

Host-switching by a vertically transmitted rhabdovirus in *Drosophila*

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A diverse range of endosymbionts are found within the cells of animals. As these endosymbionts are normally vertically transmitted, we might expect their evolutionary history to be dominated by host-fidelity and cospeciation with the host. However, studies of bacterial endosymbionts have shown that while this is true for some mutualists, parasites often move horizontally between host lineages over evolutionary timescales. For the first time, to our knowledge, we have investigated whether this is also the case for vertically transmitted viruses. Here, we describe four new sigma viruses, a group of vertically transmitted rhabdoviruses previously known in *Drosophila*. Using sequence data from these new viruses, and the previously described sigma viruses, we show that they have switched between hosts during their evolutionary history. Our results suggest that sigma virus infections may be short-lived in a given host lineage, so that their long-term persistence relies on rare horizontal transmission events between hosts.

Keywords: rhabdovirus; *Drosophila*; host-shift; sigma virus; phylogeny; vertically transmitted

1. INTRODUCTION

Many animals have intimate associations with protists, bacteria and viruses, which live within the cytoplasm of their cells and are transmitted vertically between generations [1]. Vertical transmission and an inability to survive for long outside of the host mean that endosymbionts might be expected to show extreme host-fidelity and cospeciate with their hosts. Indeed, phylogenies of bacterial endosymbionts show obligate mutualists have remarkably stable associations with their hosts. For example, *Buchnera* bacteria, which synthesize amino acids lacking from the diet of aphids, have been stably vertically transmitted for approximately 150–250 Myr [2]. Similar patterns have been found in other mutualists such as *Wigglesworthia* in tsetse flies [3], *Blochmannia* in carpenter ants [4] and *Blattabacterium* in cockroaches and termites [5]. By contrast, parasitic endosymbionts persist for relatively short periods in a given host lineage and

frequently switch host species. For example, there is little or no congruence between the phylogenies of *Wolbachia*, [6], *Rickettsia* [7] and *Spiroplasma* bacteria [8] and their arthropod hosts. These associations may be unstable as hosts can evolve resistance and drive the parasite to extinction [9].

In contrast to bacterial endosymbionts, little is known about the evolutionary history of vertically transmitted viruses. Sigma viruses are vertically transmitted rhabdoviruses previously known from three species of *Drosophila*—*Drosophila melanogaster* (DMelSV) [10], *Drosophila obscura* (DObsSV) and *Drosophila affinis* (DAffSV) [11]. These viruses are unusual in that they are transmitted vertically through both eggs and sperm [10,12]. Here, we describe four new sigma viruses that each infect a different species of *Diptera*, and use a phylogenetic approach to show that sigma viruses have switched between host species during their evolution.

2. MATERIAL AND METHODS

(a) *Viral discovery and sequencing*

We collected *Drosophila tristis* in Derbyshire, UK; *Drosophila immigrans* in Marktredwitz, Germany; *Drosophila ananassae* in Kilifi, Kenya; and *Muscina stabulans* in Cambridge, UK. Infected flies were detected by exposing them to pure CO₂ at 12°C for 15 mins. Uninfected flies recover after approximately 30 mins while infected flies remain paralysed [10]. RNA was extracted from paralysed flies, reverse transcribed (see [11]), and amplified by PCR using multiple degenerate primers targeted to conserved regions of the viral RNA-dependent RNA polymerase gene (RDRP) (electronic supplementary material, table S1). PCR products were sequenced using BigDye reagents (GenePool facility, University of Edinburgh, UK) and once a small region of the RDRP gene had been sequenced, 3' RACE (rapid amplification of cDNA ends) was used to obtain further sequence (see [11]). To obtain high-quality sequences, new primers were designed to amplify the fragment sequenced by RACE, and this was re-sequenced in both directions. The host species was confirmed by sequencing mitochondrial *COI* and/or *Cytb* genes. Additional species were also collected and tested with the CO₂ assay, but we only report those species from which we were able to amplify a sigma virus.

(b) *Inferring the virus phylogeny*

The nucleotide sequence of the RDRP genes from sigma viruses and other rhabdoviruses was aligned based on the translated amino acid sequence using CLUSTALW. Alignments were trimmed to contain only a conserved region of the RDRP that could be robustly aligned. Phylogenies were inferred using maximum-likelihood (ML) (PAUP [13]) and Bayesian (MRBAYES [14]) methods. The ML analysis used a heuristic search with a nearest neighbour interchange algorithm and a general time reversible model with a gamma-distributed rate variation and a proportion of invariable sites. This model of sequence evolution was selected by comparing alternative models using Akaike information criterion in MODEL TEST [15]. Node-support was estimated by non-parametric bootstrapping. The Bayesian analysis used the same model of sequence evolution and the Markov chain Monte Carlo was run for 1 million generations, sampled every 100 steps with the first 25 per cent of samples being discarded as burn-in.

(c) *Detecting incongruent tree topologies*

To detect topological incongruence between host and parasite phylogenies, we used a Shimodaira–Hasegawa test (SH-test) [16], which compares the likelihood of the viral phylogeny inferred from the data with one constrained to match the host topology [17,18]. We also used a Bayesian approach that identifies the proportion of the posterior sample of viral topologies that match the host phylogeny (e.g. [19]). As these approaches compare only topologies (and not branch lengths), they are a conservative test for host switching. Even when topologies are incongruent, some cospeciation or switching between related hosts may make host and virus topologies more similar than expected by chance. To test for topological similarity, we compared the distribution of Robinson-Foulds [20] distance metrics provided by 10⁴ random viral topologies to that derived from the posterior sample of viral topologies.

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only persist in the long-term by switching between host species. This appears to be a general phenomenon among vertically transmitted parasites, as similar patterns are seen among bacterial endosymbionts (see §1) [6–8], and genomic parasites such as transposable elements [27] and homing endonucleases [28]. Although the transfer mechanism is unclear, we have previously suggested that parasitic mites could act as vectors of sigma viruses [11], and arthropod vectors may be responsible for other endosymbionts and genomic parasites switching between host lineages [27,29,30].

A sigma-like virus outside of the genus *Drosophila* suggests that these viruses may be widespread in Diptera, if not insects as a whole. Unlike bacterial endosymbionts, the rapid evolution of the sigma virus genome makes it impossible to design a single pair of diagnostic PCR primers that can be used to test for new strains of the virus. In the course of this study, we encountered CO₂-sensitive individuals of other species of flies from which we were unable to amplify virus using our primers, and these may harbour other sigma-like viruses. CO₂ sensitivity has also been reported in 13 other *Drosophila* species [10], and in *Culex* mosquitoes [23]. Additionally, rhabdovirus sequences have inserted into the genomes of various insect species [31] and rhabdovirus-like particles have been found in firebug testes [32]. The non-Drosophilid sigma virus we found is of particular interest, as the closely related Dimarhabdoviruses are vector-borne diseases of vertebrates (some of which are vectored by other dipterans) [22]. The discovery of other rhabdoviruses in insects that do not blood-feed may make it possible to understand how viruses may have switched between being vector-borne pathogens of vertebrates and being purely entomopathogenic.

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