190.

Loker, E. S., Adema, C. M., Zhang, S.-M. & Kepler, T. B. 2004 Invertebrate immune systems—not homogenous, not simple, not well understood. *Immunol. Rev.* **198**, 10–24. (doi:10.1111/j.0105-2896.2004.0117.x)

Lopanik, N., Gustafson, K. R. & Lindquist, N. 2004*a* Structure of bryostatin 20, a symbiont-produced chemical defense for larvae of the host bryozoan *Bugula neritina*. *J. Nat. Prod.* **67**, 1412–1414. (doi:10.1021/np040007k)

Lopanik, N., Lindquist, N. & Targett, N. 2004*b* Potent cytotoxins produced by a microbial symbiont protect host larvae from predation. *Oecologia* **139**, 131–139. (doi:10.1007/s00442-004-1487-5)

Louis, C. & Nigro, L. 1989 Ultrastructural evidence of *Wolbachia* Rickettsiales in *Drosophila simulans* and their relationship with unidirectional cross-incompatibility. *J. Invert. Pathol.* **54**, 39–44. (doi:10.1016/0022-2011(89)90137-7)

Mattila, J. T., Munderloh, U. G. & Kurtti, T. J. 2007 *Rickettsia peacockii*, an endosymbiont of *Dermacentor andersoni*, does not elicit or inhibit humoral immune responses from immunocompetent *D. andersoni* or *ixodes scapularis* cell lines. *Dev. Comp. Immunol.* **31**, 1095–1106. (doi:10.1016/j.dci.2007.01.011)

Mattila, J. T., Munderloh, U. G. & Kurtti, T. J. In press. Phagocytosis of the Lyme disease spirochete, *Borrelia burgdorferi*, by cells from the ticks, *Ixodes scapularis* and *Dermacentor andersoni*, infected with an endosymbiont, *Rickettsia peacockii*. *J. Insect Sci.*

McGraw, E. A. & O'Neill, S. L. 2004 *Wolbachia pipentis*, intracellular infection and pathogenesis in *Drosophila*. *Curr. Opin. Microbiol.* **7**, 67–70. (doi:10.1016/j.mib.2003.12.003)

Montllor, C. B., Maxmen, A. & Purcell, A. H. 2002 Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecol. Entomol.* **27**, 189–195. (doi:10.1046/j.1365-2311.2002.00393.x)

Moran, N. A. 2006 Symbiosis. *Curr. Biol.* **16**, R866–R871. (doi:10.1016/j.cub.2006.09.019)

Moran, N. A., Degnan, P. H., Santos, S. R., Dunbar, H. E. & Ochman, H. 2005 The players in a mutualistic symbiosis, Insects bacteria, viruses, and virulence genes. *Proc. Natl Acad. Sci. USA* **102**, 16 919–16 926. (doi:10.1073/pnas.0507029102)

Oliver, K. M., Russell, J. A., Moran, N. A. & Hunter, M. S. 2003 Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proc. Natl Acad. Sci. USA* **100**, 1803–1807. (doi:10.1073/pnas.0335320100)

Oliver, K. M., Moran, N. A. & Hunter, M. S. 2005 Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proc. Natl Acad. Sci. USA* **102**, 12 795–12 800. (doi:10.1073/pnas.0506131102)

Pankewitz, F., Zöllmer, A., Hilker, M. & Gräser, Y. 2007 Presence of *Wolbachia* in insect eggs containing antimicrobially active anthraquinones. *Microb. Ecol.* **54**, 713–721. (doi:10.1007/s00248-007-9230-5)

Perlman, S. J., Hunter, M. S. & Zchori-Fein, E. 2006 The emerging diversity of *Rickettsia*. *Proc. R. Soc. B* **273**, 2097–2106. (doi:10.1098/rspb.2006.3541)

Piel, J. 2002 A polyketide synthase–pepetide synthetase gene cluster from an uncultured bacterial symbiont of *paederus* beetles. *Proc. Natl Acad. Sci. USA* **99**, 14 002–14 007. (doi:10.1073/pnas.222481399)

Piel, J., Hofer, I. & Hui, D. 2004*a* Evidence for a symbiosis island involved in horizontal acquisition of pederin biosynthetic capabilities by the bacterial symbiont of *Paederus fuscipes* beetles. *J. Bacteriol.* **186**, 1280–1286. (doi:10.1128/JB.186.5.1280-1286.2004)

Piel, J., Hui, D., Wen, G., Butzke, D., Platzer, M., Fusetani, N. & Matsunaga, S. 2004*b* Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. *Proc. Natl Acad. Sci. USA* **101**, 16 222–16 227. (doi:10.1073/pnas.0405976101)

Poulin, R. 2007 *Evolutionary ecology of parasites*, 2nd edn. Princeton, NJ: Princeton University Press.

Rigaud, T. & Haine, E. R. 2005 Conflict between co-occurring parasites as a confounding factor in manipulation studies? *Behav. Process.* **68**, 259–262. (doi:10.1016/j.beproc.2004.09.005)

Rio, R. V. M., Wu, Y.-N., Filardo, G. & Aksoy, S. 2006 Dynamics of multiple symbiont density regulation during host development, tsetse fly and its microbial flora. *Proc. R. Soc. B* **273**, 805–814. (doi:10.1098/rspb.2005.3399)

Scarborough, C. L., Ferrari, J. & Godfray, H. C. J. 2005 Aphid protected from pathogen by endosymbiont. *Science* **310**, 3781. (doi:10.1126/science.1120180)

Schmid-Hempel, P. 2003 Variation in immune defence as a question of evolutionary ecology. *Proc. R. Soc. B* **270**, 357–366. (doi:10.1098/rspb.2002.2265)

Schulenberg, H., Hurst, G. D. D., Huigens, T. M. E., van Meer, M. M. M., Jiggins, F. M. & Majerus, M. E. N. 2000 Molecular evolution and phylogenetic utility of *Wolbacha ftsZ* and *wsp* gene sequences with special reference to the origin of male-killing. *Mol. Biol. Evol.* **17**, 584–600.

Schulenburg, J. H. G. v. d., Habig, M., Sloggett, J. J., Webberley, K. M., Bertrand, D., Hurst, G. D. D. & Majerus, M. E. N. 2001 Incidence of male-killing *Rickettsia* spp. (α-Proteobacteria) in the ten-spot ladybird beetle *Adalia decempunctata* L. (Coleoptera: Coccinellidae). *Appl. Environm. Microbiol.* **67**, 270–277. (doi:10.1128/AEM.67.1.270-277.2001)

Siva-Jothy, M. T., Moret, Y. & Rolff, J. 2005 Insect immunity: an evolutionary ecology perspective. *Adv. Insect Physiol.* **32**, 1–48. (doi:10.1016/S0065-2806(05)32001-7)

Stouthamer, R., Breeuwer, J. A. & Hurst, G. D. 1999 *Wolbachia pipientis*, microbial manipulator of arthropod reproduction. *Ann. Rev. Microbiol.* **53**, 71–102. (doi:10.1146/annurev.micro.53.1.71)

Suguraman, M. 2002 Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. *Pigment Cell Res.* **15**, 2–9. (doi:10.1034/j.1600-0749.2002.00056.x)

Tain, L., Perrot-Minnot, M. J. & Cezilly, F. 2006 Altered host behaviour and brain serotonergic activity caused by acanthocephalans, evidence for specificity. *Proc. R. Soc. B* **273**, 3039–3045. (doi:10.1098/rspb.2006.3618)

Terry, R. S. *et al.* 2004 Widespread vertical transmission and associated sex-ratio distortion within the eukaryotic phylum Microspora. *Proc. R. Soc. B* **271**, 1783–1789. (doi:10.1098/rspb.2004.2793)

Theopold, U., Schmidt, O., Soderhall, K. & Dushay, M. S. 2004 Coagulation in arthropods, defence, wound closure and healing. *Trends Immunol.* **25**, 289–294. (doi:10.1016/j.it.2004.03.004)

Tiboni, O., Parisi, B. & Ciferri, O. 1968 The mode of action of pederin, a drug inhibiting protein synthesis in eukaryotic organisms. *Giorn. Bot. Ital.* **102**, 337–345.

Trachtenberg, S. 1998 Mollicutes—wall-les bacteria with internal cytoskeletons. *J. Struct. Biol.* **124**, 244–256. (doi:10.1006/jsbi.1998.4063)

Tsuchida, T., Koga, R., Shibao, H., Matsumoto, T. & Fukatsu, T. 2002 Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum*. *Mol. Ecol.* **11**, 2123–2135. (doi:10.1046/j.1365-294X.2002.01606.x)

Warr, E., Aguilar, R., Dong, Y., Mahairaki, V. & Dimopoulos, G. 2007 Spatial and sex-specific dissection of the *Anopheles gambiae* midgut transcriptome. *BMC Genomics* **8**, 37. (doi:10.1186/1471-2164-8-37)

Weeks, A. R., Marec, F. & Breeuwer, A. J. 2001 A mite species that consists entirely of haploid females. *Science* **292**, 2479–2482. (doi:10.1126/science.1060411)

Weeks, A. R., Velten, R. & Stouthamer, R. 2003 Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthroods. *Proc. R. Soc. B* **270**, 1857–1865. (doi:10.1098/rspb.2003.2425)

Werren, J. H. & O'Neill, S. L. 1997 The evolution of heritable symbionts. In *Influential passengers, inherited microorganisms and arthropod reproduction* (eds S. L. O'Neill, A. A. Hoffmann & J. H. Werren), pp. 1–41. Oxford, UK: Oxford University Press.

Werren, J. H., Windsor, D. & Guo, L. 1995 Distribution of *Wolbachia* among neotropical arthropods. *Proc. R. Soc. B* **262**, 197–204. (doi:10.1098/rspb.1995.0196)

Yen, J. H. & Barr, A. R. 1971 New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens*. *Nature* **232**, 657–658. (doi:10.1038/232657a0)

Zasloff, M. 2002 Antimicrobial peptides of multicellular organisms. *Nature* **415**, 389–395. (doi:10.1038/415389a)

Zchori-Fein, E. & Perlman, S. J. 2004 Distribution of the bacterial symbiont *Cardinium* in arthropods. *Mol. Ecol.* **13**, 2009–2016. (doi:10.1111/j.1365-294X.2004.02203.x)