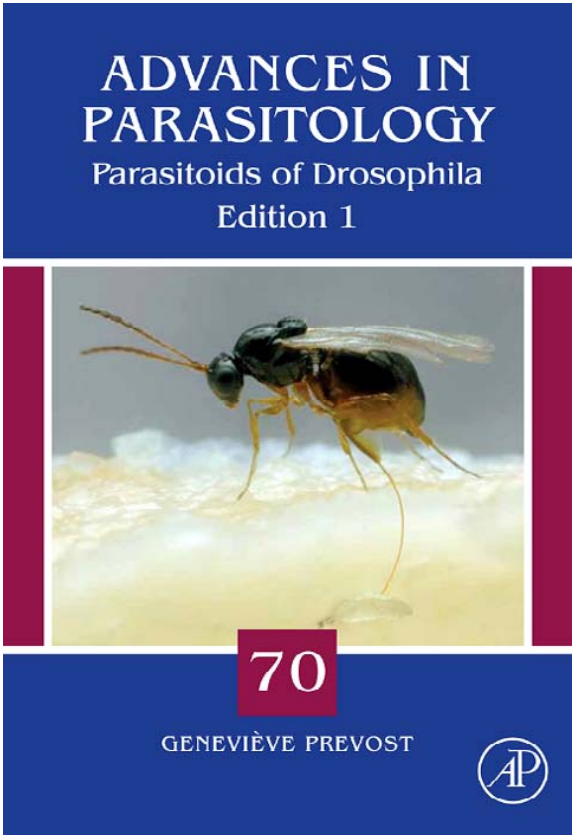

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CHAPTER 13

A Virus-Shaping Reproductive Strategy in a *Drosophila* Parasitoid

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Abstract

Insect parasitoids are often infected with heritable viruses. Some of them, such as polydnaviruses, have evolved toward an obligatory relationship with the parasitoid because they are necessary to protect the parasitoid egg from the host immune reaction.

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However, recent and past discoveries have revealed the presence of facultative inherited viruses in parasitoids for which no clear phenotypic effect was observed. In this chapter, we present how such an inherited virus was recently discovered in the *Drosophila* parasitoid, *Leptopilina boulardi*. We show that this virus is responsible for an increase in the superparasitism tendency of the infected females. This alteration is beneficial for the virus, since superparasitism conditions permit the horizontal transmission of the virus. We review theoretical developments suggesting that this leads to a conflict of interest between the parasitoid and the virus. The direct and indirect influence of the virus on several other fitness traits has also been studied both empirically and theoretically, in particular the egg load. Finally, because the frequency of horizontal transmission is a crucial parameter for the evolution of the superparasitism manipulation, we present an attempt to select the virus for high or low manipulation intensity.

13.1. INTRODUCTION

During the last decades, it has been discovered that host–parasitoid interactions are often directly or indirectly influenced by symbiotic organisms, such as bacteria and viruses. For instance, the symbiotic bacteria *Hamiltonella* infecting aphids confers resistance against parasitoid attack (Oliver et al., 2003, 2007). Although such phenomenon has not been documented to date in *Drosophila* spp., it surely indicates that symbionts have to be taken into account when studying *Drosophila*–parasitoid interactions. This idea finds further support in the recent literature, since symbiotic *Wolbachia* infecting *Drosophila melanogaster* have been found to confer resistance against viral pathogens. It is worth mentioning that this result has been obtained independently by two research groups (Hedges et al., 2008; Teixeira et al., 2008). Parasitoids have also evolved intimate associations with symbiotic bacteria (reviewed in Chapter 12 for *Drosophila*–parasitoids) deeply affecting their reproductive behavior. However, one of the most outstanding mutualistic relationships in parasitoids involves viral particles. Indeed, seven monophyletic subfamilies of Braconidae (the microgastroid complex), and two subfamilies of Ichneumonidae are associated with polydnaviruses (PDV), which replicates in females' reproductive organs without any detrimental effects to the wasp (Glatz et al., 2004). PDVs are injected into the parasitoid host during oviposition and alter host physiology thus allowing parasitoid larvae to circumvent the host immune reaction. It is likely that PDV symbiosis have arisen three times independently (giving rise to Bracovirus, Espagne et al., 2004; Ichnovirus and to a new genus recently proposed, Lapointe et al., 2007), afterward leading to long-standing coevolution between the ancestral viruses and the parasitoids.

Nowadays, all wasp species of these groups have obligate associations with PDVs. PDVs have completely lost their infectious capacity and are only vertically transmitted as an autosomal locus because of their integration within the wasp genome. The origin of PDVs have been debated since they were discovered. Recently, the ancestral bracovirus has been identified as a nudivirus, based on the expression of a large set of nudivirus related genes in the braconid wasp ovaries (Bezier et al., 2009). The ancestral state of the other PDVs is still to be determined. Although PDVs have not been found in *Drosophila* parasitoids, some proteins showing viral-like structure are also injected into the host haemolymph by *Leptopilina* spp. (Dupas et al., 1996; Rizki and Rizki, 1990). Although they do not contain deoxyribonucleic acid (DNA; as opposed to PDVs), these virus-like particles (VLPs) also circumvent the host immune reaction and may have a viral evolutionary origin. To understand the origin and mechanisms of virus or VLP incorporation into the wasps' genomes, it may be useful to study nowadays infectious viruses that are able to infect parasitoids. In *Leptopilina boulardi*, we have found that some females are infected by an inherited virus that manipulates the behavior of the wasp (Varaldi et al., 2003, 2006b). This virus, called LbFV for *L. boulardi* filamentous virus, forces the infected females to accept to lay their eggs in already parasitized hosts (a behavior called superparasitism). This behavioral manipulation benefits to the virus spread since superparasitism allows its horizontal transmission (transmission between unrelated parasitoid lineages). The peculiar transmission mode of this virus allows it to maintain and reach high frequencies in natural populations. The present chapter reviews the different features of this parasitoid/virus association.

13.2. MAIN EFFECT AND TRANSMISSION OF LbFV

As mentioned in previous chapters, all *Drosophila* parasitoids are solitary parasitoids, meaning that one *Drosophila* larva allows the development of a single parasitoid, whatever the number of parasitoid eggs. Females are usually able to recognize parasitized from unparasitized hosts (host discrimination) and normally avoid laying eggs in already parasitized host. If a female oviposits in a parasitized host, a behavior called superparasitism, parasitoid larval competition ends up in the death of all but one larva. Usually the second larva is most likely to be out-competed and its survival depends on the interval between the first and second ovipositions (van Alphen and Visser, 1990). If a parasitoid female accepts several times the same host (a behavior called self-superparasitism), she will waste some eggs since brothers and sisters will compete for the possession of the host until all but one die. Superparasitism is thus expected to be strongly counter selected in most ecological conditions. One

remarkable feature of *L. boulardi* was that in some populations, females showed a huge tendency to superparasitize, while in others most females laid only one egg per host. In the related *L. heterotoma*, however, few superparasitism was observed (Varaldi et al., 2005b). In *L. boulardi*, we were thus able to derive stable ''nonsuperparasitizing'' lines (NS) and ''superparasitizing'' lines (S). From these lines, we studied the genetic determinism. Surprisingly, the variations in the superparasitism phenotype were strictly maternally inherited: whatever the nuclear genotype, females adopted the phenotype of their mother. Furthermore, when both S and NS lines laid their eggs inside the same host, in the case where NS lines won the within-host competition, the emerging (female) offspring did adopt the ''superparasitizing'' phenotype, despite the NS phenotype of its line of origin (Varaldi et al., 2003)! All is happening as if some unknown infectious element was causing the ''superparasitizing'' phenotype and was passed from S-infected lines to NS-uninfected lines during the short time they coexisted inside the *Drosophila* larva. The newly acquired S phenotype was stably transmitted over generations (Varaldi et al., 2006b). The infectious nature of the S-inducing element was further confirmed by injecting solutions derived from S individuals into *Drosophila* larvae parasitized by NS females. Solutions of S females proved its ability to induce the S phenotype on the emerging parasitoid females (originating from an NS line), whereas NS control injections did not induce any behavioral change (Varaldi et al., 2006b). The hypothesis that the causative agent was a bacterium was tested and clearly ruled out using antibiotic treatments (Varaldi et al., 2006b). The nature of the infectious element was finally determined by electron microscopy investigations inside the ovaries of *L. boulardi* females. It was evident that in S lines, a virus was replicating in cells bordering the lumen of the oviduct, contrary to NS females (Varaldi et al., 2003, 2006b). Based on its morphology, the superparasitism-inducing virus was called LbFV (for *Leptopilina boulardi* Filamentous Virus). The virus LbFV is thus vertically transmitted through the female line, and also horizontally in conditions of superparasitism.

To date, the precise means of transmissions are not known, but our working hypothesis is that the virus is injected in addition to the egg into the host during oviposition and that it infects the emerging parasitoid during its larval life (during which the parasitoid consumes the infected host hemocoel). If the infected parasitoid develops alone, then vertical transmission occurs (with a very efficient rate, near 100% under laboratory conditions), while if superparasitism occurs, horizontal transmission may occur. We suspect that the efficiency of the horizontal transmission depends critically on the delay between successive ovipositions: if an S female superparasitizes soon after an NS female has laid her egg, then the efficiency will be high, while if this delay is important the efficiency drops (Varaldi et al., 2006c). Accordingly, when we inject extracts of

S ovaries inside *Drosophila* larvae previously parasitized by NS females, the efficiency of the contamination is high if the delay is low (<24 h: 44% ($n = 9$)), and drops to zero when we increased the delay (24 – 48 h, 0% ($n = 17$); 48–72 h: 0% ($n = 21$) temperature: 26 °C).

LbFV has been discovered using electron microscopy and thus we lacked any genomic data. This precludes from identifying its phylogenetic position and from developing molecular tools, such as markers. Since LbFV could be either a DNA or ribonucleic acid (RNA) virus, we focused our attention on the identification of viral messenger RNA (mRNA; because both viral types should produce mRNAs). We performed a suppressive subtractive hybridization (SSH) between two lines sharing the same genotypic background but differing in their superparasitism behavior. This work permitted to identify an 809 base pairs (bp) mRNA that was S specific. From this mRNA sequence, we derived a simple polymerase chain reaction (PCR) test that showed amplification on all 14 independent S lines whereas no amplification was observed for all 11 independent NS lines, starting with DNA extracts as templates (Patot et al., 2009). This perfect correlation between superparasitism phenotype and PCR-amplification validates the viral origin of this sequence. Furthermore, it shows that LbFV has at least an intermediate DNA step during its replication cycle or that, more likely, LbFV has a DNA genome. This is consistent with the electron microscopy investigations showing apparent viral replication within the nuclei of the cells. This work (Patot et al., 2009) also indicates that the virus reaches very high prevalence in natural populations (around 70% in both sampled populations in the South of France), despite the fact that the penetrance of the extended-phenotype was incomplete (only 80% of the infected females expressed signs of behavioral modification).

13.3. ADAPTIVE SIGNIFICANCE OF SUPERPARASITISM ALTERATION: A MODELIZATION APPROACH

The vertical transmission of the virus implies that the virus and the parasitoid share some fitness components (they both benefit from female fecundity). It thus remains unclear whether this induced superparasitism behavior is actually adaptive for the virus (Gandon, 2005; Varaldi et al., 2003). To demonstrate the adaptive nature of the alteration of the parasitoid behavior one must show that a virus increasing superparasitism can invade a virus population that does not alter the behavior of its parasitoid host. In other words, one must demonstrate that the evolutionarily stable strategy (ESS) of superparasitism for the virus is higher than the ESS superparasitism for the parasitoid (in the absence of the virus). To address this question, we developed a model that allows to analyze

both the dynamics and the evolution of a population of parasitoids (a proovigenic and solitary species) parasitizing a population of hosts (Gandon et al., 2006). This model includes the potential benefit of superparasitism (the possibility that parasitoid larvae developing in an already parasitized host win the within-host competition) and both the classical costs of superparasitism (the costs of time and the cost of eggs). We first used this model in the absence of any virus, to predict the fate of a mutant parasitoid with superparasitism strategy s^* appearing in a parasitoid population dominated by a resident with strategy s (where s indicates the rate of acceptance of parasitized hosts). As expected, the model predicts that the ESS of superparasitism is zero when the probability to win the within-host competition (c) is low but increases with an increase in c. This further confirmed previous models showing the potential adaptive value of superparasitism under conditions of host scarcity (van Alphen and Visser, 1990).

We extended the model to include a virus, based on LbFV biology. When females are infected, it is assumed that the parasitoid behavior is strictly under the control of the virus. In other words, the rate of acceptance of parasitized hosts of an infected female is no more s (the superparasitism strategy when the female is uninfected), but instead σ which is a feature of the virus. The virus is vertically transmitted with a rate of tv (<1), and will gain extra routes of transmission via the potential horizontal transmission that may occur between a larva infected with the virus and an uninfected larva (with probability τ_h). To allow direct competition between viral strains, it is assumed that a viral strain can replace another one when they compete inside the same *Drosophila* larva with a probability ε. However, no multi-infections at the adult stage are allowed. The model can be used to derive an expression of the fitness of a mutant virus with a strategy σ^* appearing in a population dominated by a resident virus with strategy σ, at the epidemiologic equilibrium set by the resident virus and the strategy s adopted by the host. Note that here, only the virus is allowed to evolve, not the parasitoid (s is fixed). In a first part, we fixed $\varepsilon = 0$, that is, a viral strain is not able to replace a resident viral strain in competition within *Drosophila* larvae. The results indicate that the ESS superparasitism is always higher for the virus than that observed for the parasitoid (allowed to evolve to its optimal strategy in the absence of the virus) demonstrating the adaptive value of the behavioral modification from the virus point of view. The virus is always selected to increase the natural superparasitism tendency of the parasitoid. The presence of the virus thus induces an evolutionary conflict of interest between the parasitoid and the virus on superparasitism behavior (Fig. 13.1A). The intensity of the evolutionary conflict is even increased if both the virus and the parasitoid are allowed to coevolve: after coevolution, uninfected females (that are produced even in infected populations, due to imperfect vertical

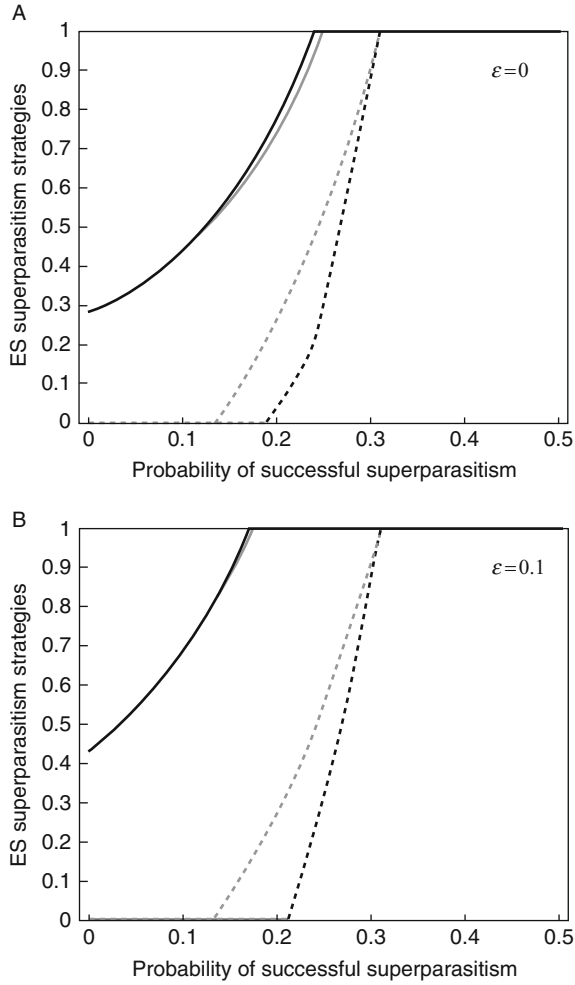


FIGURE 13.1 Evolutionarily stable superparasitism strategies of the virus (solid lines) and the parasitoid (dotted lines) versus the probability of successful superparasitism. In (A) $\varepsilon = 0$, in (B) $\varepsilon = 0.1$. The gray lines indicate a situation where the parasitoid does not coevolve with the virus. The black lines indicate the coevolutionary stable strategies of the virus and the parasitoid. Parameter values: $d = 0.2$; $e = 0.2$; $m = 0.1$; $t1 = 0$; $t2 = 0.1$; $a = 0.01$; $x_{tot} = 100$; tv $= 0.95$; th $= 0.75$; $e_{max} = 15$. (See Gandon et al., 2006 for details on parameter values).

transmission) should less superparasitize than uninfected females that did not coevolve with the virus (Fig. 13.1A). This shows that the presence of the virus in a population should indirectly modify the ESS of a trait for uninfected females. When we allowed direct competition between viral strains within *Drosophila* larvae ($\varepsilon > 0$), we found that the virus is even

selected for much higher superparasitism strategies, thus strongly increasing the conflict of interest between the parasitoid and the virus (Fig. 13.1B). Coevolution between the virus and the parasitoid further increased the conflict of interest as has been found with $\varepsilon = 0$ (Fig. 13.1B). These results clearly show that increasing the superparasitism strategy of the parasitoid is an adaptive strategy from the virus point of view (whatever ε). To say it differently, there is a conflict of interest between the virus and the parasitoid on superparasitism behavior. However, the intensity of the conflict of interest depends critically on the ability of mutant virus strains to replace resident strains inside *Drosophila* larvae (ε) and also on coevolutionary processes.

13.4. EFFECT OF LbFV ON OTHER PHENOTYPIC TRAITS

It may be argued that *L. boulardi* females infected with LbFV adopt an aberrant behavior without any adaptive significance (neither for the host nor for the virus), because the virus disrupts indifferently several cognitive and possibly physiological properties (Poulin, 1995). However, it has been found that LbFV infection has no effect on parasitoid survival of females (but a negative impact on male survival), and only a slight negative impact on size (tibia length is reduced by 2%), and developmental speed (increased by 3% for both sexes). Nevertheless, the overall locomotor activity of infected females is reduced by 45% while no effect was detected on males. Interestingly, we found that egg load was even increased for infected females (+11%) compared to uninfected females (Varaldi et al., 2005a). Overall, the effect of LbFV on various traits is relatively moderate (except for locomotor activity) or even positive (egg load; Table 13.1). This surprising beneficial effect on egg load will be discussed in detail in the next section.

The influence of LbFV on several behaviors (apart from superparasitism) has also been investigated (Varaldi et al., 2006a). The behavioral components studied included sexual communication, circadian rhythms, ability of females to detect odors of hosts and trajectometric parameters of foraging females. None of these behavioral repertoires seemed to be perturbed by LbFV infection, demonstrating a specific action of LbFV on superparasitism behavior (Table 13.1).

How does the virus manage to have such a specific action? *L. boulardi* females need to pierce the skin of the host larvae with their ovipositor to detect chemical cues associated with a previous infestation. In effect, the ovipositor of parasitoids harbors chemoreceptors that are probably (all or some of them) involved in host discrimination. Their distribution and putative function has been investigated in great details on the related species *L. heterotoma* (van Lenteren et al., 2007). This species also needs to

TABLE 13.1 Effect of LbFV on several general traits and behavioral traits

		LbFV effect (%)	Ref
Physiology	Survival	0	1
	Size	–2	1
	Development speed	+3	1
	Egg load	+11	1
	Sex ratio	0	1
	Locomotor activity	–45	1
Behavior	Superparasitism	+++	3
	Circadian rhythm	0	2
	Perception of host odors by females	0	2
	Female searching paths	0	2
	Female interspecific discrimination	0	4
	Male detection of pheromones	0	2

Notes: 1: Varaldi et al. (2005a); 2: Varaldi et al. (2006a); 3: Varaldi et al. (2003); 4: this study.

pierce the skin of the host to detect the presence of a previous infestation. The authors found seven chemoreceptors at the tip of the ovipositor that come into contact with the *Drosophila* haemolymph during host probing. One single chemoreceptor was found on the unpaired valve, and three on each paired valve. Each chemoreceptor is innervated with six neurons. One tempting hypothesis would be that LbFV injures these neurons involved in the transmission of the nervous flux, either through cell lysis or through manipulation of gene expression. However, based on the work done in *L. heterotoma* (van Lenteren et al., 2007), it is unlikely that the gustatory receptor situated on the unpaired valve is the target of LbFV action since electrophysiologic investigations suggest that it is not involved in host discrimination. The perception of previous infestations is thus probably assured by some or all of the remaining six chemoreceptors present on the paired valve, and LbFV may interfere with some of them.

In addition to discriminating between parasitized and unparasitized hosts, female parasitoids usually make selective host choices when several potential related host species are available in the environment. The value of these different host species may differ in terms of parasitoid fitness and we expect that female parasitoids discriminate among them by preferentially laying their offspring in the most profitable host species. We would also predict that the virus should not interfere with this decision since both the virus and the parasitoid has interest in developing in a good host. However, the sensory capacities of the ovipositor are also probably involved in this decision process. In order to test

(1) whether *L. boulardi* females discriminate between good and bad host species, and (2) whether LbFV interferes with this ovipositor-based decision, we conducted a choice experiment in which we proposed a mix of *D. melanogaster* and *D. subobscura* to *L. boulardi* females. Both *Drosophila* spp. can be found in the same microhabitat, although *D. subobscura* is less frugivorous than *D. melanogaster*. While *D. melanogaster* offers a very good host for the development of *L. boulardi*, *D. subobscura* is reputed to be an unfavorable host (Carton et al., 1986). Indeed, based on the protocol described in Varaldi et al. (2005a), we estimated the preimaginal survival (probability of an egg to reach adulthood) of *L. boulardi* (strain Antibes) as 0.74 ± 0.09 (mean $\pm$ standard error, $n = 10$) on *D. melanogaster* and only 0.14 ± 0.09 ($n = 12$) on *D. subobscura* (at 25 °C). To test whether *L. boulardi* discriminates between *Drosophila* spp. and whether LbFV interferes with this decision, we did the following experiment. Isolated *L. boulardi* females (either infected or not, but sharing the same nuclear background as in Varaldi et al., 2005a) were provided with a mix of larvae that hatched from 75 *D. melanogaster* and 75 *D. subobscura* eggs in standard rearing tubes (at 21 °C). Because *D. subobscura* eggs needs more time to hatch than D. *melanogaster* and *D. subobscura* larvae grow slower than *D. melanogaster*, we used *D. subobscura* eggs collected 24 h before *D. melanogaster eggs*. Consequently, at the time that we added the parasitoid female within the tube, *D. melanogaster* were 24 h old (time since eggs were deposited within tubes), whereas *D. subobscura* were 48 h old. In these conditions, the size of larvae of both species is comparable (Varaldi et al., 2005b). Females were allowed to parasitize the larvae for 24 h. Starting from the moment at which the females were added to the vials, they were transferred at 24 °C ($\pm$ 1 °C) until the end of the experiment (this temperature was chosen because it was suitable for *D. melanogaster*, low enough for *D. subobscura* and was high enough to prevent the diapause of *L. boulardi*). Sixteen replicates of each test modality were simultaneously conducted, in addition to 12 controls kept without parasitoids that were manipulated exactly in the same way as test tubes. For each of the 44 tubes, we scored the number and identity of the *Drosophila* reaching adulthood, and the number of emerging *L. boulardi* in test tubes.

The choice of each female was indirectly measured by first calculating the parasitoid-induced mortality on each *Drosophila* spp. which is a measure of the attack rate (a). Indeed, neither *D. subobscura* nor this strain of *D. melanogaster* are able to get rid of parasitoids by mounting an efficient immune reaction, thus the parasitoid-induced mortality is a good estimation of the proportion of *Drosophila* spp. that have been attacked and parasitized (in accordance with this hypothesis, we found no capsule on all emerging adult *Drosophila*). Attack rates against each *Drosophila* species were then defined for each parasitoid female as:

$$a_{\text{mel}}i = (\text{mean number of } D.\ melanogaster \text{ in controls} - \text{number of } D.\ melanogaster \text{ in test tube } i)/\text{mean number of } D.\ melanogaster \text{ in controls}$$

$$a_{\text{sub}}i = (\text{mean number of } D.\ subobscura \text{ in controls} - \text{number of } D.\ subobscura \text{ in test tube } i)/\text{mean number of } D.\ subobscura \text{ in controls}$$

Based on this, we derived a choice index calculated for each female. There was a slight difference in the survival of *D. melanogaster* and *D. subobscura*, since a mean of 58.72 *D. melanogaster* emerged from the controls (without parasitoid) versus 45.45 *D. subobscura* (out of 75 eggs initially deposited). We made the assumption that the mortality occurred before *Drosophila* eggs were exposed to the wasps (considering that the mortality occurred after the exposition to the wasp gave very similar results). Thus in each test tube, we estimated that the parasitoid female was provided approximately $58.72 + 45.45 = 104.17$ *Drosophila* larvae, including 56% (58.72/104.17) *D. melanogaster*. For each female i, we calculated the whole rate of attack of both *Drosophila* spp. as:

$$a_{\text{global}}i = (a_{\text{mel}}i \times 58.72 + a_{\text{sub}}i \times 45.45)/104.18$$

To quantify the choice of each female, we derived an index, using an analogy with the calculation of the linkage disequilibrium in population genetics: on the one hand, we know the proportion of both *Drosophila* spp. in tubes (56% *D. melanogaster* and 44% *D. subobscura*) and, on the other hand, we know for each female *i* the whole attack rate ($a_{\text{global}}i$). Under the hypothesis h0 that wasp attacks are randomly distributed among *Drosophila* spp., then we expect for a female *i*:

$$\begin{aligned}\text{Proportion of } D.\ mel \text{ attacked} &= 0.56 \times a_{\text{global}}i\\ \text{Proportion of } D.\ mel \text{ nonattacked} &= 0.56 \times (1 - a_{\text{global}}i)\\ \text{Proportion of } D.\ sub \text{ attacked} &= (1 - 0.56) \times a_{\text{global}}i\\ \text{Proportion of } D.\ sub \text{ nonattacked} &= (1 - 0.56) \times (1 - a_{\text{global}}i).\end{aligned}$$

We can then calculate a deviation from this null model by subtracting for instance the proportion of *D. melanogaster* effectively attacked by the wasp in tube *i* with the expected proportion of attacks on *D. melanogaster* under h0:

$$c = a_{\text{mel}}i - 0.56 \times a_{\text{global}}i.$$

If this choice index (c) is positive then the female preferentially attacked *D. melanogaster*, whereas if this is negative, the female preferentially attacked *D. subobscura*. Because the range of variations for this index

may vary between females (because their whole attack rates vary), we scaled it to range between –1 and +1 for all females (as is done for the calculation of D′ in population genetics) by dividing the choice index by its minimal value (if negative) or maximal value (if positive). Minimal and maximal values can be obtained this way:

$$c_{\min} = \min\left(0.56, 1 - a_{\text{global}} i\right) - 0.56 \times \left(1 - a_{\text{global}} i\right)$$
$$c_{\max} = \min\left(0.56, a_{\text{global}} i\right) - 0.56 \times \left(a_{\text{global}} i\right).$$

And finally, the scaled choice index can be obtained this way:

$$\begin{aligned} c^* &= c/c_{\min} \quad \text{if } c < 0 \\ &= c/c_{\max} \quad \text{if } c > 0. \end{aligned}$$

This scaled choice index (c^*) varies between –1 when the female concentrated to the best her attacks on *D. subobscura*, and +1 when the female concentrated to the best her attacks on *D. melanogaster*, and equals zero when the female do not show any preference.

The survival from egg to adulthood was also estimated for the offspring of each parasitoid female (each test vial):

$$\text{Offspring survival} = \text{number of parasitoid reaching adulthood in tube } i / \text{number of } \textit{Drosophila}\ (\textit{mel} + \textit{sub}) \text{ killed due to parasitism in tube } i$$

where number of *Drosophila* (*mel* + *sub*) killed due to parasitism in tube i = (mean number of *D. melanogaster* in controls – number of *D. melanogaster* in tube i) + (mean number of *D. subobscura* in controls – number of *D. subobscura* in tube i).

The number of *Drosophila* emerging in each vial is plotted in Figure 13.2A. *D. melanogaster* had a higher preimaginal survival than *D. subobscura* and the parasitoids induced a significant mortality on both *Drosophila* spp. indicating that both species were attacked by the parasitoids. The results indicate that *D. melanogaster* suffered a higher parasitoid-induced mortality than *D. subobscura*, suggesting a choice in the direction of the former. This trend was confirmed by the calculation of the choice index, which was significantly above 0 for both infection status (Student t test respectively 6.52 and 6.13 for uninfected and infected wasps, degrees of freedom (df) = 14 and 15, both $P < 0.00001$, see Fig. 13.2B). Importantly, the choice indexes obtained for infected or uninfected wasps were very similar ($t = 0.62$, 29, $P = 0.27$). First, the results show that *L. boulardi* is able to discriminate between both *Drosophila* spp. This can be due to the perception at distance of larval kairomones (odors produced by the larva) differences or to contact differences. Since both species were mixed within the tubes, *Drosophila* odors should also mix and it is unlikely that *L. boulardi* was able to use volatile components to

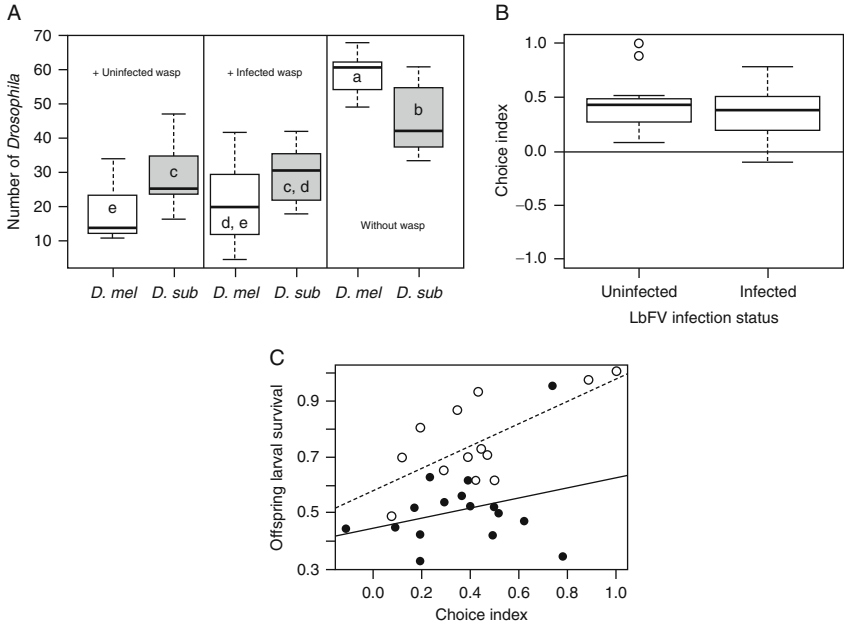


FIGURE 13.2 Host choice in *L. boulardi* and effect of LbFV. (A) Number of *Drosophila* emerging from vials containing initially 75 eggs of *D. melanogaster* (*D. mel*) and 75 eggs of *D. subobscura* (*D. sub*). Boxes with different letters (a–e) are significantly different at 0.05. (B) Choice index: negative values indicate preference for *D. subobscura*, whereas positive values indicate preference for *D. melanogaster*. (C) Open circles: uninfected, closed circles: infected. Relation between host choice and offspring survival.

discriminate in these conditions. Instead, the females probably used information obtained with their ovipositor, either by probing the medium close to the larvae or directly the larvae. There was a clear choice for *D. melanogaster* which is the most profitable host, suggesting an adaptive value for this trait. This conclusion was further supported by the global positive correlation between the choice index and the offspring larval survival ($F(1,27) = 6.91$, $P = 0.014$, Fig. 13.2C). The correlation was, however, only significant for uninfected wasps ($F(1,11) = 9.68$, $P < 0.01$ for uninfected and $F(1,14) = 1.35$, $P = 0.26$ for infected wasps) but the tendency was the same for both infection status (Fig. 13.2C). The more females chose *D. melanogaster*, the higher was their offspring survival. This confirmed previous results showing adaptive host choice obtained on *L. boulardi* or related species (Dubuffet et al., 2006; Pannebakker et al., 2008). Importantly, LbFV did not alter this adaptive host selection decision. This suggests that LbFV specifically impairs perception skills involved in superparasitism avoidance (possibly chemoreceptors)

without impairing receptors involved in the discrimination among different host species, which is quite remarkable since both perception skills are probably due to chemoreceptors innervating the ovipositor.

13.5. ADAPTIVE SIGNIFICANCE OF THE PHENOTYPIC ALTERATION INDUCED (EXCEPT SUPERPARASITISM)

In Section 13.3, we presented a theoretical approach that shows that the viral-induced modification of superparasitism behavior is an adaptive trait for the virus. This conclusion is further supported by the fact that no other behavioral component is modified by the virus (Table 13.1), underlying the specificity of the behavioral modification. A conflict of interest arises between the parasitoid and the virus since they are selected for divergent superparasitism strategies. Consequently, both partners are in conflict of interest from an evolutionary point of view. What about other traits? ''Physiology''-related traits appear to be relatively poorly affected by the virus except for locomotor activity which is reduced by 45%. This may result from an energetic cost induced by the replication of the virus, which may reduce the energy available for the insect movement. One surprising result concerns the egg load. How can the observed increase of egg load in LbFV-infected females be explained? Is it an adaptation of the parasitoid, in response to virus infection or an adaptation (another way to manipulate the reproductive behavior of the parasitoid) of the virus to increase its own transmission?

To address this question, we modified the model used to study the evolution and the manipulation of superparasitism (Gandon et al., in press). In this model, each parasitoid female is born with a fixed number of eggs and lacks the ability to mature additional oocytes later on (i.e., strictly proovigenic parasitoid). The initial egg load may be modified by the presence of the virus (either caused by a manipulation induced by the virus or by a plastic response of the host) and Ez and E refer to the egg load at emergence of infected and uninfected females, respectively. The evolution of the egg load of proovigenic parasitoid species, like any other life history trait, can be viewed as a resource allocation problem. Producing more eggs will divert resources from other important life history traits. In our model, we consider various tradeoffs between egg load and the probability of emergence, and adult survival. The ESS resource allocation strategy is the one that balances the benefits and the costs of producing more eggs.

This model can be used to study the evolution of egg load in the absence of a virus manipulating the behavior of the females. In this simple scenario, we recovered the main result of Rosenheim (1996) that the evolutionarily stable egg load increases with the rate of oviposition thus limiting the risk of egg limitation (i.e., the probability to exhaust its

total number of eggs before dying). We can also use this model to consider the situation where a virus manipulating the superparasitism behavior is present in the population (and has reached an endemic equilibrium). In this case, the parasitoid population becomes heterogeneous. Some individuals are uninfected and have a low probability of superparasitism, while other individuals are infected by the virus and have large probabilities of superparasitism. We use our model to analyze different scenarios depending on the ability of the parasitoid females to adopt plastic strategies with regard to viral infection.

First, we consider that the egg load of the females is only determined by the female but not by the virus. If egg load is allowed to be conditional on the infectious status (i.e., two different egg loads may be expressed, depending on whether or not the female is infected), we found that the ESS egg load is to increase egg load when the female is infected. This is due to the fact that infected females lay a higher number of eggs because they also lay eggs in already parasitized hosts (because females infected by the virus are assumed to always superparasitize). They thus have a higher chance of being egg limited (to run out of eggs before dying) than uninfected hosts, and this is why they evolve higher egg loads. Second, if the egg load is assumed to be a fixed strategy (independent on whether or not the female is infected) we found that the evolution of the parasitoid egg load is mainly driven by the selection acting on infected parasitoids because of the often large prevalence of the virus in the population (due to high rates of vertical and horizontal transmission). As a consequence, the unconditional ESS is close to the conditional ESS of infected females, and is thus increased by the presence of the virus in the population.

Then we also considered the scenario where the egg load of infected females is actually governed by the virus, not the parasitoid. When the virus is allowed to manipulate parasitoid egg load we find that it always increases the number of eggs above the ESS level in the absence of the virus. Thus, the fact that infected females of *L. boulardi* tend to have a higher egg load than uninfected females could be explained by two adaptive scenarios. Under the first scenario, *L. boulardi* females have evolved the ability to increase their egg load only when they are infected. Indeed, infected wasps have a higher rate of oviposition (and higher risk of egg limitation) than uninfected ones due to the manipulation of superparasitism. It is thus adaptive for infected females to produce more eggs to reduce the risk of egg limitation (increased by superparasitism). This situation thus corresponds to adaptive phenotypic plasticity of the parasitoid. Under the second scenario, this increase of egg load is induced by a manipulation of the virus. For the virus, higher egg load is also adaptive because it offers additional opportunities of vertical and horizontal transmission. This increase in egg load would thus correspond to another side of the manipulation of the parasitoid phenotype by the virus. The only

way to distinguish between the two alternatives would require an examination of the mechanism responsible for the shift in egg load. For example, one could demonstrate that it is a conditional response if it was possible to see a change in egg load in exposed-but-not-infected females (see Minchella, 1985, for a similar experiment in snails and trematodes).

Interestingly, thus, in contrast with our analysis of the evolution of superparasitism, the analysis of this model does not allow us to determine if the higher egg loads are an evolutionary response of the host or a manipulation by the virus. This results from the fact that there is no real conflict over the evolution of this trait between the parasitoid and the virus. Given that the virus manipulates the superparasitism of infected females, both partners benefit from increasing the egg load above the level in the absence of the virus. Another consequence of this alignment of interests can be seen when the parasitoid and the virus are allowed to coevolve. The optimal egg load strategies of the virus and of the uninfected females tend to be closer after coevolution. Again, this contrasts with the adaptive dynamics of superparasitism (Gandon et al. 2006), where coevolution increases the difference between the virus and the parasitoid strategies (Fig. 13.1).

13.6. EVOLUTION IN RELATION TO THE FREQUENCY OF HORIZONTAL VERSUS VERTICAL TRANSMISSION

The mode of transmission of a pathogen has long been recognized as a critical feature to consider in order to understand and predict its evolution (Ewald, 1987). It is clear that for a parasite with strict vertical transmission, host and parasite fitness are strongly correlated and any parasite feature that decrease host fitness will be counter selected. Consequently, vertical transmission is usually associated with low virulence or even mutualism (see however Chapter 12 by Vavre et al. for the special case of reproductive parasites). However, when a parasite is horizontally transmitted, host and parasite fitness are no more correlated and selection may promote highly virulent parasites, if increased virulence favors transmission. In the LbFV/*L. boulardi* system, both transmission modes may occur. Furthermore, depending on ecological conditions such as the ratio of parasitoids–hosts, the opportunities of horizontal or vertical transmission may vary. Indeed, if this ratio is low (numerous hosts for few parasitoids), then there will be few superparasitism and low horizontal transmission opportunities, whereas if this ratio is high (numerous parasitoids for few hosts), opportunities for horizontal transfer may be high (we will see later that this simple view is partly caricatural). This raises the question of the consequence of such ecological changes on the evolution of LbFV, and in particular on the evolution of superparasitism behavior. Based on the model described in Section 13.3 and in Gandon et al. (2006), we studied

the ESS of superparasitism of the manipulating virus, as a function of th, which measures the probability of horizontal transmission between infected and uninfected parasitoids sharing the same host (superparasitism). Note that in the model, we assumed that the outcome of the competition between the resident and the newly arrived parasitoid larva is determined very rapidly. Consequently, the model does not keep track of superparasitized hosts because in those hosts, soon after superparasitism, only a single larva remains alive. In the model, parasitized hosts thus regroup hosts that have been parasitized once or several times. We identified two situations, depending on the value of ε, which measures the probability that a viral strain A replaces a resident viral strain B during the short period where both strains compete within the same superparasitized host (superinfection). Because we do not have any indication to date on the value of ε in reality, we derived the ESS of superparasitism for $\varepsilon = 0$, which corresponds to the case where no superinfection can occur and $\varepsilon = 0.5$, which corresponds to the situation where a supernumerary virus strain can outcompete the resident virus in 50% of the cases.

First, in Figure 13.3, it can be noted that below a certain value of th (0.2), the virus cannot maintain in the wasp population. This is due to the fact that at each generation, infected females produce only 95% infected offspring due to the incomplete vertical transmission. We have clear indications that vertical transmission is very efficient but imperfect (Varaldi et al., 2006c). In the absence of horizontal transmission or any

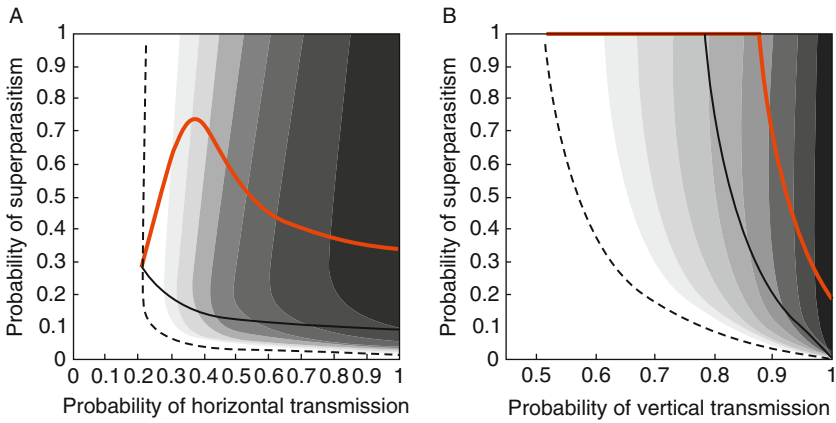


FIGURE 13.3 (A) Evolutionarily stable strategy (ESS) of superparasitism for the virus against the probability of horizontal transmission (th). (B) ESS of superparasitism for the virus against the probability of vertical transmission (tv). The shades of gray indicate the prevalence of the virus in the population, darker gray indicates more virus (10% difference in prevalence between each shade of gray). The dashed line indicates the threshold below which the virus goes extinct. Black line: $\varepsilon = 0$ (no superinfection). Gray line: $\varepsilon = 0.5$ (some superinfection may occur).

fitness advantage to being infected, because uninfected females will obviously produce 100% uninfected females, the frequency of infection in the whole population should decrease by a factor 0.95 from one generation to the next until disappearance. This verbal argument (but see Lipsitch et al., 1995 for a modelization of this simple problem) shows that without other compensating mechanism the virus cannot maintain in populations. The mechanism compensating for this incomplete vertical transmission is precisely the horizontal transmission, but in this situation (th < 0.2), it is not sufficient to compensate the incomplete vertical transmission and the virus is ousted from the population.

In the simplest situation where $\varepsilon = 0$ (no superinfection), increasing the probability of horizontal transfer (starting from 0.20) decreases the ESS of superparasitism for the virus (black line in Fig. 13.3A). This result may sound counterintuitive because it means that even if the probability of horizontal transfer is increased, the virus is selected for lower superparasitism, although superparasitism is precisely the mechanism necessary for horizontal transfer. The explanation lies in the fact that an increase in th has important epidemiologic consequences. Increasing the probability of horizontal transfer leads to a better diffusion of the virus between unrelated parasitoids and consequently to higher prevalence at the epidemiologic equilibrium (gray shading in Fig. 13.3A). Furthermore, an increase in the virus prevalence leads to an increase of the aggregation of wasp eggs inside *Drosophila* larvae and thus to a decrease in the proportion of parasitized hosts (with or without virus). Thus, increasing the probability of horizontal transmission has two main consequences. First, it decreases the number of parasitized hosts and thus limits the benefits of superparasitism. Second, it increases the prevalence of the virus among those hosts that are parasitized. This also selects against superparasitism when $\varepsilon = 0$ because no horizontal transmission can take place in this situation. Thus both these effects go in the same direction and explain why a small increase in th can lead to a decrease in the ESS superparasitism of the virus.

In contrast, if some superinfection is allowed (i.e., $\varepsilon > 0$) the pattern can be very different because horizontal transmission can take place even if the parasitoid already present in the host is infected by another strain of the virus. First, all parameter sets led to higher ESS values with $\varepsilon = 0.5$ than with $\varepsilon = 0$. This result makes sense since with $\varepsilon = 0.5$ an already parasitized host represents a potential wasp to colonize for a mutant virus even if it is already infected by a resident virus (contrary to the case where $\varepsilon = 0$). This leads to an increase in the payoff from superparasitism from the virus point of view. This result also confirms that increasing ε also increases the intensity of the conflict of interest between the parasitoid and the virus (see also fig. 5c and d in Gandon et al., 2006 and Fig. 13.1). With $\varepsilon = 0.5$, the ESS of superparasitism takes a humped shape, with an increase for low prevalence (or low probability of horizontal transmission)

and a subsequent decrease for higher prevalences (high probability of horizontal transmission). The interpretation of this result also implies the correlative change in the viral prevalence. For low probability of horizontal transmission (but >0.2), the virus maintains at relatively low frequency (below 20%), and there is lots of opportunities for horizontal transfer. Conversely to the case where $\varepsilon = 0$, increasing the probability of horizontal transmission also increases the opportunities for horizontal transmission even at the epidemiologic equilibrium (where the prevalence reaches its equilibrium value) because one viral strain can replace another one within the host. This selects for higher superparasitism until a critical prevalence value is reached (about 20% with this parameter set) where the environment starts to saturate with the virus (which reduces the proportion of parasitized hosts due to egg aggregation), reducing drastically the opportunities for viral horizontal transfer (at the epidemiologic equilibrium) and also reducing the opportunities for vertical transmission during superparasitism. Consequently, this selects for reduced superparasitism.

When we fixed the probability of th, and varied the value of tv, the interpretation was much simpler (Fig. 13.3B). Here again, there was a minimal value for tv for the virus to maintain in the population (0.65). Above this threshold, the viral prevalence increased monotonously with an increase of tv. As expected, the ESS for the virus was high when tv is low and decreased afterward. This pattern was observed for both situations ($\varepsilon = 0$ and $\varepsilon = 0.5$). In Section 13.3, we have shown that the virus is selected for higher ESS values of superparasitism than the parasitoid (except in some peculiar combination of parameter sets, e.g., $\text{tv} = 1$ and $\varepsilon = 0$). In other words, the virus reduces the fitness of the parasitoid due to the wastage of eggs induced by the manipulation (classical cost of superparasitism). Consequently, increasing tv also increased the correlation of the fitness of both the virus and the parasitoid, thus reducing the conflict of interest. This selects for a reduction of the ESS of the virus. In the special case where $\text{tv} = 1$, the virus is selected to adopt the same strategy as the parasitoid (with this parameter set, the ESS for the parasitoid was to never superparasitize, not shown). However, this is true only with $\varepsilon = 0$, that is, when no superinfection is allowed. With superinfection ($\varepsilon = 0.5$), the conflict of interest between the virus and the parasitoid still holds.

The model shows three important features of the LbFV/L. *boulardi* system. The first is the importance of epidemiologic feedbacks. It was particularly visible when we varied the probability of horizontal transmission. Indeed the predictions were counterintuitive due to the indirect effect (i.e., epidemiologic effect) of an increase in th, through a decrease in the number of parasitized hosts, and an increase in the prevalence of the virus in the parasitoid population. Paradoxically, within a population with a high intensity of superparasitism and high viral prevalence, the frequency of horizontal transfer may be lower than within a population

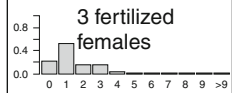
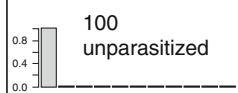
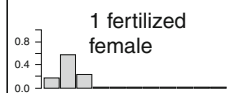
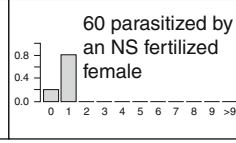
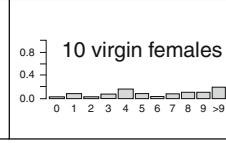
with fewer superparasitism but lower viral prevalence. However, this conclusion is deeply influenced by the superinfection parameter (ε). In this model, we were interested in epidemiologic equilibrium. However, the relative contribution of horizontal and vertical transmission in the course of the invasion process change substantially, with strong contribution of horizontal transmission at the beginning and a reduction with an increase in prevalence. This problem has been addressed in a general context in Lipsitch et al. (1996). Thus, highly manipulative strains are selected for at the beginning of the invasion process and less manipulative at the epidemiologic equilibrium. Another conclusion that can be drawn from the model, is that the value taken by the superinfection parameter (ε) is critical. In both Figures 13.3A and B, we found that increasing ε strongly increased the ESS of superparasitism and also modified the form of the relation between ESS and th. It is evident that the value of this parameter needs to be estimated in this system in order to predict correctly the ESS in natural populations.

13.7. EXPERIMENTAL EVOLUTION IN RELATION TO TRANSMISSION TYPE (HORIZONTAL OR VERTICAL)

The previous section showed how transmission type (horizontal or vertical) is a critical factor governing the evolution of the virus-induced superparasitism phenotype within natural populations. In this section, we describe an experiment in which we manipulated the transmission of the virus, either forcing it to spread vertically but not horizontally or forcing it to spread exclusively horizontally. Contrary to the model described above, this experiment did not include any epidemiologic feedback but only asked whether changing the transmission mode will select for alternative viral strategies. Our prediction was that forcing the virus to propagate exclusively by vertical means should select for lower superparasitism strategy, whereas forcing horizontal transmission should select for higher superparasitism strategy. In standard rearing conditions, three females are used to parasitize about 150 hosts in each vial. In these conditions, moderate superparasitism do occur (Varaldi et al., 2005a). Consequently, when the females are infected, it is likely that both vertical transmission (from mother to offspring) and horizontal transmission occur (horizontal transmission may occur if one viral strain is able to replace one other strain inside the *Drosophila* larva, e.g., $\varepsilon > 0$ in the previous model). However, in standard rearing tubes, if we use only a single female, then only vertical transmission will occur. Conversely, we can provide hosts already parasitized by uninfected females to (superparasitizing) infected females to maximize horizontal transfer. Under this condition, the offspring of uninfected females may become infected at the

next generation. In this species, the reproduction is arrhenotokous parthenogenesis (males are haploid and obtained from unfertilized eggs whereas females are diploid and obtained from fertilized eggs). It is very simple to be sure that *all* transmission events are horizontal, by taking advantage of the fact that unfertilized females will only lay sons whereas fertilized females will lay sons and daughters. Consequently, by exposing hosts first to uninfected and fertilized females and subsequently to virgin infected females, we have the certainty that all infected female offspring is obtained through horizontal transfer. Based on this idea, we did the following experiment using an infected strain originating from Sienna, Italy (described in Varaldi et al., 2003; see Table 13.2). One hundred unparasitized *D. melanogaster* larvae were offered to three fertilized infected females in standard rearing tubes. At each generation, three emerging females were randomly selected and allowed to mate and used to maintain the line. Ten independent replicates were performed in parallel. At each generation, the superparasitism phenotype of two females emerging from each tube was tested according to a standard procedure (female isolated on 10 *D. melanogaster* larvae, see Varaldi et al., 2006b for details). This condition constitutes the control conditions where both vertical and horizontal transmission are likely to occur, because some superparasitism occurs (Table 13.2). A second modality forcing vertical transmission was performed, where a single female was

TABLE 13.2 Description of the experimental setup

	Hosts	Wasps	N lines	Tests per line
Control			10	2
Vertical			30	1
Horizontal			8	2

In each cell is indicated the frequency distribution of wasp eggs inside *Drosophila* larvae (left column: before infected wasp(s) were added, right column: after infected wasp(s) were removed).

provided with 100 unparasitized hosts. To allow a selective process to occur, we prepared 30 independent tubes, mixed all the emerging offspring (from all 30 tubes) at each generation and randomly selected 30 (fertilized) females to establish the next generation. The idea was that a virus that induces a low fitness cost and especially that induces few superparasitism will be selected since the infected wasp will not waste its eggs in (self-) superparasitism and will contribute more to the pool of emerging wasps. At each generation, the superparasitism phenotype of one female per line was tested. Finally, we provided 60 hosts already parasitized by an uninfected fertilized female to 10 virgin infected females. In this situation, harsh superparasitism occurs (Table 13.2), favoring horizontal transfer. To standardize the whole number of *Drosophila* larvae in the tubes for the three modalities (a total of 100), we added 40 unparasitized *Drosophila* larvae to the 60 (super)parasitized hosts. From the emerging wasp offspring, two females were used to test their superparasitism phenotype and 10 virgin females were used to continue the protocol (again they were provided with 60 hosts already parasitized by an uninfected fertilized female). Eight independent lines were performed (horizontal).

The controls (three fertilized females for 100 hosts) showed the expected superparasitism phenotype with a mean number of eggs per host between two and four (Fig. 13.4). Also, the horizontal transfers that were expected in the modality ''horizontal'' were evident, since the mean number of eggs per hosts was 1.54 and 4.63, compared with the phenotype

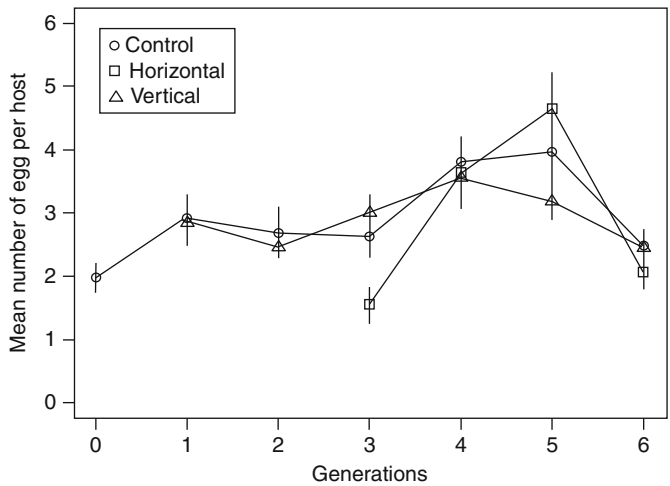


FIGURE 13.4 Experimental evolution of the intensity of the manipulation induced by LbFV.

of the uninfected line (mean = 1.07, $n = 5$). The superparasitism phenotype was tested in all three modalities (control, strict vertical and strict horizontal) starting from the third generation of selection (from generations three to six). In these data, there was evidence of between-generation variations ($F(3,199) = 9.81$, $P < 0.0001$), but no evidence of the type of transmission ($F(2,199) = 0.27$, $P = 0.75$) and no interaction between generation and the type of transmission ($F(6,199) = 2.12$, $P = 0.052$). As a conclusion, there was no evidence of evolution in this dataset. Several hypotheses can be formulated to explain this absence of response. The first is that the selective differential is not sufficient to observe a change in only six generations because of sampling errors. This explanation probably holds for the vertical transmission modality, because only 30 females out of around 180 (each of the 30 tubes produced around 60 females) were randomly selected at each generation to continue the experiment. Consequently, even if a female contributed more than the others to the whole emerging population (because its virus was more benevolent, induced less superparasitism), sampling errors may have cancelled the initial overrepresentation of this peculiar virus strain. However, this explanation is unlikely to hold for the horizontal transmission modality since at each generation, 10 wasps were randomly selected in a pool of only about 20 emerging females. This low number of emerging females (we recall that only 60 hosts were superparasitized in this modality) was due to the fact that when strong superparasitism occurs (as it is the case in this modality, see Table 13.2), both the host and the parasitoid incur a high risk of dying during the development (Varaldi et al., 2005a). This phenomenon constitutes a potential cost to the spread of highly manipulative strains and may explain part of the absence of response in the horizontal modality. Finally, one trivial hypothesis that may explain the absence of any selective response neither in the vertical nor the horizontal modality is that there was no sufficient genetic variability of the virus at the beginning of the experiment and that mutation alone did not generate enough polymorphism in the course of the experiment.

13.8. OTHER VIRUSES IN THE *DROSOPHILA*–PARASITOID COMMUNITY

Due to their very diverse genomic structure (DNA, RNA, single or double stranded) and to their high mutation rates, no simple systematic methods (such as PCR based) are available to detect the presence of viruses or even to detect all members of a given family. However, several viruses have been regularly discovered in several *Drosophila* spp., especially *D. melanogaster*. It is reasonable to think that most of these viruses have been discovered because *D. melanogaster* is a model system since the beginning of the twentieth century and has been extensively studied from all aspects of its biology

(including immunity). L'Heritier and Teissier (1937) for instance discovered the σ virus because certain strains showed atypical (virus-induced) CO_2 sensitivity. Recently, molecular techniques have also provided additional means to reveal their presence. For instance, Asling et al. (1995) were interested in comparing the transcriptomes of *D. melanogaster* either ''uninfected'' or ''challenged'' with a pathogenic bacteria in order to identify immunity-related genes. They did found the induction of an antimicrobial peptide but they also detected an induced band presenting sequence similarity with viruses. They were in fact discovering a new single-stranded RNA (ssRNA) virus (picorna-like) apparently asymptomatic, called Nora virus (Habayeb et al., 2006). The virus was first detected in a huge quantity of fly stocks, but it was later found that a technical bias led to an overestimation of its prevalence (Habayeb et al., 2007). Table 13.3 presents a comprehensive list of the identified viruses infecting *Drosophila* spp. One striking pattern is that all of them are RNA viruses (or likely to be, when no genomic information are available), although other *Diptera* are infected by DNA viruses (Gratz, 2004). This surprising pattern remains unexplained. Understanding the biological reason for this (if any) may provide exciting insights on the enigmatic observation that the major virus genomic structures are clearly nonuniformly distributed among the main branches of hosts (Koonin et al., 2008). One other feature of *Drosophila* viruses that can be underlined is the diversity of transmission modes with strictly horizontally transmitted viruses (e.g., DCV), strictly vertically transmitted viruses (for instance σ virus) and viruses presenting both transmission modes (virus P and virus A). A remarkable feature of most of these viruses is that they have relatively mild pathological effects on their hosts. For instance, although DCV virus is highly pathogenic when artificially injected, Thomas (1974) and Gomariz-Zilber and Thomas-Orillard (1993) found that under natural infection routes (larval feeding on contaminated substrate), DCV reduces only slightly the survival of larvae and even induces an increase in the number of ovarioles and on adult longevity. However, Texeira (personal communication) found clear pathogenic effects of DCV even when larvae become infected by feeding. The reason for these somehow conflicting results remains unclear. The hereditary σ virus does not affect fertility, female longevity, but reduces egg viability (Fleuriet, 1981a) and overwintering survival probability (Fleuriet, 1981b). Sigma virus also induces CO_2 sensitivity, that is, *Drosophila* exposed for a while to CO_2 die instead of recovering from sleep. However, the ecological significance of this phenotype is probably negligible since CO_2 concentrations never reach such high concentration in the wild. It provides, however, a convenient way to identify infected flies, allowing population-level investigations (Bangham et al., 2008a,b; Carpenter et al., 2007).

A rough estimate of the overall viral prevalence in *D. melanogaster* has been given by Brun and Plus (1980). They found that among 49

TABLE 13.3 Viruses infecting *Drosophila* spp.

Virus	Host	Genome structure	Family	Genome sequence	Ref. genome	Transmission	Effects	Refs effects	Prevalences	Ref. prev
Sigma	*D. melanogaster*	ssRNA-	Rhabdoviridae	6477bp incomplete (ref genbank X91062)	1	Vertical through males and females gametes	No effect on fertility, female longevity, sexual selection and egg viability; reduced survival of eggs and overwintering survival (and CO_2 sensitivity)	1, 9	Up to 60%	17
DXV	*D. melanogaster*	dsRNA	Birnaviridae	6603bp (in 2 segments, ref genbank NC_004177, NC_004169)	2, 3	Horizontal (contact) apparently not vertical	Anoxia sensitivity reduction in survival (sometimes asymptomatic)	11	Never observed under natural conditions	
Virus C	*D. melanogaster* specific (16)	ssRNA	Dicistrioviridae	9264bp ref genbank NC_001834	4	Horizontal by feeding (adults or larvae)	Conflicting results. See text	12, 13, 14	6 populations infected out of 49	16
Virus P	*D. melanogaster*	ssRNA	Picornavirus-like superfamily	?	5	Horizontal by contact and ingestion and vertical by young females	Fitness reduction (survie, egg-laying)	15	?	
Virus A	*D. mel* but not only (16)	ssRNA	Picornavirus-like superfamily	4806bp NC_012958	19	Horizontal by contact and ingestion and vertical by young females	Low pathogeny	5	?	
Nora	*D. melanogaster*	ssRNA	Picornavirus-like superfamily	11908bp ref genbank NC_007919	6	Horizontal through feces	Slight reduction in survival and hatching	20	?	
Reovirus F	*D. mel* but not only (16)	dsRNA?	Reoviridae	?	7	Horizontal by contact, apparently not vertical	No signs	16	?	
Virus G	*D. mel* but not only (16)	RNA	?	?		Horizontal by contact, apparently not vertical	No signs	16	?	
DSV	*D. simulans*	dsRNA	Reoviridae	Around 8410bp (at least 8 segments)	8	Hereditary mainly maternal	Modification of cuticule (bristle) negative effects on fitness	18	?	
Iota virus	*D. immigrans*	RNA	Picornavirus-like superfamily	?		Transovarian	No signs. Induce CO_2 sensitivity in *D. melanogaster*	16	Up to 100%	16
RS virus	*D. ananassae D.montium*	?	?	?		?	?	16	?	

Notes: 1: Landès-Devauchelle et al. (1995); 2: Shwed et al. (2002); 3: Chung et al. (1996); 4: Johnson and Christian (1998); 5: Plus et al. (1976); 6: Habayeb et al. (2007); 7: Plus et al. (1981); 8: López-Ferber et al. (1989); 9: Fleuriet (1981a); 10: Fleuriet (1981b); 11: Teninges et al. (1979); 12: Thomas (1974); 13: Gomariz-Zilber (1993); 14: Jousset and Plus (1975); 15: David and Plus (1971); 16: Brun and Plus (1980); 17: Fleuriet and Periquet (1993); 18: Louis et al. (1988); 19: Ambrose et al. (in press); 20: Habayeb et al. (2009).

populations originating from Europe, Africa, North and South America, 19 populations were infected by at least one virus (39%). More detailed investigations have been done on the σ virus. The hereditary σ virus showed a frequency of up to 65% in some French populations (Fleuriet and Periquet, 1993), while a more recent study revealed that σ virus was present in five populations out of 12 originating from Greece, United Kingdom, Polynesia, United States of America, Kenya, Spain and Austria, with frequencies reaching 15% (Carpenter et al., 2007). These relatively high frequencies make them potential factors influencing the ecology and evolution of their hosts. It is interesting to note that in the aphid *Acirtosiphon pisum* several maternally transmitted bacterial secondary symbionts (facultative endosymbionts) reach high prevalence (but not fixation) in natural populations (Oliver et al., 2006). The ecological factors explaining their distribution has been elusive for a while. However, it has been shown that the secondary symbionts may increase the fitness of their aphid host in certain environments, because they confer resistance against heat stress, resistance to fungal pathogens, adaptation to host plant or protection against parasitoids (*Hamiltonella defensa*). However, they may be costly under alternative environments (Oliver et al., 2007; Russell and Moran, 2006), providing an explanation for their intermediate frequencies. In addition, secondary symbionts may benefit from natural horizontal transfer for instance during copulation (Moran et al., 2006), favoring the spread of infection and the occurrence of coinfection. There are evident similarities between both model systems (aphid secondary symbionts and *Drosophila* viruses) and we can ask whether some of these viruses have anything to do with the adaptation of *Drosophila* to their local environment, and especially to the presence of parasitoids. On this scale, it is interesting to note that the protective effect conferred by *Hamiltonella defensa* to its aphid host is probably caused by the presence of specific toxins encoded by its bacteriophage (Degnan and Moran, 2008).

It is clear that the parasitoids attacking *Drosophila* spp. have received much less attention than *Drosophila*. To our knowledge, the only virus described to date in *Drosophila* parasitoid is LbFV, apart from VLPs that may have a viral evolutionary origin. We argue that this apparent asymmetry between *Drosophila* and their parasitoids is probably a sampling bias, and we suspect that several other parasitoid viruses will be described in the near future. New molecular tools that are now available, especially high-throughput sequencing (Marioni et al., 2008; Vera et al., 2008) allowing for metagenomic analysis (Cox-Foster et al., 2007), will provide evidence of new infectious and/or heritable viruses in parasitoids. We can mention that another RNA virus have been fortuitously discovered in the Lepidoptera parasitoid wasp *Venturia canescens*, using transcriptomic analysis exactly the same way as was discovered the *Drosophila* Nora virus (Reineke and Asgari, 2005). Finally, it has been

recently found that bacterial symbiont can confer protection against virus infection, suggesting possible interactions between virus and bacterial endosymbionts (Hedges et al., 2008; Teixeira et al., 2008). This result is particularly interesting since the phylogeny of *Leptopilina* spp. reveals that all *Leptopilina* spp. are infected by the endosymbiont *Wolbachia* (see Chapter 12), at the exception of *L. boulardi* where was found the manipulating virus (Allemand et al., 2002).

13.9. CONCLUSION

Viruses are ubiquitous. The *Drosophila*–parasitoids community is not an exception as several (RNA) viruses infecting *Drosophila* spp. have been identified. We discovered a new virus (probably a DNA virus) in the parasitoid wasp *Leptopilina boulardi* and we suspect that new viruses will be discovered in the near future, especially in parasitoids because the sampling effort in this group has been relatively low until now. Viruses may reach high prevalence in natural populations and are thus important players in the ecology and evolution of their hosts and on host–parasitoid interactions. Their possible ecological and evolutionary implications are illustrated by the LbFV/parasitoid interaction. Indeed, this virus specifically affects a critical foraging component of the wasp (superparasitism), allowing the virus to be horizontally transferred and to spread within wasp population. The behavior of most of the females of a population may then be deeply modified. This indirectly selects for different superparasitism strategies in uninfected females (Section 13.3), and also for higher investment in the egg load of infected females (Section 13.5). Because both the virus and the parasitoid share some fitness components due to vertical transmission, specific parasitoid virus combinations may be the target of selection, possibly leading to coadaptation and evolutionary innovation. In this respect, the discovery of LbFV may provide insights into the symbiogenesis at the origin of PDVs that protect parasitoids from the host immune response. Future investigations will target the molecular mechanisms allowing the virus to be maintained in wasp populations (superparasitism manipulation), the genetic response of their hosts and the ecological consequences on interspecific interactions.

REFERENCES

Allemand, R., Lemaitre, C., Frey, F., Boulétreau, M., Vavre, F., Nordlander, G., et al., 2002. Phylogeny of six African *Leptopilina* species (Hymenoptera: Cynipoidea, Figitidae), parasitoids of *Drosophila*, with description of three new species. Ann. Soc. Entomol. Fr. 38, 319–332.

Ambrose, R.L., Lander, G.C., Maaty, W.S., Bothner, B., Johnson, J.E., Johnson, K.N., in press. Drosophila A virus is an unusual RNA virus with a T = 3 icosahedral core and permuted RNA-dependent RNA-polymerase. J. Gen. Virol.

Asling, B., Dushay, M.S., Hultmark, D., 1995. Identification of early genes in the *Drosophila* immune response by PCR-based differential display: the Attacin A gene and the evolution of attacin-like proteins. Insect Biochem. Mol. Biol. 25, 511–518.

Bangham, J., Kim, K.W., Webster, C.L., Jiggins, F.M., 2008a. Genetic variation affecting host–parasite interactions: different genes affect different aspects of sigma virus replication and transmission in *Drosophila melanogaster*. Genetics 178, 2191–2199.

Bangham, J., Knott, S.A., Kim, K.W., Young, R.S., Jiggins, F.M., 2008b. Genetic variation affecting host–parasite interactions: major-effect quantitative trait loci affect the transmission of sigma virus in *Drosophila melanogaster*. Mol. Ecol. 17, 3800–3807.

Bezier, A., Annaheim, M., Herbinière, J., Wetterwald, C., Gyapay, G., Bernard-Samain, S., et al., 2009. Polydnaviruses of braconid wasps derive from an ancestral nudivirus. Science 323, 926–930.

Brun, P., Plus, N., 1980. The viruses of *Drosophila*. In: Ashburner, Wright, (Eds.), The Genetics and Biology of *Drosophila*. Academic Press, London, pp. 626–702.

Carpenter, J.A., Obbard, D.J., Maside, X., Jiggins, F.M., 2007. The recent spread of a vertically transmitted virus through populations of *Drosophila melanogaster*. Mol. Ecol. 16, 3947–3954.

Chung, H.K., Kordyban, S., Cameron, L., Dobos, P., 1996. Sequence analysis of the bicistronic *Drosophila* X virus genome segment A and its encoded polypeptides. Virology 225, 359–368.

Carton, Y., Boulétreau, M., Van Alphen, J.J. M., Van Lenteren, J.C., 1986. The *Drosophila* parasitic wasps. In : The Genetics and Biology of *Drosophila*. Ashburner, Carson, Thompson, (Eds.), Academic Press, Orlando, pp. 347–394.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., et al., 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318, 283–287.

David, J., Plus, N., 1971. Le virus P de la drosophile: comparaison de la longevité et de la fecondité des mouches infectées par injection ou par contamination naturelle. Ann. Institut. Pasteur 120, 107–119.

Degnan, P.H., Moran, N.A., 2008. Diverse phage-encoded toxins in a protective insect endosymbiont. Appl. Environ. Microbiol. 74, 6782–6791.

Dubuffet, A., Alvarez, C.I., Drezen, J.M., van Alphen, J.J.M., Poirie, M., 2006. Do parasitoid preferences for different host species match virulence? Physiol. Entomol. 31, 170–177.

Dupas, S., Brehelin, M., Frey, F., Carton, Y., 1996. Immune suppressive virus-like particles in a *Drosophila* parasitoid: significance of their intraspecific morphological variations. Parasitology 113, 207–212.

Espagne, E., Dupuy, C., Huguet, E., Cattolico, L., Provost, B., Martins, N., et al., 2004. Genome sequence of a polydnavirus: insights into symbiotic virus evolution. Science 306, 286–289.

Ewald, P.W., 1987. Transmission modes and evolution of the parasitism-mutualism continuum. Ann. N. Y. Acad. Sci. 503, 295–306.

Fleuriet, A., 1981a. Comparison of various physiological traits in flies (*Drosophila melanogaster*) of wild origin, infected or uninfected by the hereditary rhabdovirus sigma. Arch. Virol. 69, 261–272.

Fleuriet, A., 1981b. Effect of overwintering on the frequency of flies infected by the rhabdovirus sigma in experimental populations of *Drosophila melanogaster*. Arch. Virol. 69, 253–260.

Fleuriet, A., Periquet, G., 1993. Evolution of the *Drosophila melanogaster*-sigma virus system in natural populations from Languedoc (southern France). Arch. Virol. 129, 131–143.

Gandon, S., 2005. Parasitic manipulation: a theoretical framework may help. Behav. Process. 68, 247–248.
Gandon, S., Rivero, A., Varaldi, J., 2006. Superparasitism evolution: adaptation or manipulation? Am. Nat. 167, E1–E22.
Gandon, S., Varaldi, J., Fleury, F., Rivero, A., in press. Evolution and manipulation of parasitoid egg load. Evolution.
Glatz, R.V., Asgari, S., Schmidt, O., 2004. Evolution of polydnaviruses as insect immune suppressors. Trends Microbiol. 12, 545–554.
Gomariz-Zilber, E., Thomas-Orillard, M., 1993. *Drosophila* C virus and *Drosophila* hosts: a good association in various environments. J. Evol. Biol. 6, 677–689.
Gratz, N.G., 2004. Critical review of the vector status of *Aedes albopictus*. Med. Vet. Entomol. 18, 215–227.
Habayeb, M.S., Cantera, R., Casanova, G., Ekström, J., Albright, S., Hultmark, D., 2009. The *Drosophila* Nora virus is an enteric virus, transmitted via feces. J. Invertebr. Pathol. 101, 29–33.
Habayeb, M.S., Ekengren, S.K., Hultmark, D., 2006. Nora virus, a persistent virus in *Drosophila*, defines a new picorna-like virus family. J. Gen. Virol. 87, 3045–3051.
Habayeb, M.S., Ekengren, S.K., Hultmark, D., 2007. Nora virus, a persistent virus in *Drosophila*, defines a new picorna-like virus family. J. Gen. Virol. 88, 3493.
Hedges, L.M., Brownlie, J.C., O'Neill, S.L., Johnson, K.N., 2008. *Wolbachia* and virus protection in insects. Science 322, 702.
Johnson, K.N., Christian, P.D., 1998. The novel genome organization of the insect picorna-like virus *Drosophila* C virus suggests this virus belongs to a previously undescribed virus family. J. Gen. Virol. 79, 191–203.
Jousset, F.X., Plus, N., 1975. Study of the vertical transmission and horizontal transmission of ''*Drosophila melanogaster*'' and ''*Drosophila* immigrans'' picornavirus. Ann. Microbiol. (Paris) 126, 231–249 [In French].
Koonin, E., Wolf, Y., Nagasaki, K., Dolja, V., 2008. The Big Bang of picorna-like virus evolution antedates the radiation of eukaryotic supergroups. Nat. Rev. Microbiol. 6, 925–939.
L'Heritier, P.H., Teissier, G., 1937. Une anomalie physiologique héréditaire chez la *Drosophile*. C. R. Acad. Sci. Paris 206, 1683–1685.
Landès-Devauchelle, C., Bras, F., Dezélée, S., Teninges, D., 1995. Gene 2 of the sigma rhabdovirus genome encodes the P protein, and gene 3 encodes a protein related to the reverse transcriptase of retroelements. Virology 213, 300–312.
Lapointe, R., Tanaka, K., Barney, W.E., Whitfield, J.B., Banks, J.C., Béliveau, C., et al., 2007. Genomic and morphological features of a banchine polydnavirus: comparison with bracoviruses and ichnoviruses. J. Virol. 81, 6491–6501.
Lipsitch, M., Nowak, M.A., Ebert, D., May, R.M., 1995. The population dynamics of vertically and horizontally transmitted parasites. Proc. Biol. Sci. B 260, 321–327.
Lipsitch, M., Siller, S., Nowak, M.A., 1996. The evolution of virulence in pathogens with vertical and horizontal transmission. Evolution 50, 1729–1741.
López-Ferber, M., Veyrunes, J.C., Croizier, L., 1989. *Drosophila* S virus is a member of the Reoviridae family. J. Virol. 63, 1007–1009.
Louis, C., Lopez-Ferber, M., Comendador, M., Plus, N., Kuhl, G., Baker, S., 1988. *Drosophila* S virus, a hereditary reolike virus, probable agent of the morphological S character in *Drosophila simulans*. J. Virol. 62, 1266–1270.
Marioni, J.C., Mason, C.E., Mane, S.M., Stephens, M., Gilad, Y., 2008. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Res. 18, 1509–1517.
Minchella, D.J., 1985. Host life history variation in response to parasitism. Parasitology 90, 205–216.

Moran, N.A., Dunbar, H.E., 2006. Sexual acquisition of beneficial symbionts in aphids. Proc. Natl Acad. Sci. USA 103, 12803–12806.
Oliver, K., Campos, J., Moran, N., Hunter, M., 2007. Population dynamics of defensive symbionts in aphids. Proc. R. Soc. B 275, 293–299.
Oliver, K.M., Moran, N.A., Hunter, M.S., 2006. Costs and benefits of a superinfection of facultative symbionts in aphids. Proc. Biol. Sci. 273, 1273–1280.
Oliver, K.M., Russell, J.A., Moran, N.A., Hunter, M.S., 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc. Natl. Acad. Sci. USA 100, 1803–1807.
Pannebakker, B.A., Garrido, N.R.T., Zwaan, B.J., van Alphen, J.J.M., 2008. Geographic variation in host-selection behaviour in the *Drosophila* parasitoid *Leptopilina clavipes*. Entomol. Exp. Appl. 127, 48–54.
Patot, S., Lepetit, D., Charif, D., Varaldi, J., Fleury, F., 2009. Molecular detection, penetrance and transmission of an inherited virus responsible for a behavioral manipulation of an insect parasitoid. Appl. Environ. Microbiol. 75, 703–710.
Plus, N., Croizier, G., Veyrunes, J.C., David, J., 1976. A comparison of buoyant density and polypeptides of *Drosophila* P, C and A viruses. Intervirology 7, 346–350.
Plus, N., Gissman, L., Veyrunes, J.C., Pfister, H., Gateff, E., 1981. Reoviruses of *Drosophila* and *Ceratitis* populations and of *Drosophila* cell lines: a new genus of the Reoviridae family. Ann. Virol. (Inst. Pasteur) 132E, 261–270.
Poulin, R., 1995. ''Adaptive'' changes in the behaviour of parasitized animals: a critical review. Int. J. Parasitol. 25, 1371–1383.
Reineke, A., Asgari, S., 2005. Presence of a novel small RNA-containing virus in a laboratory culture of the endoparasitic wasp Venturia canescens (Hymenoptera: Ichneumonidae). J. Insect Physiol. 51, 127–135.
Rizki, R.M., Rizki, T.M., 1990. Parasitoid virus-like particles destroy *Drosophila* cellular immunity. Proc. Natl. Acad. Sci. USA 87, 8388–8392.
Rosenheim, J.A., 1996. An evolutionary argument for egg limitation. Evolution 50, 2089–2094.
Russell, J.A., Moran, N.A., 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. Proc. Biol. Sci. 273, 603–610.
Shwed, P.S., Dobos, P., Cameron, L.A., Vakharia, V.N., Duncan, R., 2002. Birnavirus VP1 proteins form a distinct subgroup of RNA-dependent RNA polymerases lacking a GDD motif. Virology 296, 241–250.
Teixeira, L., Ferreira, A., Ashburner, M., 2008. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. PLoS Biol. 23, E2.
Teninges, D., Ohanessian, A., Richard-Molard, C., Contamine, D., 1979. Isolation and biological properties of *Drosophila* X virus. J. Gen. Virol. 42, 241–254.
Thomas, M., 1974. Contribution à l'Etude du Déterminisme du Nombre d'Ovarioles et de Quelques Autres Caractères Quantitatifs Chez *Drosophila melanogaster* Meigen [Thesis]. University Paris VI.
van Alphen, J.J., Visser, M.E., 1990. Superparasitism as an adaptive strategy for insect parasitoids. Annu. Rev. Entomol. 35, 59–79.
van Lenteren, J.C., Ruschioni, S., Romani, R., van Loon, J.J., Qiu, Y.T., Smid, H.M., et al., 2007. Structure and electrophysiological responses of gustatory organs on the ovipositor of the parasitoid *Leptopilina heterotoma*. Arthr. Struct. Dev. 36, 271–276.
Varaldi, J., Boulétreau, M., Fleury, F., 2005a. Cost induced by viral particles manipulating superparasitism behaviour in the parasitoid *Leptopilina boulardi*. Parasitology 131, 161–168.
Varaldi, J., Fouillet, P., Boulétreau, M., Fleury, F., 2005b. Superparasitism acceptance and patch-leaving mechanisms in parasitoids: a comparison between two sympatric wasps. Anim. Behav. 69, 1227–1234.
Varaldi, J., Fouillet, P., Ravallec, M., López-Ferber, M., Boulétreau, M., Fleury, F., 2003. Infectious behavior in a parasitoid. Science 302, 1930.

Varaldi, J., Gandon, S., Rivero, A., Patot, S., Fleury, F., 2006c. A newly discovered virus manipulates superparasitism behavior in a parasitoid wasp. In: Bourtzis, Miller, (Eds.), Insect symbiosis, Vol. 2. CRC Press, Boca Raton, FL, pp. 119–139.

Varaldi, J., Petit, S., Boulétreau, M., Fleury, F., 2006a. The virus infecting the parasitoid *Leptopilina boulardi* exerts a specific action on superparasitism behaviour. Parasitology 132, 747–756.

Varaldi, J., Ravallec, M., Labrosse, C., Lopez-Ferber, M., Boulétreau, M., Fleury, F., 2006b. Artifical transfer and morphological description of virus particles associated with superparasitism behaviour in a parasitoid wasp. J. Insect Physiol. 52, 1202–1212.

Vera, J.C., Wheat, C.W., Fescemyer, H.W., Frilander, M.J., Crawford, D.L., Hanski, I., et al., 2008. Rapid transcriptome characterization for a nonmodel organism using 454 pyrosequencing. Mol. Ecol. 17, 1636–1647.