Supplementary methods

Species	Food
D.americana	malt
D.arizonae	banana
D.euronotus	cornmeal
D.lummei	malt + yeast
D.mauritiana	proprionic
D.melanogaster	cornmeal
D.mojavensis	banana
D.montana	malt + yeast
D.paramelanica	cornmeal
D.persimilis	malt
D.prosaltans	proprionic
D.pseudoobscura	banana
S.lebanonensis	proprionic
S.pattersoni	Banana
D.santomea	cornmeal
D.sechellia	proprionic
D.simulans	cornmeal
D.teissieri	cornmeal

Table S1: Fly species and food reared on. Food recipes can be found in [1, 2]

Primer pair code	Primer	Sequence	Annealing temperature °C
A	DCV62F	GCCTGCGGTCCCTAATTGTTGAAT	69
	DCV1245R	CATTCATCTGTTCAGAGCCAACACCAT	
B	DCV1131F	GAGTTGCGTCGACAAATCAAAAATAGGAA	69
	DCV2330R	CTGAGGAAAAGTGTTGCAAGAGCGAAT	
C	DCV2243F	TGATGATGCGTTTCAGAAGAAAGACGA	69
	DCV3406R	GTAGACCACTGGGCAAGTTCACGTTTC	
D	DCV3290F	CGAATGTGGTATGCATAGGATGTCTGC	69
	DCV4468R	ACTGCCTGTCCCACACGAATAGATGAC	
E	DCV4377F	CCCAATATGGTTGATCCACTTGGTGAT	69
	DCV5495R	AAATTTCTCCATCACACGAATCCACGA	
F	DCV5405F	ACCTTCCGGCAACCCTTTCACTGTTAT	69
	DCV6553R	GACTAAAGTTGCTCGCAAACCCACAAA	
G	DCV6423F	CTGGTCTTTGGTCTTCCGCTACAACTG	69
	DCV7693R	GCGAGAAACATCGGGAACTCCTGTAG	
H	DCV7449F	TACTTGGAACACAACCGATGCCACTAG	71
	DCV8902R	AGGATCCAGAATAGTAAGGGAGTTAGGGT	
I	DCV8153F	CATGGTGTTCATCCAATGACCATAGACAC	70
	DCV9039R	CCGTGTAAGCAGGGCAGATAGTTACTGAA	

Table S2: Primers used to amplify DCV genome. PCR Cycle: 98°C 30sec, (98°C 30sec, 69-71°C 30sec, 72°C 45sec) x 35 cycles, 72°C 2min

Supplementary results

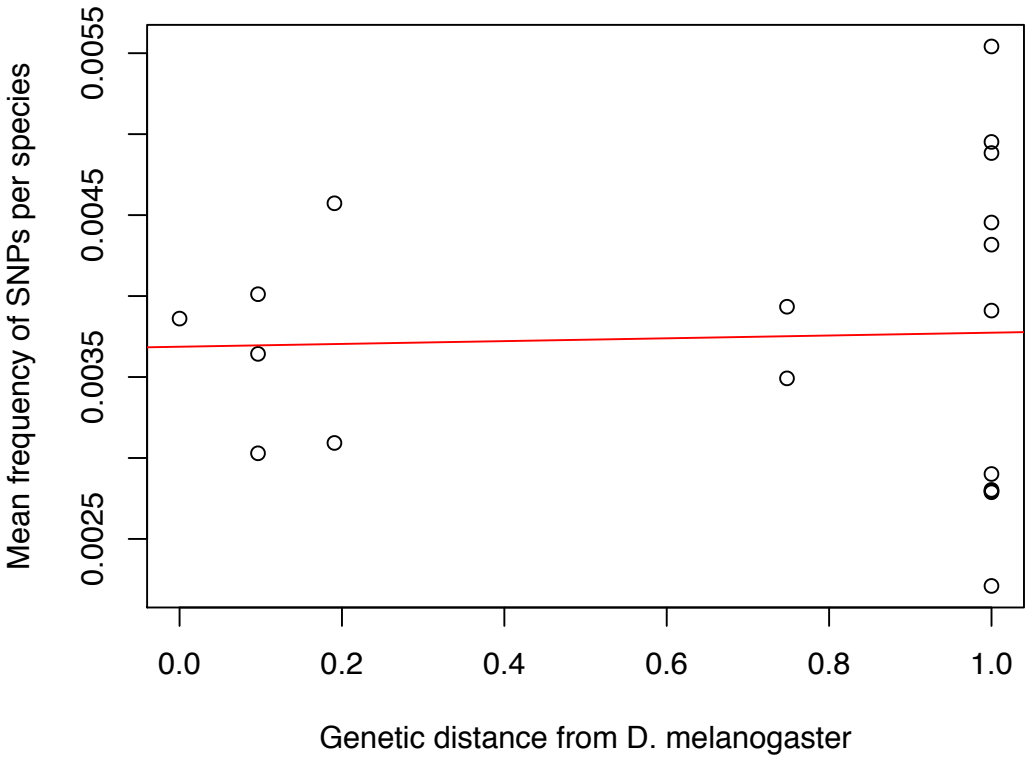

Figure S1: Correlation between mean SNP frequency and distance from *D. melanogaster.* We calculated the mean frequency of SNPs across the viral genome for each host species and examined if viruses showed higher rates of molecular evolution with increasing distance from *D. melanogaster*; the host DCV was isolated from and naturally infects. We found no significant change in mean SNP frequency with distance from *D. melanogaster* (phylogenetic mixed model implemented in MCMCglmm package in R as in [1], correlation= 0.0006 (CIs= -0.0176, 0.0188).

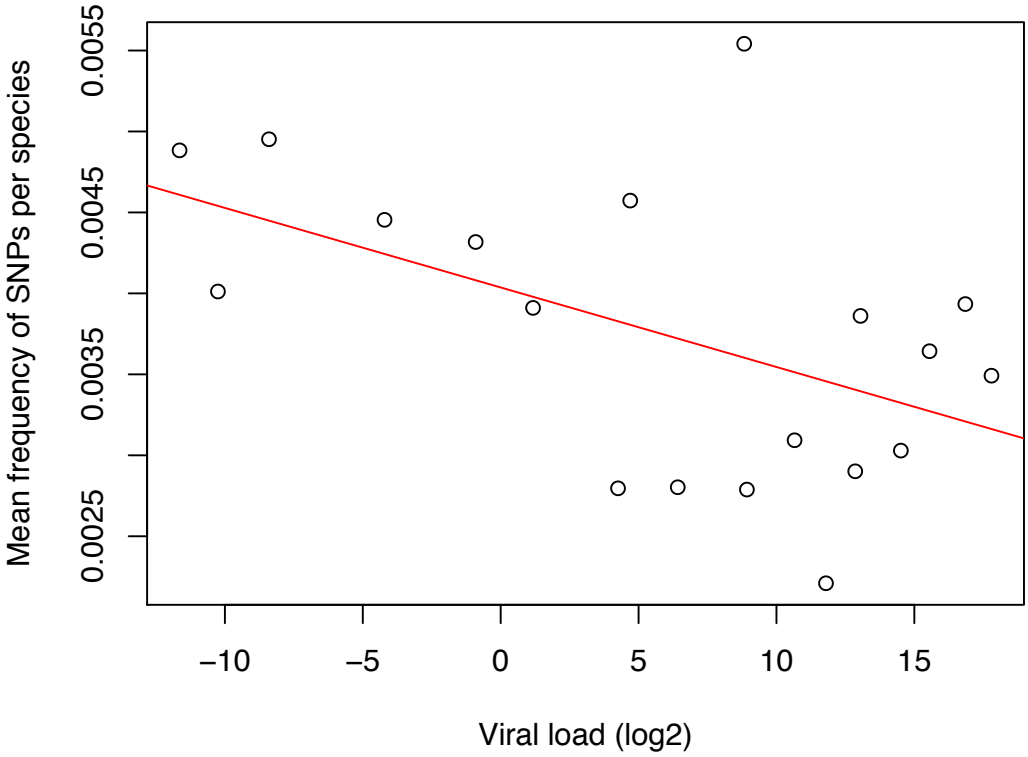

Figure S2: Correlation between mean SNP frequency and RNA viral load.
We examined if mean SNP frequency in a species was affected by viral population size. We used RNA viral load calculated by qRT-PCR as in [1] at passage 10 as a proxy for viral population size (viral load did not change significantly between passages 3 and 10). We found no significant relationship between mean SNP frequency and viral load (phylogenetic mixed model implemented in MCMCglmm package in R as in [1], correlation= -0.00006 (CIs= -0.00072, 0.00054).

Supplementary references

1. Longdon B, Hadfield JD, Day JP, Smith SC, McGonigle JE, Cogni R, et al. The Causes and Consequences of Changes in Virulence following Pathogen Host Shifts. PLoS Pathog. 2015;11(3):e1004728. Epub 2015/03/17. doi: 10.1371/journal.ppat.1004728. PubMed PMID: 25774803.
2. Longdon B, Hadfield JD, Webster CL, Obbard DJ, Jiggins FM. Host phylogeny determines viral persistence and replication in novel hosts. PLoS Pathogens. 2011;7((9)):e1002260. doi: 10.1371/journal.ppat.1002260.