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Review

Vertically transmitted viral endosymbionts of insects: do sigma viruses walk alone?

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Insects are host to a wide range of vertically transmitted bacterial endosymbionts, but we know relatively little about their viral counterparts. Here, we discuss the vertically transmitted viral endosymbionts of insects, firstly examining the diversity of this group, and then focusing on the well-studied sigma viruses that infect dipterans. Despite limited sampling, evidence suggests that vertically transmitted viruses may be common in insects. Unlike bacteria, viruses can be transmitted through sperm and eggs, a trait that allows them to rapidly spread through host populations even when infection is costly to the host. Work on *Drosophila melanogaster* has shown that sigma viruses and their hosts are engaged in a coevolutionary arms race, in which the spread of resistance genes in the host population is followed by the spread of viral genotypes that can overcome host resistance. In the long-term, associations between sigma viruses and their hosts are unstable, and the viruses persist by occasionally switching to new host species. It therefore seems likely that viral endosymbionts have major impacts on the evolution and ecology of insects.

Keywords: sigma virus; sweep; host shift; rhabdovirus; biparental transmission; *Wolbachia*

1. INTRODUCTION

Insects are host to a wide range of vertically transmitted endosymbionts; micro-organisms that reside inside their insect host and are passed vertically from parent to offspring. These endosymbionts can sit anywhere on a scale from mutualist (the host derives a fitness benefit) to parasitic (the host suffers a decline in fitness) [1,2]. Bacterial endosymbionts are estimated to infect the majority of all insect species [3,4], and are usually transmitted vertically by females through their eggs [5]. Insects can also be host to viruses that are transmitted vertically as viral particles from parent to offspring, and we refer to these as viral endosymbionts.

While bacterial endosymbionts of insects have been relatively well studied, our understanding of their viral counterparts is lacking. The reason why relatively few viral endosymbionts have been described is likely due in part to the difficulty in detecting them. Unlike bacterial endosymbionts, where often a single set of conserved PCR primers can be used to test insects for infection [6], the high levels of sequence divergence between virus groups, coupled with the poor sampling of viruses in invertebrates, makes it difficult to rapidly detect new viruses.

Bacterial endosymbionts have evolved a suite of different strategies to spread in host populations, and as a result of this they can have important impacts on host biology. For example, symbionts can protect insects against natural enemies [7], allow them to feed on particular plants [2], distort the sex ratio to such an extent that sexual behaviour changes [8] and potentially promote speciation by inducing reproductive incompatibilities [9]. In other taxa viral endosymbionts can have important consequences for their hosts. For example, a viral endosymbiont that infects a fungus of panic grass confers thermo-tolerance to both the fungi and its plant host, and a viral endosymbiont in clover can suppress root nodulation when sufficient nitrogen is present [10].

However, we know relatively little about the impacts of viral endosymbionts on insect biology. In this paper, we aim to address this by reviewing what is known about the vertically transmitted viruses of insects, in particular, the well-studied sigma viruses that infect *Drosophila*, highlighting the biology of these viruses and the impacts they have on their hosts, while comparing and contrasting them to bacterial endosymbionts.

2. THE DIVERSITY OF VERTICALLY TRANSMITTED INSECT VIRUSES

One way in which viruses can be vertically transmitted is by integrating into the host genome. It is common to find retroviruses that have integrated into the host germ-line and are therefore stably inherited, but most of these endogenous retroviruses are no longer able to produce viable virions [11]. A curious exception to this pattern are polydnaviruses, which are transmitted as proviruses integrated into the genomes of ichneumonid and braconid parasitoid wasps, but are still able to produce viral particles [12]. The ancestor of polydnaviruses in braconid wasps was a nudivirus, a genus of arthropod viruses with double-stranded DNA genomes [13], which integrated into the wasp genome [14]. The viral genome is copied into multiple double-stranded DNA circles and packaged into viral particles that are injected into the lepidopteran larval hosts that the wasps parasitize [12]. Here, the virus enters the lepidopteran cells and the viral genes are expressed (although the virus does not

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replicate), suppressing the lepidopteran immune system [12]. It is therefore believed that these viruses are spread through wasp populations as mutualists [10].

A second class of vertically transmitted viruses can be transmitted both as infectious virions and as a provirus integrated into the insect genome. The only virus we are currently aware of that has evolved this strategy is the *gypsy* element in *Drosophila melanogaster* [15], which can produce infectious virions that are transmitted to the next generation in eggs, enabling the virus to spread through populations as a selfish genetic element [16]. Gypsy elements are widely distributed in other species of Drosophilidae and show evidence of switching between host species, suggesting that they may also produce infectious particles [17]. Viruses with similar modes of transmission may be widespread, as other insect endogenous retroviruses have been shown to express envelope proteins (e.g. *zam* in *D. melanogaster*, *tom* in *Drosophila ananassae* and *TED* in the lepidopteran *Trichoplusia ni*), although none of these have been shown to produce infectious particles [18–20]. Viruses that are transmitted while integrated into the host genome are not the focus of this review, but they have been discussed in detail elsewhere [12,15].

Other insect viruses are transmitted maternally, but also rely on horizontal transmission between individuals to be sustained in the host population. These include *Leptopilina boulardi* filamentous virus, a DNA virus that infects a parasitoid wasp [21]. When both an infected and a uninfected wasp lay eggs in the same *Drosophila* larvae, the virus can be transmitted horizontally to the offspring of the uninfected parent [21,22]. The wasps normally avoid laying eggs in already parasitized *Drosophila* larvae (superparasitism), but the virus can suppress this behaviour, increasing the superparasitism rate and therefore the rate of horizontal transmission [21]. In the moth *Homona magnanima*, what is thought to be a novel type of RNA virus establishes apparently benign infections in females and is transmitted vertically through eggs [23]. In males, it kills larvae late in their development, apparently to release viruses to infect other hosts. Other insect-infecting viruses, such as dengue virus and tsetse salivary gland hypertrophy virus, are primarily transmitted horizontally, but can also be maternally transmitted [24,25]. The focus of this review, however, are viruses that are only transmitted vertically and are not integrated into the host genome. To our knowledge, there are few reports of such viruses in the literature. In *Drosophila simulans*, a virus named DSV that causes bristle abnormalities, can be transmitted vertically by both males and females [26]. A similar reovirus-like particle has been described in other invertebrates (isopods) and is also transmitted vertically by both sexes [27]. However, apart from their initial characterization no further research has been published on these viruses.

The best-studied group of purely vertically transmitted viral endosymbionts are the sigma viruses, a group of rhabdoviruses that infect dipterans (figure 1). Perhaps, part of the reason for this is that they are easy to detect as they cause flies to become paralysed and die on exposure to CO_2 [29–31], a trait that seems to be a common effect of rhabdovirus infection in insects [32–34]. They also have relatively small genomes (approx. 12.5 kb) composed of six genes, meaning the population genetics of the virus can be practically studied.

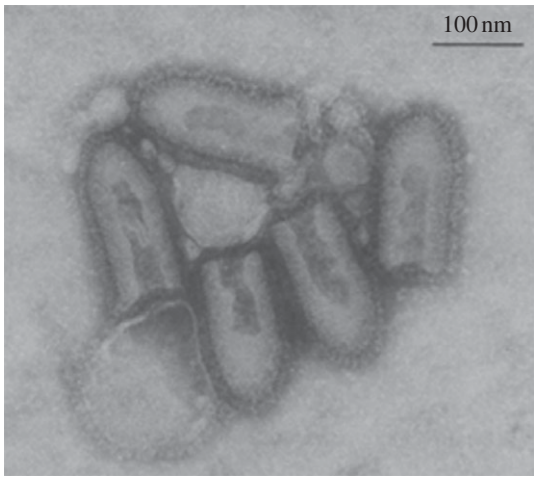

Figure 1. Electron micrograph of negatively stained DMelSV particles grown in cell culture. It was initially unclear what was causing the vertically transmitted CO_2 sensitivity in *D. melanogaster* and the agent 'sigma' was only later identified as a rhabdovirus based on its morphology. Image reprinted with permission from Elsevier from [28] copyright (1988).

The sigma virus of *D. melanogaster* (DMelSV) has been studied for over 70 years [29] resulting in a wealth of information that will be the focus of this review.

3. SIGMA VIRUSES ARE COMMON VIRAL ENDOSYMBIONTS

When the sigma virus was first discovered via its CO_2 sensitivity trait in a strain of *D. melanogaster* from Paris [29], it was assumed to be such a rare trait that considerable effort was made to maintain the fly stock during the German occupation of France during the Second World War [35]. However, when further lines of *D. melanogaster* were collected from the wild, it was discovered that the infection was common—typically about 0–30% of the population is infected [36–39].

More recently, it has become clear that sigma-like viruses are probably common endosymbionts of insects, if not arthropods as a whole. Recently, sigma viruses have been isolated that infect five additional species of *Drosophila* (*Drosophila affinis*, *Drosophila ananassae*, *Drosophila immigrans*, *Drosophila obscura* and *Drosophila tristis*) and one species of Muscid fly (*Muscina stabulans*) [40,41]. By sequencing partial or complete genome sequences, it was found that these viruses form a major clade of dipteran-infecting viruses [31,40] that has been proposed as a new genus in the family Rhabdoviridae. Additionally, the characteristic symptom of infection, CO_2 sensitivity, has been reported in 13 additional species of *Drosophila* and in *Culex quinquefasciatus* mosquitoes [30,34]. It is likely that sigma viruses may extend to other insect orders, as rhabdovirus-like particles have been detected in the testes of firebugs [42], and a maternally transmitted rhabdovirus has been observed in a parasitoid wasp [43]. Furthermore, fragments of rhabdovirus genomes have been found to be integrated into a wide array of different insect (and other arthropod) genomes, including in *Drosophila* and *Culex* mosquitoes [44,45], suggesting these insects have been—or are—infected with rhabdoviruses.

4. BIPARENTAL TRANSMISSION OF SIGMA VIRUSES

All of the sigma viruses that have had their mode of transmission examined (*D. melanogaster*, *D. obscura* and *D. affinis* sigma viruses, hereafter: DMelSV, DObsSV and DAffSV) have been found to be transmitted by both males and females to their offspring (i.e. through both eggs and sperm) [30,31]. Additionally, the trait of being paralysed by CO_2 that has been reported in *Drosophila athabasca* and *C. quinquefasciatus* is transmitted vertically in the biparental manner that is characteristic of sigma viruses [34,46]. Together these observations suggest that the sigma viruses are probably a clade of biparentally transmitted viruses.

The paternal transmission of sigma viruses is unusual among parasites and endosymbionts and, to our knowledge, only viruses are known to be transmitted directly in or on sperm [47]. Most bacterial endosymbionts are only transmitted maternally through eggs, and this is probably because most of the sperm cell's cytoplasm is eliminated during spermatogenesis [48], which will also act to remove bacterial endosymbionts. Viruses, however, appear to be small enough to persist in sperm cells. In the case of sigma viruses, it is unclear how they are transmitted by males to their offspring, but DMelSV particles have been observed in sperm cells of adult flies [49].

An unusual property of sigma virus transmission is that the virus cannot be transmitted through the male lineage for two consecutive generations. A fly infected by its mother will transmit the virus very efficiently; in the case of DMelSV, a female will often pass the virus on to close to 100 per cent of the subsequent generation while a male will typically infect about 65 per cent of its offspring [36]. However, a fly infected by its father will transmit the virus far less efficiently; in the case of DMelSV, females typically infect about 80 per cent of their offspring, and males infected by their fathers do not seem to transmit the virus at all [36] (although see [37] for a single exception of this). With minor differences, similar patterns of transmission are found in DObsSV and DAffSV [31]. This reduced rate of transmission by flies infected by their father seems to be due to sperm transferring a lower viral titre to the developing embryo [31,50,51]. As a result of this, it is thought the virus fails to infect the early-stage germ line, preventing it from infecting the gametes, even though it may reach high levels in somatic tissues in adult flies [50,51].

5. POPULATION DYNAMICS AND THE COST OF INFECTION

Biparental vertical transmission allows sigma viruses to rapidly spread through insect populations, even if the infection is costly to its host [51,52]. The spread of a sigma virus is similar to the spread of selfish genetic elements, as a single infected parent can transmit the virus to more than half of its offspring. For example, if a sigma virus infects a previously uninfected population and is transmitted on average to three quarters of the offspring of an infected insect, then the prevalence will initially increase by 50 per cent every generation. As infected individuals produce uninfected gametes, the prevalence will eventually stabilize at an intermediate equilibrium prevalence.

The speed with which sigma viruses can spread is illustrated by recent work on DObsSV in populations of *D. obscura* [31]. By sequencing viral isolates collected from across the UK, it was found that the viral population shows clear evidence of a recent expansion, with low genetic diversity and an excess of rare polymorphisms. Using this RNA sequence data, it was possible to reconstruct the viral population history, and estimate that the viral population size had doubled approximately every nine months, and within approximately the last 10 years it had swept across the UK to infect 39 per cent of flies. By measuring the transmission rate of the virus in the laboratory, it was found that such a spread could occur in this timescale even if the virus caused about a 10 per cent reduction in fecundity in infected hosts (figure 2) [31]. Although it is unclear, whether this virus was spreading through a previously uninfected host population or was replacing pre-existing strains, these results demonstrate that biparental transmission is a highly effective way for a virus to spread through host populations.

Sigma viruses are not unique in showing rapid sweeps through host populations, and similar patterns have been documented in vertically transmitted bacterial endosymbionts. For example, a strain of *Wolbachia* was observed to spread through populations of *D. simulans* on the west coast of the USA at a rate of 100 km yr^{-1} [54]. Similarly, a strain of *Spiroplasma* has been observed to be sweeping through populations of *Drosophila neotesteca* in North America [55], and in the whitefly *Bemisia tabaci* from the Southwestern US, a strain of *Rickettsia* swept from 1 per cent to close to 100 per cent in just 6 years [56]. In *D. melanogaster*, one strain of *Wolbachia* has spread through global populations in the last century, replacing all other strains [57]. Further examples have been detected by examining patterns of genetic variation in the mitochondrial DNA of insects, which is tightly linked to the maternally transmitted bacteria [58]. Why do endosymbionts show evidence of such rapid sweeps through host populations? One possibility is that endosymbionts are frequently lost from host populations, for example, when hosts evolve resistance to parasites, and this leaves the uninfected population vulnerable to invasion by endosymbionts from other host species. The long-term persistence of endosymbionts may therefore rely on the frequent invasion of new host populations or species [59].

Although there are superficial similarities between the spread of bacterial endosymbionts and sigma viruses, their different modes of transmission underlie important differences in their biology. The sigma viruses can spread solely by virtue of biparental transmission [51]. In contrast, the pure maternal transmission seen in bacterial endosymbionts does not by itself allow an endosymbiont to spread, as if the infection is at all costly to its host it will lead to a parasite's decline and extinction [51,52]. This has led bacterial endosymbionts to evolve elaborate strategies to enhance their transmission to future generations that range from providing benefits to the host to manipulation of host reproduction—such as causing cytoplasmic incompatibilities between infected and uninfected individuals or distorting the sex ratio towards females [2,5,7].

Given the fitness of a vertically transmitted parasite is closely linked to that of its host, it may be expected that sigma viruses cause low virulence [52]. At first glance,

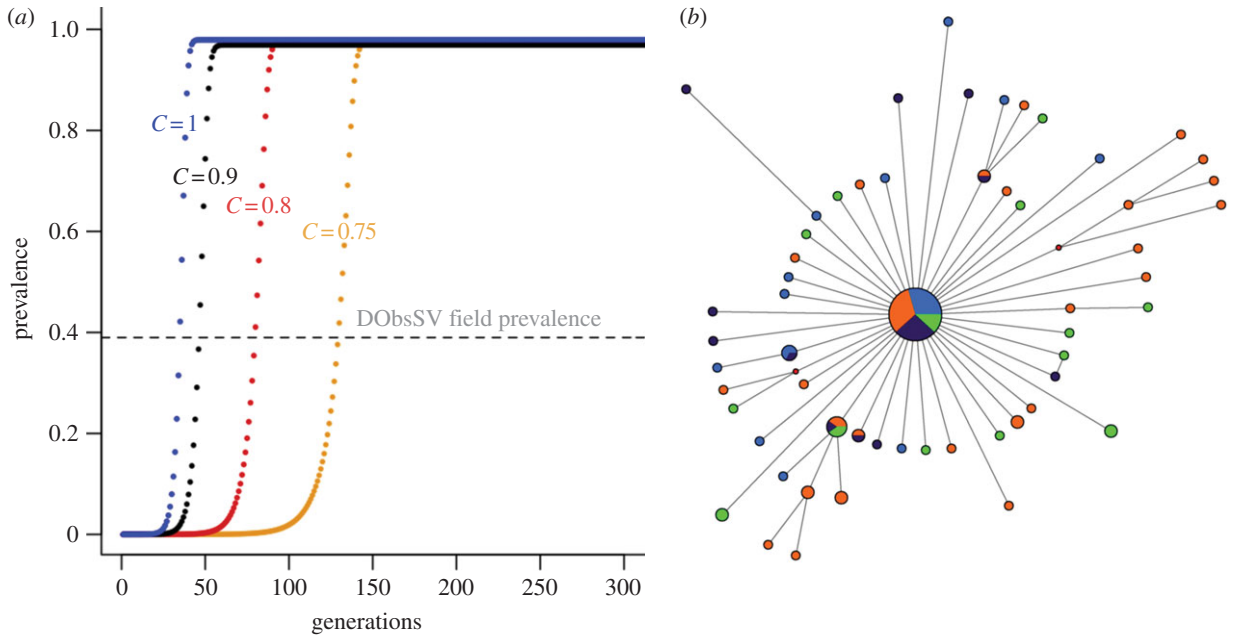

Figure 2. The rapid spread of DObsSV though natural populations of *Drosophila obscura*. (*a*) Simulations of the virus spreading based on laboratory estimates of transmission rates and a range of possible fertility reductions. Colours represent the different fertilities of infected flies relative to uninfected flies (C). The virus failed to invade if fertility is reduced by more than 25% ($C < 0.75$). The dashed horizontal line represents the mean prevalence of DObsSV in UK populations. In the UK, it is estimated there are three to four generations of *D. obscura* each year [53]. (*b*) Phylogenetic network of DObsSV sequences from the UK, showing an excess of rare polymorphisms (star-shaped network). Nodes are colour-coded (blue, Bristol; orange, Derbyshire; purple, Kent; green, Sussex) based on location and their size is proportional to the frequency of viral sequences. Branches are approximately sized to the number of mutations. Adapted from [31]. Copyright Genetics Society of America.

this seems to be the case, as normally laboratory stocks of *D. melanogaster* infected with DMelSV appear healthy. However, closer investigation has shown DMelSV is costly to its host. The virus will rapidly spread through fly populations kept at low density in the laboratory, but if the flies are placed under higher density conditions the virus shows a decline in frequency, suggesting the infected flies are out competed by their uninfected counterparts [60]. From these experiments, it has been estimated that infected flies suffer a reduction in fitness of approximately 20–30% in the laboratory [60]. It is likely the virus is costly to flies in the wild as well, as it has been calculated that the low prevalence of the virus in the field (0–30%) is only likely if the infection reduces the fitness of flies [36–38]. The basis of this reduction in fitness may include a reduction in egg viability [61,62], delayed egg development [63], a possible reduction in over-winter survival [64] and infected flies having increased mortality when co-infected with a fungal pathogen [65].

6. RESISTANCE TO SIGMA VIRUSES

As sigma viruses are common and costly parasites of *D. melanogaster*, there is selection for flies to evolve resistance. In natural populations of *D. melanogaster*, there is considerable genetic variation in the susceptibility of flies to infection by sigma viruses, and genetic mapping studies have shown that there are a number of major effect resistance polymorphisms segregating in natural populations [66,67]. Two of the loci that provide resistance to DMelSV have been identified at the molecular level—*CHKov* and *Ref*(2)*P* [66–70].

The first of the polymorphisms that provides resistance to DMelSV involves the *CHKov1* and *CHKov2* genes [69]. Three different alleles of this pair of paralogous genes occur in *D. melanogaster*, each conferring differing levels of protection to DMelSV infection. The most susceptible individuals have the ancestral state of fully intact genes. The second allele has a transposable element insertion in the coding region of *CHKov1* that is associated with increased resistance to DMelSV (figure 3). The insertion results in a truncated mRNA, leading to a shorter protein than the ancestral allele. This is the most common allele in North American populations, being found at a frequency of 0.82 in North Carolina. Finally, the third allele is the result of two duplications, with three copies of both the truncated *CHKov1* allele and *CHKov2* (one of which is also truncated). These flies have the greatest level of resistance but the allele appears relatively rare in nature, having only been found in a single fly stock from France [69].

Contrary to the general consensus that resistance to infection tends to be a costly trait to evolve [71], the transposable element insertion that confers resistance has pleiotropic effects that are beneficial rather than costly to the host, as it also provides resistance against organophosphate insecticides [72]. Interestingly, the insertion appears to predate the use of insecticides [72] suggesting it first played a role in antiviral defence before, by chance, conferring protection from insecticides.

The mechanism by which the *CHKov* genes confer protection is less clear, and they do not appear to be part of the induced immune response [73]. Both *CHKov* genes are putative choline kinases and may affect the function of choline esterase (which is the

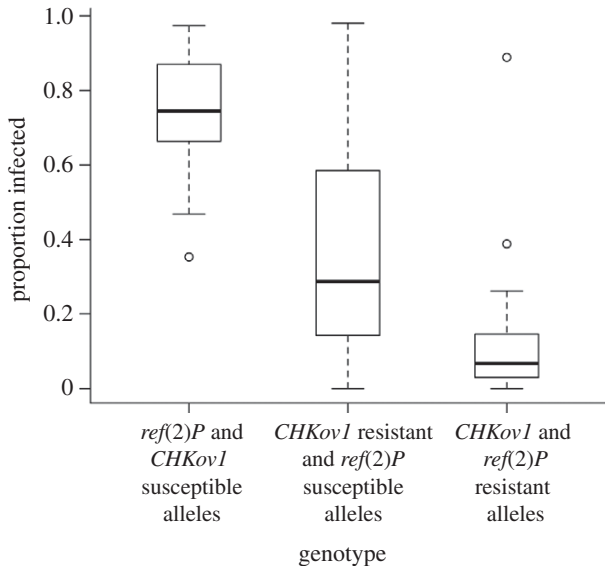

Figure 3. Box plot showing the effect of two polymorphic resistance genes on infection rates in 176 inbred lines of *D. melanogaster* (number of lines tested for each genotype were 27, 126 and 23, respectively). Flies were injected with DMelSV and classified as infected or uninfected using the CO_2 sensitivity assay. Details of the experiment are described in Magwire *et al.* [69].

target of organophosphates) or choline metabolism in general [72]. As other rhabdoviruses use acetylcholine receptors to enter host cells [74], it is possible this presents a potential mechanism of DMelSV resistance also.

The other resistance gene that has been identified is *ref*(2)*P* [75,76]. Here, a mutation from a Gln–Asn to a single Gly in the PB1 domain (a protein interaction module) of the protein confers resistance to DMelSV (figure 3) [68,70,77–79]. Vertical transmission of the virus to offspring is considerably reduced in resistant flies. This effect is greatest in females, where transmission rates drop from 99 to 8 per cent when the offspring are homozygous for the resistant allele [66]. Furthermore, reduced transmission is also seen in wild flies carrying the resistance allele [38]. The *ref*(2)*P* resistance allele is currently found at frequencies of 24 per cent in natural populations [38,77], so only about 5 per cent of flies will be homozygous. As the rate of vertical transmission is only slightly reduced in heterozygotes, the effect of this gene on transmission rates in wild populations might be quite small [77].

The mechanism by which *ref*(2)*P* confers resistance to DMelSV is unknown, but there is evidence to suggest that it may be involved in autophagy. Autophagy is a process by which cytoplasmic components are encapsulated inside double membrane bound vesicles known as autophagosomes, which fuse with lysosomes and are then degraded [80]. Recently, it has been shown autophagy is a defence measure against intracellular parasites [81,82]. Interestingly, vesicular stomatitis virus, which is a fairly close relative to the sigma viruses, activates autophagy in *D. melanogaster*, which protects the flies against the virus [82]. Ref(2)P is an adaptor protein which selectively targets polyubiquitinated substrates for degradation by autophagy [83], and its mammalian homologue, p62, has been found to target bacteria for degradation by autophagy [84]. It therefore seems likely the involvement of ref(2)P in the autophagy pathway protects *D. melanogaster* against sigma virus infection.

7. COEVOLUTION AND VIRAL COUNTERMEASURES

Patterns of genetic variation around the resistant alleles of both *ref*(2)*P* and *CHKov1* indicate that these resistance genes have been driven to a high frequency by natural selection [69,70,72,77]. In both cases, there is extended linkage disequilibrium and low genetic variation around the resistant alleles, and it has been estimated that *ref*(2)*P* has experienced a selective sweep within the last 1000–7000 years, while *CHKov1* swept to a higher frequency within the last few hundred years (although the allele may be much older) [72,77,85]. Therefore, it seems DMelSV has selected for new resistance mutations that are sweeping through the population under directional selection.

The spread of the *ref*(2)*p* allele has led to the reciprocal evolution of a DMelSV viral type that can infect resistant flies. Current natural populations of DMelSV contain two viral types, one that can infect *ref*(2)*P* resistant flies ('insensitive' viruses) and one that cannot infect resistant flies ('sensitive' viruses) [36,86]. It seems that the insensitive viral type has arisen very recently, as European populations of DMelSV—which contain both viral types [87]—shared a common ancestor about 200 years ago [39]. The spread of the insensitive viral type was documented in France and Germany over approximately 10 years in the 1980s, with the insensitive virus largely replacing the sensitive viral type over this period [88,89]. Are sigma viruses adapting to overcome the other resistance genes that have been found in host populations [66,67]? Two experiments on paternal transmission-blocking genes have produced contrasting results. The first of these studies found a similar situation to *ref*(2)*P*, where host resistance was only effective against certain viral isolates [90], while a second study found that all of the 95 viral isolates tested were affected by the host resistance gene [87]. The great speed with which these arms races occur means that it may be difficult to witness selective sweeps in progress, and genetic variation in the viral population that interacts with other host resistance genes may exist only for short periods of time [87].

Viral endosymbionts therefore do not remain stationary in the face of host resistance, with the spread of alleles that increase host resistance being followed by the spread of parasite genotypes that can overcome these host defences as part of a coevolutionary arms race [91]. The high mutation rates found in RNA viruses [92], coupled with biparental transmission, can allow the rapid evolution and spread of resistance-breaking viral types. Such rapid coevolutionary arms races, involving sweeps of endosymbionts and host resistance genes have also been observed in bacterial endosymbionts [93–95].

8. HOST SHIFTS

Despite the virus being vertically transmitted, the host and sigma virus phylogenies are incongruent, indicating that over evolutionary time sigma viruses switch between host species rather than cospeciate with their hosts [41]. This pattern is very similar to that observed in many bacterial endosymbionts [96–98], and it may be linked to the

rapid loss (and gain) of parasitic endosymbionts due to the evolution of host resistance, as the only endosymbionts that show high levels of host fidelity and cospeciation are mutualists [99–102]. Therefore, the ability of sigma viruses to survive over evolutionary timescales may rely on horizontal transmission events between host lineages, perhaps when vectors such as parasitic mites carry the virus between host species [40].

Which species do endosymbionts switch between? Sympatry is clearly essential, but once contact is accounted for it has been suggested that parasites may be more likely to switch between closely related species, as they offer a more similar environment for the parasite [103]. Cross-infection experiments suggest bacterial endosymbionts may be more successful in shifting between closely related species (reviewed in [104]), and data from natural populations are consistent with such patterns [104–106].

Sigma viruses show similar trends; a recent cross-infection experiment using 51 species of Drosophilidae found that viruses were less successful at persisting and replicating in hosts more distantly related to the viruses' natural host, when the non-independence of host species was accounted for [107]. However, after accounting for the effect of genetic distance from the natural host, there was a strong effect of the host phylogeny that was found to explain almost all of the remaining variation in a viruses' ability to infect a novel host. Therefore, groups of closely related hosts tend to have similar levels of susceptibility [107]. This could be due to certain host clades having lost or gained immune or cellular components affecting general susceptibility to all sigma viruses [108–110].

What does this mean for host switching of viral endosymbionts? Endosymbionts will often jump between closely related species [103,104]. However, in some cases, groups of hosts distantly related to the natural host may be particularly susceptible. This means that the host phylogeny may represent a patchwork of host clades with varying degrees of susceptibility. Consequently, endosymbionts may sometimes successfully jump into distantly related—but susceptible—species, where they can then spread through that clade of susceptible hosts.

9. CONCLUSIONS

Evidence suggests vertically transmitted viral endosymbionts—in particular rhabdoviruses—may be common parasites of insects. But how common are they? Are they the viral equivalent to *Wolbachia* [3], infecting a high proportion of insect species? Further sampling across a wide range of insects is required to uncover the diversity and incidence of viral endosymbionts, but the lack of diagnostic PCR tests for these viruses coupled to the fact they can occur at a low prevalence makes this a difficult task. The availability of low-cost high-throughput sequencing technologies will hopefully allow new viral endosymbionts to be more readily discovered (for e.g. [111]). Searches of insect expressed sequence tag libraries may also unearth viral sequences, and comparisons with genome sequences will help determine whether they are integrated into the host genome [43]. Additionally, storing of insect samples in such a way that RNA is preserved is also important to allow future screening for newly discovered viral endosymbionts, and will also allow the study of the ecological and evolutionary changes in viral endosymbionts over time.

A key difference between viral and bacterial endosymbionts is that viruses can be transmitted through sperm as well as eggs. This not only allows them to rapidly invade and spread through insect populations when the infection is costly to the host [51], but may also mean that the phenotypic effects of viral endosymbionts on their hosts may be different from bacterial endosymbionts. The range of phenotypic effects that viral endosymbionts have on their hosts is poorly understood. For example, to what extent do viral endosymbionts act as mutualists by providing benefits to the host as has been found with bacterial endosymbionts [7]? Viruses have been shown to be capable of such phenotypes [10]; for example, polydnaviruses preventing the larvae of parasitoid wasps from being encapsulated in their host. It seems possible that viral endosymbionts may also act as reproductive manipulators, as in the case of the RNA virus found in the moth *H. magnanima* that appears to cause late male killing [23]. However, as viral endosymbionts can be transmitted via sperm as well as eggs it means that the complex strategies used by maternally transmitted parasites are not required to spread in host populations. Although RNA viruses can have complex effects on their hosts, their relatively small genomes compared with bacteria and DNA viruses, may prevent them evolving some of the complex phenotypes seen in other insect endosymbionts (for example, synthesizing essential amino acids is unlikely to be possible for a virus with six genes). However, as RNA viruses in other taxa can have major effects on their host's phenotype it seems possible that they could have such effects in insects also [9]. Therefore, a major question is: what are the phenotypic effects of viral endosymbionts on their insect hosts, and how do they aid the spread of these endosymbionts through insect populations?

Viral endosymbionts have very dynamic relationships with their hosts, involving selective sweeps of host resistance genes and viral countermeasures, together with occasional host shifts when the virus jumps into a new species. It is likely that these two phenomena are linked, perhaps owing to the evolution of host resistance driving the virus extinct in a given host, leaving it vulnerable to invasion by viruses from other species—meaning viruses must invade new host populations if they are to persist over evolutionary timescales. In conclusion, viral endosymbionts may be an important but overlooked force in host evolution, much as bacterial endosymbionts were 25 years ago.

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