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Current Opinion in Virology

# The genetics of host–virus coevolution in invertebrates

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Although viral infection and antiviral defence are ubiquitous, genetic data are currently unavailable from the vast majority of animal phyla — potentially biasing our overall perspective of the coevolutionary process. Rapid adaptive evolution is seen in some insect antiviral genes, consistent with invertebrate-virus 'arms-race' coevolution, but equivalent signatures of selection are hard to detect in viruses. We find that, despite the large differences in vertebrate, invertebrate, and plant immune responses, comparison of viral evolution fails to identify any difference among these hosts in the impact of positive selection. The best evidence for invertebrate-virus coevolution is currently provided by large-effect polymorphisms for host resistance and/or viral evasion, as these often appear to have arisen and spread recently, and can be favoured by virus-mediated selection.

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**Current Opinion in Virology** 2014, **8**:73–78

This review comes from a themed issue on **Virus evolution**

Edited by **Michael Brockhurst** and **Scott Hensley**

For a complete overview see the Issue and the Editorial

Available online 24th July 2014

**http://dx.doi.org/10.1016/j.coviro.2014.07.002**



## Background

Viral infection and antiviral defence are universal phenomena [1] and viral infections are reported across the metazoa [e.g. 2–4]. However, research tends to focus more on the coevolution of vertebrates (and plants) and their viruses than on invertebrates and their viruses, and relevant genetic data on viruses and antiviral resistance are lacking for almost all invertebrate phyla. If major lineages differ systematically in their molecular or ecological interaction with viruses, as might be expected given the differences in immune mechanisms, then the research bias could skew our overall perspective of host–virus (co)evolutionary process [e.g. 5•].

In this review we present data from arthropods that broadly suggest viruses do indeed drive invertebrate evolution — selective sweeps, resistance polymorphisms, and elevated rates of protein evolution have all been attributed to virus-mediated selection. However, whether this is part of a strict coevolutionary process [6,7] is less clear: viruses certainly evolve in response to invertebrate hosts, but as yet there is relatively little evidence demonstrating that this occurs as part of a reciprocal selective process.

## Virus-driven invertebrate evolution

Selection by viruses could drive frequent and rapid fixations in invertebrate populations, reducing genetic diversity at the selected loci and elevating divergence between species. Selection on amino-acid sequences, which may be common for antagonistic host–virus interaction, could additionally elevate the rate of non-synonymous substitution (*dN*). Comparison of such 'footprints of selection' between immune genes and genes with other functions argues in favour of pathogen-mediated selection in arthropods generally [e.g. 8–11], and identifies the antiviral RNAi pathway as a potential coevolutionary hotspot in *Drosophila* [9,12•,13]. Genes mediating antiviral RNAi [Ago2 and Dcr2, reviewed in 14] are among the fastest evolving 3% of protein sequences across *D. melanogaster* and *D. simulans*, with adaptive amino-acid fixations in this pathway estimated to happen every 10–40 thousand years [15]. Moreover, there is evidence for positive selection and recent selective sweeps in antiviral RNAi genes from multiple *Drosophila* lineages, while homologous 'housekeeping' genes do not show this pattern [12•,15,16].

The hypothesis that this is driven by a molecular 'arms race' with viruses is appealing [15], first because virus-encoded suppressors of RNAi (VSRs) are widespread among RNA viruses [reviewed in 17], second because some VSRs are known to interact directly with AGO2 and DCR2 [e.g. 18–20], and third because VSRs from Drosophila Nora viruses can be highly specific to the host species' AGO2 [21••]. However, other invertebrate antiviral genes are not reported to display extensive positive selection, and it remains possible that selection on *Drosophila* RNAi genes has been mediated by other selective agents [22]. To test whether such potential 'hot spots' of immune system evolution are a general phenomenon will require data from a wider range of invertebrate taxa, and based on sequence analysis alone it will remain hard to attribute selection to the action of viruses.

Virus-mediated selection may also be inferred using high-frequency large-effect host resistance polymorphisms, as these can result from negative frequency dependent

selection (i.e. when rare alleles have higher fitness) or incomplete/ongoing selective sweeps [reviewed in 7]. A large-effect polymorphism in the *D. melanogaster* autophagy-pathway gene *ref(2)P* conveys resistance to the vertically-transmitted *Drosophila melanogaster* Sigma Virus (DMelSV), with the resistant allele reducing viral transmission by ~90% in females and ~60% in males [reviewed in 23]. The resistant allele occurs at 25–35% in European populations, and population-genetic analyses suggest it arose roughly 1–10Kya and has increased in frequency recently [24,25]. A second large-effect DMelSV resistance polymorphism comprises a natural *Doc* transposable element insertion into *CHKov1* followed by a partial duplication and inversion involving *CHKov1* and *CHKov2*. The *Doc* insertion exists at high frequency (80% in a North American population) and reduces infection rates by ~50%. The subsequent rearrangement gave rise to a virus-inducible *CHKov2* transcript associated with an 80–140 fold decrease in viral titre [26]. Again, population genetic analyses of this locus suggest resistance is derived and has recently increased in frequency [26,27]. Resistance to Drosophila C virus (DCV) is associated with segregating variants in *pastrel* (~50% increase in survival time) and *Anaphase promoting complex 7* (>100% increase, but this currently lacks experimental verification [28••]), although both resistant alleles are currently rare [15% and 3% of surveyed alleles in the wild, see 28••]. Finally, experimental evolution under recurrent challenge with DCV also identified functional polymorphism in *pastrel*, and further identified virus-resistant alleles segregating in *Ubc-E2H* and *CG8492*. The DCV-resistant alleles of *pastrel* and *Ubc-E2H* respectively displayed a 24% and 14% selective advantage under experimental conditions, and knock-downs of gene expression reduced survival after challenge [29••].

High-frequency large-effect viral resistance polymorphisms have also been reported from other invertebrates. For example, segregating resistance to the Orsay Virus in the nematode *Caenorhabditis elegans* maps to a non-functional truncation of *Drh-1*, one of three dicer-related helicases involved in RNAi [30•]. Here the susceptible allele is derived, but is nevertheless found at a global frequency of 23% and appears to have spread recently, perhaps suggesting the action of selection at a linked locus [30•]. Polymorphism in the antiviral RNAi pathway (*Dicer-2*) has also been proposed to underlie some of the genetic variance for resistance to Dengue virus in the mosquito *Aedes aegypti* [31]. In other cases the mechanism for resistance is unknown. For example, some populations of the pest moth *Cydia pomonella* have recently evolved resistance to its Granulosis virus, via a single dominant sex-linked allele that blocks viral replication [32,33]. Similarly, resistance to White Spot Syndrome Virus in the shrimp *Penaeus monodon* has been mapped to single marker associated with a ~2000-fold reduction in viral titre [34], which occurs at a frequency of 40–60% [35].

Such polymorphisms are consistent with negative frequency dependent selection or with incomplete/ongoing selective sweeps [e.g. 28••], but because the resistant allele is often recently derived and increasing in frequency, it seems likely that many may be in the process of fixing. However, robustly attributing evolution to virus-mediated selection is challenging, and selection by other agents [e.g. *Doc* insertion in *CHKov1*; 27], and at linked loci [e.g. *drh-1* deletion; 30•] have been proposed in some cases. Nevertheless, experimental evolution shows that virus-mediated selection can lead to a rapid evolutionary response in *Drosophila* and can select for segregating variants such as *pastrel* [29••] and *ref(2)P* [36].

## Invertebrate-driven virus evolution

It seems certain that viral evolution occurs in response to invertebrates, if only because hosts always dominate the viral environment. For example, viral adaptation may underlie host-specificity seen in some insect viruses [e.g. 21,37,38], and adaptation to the invertebrate host has been attributed to specific amino-acid changes in several invertebrate-vectored viruses, including Chikungunya Virus, Venezuelan equine encephalitis virus, and West Nile Virus [39–41]. Such adaptation to the host may also be reflected by the tendency for Sigma Viruses to replicate more effectively in closer relatives of their natural hosts [42].

Given this, it is interesting to ask whether virus evolution occurs in response to specific host immune mechanisms. Genotype by genotype interactions — with host polymorphism for resistance and viral polymorphism for overcoming that resistance — may be indicative of negative frequency-dependent selection or incomplete on-going selective sweeps in the virus, driven by selection mediated by host resistance. For example, genotype by genotype interactions have been reported between Dengue Virus 1 and *Aedes aegypti* mosquitoes [e.g. 43,44]. The best-studied invertebrate case may be the interaction between *ref(2)P* and DMelSV [reviewed in 23,45], where a viral lineage capable of overcoming *ref(2)P* resistance arose a few hundred years ago and subsequently spread to become the most common form [46,47]. The rapid spread of this resistance-insensitive virus was documented as it occurred in two European populations [48,49], and experiments suggest that the *ref(2)P*-insensitive virus can replace the sensitive virus in a resistant *ref(2)P* host background — indicating that host resistance may indeed drive viral evolution [36]. The rapid spread of a viral lineage may often be indicative of a selective sweep, and such expansions have also been seen in the Sigma virus of *D. obscura* [50]. However, without additional evidence of pre-sweep genotypes or genomic regions such potential sweeps cannot be differentiated from expansions [e.g. an epidemic, 51], and cannot be attributed to host-mediated selection.