

Rapid accumulation of a vertically transmitted parasite triggered by relaxation of natural selection among hosts

Lev Yu. Yampolsky, Colleen T. Webb, Svetlana A. Shabalina and Alexey S. Kondrashov*

Section of Ecology and Systematics, Cornell University, Ithaca, NY 14853, USA

ABSTRACT

The fate of a parasite with exclusively vertical transmission depends critically on its virulence, the magnitude of its negative impact on fitness of the infected host. Relaxation of natural selection within the host population should favour the parasite by reducing this impact. We studied the dynamics of the sigma virus, a vertically transmitted parasite of *Drosophila melanogaster*, within a captive host population kept under relaxed natural selection. The frequency of infected flies in the population grew from 5% to 70% in just 15 generations. In contrast, strong natural selection among the host curbed the propagation of the virus. Two of 15 populations originating from a pair of infected parents and kept under intense competition for 10 generations have lost the virus completely. The implications of these findings for host–parasite co-evolution are discussed.

Keywords: *Drosophila*, host–parasite co-evolution, sigma virus, vertical transmission.

INTRODUCTION

Sigma virus is a single-stranded RNA rhabdovirus of *Drosophila* with exclusively vertical transmission through both sexes of the host (see Bras *et al.*, 1994). The virus is present at low frequencies in many natural populations of *D. melanogaster* (L'Heritier, 1970; Fleuriet, 1988). The most conspicuous manifestation of the infection is sensitivity of the host to high concentrations of CO₂ (L'Heritier, 1970; Brun and Plus, 1980). Infected flies become paralysed and later die after a short exposure to CO₂ at concentrations used for anaesthesia. At ambient CO₂ concentrations, the infection does not affect the host in any obvious way, although a decrease of egg viability and of some other fitness-related traits has been detected (Fleuriet, 1988, 1996).

Vertically transmitted parasites survive or die together with their hosts. Thus, virulence – the impact of the parasite on host fitness – must be critical to parasite dynamics (Fine, 1975; Ebert and Herre, 1996). In fact, only non-virulent parasites can be maintained by exclusively vertical transmission if the host is asexual or if transmission is strictly confined

* Author to whom all correspondence should be addressed. e-mail: ask3@cornell.edu

to one sex (Lipsitch *et al.*, 1995a; Mangin *et al.*, 1995). However, a vertically transmitted parasite can be virulent if both sexes of the host participate in transmission. Obviously, if transmission is complete through both sexes, a rare exclusively vertically transmitted parasite can persist as long as it decreases the host fitness by no more than a factor of two. Non-zero virulence can evolve, for example, if it is positively correlated with efficient transmission (Bull, 1994). However, an increased level of vertical transmission generally favours lower virulence (Lipsitch *et al.*, 1996), so that we can expect successful vertically transmitted parasites to be only moderately virulent.

It was reported for various situations that harsh, competitive conditions magnify the variance in fitness (e.g. Kondrashov and Houle, 1994; Ritland, 1996). Thus, it is natural to assume that, under benign, non-competitive conditions, the virulence is less than under harsh, competitive conditions. Therefore, relaxation of competition and natural selection among the host should favour a vertically transmitted parasite. In this paper, we report fast accumulation of the sigma virus in a *D. melanogaster* population kept under relaxed selection, followed in some cases by the secondary loss of the virus when intense competition among the hosts was restored.

METHODS AND MATERIALS

Accumulation of the virus

In July 1995, we sampled 160 mated females from a large wild synanthropic population of *D. melanogaster* near Ithaca, NY. Their virgin offspring were placed in a cage. The experimental population was started from the set of 100 mated females sampled from the cage and was kept for 30 generations using 'middle class neighbourhood' (MCN) experimental design. Each generation, a population consisted of 100 randomly formed mating pairs, each occupying a separate vial, and care was taken to reduce egg-to-adult mortality. All fecund individuals contributed equally to the next generation, because one daughter and one son were recruited randomly from each sibship. A few extra flies were recruited from randomly chosen sibships to reduce absent offspring from rare sterile vials (see Shabalina *et al.*, 1997, for details).

The sum of egg-to-adult mortality and sterility rates was ~20%, while the heritability of mortality was ~0.20, so that the genetic load was below 5% (see fig. 1 in Shabalina *et al.*, 1997). Thus, the MCN design ensured that there was little room for natural selection. Therefore, as long as the flies are not exposed to high CO₂ concentrations, sigma virus behaves as a vertically transmitted, selectively neutral commensal, regardless of the deleterious effects it may have on the host under competitive conditions.

The presence of the sigma virus in our population was detected in generation 15. After this, we switched from CO₂ anaesthesia to cold anaesthesia, which does not adversely affect infected flies. Simultaneously, we began measuring the frequency of the flies sensitive to 5 min exposure to a high concentration of CO₂. Each generation, the sibs of all the individuals recruited to establish the next generation were assayed. Each sibship, usually consisting of at least 20 flies, was assigned an infection score from 0 (no effect) to 4 (all flies dead). The presence of the sigma virus in CO₂-sensitive flies was confirmed by the endpoint dilution method (Contamine, 1981; Coulon and Contamine, 1982; D. Contamine, personal communication) and by reverse transcription PCR (RT-PCR; see below).

Purging of the virus

After generation 30, fifteen isofemale populations were established from the experimental population. Each population was started from a female and a male which later died after the CO₂ treatment. These populations were kept for 10 generations under high density (~200–300 flies per vial) in separate vials. Each generation, all flies from a vial were allowed to lay eggs for 2 days in a fresh vial, resulting in several thousand eggs per vial. Sigma virus is usually under-replicated (and therefore does not cause CO₂ sensitivity) in flies reared under harsh conditions (D. Contamine, personal communication). Therefore, to assay the presence of the virus, starting with generation 8, a few young larvae from each population were placed in separate vials, developed there under benign conditions, used in the CO₂ sensitivity test and then discarded.

Reverse transcription PCR

The presence or absence of sigma virus was assayed by RT-PCR. Before RNA extraction, the flies were kept for several days under benign conditions. Total RNA was isolated following standard procedures using 50 flies per 1 ml TRIzol (Life Technologies). We performed RT-PCR on the total fly RNA using the GeneAmp RNA PCR kit (Perkin Elmer) with primers specific to the N gene of sigma virus (accession #X77038 in the NCBI Entrez database). The length of this sequence was 598 bp. Reverse transcription was performed using synthetic oligo d(T) and a synthetic oligonucleotide TTTCATGAG-CTGTCACACCC as 3' primers.

cDNA was then amplified with synthetic oligonucleotide primer ATTTGCAG-GGTGCCTAGTGT used for the 5' end. Amplification conditions were as follows: 2 min at 95°C, followed by 15 cycles of 1 min at 95°C, 1 min at 50°C and 2 min at 72°C. In the next 10 cycles, the annealing temperature was increased to 55°C and then to 60°C for another 10 cycles. The final step was 7 min at 72°C.

DNA for sequencing was obtained by gel purification. We removed the 600 bp band from the gel and used Qiaex II beads (Qiagen) to purify the DNA. The purified DNA was then sequenced using a Thermo Sequenase radiolabelled terminator cycle sequencing kit (Amersham Life Science).

RESULTS

Infection rate in nature

Another MCN population, founded from a separate set of 100 mated females sampled from the same cage from which the experimental population was established (Shabalina *et al.*, 1997), did not display any CO₂ sensitivity and did not contain sigma virus (D. Contamine, personal communication). This indicates a low frequency of the virus (frequencies of 5% or more can be rejected with 99% confidence) in the Ithaca natural population in July 1995. In September 1996, thirteen of 747 flies caught in the same place and kept under good conditions for several days before testing were sensitive to CO₂, implying a 1.7% infection rate. No CO₂-sensitive flies were detected among 120 flies sampled in the same place in September 1997. Thus, while the infection rate grew rapidly in the experimental MCN population, it remained low in the wild.

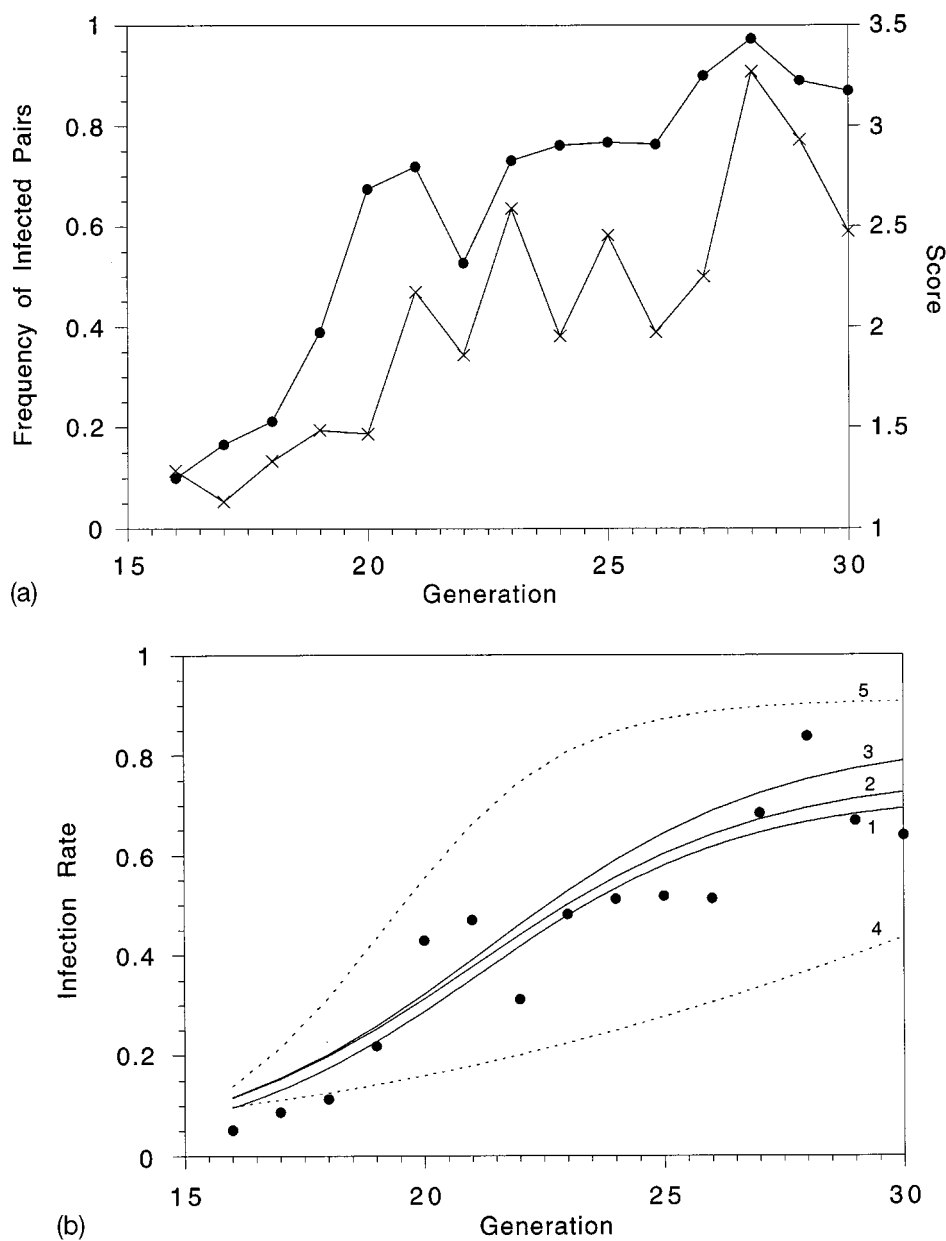


Fig. 1. (a) Increase of P , the frequency of pairs that produce at least one infected offspring (sibships with scores 1–4) (●), and of mean CO₂ sensitivity score among the infected sibships (×). (b) Increase of the estimated frequency of infected flies F (●), together with its various logistic approximations (see text).

Accumulation of the virus in the experimental population

The dynamics of the frequency of CO₂-sensitive sibships P and of the mean sensitivity score of such sibships are shown in Fig. 1a. The frequency of sigma virus infection F in the population (Fig. 1b) was calculated from P assuming that, if at least one parent was infected, some sensitive offspring will be detected in the sibship, so that:

$$F = 1 - \sqrt{1 - P} \quad (1)$$

Assuming invariant transmission efficiencies by females and males, e_f and e_m , the dynamics of a vertically transmitted selectively neutral parasite in a population of hosts with non-overlapping generations is described by:

$$F_{n+1} - F_n = F_n(e_m + e_f) - e_f e_m F_n^2 \quad (2)$$

where F_n is the frequency of infected individuals in generation n . This model is a particular case of a more general model of L'Heritier (1970), which also allowed for variability in transmission efficiencies and for selection against the infected hosts (see also Régnière, 1984; Busenberg and Cooke, 1993). The continuous time t approximation to the solution of (2) is a logistic function:

$$F(t) = \frac{K}{1 + e^{-rt}(K - F_0)/F_0} \quad (3)$$

where $K = (e_m + e_f - 1)/e_m e_f$ is the equilibrium infection frequency, $r = e_m + e_f - 1$ is the intrinsic rate of increase of infection frequency, and F_0 is the initial infection frequency. Obviously, the growth of F_n with time implies that $e_f + e_m > 1$, while $K < 1$ implies that e_f and $e_m < 1$.

Fitting equation (3) to the data results in $K = 0.72 \pm 0.08$, $r = 0.37 \pm 0.11$ and $F_0 = 0.07 \pm 0.04$ ($R^2 = 0.88$) (Fig. 1b, curve 1). These values imply complex e_f and e_m . However, the same F_0 with $K = 0.76$, $r = 0.34$ (Fig. 1b, curve 2) implies real values of transmission efficiencies $e_f = e_m = 0.67$, while the fit remains very good. Because of the short duration of the experiment, our data provide more information on r than on K , so that we have little power to estimate e_f and e_m separately, while the estimate of r and, thus, of their sum, is much more reliable. For example, the assumption of $e_f = 0.87$ and $e_m = 0.47$ (so that r is still 0.34, while $K = 0.83$, $e_f > e_m$; Fleuriet, 1988) yields a logistic curve that fits the data well (Fig. 1b, curve 3). In contrast, with $r = 0.14$ and $K = 1.00$ (this requires maximally different e_f and e_m and results in the fastest growth of F for a given r), the growth is too slow (Fig. 1b, curve 4); with $r = 0.54$ and $K = 0.91$ (this requires $e_f = e_m$ and results in the slowest growth of F for a given r), the growth is too fast (Fig. 1b, curve 5). Thus, we can conclude with confidence that r is much higher than 1, and is perhaps confined within the range 1.25–1.45.

These estimates are consistent with the data obtained on the isofemale populations: the expected frequency of the infected offspring whose parents were both infected with our estimates of e_m and e_f is 0.9, while the observed frequency in the first generation of the isofemale experiment was 0.863 ± 0.04 .

Loss of the virus in two isofemale populations

After 10 generations under high density, sensitivity to CO₂ became rather variable among the isofemale populations (Fig. 2). Two populations lost CO₂ sensitivity completely, 10 populations showed intermediate levels of sensitivity, and three populations remained 100%

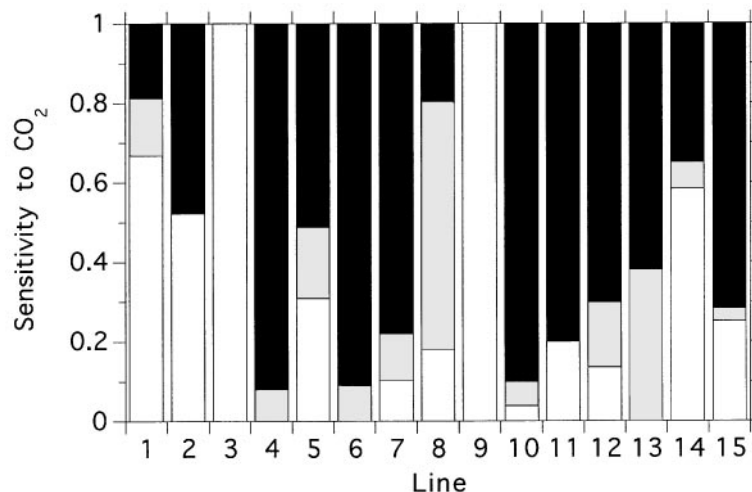


Fig. 2. Sensitivity to CO₂ in individual isofemale populations in generation 10. Black, grey and white indicate the fractions of dead, paralysed and unaffected flies, respectively. In total, approximately 50 flies were tested in each population.

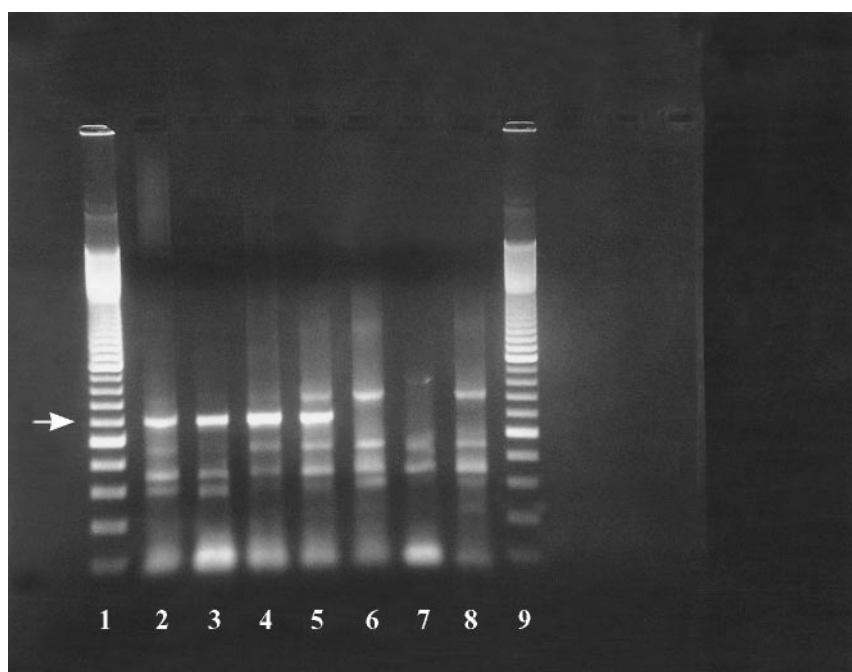


Fig. 3. Results of RT-PCR. The presence of a band at ~600 bp indicates the presence of sigma virus. Lanes (1) and (9) 100 bp DNA ladder; lane (2) MCN – virus accumulation population; lane (3) isofemale population 1, 67% sensitive; lane (4) isofemale population 6, 100% sensitive; lane (5) isofemale population 10, 96% sensitive; lane (6) isofemale population 9, 0% sensitive; lane (7) isofemale population 3, 0% sensitive; lane (8) negative control, laboratory stock maintained using CO₂ anaesthesia for many generations.

sensitive. RT-PCR demonstrated that the populations that lost CO₂ sensitivity did not contain any detectable amount of sigma virus (Fig. 3).

We sequenced 558 bp of our PCR product. It matched the published sequence of the N gene of sigma virus (Bras *et al.*, 1994; accession #X77038 in the NCBI Entrez database) except for two silent substitutions in nucleotide positions 1062 (C → T) and 1167 (A → G).

DISCUSSION

Our estimates of maternal and paternal efficiencies of the virus transmission, e_f and e_m , are only approximate due to high standard errors of the regression parameters. Values of e_f and e_m deviating by, say, 0.1 from these estimates will also produce an acceptable fit (data not presented). Moreover, we have only estimated the average values of e_f and e_m within the experimental population, while the transmission efficiencies were almost certainly variable among the flies (and may have actually changed from generation to generation) for at least three reasons.

First, there may be two types of infected flies, stabilized and non-stabilized. Stabilized individuals transmit the virus more efficiently, and infection in the offspring of stabilized mothers is usually stabilized (L'Heritier, 1970; Fleuriet, 1988). The frequency of stabilized individuals among the infected flies was probably high, at least by the end of the experiment (L'Heritier, 1970), because infection did not seriously impair host fitness during the experiment and the transmission efficiency by the stabilized flies was close to 1 (Fleuriet, 1988). Second, the virus may be genetically heterogeneous. Indeed, in non-stabilized flies, different strains of the virus differ in transmission efficiency (Fleuriet, 1988). Third, transmission efficiency depends on the genetics of the host (Fleuriet *et al.*, 1990; Fleuriet and Periquet, 1993; Wayne *et al.*, 1996). Resistance alleles of the *ref(2)P* locus restrict the multiplication and the transmission efficiency of most of the strains of sigma virus (Nakamura *et al.*, 1986; Gay and Contamine, 1993). Individual infected pairs in our population produced from 0 to 100% infected offspring (data not reported). Variability at the resistance loci was noted in flies in our experimental population by crossing with reference infected flies (D. Contamine, personal communication), but no quantitative measurement of it was attempted.

Nevertheless, very rapid accumulation of sigma virus in a *D. melanogaster* population with relaxed natural selection proves that $e_f + e_m > 1$ (Fig. 1b). Fleuriet (1982) observed a much slower accumulation of sigma virus in a cage population of *D. melanogaster* where selection was not excluded; the frequency of the infected flies increased from ~5% to ~90% in 30 months (i.e. in ~60 generations).

In contrast, we observed elimination of the virus from some isofemale populations maintained under high density and tough competition. To our knowledge, this is the first demonstration of the negative impact of competition among hosts on the prevalence of a vertically transmitted parasite. This is strikingly different from the dynamics of horizontally transmitted parasites, which often spread in high-density host populations (Burdon *et al.*, 1992; Knell *et al.*, 1996).

Our experimental population should have a very similar genetic structure to that of the natural population from which it was sampled because selection was almost absent, while the number of generations was too small for random drift to have had a significant impact. Thus, the rapid expansion of the virus in the experiment raises the following question: Why is the frequency of the virus in nature one or two orders of magnitude less than after 30 generations in the laboratory? The only explanation we can think of is that the virus

must have a strong negative impact on host fitness in nature. The rate of the expansion of the virus under relaxed selection among the hosts implies that this impact must be at least 20–30%.

This explanation is supported by the fact that fitness assayed under competitive conditions was lower in the infected experimental population than in a similar virus-free population (Shabalina *et al.*, 1997). Thus, the expansion of the virus under relaxed selection may be quite similar to accumulation of deleterious mutations under the same conditions. A much slower expansion of the virus in the cage population of Fleuriet (1982) indicates that, in her experiment, the virus impaired the fitness of the flies, although to a lesser extent than in nature. It is not clear why the virus persists in nature at a low frequency, instead of disappearing completely. Perhaps spatial structure of the natural host population should be taken into account (Herre, 1995; Lipsitch *et al.*, 1995b).

There are three possible reasons for the loss of the virus in some isofemale populations kept under high density, which are not necessarily mutually exclusive. First, uninfected or less infected flies probably had a competitive advantage. Such flies appeared in each generation due to incomplete transmission of the virus by both sexes. Second, efficiency of transmission could have gradually declined within the host populations if resistance alleles were present in a heterozygous state in their founders, because the hosts with decreased transmission of a virulent parasite have an advantage. This can lead to the increased frequency of these alleles, perhaps even to their fixation, and could facilitate the loss of the virus. Third, because the intensity of the viral infection appears to be reduced under crowded conditions, transmission in the isofemale populations can be inefficient (Dunn and Hatcher, 1997).

Genotype $\times$ environment interactions in the intensities of selection under benign and tough conditions are important for studies of accumulation of deleterious mutations (Kondrashov and Houle, 1994) and inbreeding depression (Ritland, 1996). The results reported here indicate that they may also have a critical effect on the dynamics of vertically transmitted parasites and, perhaps, on their co-evolution with their hosts. *D. melanogaster* and sigma virus appear to be an excellent model for the relevant studies.

ACKNOWLEDGEMENTS

We are grateful to Didier Contamine for advice and for testing some of our flies. The molecular work was performed in the laboratory of Chip Aquadro, with a lot of help from Martha Hamblin, Susan Gleuk and Linda Hook. The comments of two anonymous reviewers are acknowledged with gratitude. This work was supported by NSF grant DEB-9531949.

REFERENCES

- Bras, F., Teninges, D. and Dezelle, S. 1994. Sequences of the N and M genes of the sigma virus of *Drosophila* and evolutionary comparison. *Virology*, **200**: 189–199.
- Brun, G. and Plus, N. 1980. The viruses of *Drosophila*. In *The Genetics and Biology of Drosophila* (M. Ashburner and T.R.F. Wright, eds), pp. 625–702. New York: Academic Press.
- Bull, J.J. 1994. Perspective: Virulence. *Evolution*, **48**: 1423–1437.
- Burdon, J.J., Wennstrom, A., Ericson, L., Muller, W.J. and Morton, R. 1992. Density-dependent mortality in *Pinus sylvestris* caused by the snow blight pathogen *Phacidium infestans*. *Oecologia*, **90**: 74–79.
- Busenberg, S. and Cooke, K. 1993. *Vertically Transmitted Diseases*. Berlin: Springer.

- Contamine, D. 1981. Role of the *Drosophila* genome in sigma virus multiplication. I. Role of the *ref(2)P*: Selection of host-adapted mutants at the non-permissive allele *ref(2)P^P*. *Virology*, **114**: 474–488.
- Coulon, P. and Contamine, D. 1982. Role of the *Drosophila* genome in sigma virus multiplication. II. Host spectrum variants among the haP mutant. *Virology*, **123**: 381–392.
- Dunn, A.M. and Hatcher, M.J. 1997. Prevalence, transmission and intensity of infection by a microsporidian sex ratio distorter in natural *Gammarus duebeni* populations. *Parasitology*, **114**: 231–236.
- Ebert, D. and Herre, E.A. 1996. The evolution of parasitic diseases. *Parasitology Today*, **12**: 96–101.
- Fine, P.E.M. 1975. Vectors and vertical transmission: An epidemiologic perspective. *Ann. NY Acad. Sci.*, **266**: 173–194.
- Fleuriet, A. 1982. Transmission efficiency of the sigma virus in natural populations of its host, *D. melanogaster*. *Arch. Virol.*, **71**: 155–167.
- Fleuriet, A. 1988. Maintenance of a hereditary virus. *Evol. Biol.*, **23**: 1–30.
- Fleuriet, A. 1996. Polymorphism of the *Drosophila melanogaster*–sigma virus system. *J. Evol. Biol.*, **9**: 471–484.
- Fleuriet, A. and Periquet, G. 1993. Evolution of the *Drosophila melanogaster*–sigma virus system in natural populations from Languedoc, Southern France. *Arch. Virol.*, **129**: 131–143.
- Fleuriet, A., Periquet, G. and Anxolabehere, D. 1990. Evolution of natural populations in the *Drosophila melanogaster*–sigma virus system. I. Languedoc, Southern France, *Genetica*, **81**: 21–32.
- Gay, P. and Contamine, D. 1993. Study of the *ref(2)P* locus of *Drosophila melanogaster*. II. Genetic studies of the 37df region. *Mol. Gen. Genet.*, **239**: 361–370.
- Herre, E.A. 1995. Factors affecting the evolution of virulence: Nematode parasites of fig wasps as a case study. *Parasitology*, **111**: S179–S191.
- Knell, R.J., Begon, M. and Thompson, D.J. 1996. Transmission dynamics of *Bacillus thuringiensis* infecting *Plodia interpunctella*: A test of the mass action assumption with an insect pathogen. *Proc. Roy. Soc. Lond., B*, **263**: 75–81.
- Kondrashov, A.S. and Houle, D. 1994. Genotype–environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. *Proc. Roy. Soc. Lond., B*, **258**: 221–227.
- L'Heritier, P. 1970. *Drosophila* viruses and their role as evolutionary factors. *Evol. Biol.*, **4**: 185–209.
- Lipsitch, M., Nowak, M.A., Ebert, D. and May, R.M. 1995a. The population dynamics of vertically and horizontally transmitted parasites. *Proc. Roy. Soc. Lond., B*, **260**: 321–327.
- Lipsitch, M., Herre, E.A. and Nowak, M.A. 1995b. Host population structure and the evolution of virulence: A 'law of diminishing returns'. *Evolution*, **49**: 743–748.
- Lipsitch, M., Siller, S. and Nowak, M.A. 1996. The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution*, **50**: 1729–1741.
- Mangin, K.L., Lipsitch, M. and Ebert, D. 1995. Virulence and transmission modes of two microsporidia in *Daphnia magna*. *Parasitology*, **111**: 133–142.
- Nakamura, N., Gay, P. and Contamine, D. 1986. A study on the *ref(2)P* locus of *Drosophila melanogaster*. I. Cytogenetic localization of *ref(2)P*. *Biology of the Cell*, **56**: 227–238.
- Régnière, J. 1984. Vertical transmission of diseases and population dynamics of insects with discrete generations: A model. *J. Theor. Biol.*, **107**: 287–301.
- Ritland, K. 1996. Inferring the genetic basis of inbreeding depression in plants. *Genome*, **39**: 1–8.
- Shabalina, S.A., Yampolsky, L.Yu. and Kondrashov, A.S. 1997. Rapid decline of fitness in panmictic *Drosophila* populations under relaxed selection. *Proc. Natl. Acad. Sci. USA*, **94**: 13034–13039.
- Wayne, M.L., Contamine, D. and Kreitman, M. 1996. Molecular population genetics of *ref(2)P*, a locus which confers viral resistance in *Drosophila*. *Mol. Biol. Evol.*, **13**: 191–199.