PARASITISM INCREASES AND DECREASES THE COSTS OF INSECTICIDE RESISTANCE IN MOSQUITOES

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Abstract.—Adaptations conferring resistance to xenobiotics (antibiotics, insecticides, herbicides, etc.) are often costly to the organism's fitness in the absence of the selecting agent. In such conditions, and unless other mutations compensate for the costs of resistance, sensitive individuals are expected to out-reproduce resistant individuals and drive resistance alleles to a low frequency, with the rate and magnitude of this decline being proportional to the costs of resistance. However, this evolutionary dynamic is open to modification by other sources of selection acting on the relative fitness of susceptible and resistant individuals. Here we show parasitism not only as a source of selection capable of modifying the costs of organophosphate insecticide resistance in mosquitoes, but also that qualitatively different interactions (increasing or decreasing the relative fitness of resistant individuals) occurred depending on the particular form of resistance involved. As estimates of the parasite's fitness also varied according to its host's form of resistance, our data illustrate the potential for epidemiological feedbacks to influence the strength and direction of selection acting on resistance mutations in untreated environments.

Key words.—Cost, host, insecticide, microsporidia, mosquito, parasite, resistance.

It is widely expected that mutations conferring resistance to xenobiotics (antibiotics, insecticides, herbicides, etc.) will involve some sort of cost to a resistant individual's fitness in a xenobiotic-free environment. This expectation has a sound theoretical basis (Fisher 1958; Orr 1998), and is well supported by empirical data from field and laboratory studies, involving a broad range of target organisms and a diversity of the toxins deployed against them (Glass et al. 1986a; Andersson and Levin 1999). Unless these costs are compensated for by secondary mutations (Fisher 1958; Levin et al. 2000), the frequency of resistant individuals is expected to decline in untreated environments as they are displaced by sensitive individuals having a greater reproductive success and superior rates of population growth.

Although many mutations and their functional role in conferring resistance to xenobiotics have been identified (Glass et al. 1986b; ffrench-Constant et al. 1998; Weill et al. 2003), the actual costs they impose often remains to be clarified. This deficit is partially due to difficulties in isolating variation in fitness due to the possession of a resistance mutation from that due to variation in the genetic background in which they are expressed (McKenzie et al. 1982; Schrag et al. 1997), and from variation in the environmental conditions in which these costs are measured (McKenzie 1994; Foster et al. 1996; Purrington and Bergelson 1999). Identifying components of these sources of variation and how they influence the costs of being resistant will increase our understanding of the selection pressures acting on resistance mutations and how they influence the evolutionary dynamics of resistance in untreated environments.

We investigated parasitism as a potential source of environmental variation influencing the strength of selection on resistance mutations in a xenobiotic-free environment. Parasites are recognized as being an important source of selection acting in natural populations (Price 1980), but their role in the evolutionary dynamics of resistance has received relatively little attention. Unlike abiotic components of the environment, selection pressures exerted by parasites can depend on how they interact with their host populations. Parasites may differentially affect the fitness of resistant and sensitive hosts, for example, because resistant hosts are more exposed to infection (Foster et al. 1999), or because they have higher burdens of infection, as has recently been reported for *Wolbachia* infections of insecticide resistant mosquitoes (Berticat et al. 2002a). Parasites may also gain different fitness dividends from exploiting sensitive and resistant hosts, for example, the transmission success of the filarial worm *Wuchereria bancrofti* is predicted to be lower from *Culex* mosquitoes resistant to organophosphate (OP) insecticides (Farid 1996; McCarroll et al. 2000). Therefore, the strength and direction of selection acting on resistance mutations due to parasitism has the potential to vary as a consequence of how they themselves influence the epidemiology of the host-parasite interaction.

In the following experiment, we tested for the effects of three OP insecticide resistance mutations on life-history traits of *Culex pipiens quinquefasciatus* mosquitoes when infected or not by the microsporidian parasite *Vavraia culicis*. Each resistance mutation was separately back-crossed into a common and sensitive genetic background (Berticat et al. 2002b),

thus enabling the effects of these mutations to be directly estimated and compared while controlling for the genetic environment in which they were expressed. As life-history traits of mosquitoes are known to be sensitive to density-dependent interactions among larvae (Agnew et al. 2000), we controlled for this source of environmental variation by rearing each larva in its own individual vial (Agnew et al. 2002). Larvae were also reared in conditions of high and low food availability to assess whether this environmental parameter influenced the traits measured.

MATERIALS AND METHODS

Host Material

The S-Lab strain of *C. pipiens* (Georghiou et al. 1966) was used to provide an OP insecticide sensitive background into which three resistance alleles were separately introgressed during 14–15 generations of back-crossing and selection (Berticat et al. 2002b). Each of these alleles is known to be strongly selected against in untreated areas of southern France (Lenormand et al. 1999). Resistant strains SA1 and SA4 are homozygous for the *Ester*[1] and *Ester*[4] alleles at the *Ester* locus, respectively; each allele codes for a constitutional overproduction of detoxifying esterases that degrade OP insecticides. The *Ester*[1] allele which was the first to appear in southern France provides more resistance to OP insecticides, but is more strongly selected against than the *Ester*[4] allele (Guillemaud et al. 1998). Resistant strain SR is homozygous for the allele *ace-1*[R] at the locus *ace-1* which is responsible for the production of an acetylcholinesterase insensitive to OP presence. Whereas SR is the strongest form of OP resistance, it is also estimated to be the most costly because it interferes with the general functioning of the central nervous system throughout a mosquito's life and adversely modifies behavioral traits, for example, those linked to mating success (Berticat et al. 2002b). We compared and contrasted the life-history traits of these three resistant strains (SA1, SA4, and SR), with those of the OP sensitive control strain, S-Lab.

Parasite Material

Vavraia culicis is a representative member of a diverse group of single-celled and obligate intracellular parasites that are among the most prevalent pathogens in natural populations of mosquitoes (Castillo 1980). *Vavraia culicis* itself infects the larvae of several genera of mosquitoes. It was originally described from *C. pipiens* in central Europe and has been reported from diverse locations around the globe (Weiser 1980). It has a direct life cycle in which its transmission success mainly relies upon infected hosts being killed as larvae or pupae and thus before they leave the aquatic environment as an adult mosquito (Reynolds 1972; Kelly et al. 1981). Invertebrate hosts have no documented form of immune response capable of suppressing or clearing infections by these intracellular parasites. Therefore, the influence of insecticide resistance on the mosquito's immune system is unlikely to be an important explanatory variable for our results.

The material used in this experiment was generously supplied by Dr. J. J. Becnel at the United States Department of Agriculture, Florida, and had originally been isolated from *Aedes albopictus* (Fukuda et al. 1997).

Experimental Protocol and Data Collection

Oviposition of the four parental strains of mosquito was synchronized and seven groups of 60 1st instar larvae were prepared for strains S-Lab, SA4, and SR; enough individuals were available to prepare only four groups of 60 larvae for strain SA1. Each group of larvae was transferred to a 55 mm diam. petri dish containing 10 ml of deionized water and 0.06 mg of Tetramin powdered fish food larva[-1]. For strains S-Lab, SA4, and SR, larvae in four of the seven petri dishes were exposed to infection by adding 1.2×10^7 *V. culicis* spores to each dish; two of the four petri dishes containing larvae of SA1 received the same treatment. This exposure lasted for 24 h, after which larvae were rinsed and pooled together according to their strain and infection treatment.

Larvae were then transferred to their own individual *Drosophila* tube (diam. 20 mm × 90 mm) containing 5 ml of deionized water for the rest of the experiment. Thirty six racks holding 40 of these tubes were arranged on a single shelf in an insectarium (25 ± 1°C, 12:12 h light:dark, >60% humidity) and alternately assigned to a high or low food treatment. From the initial 0.06 mg larva[-1], food was increased daily by a factor of 1.250 and 1.125 in the high and low treatments, such that pupation would occur roughly 10 and 15 days into the experiment, respectively (P. Agnew, unpubl. data). Food was provided in a 1 ml solution, prior to this 1 ml of water was removed from each tube to maintain the volume at a constant 5 ml. Larvae from each strain and infection treatment were equally distributed across the two food treatments and within each food treatment, larvae from each strain and infection treatment were randomly distributed across racks.

Larval mortality was recorded daily and the cadaver transferred to a numbered 1.7 ml vial and stored at −20°C until further treatment. Pupation was recorded every 12 h from day 6 onwards. Pupae were removed from their tubes, the tube rinsed, refilled with 5 ml of water, the pupa placed back into its tube, and the tube covered with a mesh. Day of emergence and sex of emerging adults were recorded.

No food was provided to the adults but they had access to the water in their tube; this forced them to survive by metabolizing nutritional reserves accumulated during their larval life (Briegel et al. 2001). Adult mortality was recorded daily. Dead adults were transferred to numbered 1.7 ml tubes, dried at 60°C for at least 12 h, weighed to a precision of ±1 µg by a Mettler Toledo MX5 balance (Mettler-Toledo GmbH, Greifensee, Switzerland). Subsequently one wing was removed and measured from the alula notch to the wing tip with a dissecting microscope. This measure is positively correlated with an individual's adult size and its reproductive success (Clements 1992).

Spore content of all dead individuals from infected treatments was estimated by grinding the cadaver in 0.5ml of water and counting the spores in a cell counter with a phase contrast microscope (×400). Excess larvae not required for

the experiment were rinsed, provided food ad libitum, smeared, and Giemsa (Sigma Diagnostics, St. Louis, MO) stained on day 10 of the experiment for evidence of infection. Infection rates were >90% for S-Lab, SA4, and SR (SA1 was not tested) showing equal susceptibility of these strains to infection.

In a complementary experiment to test for differences in feeding behavior among strains, 20 uninfected 4th instar larvae of each strain were fed ad libitum on a diet of green algae; the dark gut contents are visible through the cuticle to the naked eye. Series of larvae, one from each strain, were transferred to their own *Drosophila* vial, containing 5 ml of deionized water and 1 mg of brewer's yeast, and we measured the time taken for the dark gut contents to be entirely voided. Vials were subsequently covered with a mesh and the sex of the emerging adult recorded.

Statistical Analysis

Fully factorial models were used throughout the analyses, with the factor sex included for adult traits. All parameter estimates and statistics reported below are taken from such models, and thus take into account effects due to variation in treatment sample sizes and interactions among treatment effects. To help balance analyses involving adult traits, only individuals with all life-history traits measured were analyzed. Data from each combination of food and infection treatment were not always available for adults of strain SA1. In such cases, this strain was excluded from analyses and fully factorial models were applied to the remaining strains. Individuals that had been exposed to infection but did not contain spores as adults were assumed to be uninfected and eliminated from analyses. The chi-square values presented are log likelihood estimates from nominal logistic regressions. Preference was given to parametric models where the data structure allowed. Analyses were performed by JMP (SAS Institute 1995).

Results

The dataset analyzed involved a total of 1054 individuals, including 638 adults for which each life-history trait was measured. We first present results from the uninfected treatments followed by those from the infected treatments. In particular we focus on effects due to differences among strains.

Uninfected Treatments

Evidence for costs of OP insecticide resistance in uninfected mosquitoes was found for the traits of survival to adulthood and adult longevity. The survival of S-Lab across food treatments was significantly greater than for SR ($\chi_1^2 = 7.48$, $P = 0.006$) and SA1 ($\chi_1^2 = 28.50$, $P < 0.001$), but no different from SA4 ($\chi_1^2 = 0.60$, $P = 0.437$) (Fig. 1A). These differences indicate important costs of resistance for the *Ester*[1] allele at the *Ester* locus and the *ace-1*[R] allele at the *ace-1* locus in the absence of OP insecticides. Adult longevity of the S-Lab strain was also significantly greater than for each of the three resistant strains (contrast S-Lab vs. SA4/SR/SA1, $F_{1,367} = 86.65$, $P < 0.001$) (Fig. 1B), suggesting metabolic
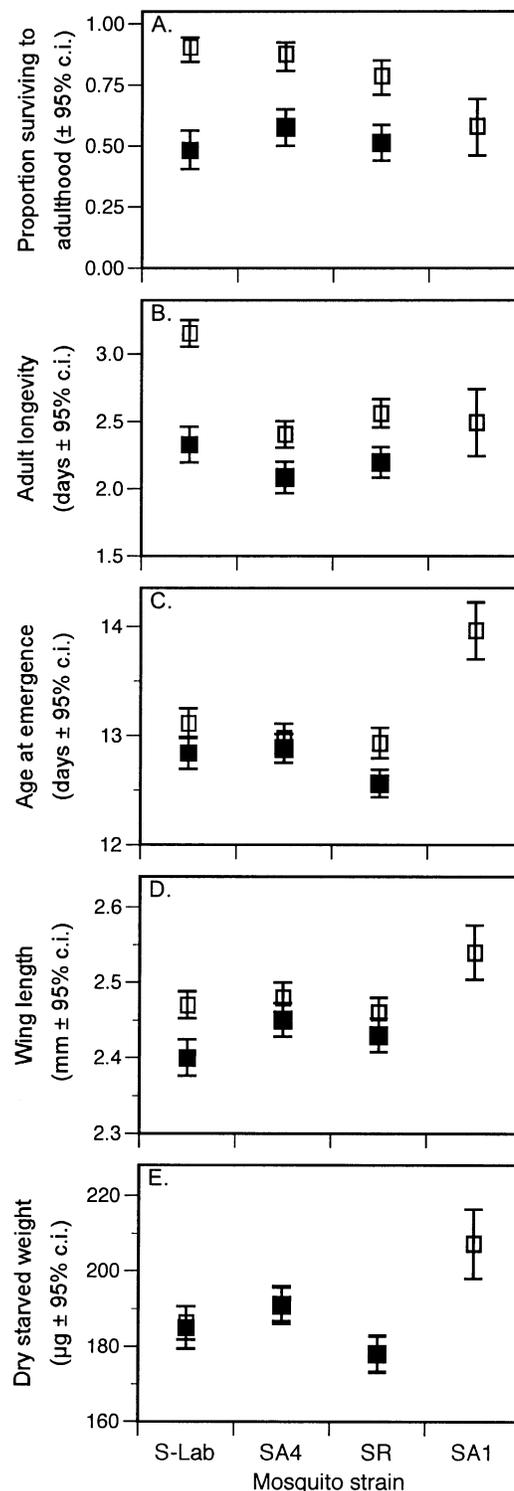

FIG. 1. Life-history traits of infected (■) and uninfected (□) mosquitoes for each strain of mosquito: (A) Proportion of individuals surviving to adulthood, (B) Adult longevity, (C) Age at emergence, (D) Wing length, (E) Dry starved weight. In each case the effects of larval food treatment have been taken into account, as has an individual's sex for adult traits. No overall estimate could be calculated for infected individuals of SA1, because no individuals survived to adulthood in the low-food treatment.

TABLE 1. Life-history traits of uninfected mosquitoes in high and low food treatments.

Trait	Food treatment		Test statistics
	High	Low	
Survival to adulthood, %[a]	71	58	$\chi^2_1 = 1.65$, $P = 0.199$
Age at emergence, days[b]	11.81	14.67	$F_{1,367} = 1049.17$, $P < 0.001$
Wing length, mm[b]	2.55	2.43	$F_{1,367} = 86.74$, $P < 0.001$
Dry starved weight, μg[b]	205	175	$F_{1,367} = 110.65$, $P < 0.001$
Adult longevity, days[b]	2.90	2.49	$F_{1,367} = 36.42$, $P < 0.001$

[a] Estimates and statistic taken from a fully factorial nominal logistic regression for the effects of strain and food treatments.
[b] Estimates and statistic taken from a fully factorial analysis of variance for the effects of strain, food, and an individual's sex.

costs may be associated with each resistance allele (see below).

Significant variation among strains was also found for age at emergence (Fig. 1C), wing length (Fig. 1D), and dry starved weight (Fig. 1E). Much of this variation was due to strain SA1, with differences disappearing (age at emergence, wing length) or being reduced (dry starved weight) upon its exclusion from analyses (details not shown). The remaining differences did not suggest any particular costs (or benefits) associated with OP resistance.

Larvae were equally likely to reach adulthood in the high- and low-food treatments, but the life-history traits of those emerging from the high food treatment indicated a greater potential reproductive success due to their faster development, greater size and increased adult longevity (Clements 1992) (Table 1). Strains responded similarly to the different food treatments, except for SA1. Its presence caused strain × food interactions due to a relatively longer time to adult emergence and a relatively heavier dry starved weight in the low food treatment ($F_{3,367} = 2.64$, $P = 0.049$ and $F_{3,367} = 3.76$, $P = 0.011$, respectively).

Infected Treatments

Parasitism by *V. culicis* was costly to mosquito fitness, but did not affect each strain equally. The costs of OP resistance expressed by strain SA1 when uninfected were increased by *V. culicis*. Nearly 40% (47 of 120) of the 1st instar larvae initially exposed to infection by the parasites' spores died during this 24 h period, whereas the loss for each other strain (and controls) was <10%. None (0 of 15) of the remaining larvae placed in tubes of the low food treatment survived to adulthood, whereas at least 35% did so for the other treatments; S-Lab (27 of 77), SA4 (33 of 88), SR (37 of 89). The 31% (8 of 26) of infected SA1 reaching adulthood in the high food treatment was markedly less than 50% achieved by the other strains; S-Lab (44 of 83), SA4 (58 of 69), SR (54 of 97). These results show that the mosquitoes with resistance allele *Ester[1]* were particularly susceptible to the costs of parasitism by *V. culicis*.

The lack of infected SA1 individuals emerging from the low food treatment precluded a full strain × infection × food analysis. With SA1 excluded, survival to adulthood was found to be lower in infected (53%) than uninfected (86%) treatments ($\chi^2_1 = 123.23$, $P < 0.001$) and less in the low (43%), rather than high (62%), food treatments ($\chi^2_1 = 21.22$, $P < 0.001$), but no food × infection or food × infection × strain interactions were found ($\chi^2_1 = 1.44$, $P = 0.230$, $\chi^2_2 = 1.76$, $P = 0.415$, respectively). There was, however, an overall strain × infection interaction ($\chi^2_2 = 6.81$, $P = 0.033$). This interaction was mainly due to differences in the survival of S-Lab and SR. In contrast to having a significantly lower probability of surviving to adulthood than S-Lab when uninfected, the survival of SR was equal to that of S-Lab when infected (Fig. 1A). If just these two strains are considered, the above interaction becomes stronger ($\chi^2_1 = 6.78$, $P = 0.009$). Thus, parasitism by *V. culicis* acted to increase the relative fitness of mosquitoes bearing the resistance allele *ace-1[R]* by erasing the survival costs experienced when uninfected.

No increase or decrease in the relative probability of surviving to adulthood was observed for strain SA4, which remained similar to S-Lab whether infected or not (strain × infection, $\chi^2_1 = 2.27$, $P = 0.132$). Thus, *V. culicis* imposed no selective effect on the relative survival of mosquitoes bearing the *Ester[4]* resistance allele. Patterns of larval and pupal mortality underlying the above results are treated in more detail further below.

The overall effects of *V. culicis* on the life-history traits of mosquitoes that survived to adulthood are summarized in Table 2; strain SA1 was not included in these analyses, as data were only available from the high food treatment. Infected mosquitoes emerged earlier than uninfected mosquitoes, but this was not due to them bringing forward their age at pupation, as reported in different conditions for this host-parasite relationship (Agnew et al. 1999). The observed pattern was due to the mortality of the more slowly developing infected individuals while still as larvae or pupae, thus eliminating their contribution towards estimates of age at emergence.

TABLE 2. Life-history traits of infected and uninfected adult mosquitoes.

Trait	Infected	Uninfected	Test statistics
Age at emergence, days[a]	12.76	12.99	$F_{1,567} = 16.89$, $P < 0.001$
Wing length, mm[a]	2.43	2.47	$F_{1,567} = 27.76$, $P < 0.001$
Dry starved weight, μg[a]	184	185	$F_{1,567} = 0.238$, $P = 0.626$
Adult longevity, days[a]	2.20	2.70	$F_{1,567} = 120.69$, $P < 0.001$

[a] Estimates and statistic taken from a fully factorial analysis of variance for the effects of strain, infection, food, and an individual's sex.
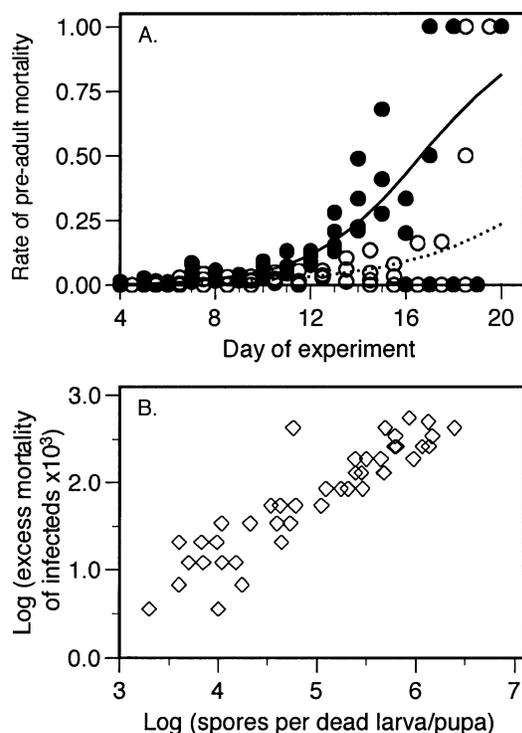
FIG. 2. (A) Observed and estimated rates of pre-adult mortality as a function of time. Observed rates of mortality in infected (●) and uninfected (○) treatments for each strain as a weighted mean of mortality in the high- and low-food treatments. Estimated values for infected (solid line) and uninfected (dashed line) treatments derived from a fully factorial nominal logistic regression on the frequency of larvae and pupae dying per day, relative to the size of the pre-adult population at the beginning of that day. (B) Relationship between the excess rate of mortality in infected treatments and the number of *Vavraia culicis*' spores per dead larva or pupa.

Vavraia culicis reduced the adult longevity of each strain (Table 2). However, the superior longevity of uninfected S-Lab mosquitoes was reduced to a greater extent than for strains SA4 or SR when they were infected (strain × infection, $F_{2,567} = 11.82$, $P < 0.001$) (Fig. 1B). Thus, parasitism by *V. culicis* acted to increase the relative fitness associated with this trait for mosquitoes bearing the resistance alleles *Ester^A* and *ace-1^R*.

No evidence was found for strain × infection interactions involving a mosquito's age at emergence (Fig. 1C), wing length (Fig. 1D), or dry starved weight (Fig. 1E) (analyses not shown).

Patterns of Mortality and Spore Production

The daily rate of preadult mortality (frequency of larvae and pupae dying/size of preadult population at the beginning of the day) was similar for infected and uninfected treatments at the beginning of the experiment (Fig. 2A), but diverged as mortality in the infected treatments increased faster than for uninfected treatments (infection × day, $\chi_1^2 = 7.08$, $P = 0.008$). The divergence in mortality between infected and uninfected treatments correlated closely with the number of *V. culicis*' spores found in infected individuals dying as larvae
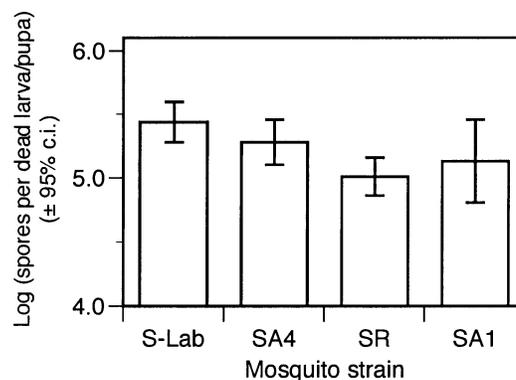

FIG. 3. Differences in the mean number of *Vavraia culicis*' spores per dead larvae and pupae for each strain of mosquito. Values are taken from a fully factorial analysis of variance for the effects of strain and food treatments.

or pupae ($r = 0.80$, $P = 0.001$) (Fig. 2B). Apart from strain SA1, which had the highest rates of pre-adult mortality both before and after spore production began, the infected treatments of S-Lab, SA4, and SR showed similar rates of pre-adult mortality ($\chi_2^2 = 0.52$, $P = 0.770$) and were equally likely to survive to adulthood (Fig. 1A).

The day at which infected larvae or pupae died did not vary among the four strains (median 12 days, nonparametric analysis of variance [ANOVA], $H_{3,239} = 0.67$, $P = 0.880$), but they varied in the amount of spores harbored by dead individuals ($F_{3,239} = 5.25$, $P = 0.002$) (Fig. 3). Differences in spore production could not be explained by *V. culicis* growing at different rates in each strain of mosquito: we removed the effect of food treatment from spore number and age at death for infected individuals dying as larvae or pupae. Residual spore numbers were regressed against residual age at death. The slopes of these regressions did not vary among strains ($F_{3,239} = 0.62$, $P = 0.603$) indicating that *V. culicis* had a common growth trajectory in each strain (estimated common $\beta = 0.261$). The intercepts of these regressions, as measured by their elevation, however, were not equal ($F_{3,242} = 13.57$, $P < 0.001$), with S-Lab and SA4 being higher than for SR and SA1 (Tukey tests, details not shown). Given that *V. culicis* developed at the same rate in each strain, these analyses suggested that an alternative hypothesis to explain the observed differences in the number of spores per strain was due to differences in the initial sizes of their infections.

To test whether this was the case, we performed a separate experiment in which we compared the time taken for uninfected 4th instar larvae to replace their gut contents. We assumed this would correlated with a larva's exposure to the parasite's spores and the size of an infection. Strains varied in their time to replace gut contents ($F_{3,39} = 20.13$, $P < 0.001$); average times in minutes ($\pm$SE) were: SR 19.2 ($\pm$1.9), S-Lab 28.0 ($\pm$1.8), SA4 33.1 ($\pm$2.0), SA1 41.6 ($\pm$2.3). Thus, strains did vary in their feeding behavior but this trait did not correlate directly with expectations for the size of an initial infection.

DISCUSSION

The aim of this study was to identify whether the type of parasite that mosquito larvae are likely to encounter in natural

environments could influence the relative fitness of mosquitoes sensitive or resistant to OP insecticides.

Host Traits

Uninfected hosts

Survival to adulthood was the life-history trait measured most directly related to an individual's fitness. In the conditions of this experiment, larval food availability did not influence the probability of uninfected mosquitoes reaching adulthood, but their resistance status did. In particular, the survival of S-Lab was significantly greater than for SR and SA1 (Fig. 1A), indicating important fitness costs for resistance alleles *ace-1*[R] and *Ester*[1] in untreated environments. These results agree well with those from field data collected in untreated areas of southern France: selection against resistance alleles *Ester*[1] and *ace-1*[R] is stronger than for *Ester*[4] (Lenormand et al. 1999). The lower costs associated with the *Ester*[4] allele than for the first esterase mutation to be detected, *Ester*[1], reveals a temporal pattern of selection that has favored the evolution of resistance mutations responsible for a reduced negative effect on the fitness of their surrounding genetic background (Guillemaud et al. 1998). However, selection does act against the *Ester*[4] allele in field conditions. Despite finding no effect of this allele on survival to adulthood, it should exert costs elsewhere.

The longevity of adult mosquitoes provided only with access to water reflects their ability to survive on nutritional resources accumulated during their larval development (Briegel et al. 2001). When uninfected, each of the three resistant strains showed a significantly shorter period of adult longevity than S-Lab (Fig. 1B), indicating there are metabolic costs associated with each resistance allele, including *Ester*[4]. The actual resources being metabolized are likely to be lipids (Timmermann and Briegel 1999), because these are the main source of reserves mobilized while adult mosquitoes are at rest (Clements 1992), as they were in the conditions of this experiment. Energetically deficient female mosquitoes often require more than one bloodmeal to mature a clutch of eggs (Briegel 1990). As blood-feeding activity is an important source of mortality for female mosquitoes (Anderson and Brust 1996), the costs of being resistant may be expressed at this point in field conditions. Alternatively, resistant mosquitoes could compensate for these costs by spending additional effort seeking other sources of energy, such as, flower nectar. These costs will apply to males and females and will be at the detriment of time they are able to invest in reproductive activity.

Besides the effects found on survival to adulthood (Fig. 1A) and adult longevity (Fig. 1B), we found no other convincing evidence for costs of resistance in uninfected mosquitoes for the traits of age at emergence (Fig. 1C), adult wing length (Fig. 1D), or their dry starved weight (Fig. 1E).

Larval food availability was an environmental parameter that had an important influence on the life-history traits of uninfected mosquitoes (Table 1), but showed little interaction with a mosquito's resistance status, other than low food conditions causing SA1 to take relatively longer to complete its larval development and to have a relatively heavy dry starved weight.

Infected hosts

Parasitism had a strongly negative impact on the fitness of infected mosquitoes. The probability of mosquitoes surviving to adulthood was markedly less in infected treatments (Fig. 1A). Of particular interest was how infection altered the relative differences between the resistant strains and S-Lab. Whereas SR had a significantly lower probability of reaching adulthood than S-Lab when these strains were uninfected, this difference disappeared when they were infected by *V. culicis*. Thus, parasitism caused a relative increase in the fitness of mosquitoes bearing the *ace-1*[R] resistance allele. In contrast, there were no differences between S-Lab and SA4 in reaching adulthood in either infected or uninfected treatments. This indicates parasitism did not alter their relative prospects of survival to adulthood and thus was an effectively neutral source of selection on this trait. The effect of *V. culicis* on the survival of SA1 was particularly strong. Nearly 40% of the SA1 larvae initially exposed to infection by the parasite's spores died during this period. Furthermore, none of the remaining larvae reached adulthood in the low food treatment conditions. Thus, relative to S-Lab, the fitness of mosquitoes bearing the *Ester*[1] allele decreased in the presence of parasitism.

Adult longevity was another fitness-related trait influenced by parasitism. Strains S-Lab, SA4 and SR showed reduced longevity when infected (Fig. 1B). However, the influence of *V. culicis* was not equal for each strain. In particular, the greater longevity of S-Lab when uninfected was reduced to a greater extent than for SA4 and SR. Consequently, the fitness advantages related to this trait for S-Lab were diminished when infected, that is, the relative fitness of individuals bearing resistance alleles *Ester*[4] or *ace-1*[R] was increased.

Infected adults may have lived for less time than uninfected adults because they were killed by their infections or because they emerged as smaller adults with fewer nutritional reserves. If they had been killed, we would expect unmetabolized reserves to contribute towards their dry weight at the time of death. A comparison of the ratio of dry weight to wing length (thus correcting for body size) found no difference between adults from infected and uninfected treatments (nonparametric ANOVA, $H_1 = 1.71$, $P = 0.191$). In contrast, the ratio of longevity to wing length was significantly less for infected mosquitoes (nonparametric ANOVA, $H_1 = 68.84$, $P < 0.001$). These results suggest that the reduced longevity of infected mosquitoes was more likely due to them emerging as smaller adults with fewer metabolic reserves than because they had been killed before exhausting such reserves.

Infection by *V. culicis* did not influence the relative differences among strains for the remaining adult life-history traits measured; age at emergence (Fig. 1C), wing length (Fig. 1D), and dry starved weight (Fig. 1E).

Parasite Traits

The proportion of hosts killed before they leave the aquatic environment as adult mosquitoes and the number of spores yielded from these individuals are key determinants of *V. culicis*' transmission success (Reynolds 1972; Kelly et al. 1981).

Infected hosts of strains S-Lab, SA4 and SR were equally likely to die before reaching adulthood (Fig. 1A) and the rate at which their mortality occurred was closely correlated with the number of spores produced by *V. culicis* (Fig. 2B). Despite there being no differences among strains for the time at which preadult mortality occurred, there were significant differences in the amount of spores they harbored (Fig. 3). In particular, the yield of spores from dead SR hosts was less than that from those of S-Lab, suggesting these resistant hosts would offer less transmission success back to *V. culicis* than OP sensitive mosquitoes. Although many infected individuals of strain SA1 failed to reach adulthood, the value of spores from these larvae and pupae needs to be discounted by the proportion of hosts dying at the time of exposure to infection: the latter hosts will offer no transmission success to the parasite.

Our data suggested the differences in the number of spores produced in each strain varied because the size of initial infections varied across strains, rather than because *V. culicis* produced spores at different rates across strains. Additional data provided partial support for the hypothesis that strains varied in their exposure to infection because of differences in their feeding behavior. It has already been demonstrated that the *ace-1*^{R} mutation responsible for increased acetylcholinesterase activity can adversely modify the behavioral traits of adult mosquitoes (Berticat et al. 2002b). However, a simple positive relation between the rate at which food passes through larval guts and initial infection size was not observed. Instead, larval mortality was greatest in the slowest feeding strain (SA1) and spore production was lowest in the fastest feeding strain (SR). These results tentatively suggest the time available for ingested spores to infect epithelial cells of the host's gut may also help determine the size of an infection.

High concentrations of OP esterases are known to exist in epithelial cells of esterase resistant mosquitoes (Pasteur et al. 2001), and have been proposed as a proximal cause in limiting the growth of filarial worm infections in OP insecticide resistant *Culex* mosquitoes in Sri Lanka (McCarroll et al. 2000). It was further suggested that the adverse effects of OP esterases might be more general and impair the growth of other parasites. The similarity of our results for infected individuals of S-Lab and the OP esterase producing strain SA4, in all but their adult longevity, does not provide support for the generality of this hypothesis.

Conclusions

Our results found there were costs of resistance associated with each resistance allele we tested and that these costs depended on the resistance allele involved, larval resource availability, whether mosquitoes were infected or not, and the particular life-history trait measured. Hence, general predictions that xenobiotic resistance will be costly in a xenobiotic-free environment and that the expression of such costs will be context-dependant were met in the conditions of our experiment.

Of particular interest was the effect of parasitism as an environmental source of biotic selection pressure acting on the fitness of sensitive and resistant populations of host mosquitoes. Although the overall effect of *V. culicis* was to reduce mosquito fitness, it acted to increase or decrease differences among traits of the resistant strains and the sensitive S-Lab strain relative to when they were uninfected. This is interesting because parasites causing such interactions have the potential to alter the strength and direction of selection against resistance mutations in untreated environments.

Our data also found that traits linked with the parasite's transmission success varied among mosquito strains. Heterogeneity in parasite fitness caused by this type of interaction could result in feedback into the dynamics of the host-parasite relationship (e.g., by influencing parasite prevalence). An epidemiological feedback of this nature would add a further dynamic to factors influencing the strength of selection acting on resistance mutations.

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