Food availability and parasite infection influence the correlated responses of life history traits to selection for age at pupation in the mosquito *Aedes aegypti*

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life history.

Abstract

We selected six lines of mosquito *Aedes aegypti* for earlier or later pupation and measured the correlated responses of several life history traits: adult size, two fecundity measures and pre-adult survival. We further examined the influence of two environmental parameters – larval food availability and infection by the microsporidian parasite *Edhazardia aedis* – on the correlated responses. Pre-adult survival did not respond to selection for age at pupation in any environment. For all of the other traits, the environment influenced the correlated response, though the contribution of the different environmental aspects differed among traits. While the correlated response of adult size was influenced only by larval food availability, the likelihood that a female laid eggs was influenced by parasite infection, and the correlated response of the number of eggs was influenced by the interaction of the two environmental parameters. Generally, a deteriorating environment moved the correlated response from one favouring later pupation to one favouring earlier pupation. Larval food availability and parasite infection also influenced the association between the mean wing length and fecundity of the selected lines. At high food availability, there was a positive relationship between adult size and fecundity, while infected mosquitoes reared at low food availability showed the opposite trend. We discuss these results in light of the coevolutionary potential of the host–parasite interaction.

Introduction

Given the importance of life history traits in determining reproductive success, it is not surprising that a host's life history can be involved in resistance against pathogens and parasites. In some cases, life history traits are genetically correlated with physiological and biochemical resistance. Thus, resistance of anopheline mosquitoes against malaria infection is negatively correlated with their developmental time and adult size (Yan *et al*., 1997), and resistance of meal moths against a granulosis virus is positively correlated with developmental time, pupal weight and egg mortality (Boots & Begon, 1993). In other cases, the life history itself may form the basis of the host's resistance, or at least tolerance, to the parasite. Two examples of this are the flower *Silene latifolia*, in which long-lived flowers are at a higher risk of contracting the fungus *M. violaceum* than short-lived ones (Shykoff *et al*., 1996), and the mosquito *Aedes aegypti*, where mosquitoes that pupate early are less likely to be killed by the microsporidian *Edhazardia aedis* than those that pupate late (Agnew & Koella, 1999). As the difference in the life histories, at least in the latter system (Koella & Agnew, 1999), is partly genetic, one would therefore expect the parasite to exert selection pressure on its host's history.

This expectation is supported by the only published mathematical model of the evolutionary response of a life history trait – age at first reproduction – to parasite

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pressure (Hochberg *et al*., 1992). The conclusion of the model is that virulent parasites would select for earlier reproduction, thereby enabling the host to diminish the amount of damage inflicted by the parasite.

However, such a response by the host's life history will only be possible if the associated costs are not too high. In *Silene*, for example, the selection pressure for short-lived flowers would be counteracted by the necessity for the female flowers to be open long enough for pollination to occur. More generally, the costs to changes in a life history trait involve genetic correlations with other aspects of the life history. In *Anopheles*, for example, selection for resistance brings with it earlier pupation, which in turn leads to smaller adults (Yan *et al*., 1997).

Thus, as in standard quantitative genetic models of life history evolution (Lande, 1982), the evolution of life history traits in response to parasite pressure becomes a problem of balancing the selection pressure due to the parasite and the constraints due to the genetic covariance matrix. One of the problems in using such models is that the genetic covariance matrix can vary. The genetic correlations can change over time because allele frequencies change during evolution, or because of genotype by environment interactions in changing environments (Turelli, 1988). They can also depend on the environmental conditions (Via, 1984), sometimes to the extent that they are positive in some environments but negative in others (Gebhardt & Stearns, 1988; Newman, 1988), leading to qualitatively different predictions about evolutionary change in different environments.

Thus, to predict the evolutionary result of parasite pressure on the life-histories of their hosts, one must understand the genetic covariance structure of life history traits (Lande, 1979) and how genetic covariances change in different environments. In particular, one must know whether parasite infection itself, as an aspect of the host's environment, affects the genetic covariances.

We have investigated this question with the mosquito *Ae. aegypti* and its microsporidian parasite *E. aedis*. The mosquito's life-history, in particular its age at pupation, influences the parasite's virulence (Agnew & Koella, 1998; Koella & Agnew, 1998). As mosquitoes that pupate early are less likely to be killed by the microsporidian than those that pupate late (Agnew & Koella, 1998; Koella & Agnew, 1998), the parasite potentially selects for earlier age at pupation. Therefore, we selected mosquito lines for early or late pupation, and investigated the correlated response of adult size and fecundity to our selection regime while varying two aspects of the mosquito's environment: food level and parasite infection.

Methods

Aedes aegypti

Aedes aegypti, the yellow fever mosquito, has been studied in detail owing to its importance in disease transmission and the ease of maintaining it in the laboratory (Christophers, 1960). For our experiments we used the Rockefeller strain obtained from Dr W. Rudin (Swiss Tropical Institute, Basel, Switzerland). We had previously selected three lines for early pupation and three lines for late pupation over seven generations (Koella & Agnew, 1998). The selection procedure was done at the food level described below as 100%

Edhazardia aedis

Edhazardia aedis is an obligate, intracellular microsporidian parasite (Becnel *et al*., 1989) and is host-species specific (Becnel & Johnson, 1993; Andreadis, 1994). Its life-cycle (Fig. 1) involves horizontal and vertical transmission with two different spore types (Becnel *et al*., 1989). Horizontal transmission occurs when larvae ingest uninucleate spores from the environment. After going through several developmental stages, the parasite produces binucleate spores. In some cases, these sporulate before the larva emerges as an adult and go on to produce a further generation of uninucleate spores, kill the larva and initiate another round of horizontal transmission. In other cases, larvae survive the infection and develop into adults. In females, the binucleate spores transmit vertically by infecting the developing eggs. These hatch and larvae develop, but rarely survive to adulthood. Instead, their death is induced by the continued development of the parasite and the production of uninucleate spores. When the larva dies, the spores are released into the environment to initiate the cycle once again.

Which transmission route is followed is partly determined by the host's age at pupation (Agnew & Koella, 1998; Koella & Agnew, 1998). In rapidly developing mosquitoes that pupate early, the parasites generally follow the route including vertical transmission. In slowly developing mosquitoes, the parasites generally bypass vertical transmission and follow the route of repeated horizontal transmission. Thus, the parasite's virulence increases with the host's age at pupation (Koella & Agnew, 1998).

The parasites, which were originally derived from specimens collected in Thailand, were obtained from Dr J. Becnel (United States Department of Agriculture, Gainesville, USA).

General procedures and treatments

We reared the mosquitoes in a climate chamber maintained at 28 (± 0.5) °C and 80 (± 5)% relative humidity with a 12 h:12 h light–dark cycle. We hatched mosquitoes synchronously by flooding them under reduced pressure for 1 h. After 24 h, we separated the larvae of each line into six treatments, each consisting of 50 individuals within a plastic cup containing 100 mL demineralized water. The treatments varied in two parameters: infection level and food regime.

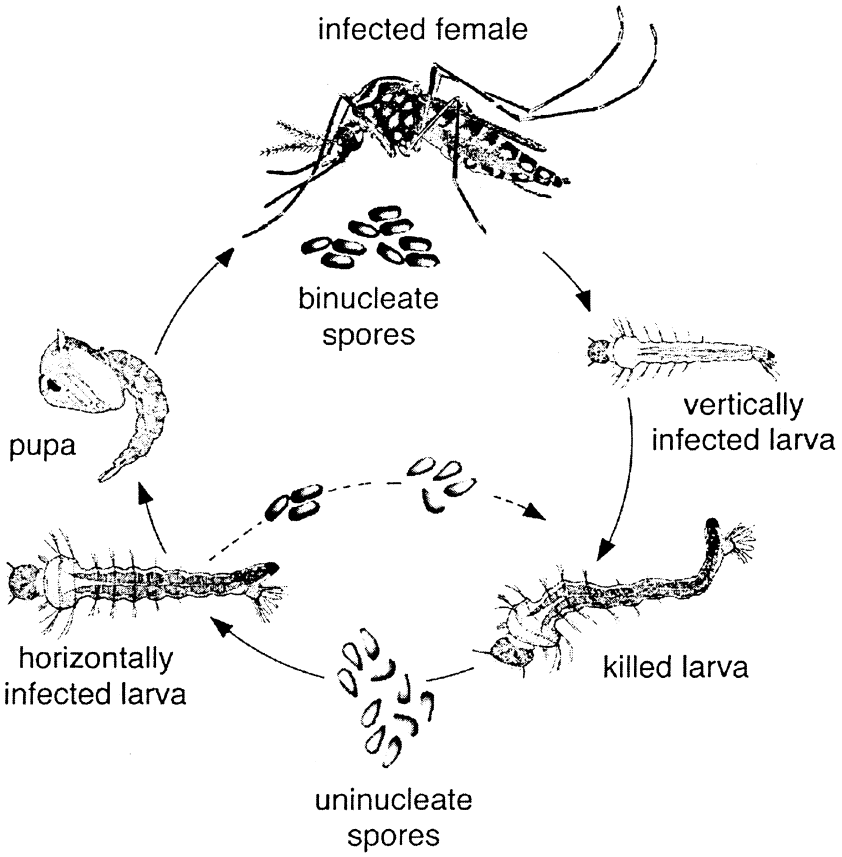

Fig. 1 Life cycle of the microsporidian parasite *Edhazardia aedis* infecting the yellow fever mosquito *Aedes aegypti*. The life cycle normally involves transovarial infection of eggs, the formation of uninucleate spores, oral infection of larvae and the formation of binucleate spores in the pupae and adults (solid line). In some mosquitoes the entire developmental cycle of the parasite – from binucleate to uninucleate spores – is completed in the larval host, leading to repeated horizontal transmission (dashed line).

Food regime was manipulated as 100%, 75% or 50% of a standardized ration of Tetra MicroMin®. The 100% ration per larva was 0.06 mg on the first day after hatching, 0.08 mg on day 2, 0.16 mg on day 3, 0.32 mg on day 4 and 0.64 mg on odd-numbered days thereafter. Daily mortality and pupation of larvae were taken account of in calculating the food given to each treatment.

Infection level was either uninfected controls or infected by adding 500 uninucleate spores mL^{-1} to the plastic cups 72 h after hatching. We had previously established that this level of infection would only rarely kill the developing larvae or pupae.

Life-history traits

We removed dead individuals and checked for pupae every 12 h. The pupae were put into a cage according to the selection line and treatment to allow the emerged adults to mate. If a cage contained only females, we added male mosquitoes from the appropriate line developing at 100% food regime parallel to the experiment. Four days after pupating (i.e. 2 days after emergence) the females were given the opportunity to feed on one of J.C.K.'s arms for 3 min. They were then placed individually into 50-mL plastic containers (3 cm Ø) containing wet filter paper for oviposition. After 5 days, we froze the mosquitoes and counted the eggs they had laid. Fecundity was analysed in two ways: first, as the proportion of mosquitoes that laid at least one egg, and second, as the number of eggs laid by these mosquitoes.

We used wing length as a measure of adult size. Both wings of each mosquito were glued onto a slide. Their lengths from the distal end of the alula to the tip of the vein R3 were measured with a dissecting scope attached to a video camera and a computer supplied with the image analysis software NIH Image 1.61 (http://rsb.info.nih.gov/nih-image/). The mean length of the two wings was used for the analyses.

We report only the results obtained on females.

Statistics

We tested for differences among treatments (food regime and infection) and selection lines of each parameter individually with a three-way ANOVA including a term for selection line within selection regime. The analyses were done with the means within selection line, food regime and infection to facilitate comparison with the analysis of the correlated response (see description of

ANCOVA below). These analyses were done with JMP 3.1.6 (SAS, 1994).

To estimate the correlated responses to selection of the continuous traits (wing length and number of eggs), we used a nested analysis of covariance (Bell, 1989; Armbruster, 1991). We calculated the among selection line components of covariance and the variance of age at pupation with the SAS procedure NESTED (SAS, 1989) and estimated each correlated response as the ratio of the covariance and the variance.

We investigated the effects of the two environmental parameters and of their interaction on the correlated responses with an analysis of covariance (ANCOVA) on the mean trait values within each treatment and selection line. Though, in contrast to the nested analysis, an ANCOVA gives biased slopes (as the variabilities within selection lines of the dependent and the independent variable are neglected), this approach appears to be the standard way to analyse correlated responses and genetic correlations (Via, 1984). The ANCOVA has two advantages over the nested analysis of covariance. First, in contrast to the latter, it allows direct investigation of the effects of the environmental factors, and second, it allows investigation of the correlated responses of the two binary traits – the proportion of mosquitoes laying at least one egg and pre-adult mortality – to selection for age at pupation with the arc-sine transformed proportion within treatment and selection line. The use of ANCOVA can be justified for the presented data, as the results of the two approaches were similar. The ANCOVA analyses were done with JMP 3.1.6 (SAS, 1994).

We further investigated the effects of food and infection on the association between wing length as a measure of body size and the two fecundity parameters: number of eggs and the likelihood that a mosquito lays at least one egg. This was also done with an ANCOVA on the mean trait values within each treatment and selection line.

In all analyses, infection and food level were treated as a nominal factor and proportions were arc-sine transformed. In the ANCOVA tests we included interaction terms up to the level indicated by previous step-wise tests.

Results

Univariate analyses

The effects of selection and treatment on life history traits are summarized in Table 1.

The mosquitoes that had been selected for early pupation developed most rapidly under all conditions (Fig. 2a). The difference between the selection regimes, however, depended strongly on the treatment conditions (Table 1); the largest difference was generated among uninfected mosquitoes developing under the lowest food level. The effect of infection on age at pupation depended on the food level and the selection regime: at 100% food, infection brought pupation forward from 7.9 to 7.4 days; at 75% food, infection delayed pupation by 0.5 days; at 50% food, the direction of the effect of infection depended on the selection regime (selection for early pupation: pupation delayed by 1 day; selection for late pupation: pupation brought forward by 1 day).

Wing length generally increased with larval food availability from 2.37 mm (with a standard deviation of 0.14 mm) at the lowest amount of food to 2.52 mm (SD 0.16 mm) at the highest amount, while infection had almost no effect on wing length (Fig. 2b).

The likelihood that a female laid at least one egg (Fig. 2c) decreased with decreasing food levels (from 38% at the highest food availability to 18% at the lowest)

Table 1 Univariate analysis of the measured life history traits. Number of eggs represents only those mosquitoes that laid at least one egg; egg laying success measures the likelihood that a mosquito laid at least one egg. Each trait was analysed with an analysis of variance of the mean values within food level, infection status and selection line; the proportions of pre-adult mortality and of mosquitoes that laid at least one egg were arc-sine transformed.

		Age at pupation (n = 676)[a]		Wing length (n = 668)		Number of eggs (n = 152)[b]		Egg laying success (n = 469)		Mortality (n = 1800)	
Source	d.f.	SS	F ratio	SS	F ratio	SS	F ratio	SS	F ratio	SS	F ratio
Selection regime	1	21.99	99.93***	0.015	5.93*	300	2.08	0.516	20.13***	0.030	1.44
Line[selection]	4	3.00	3.41*	0.115	11.01***	1204	2.08	0.163	1.59	0.026	0.30
Food level	2	17.20	39.08***	0.137	26.40***	1744	6.03**	0.587	11.44***	0.063	1.50
Infection	1	0.02	0.07	<0.001	0.02	911	6.29*	0.248	9.68**	0.006	0.31
Selection * food	2	0.74	1.68	0.001	0.26	293	1.01	0.017	0.33	0.008	0.19
Selection * infection	1	0.83	3.75†	0.001	0.57	672	4.64*	0.080	3.13†	0.004	0.17
Food * infection	2	1.68	3.83*	0.001	0.12	437	1.51	0.003	0.07	0.003	0.06
Select * food * infection	2	2.39	5.42*	0.001	0.24	399	1.38	0.008	0.17	0.028	0.65
Error	20	4.40		0.052		2605[b]		0.513		0.424	

[a] The numbers in parentheses are the number of mosquitoes used in each analysis. [b] In two treatments, none of the mosquitoes laid eggs; the error degrees of freedom for the number of eggs is therefore only 18. †$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

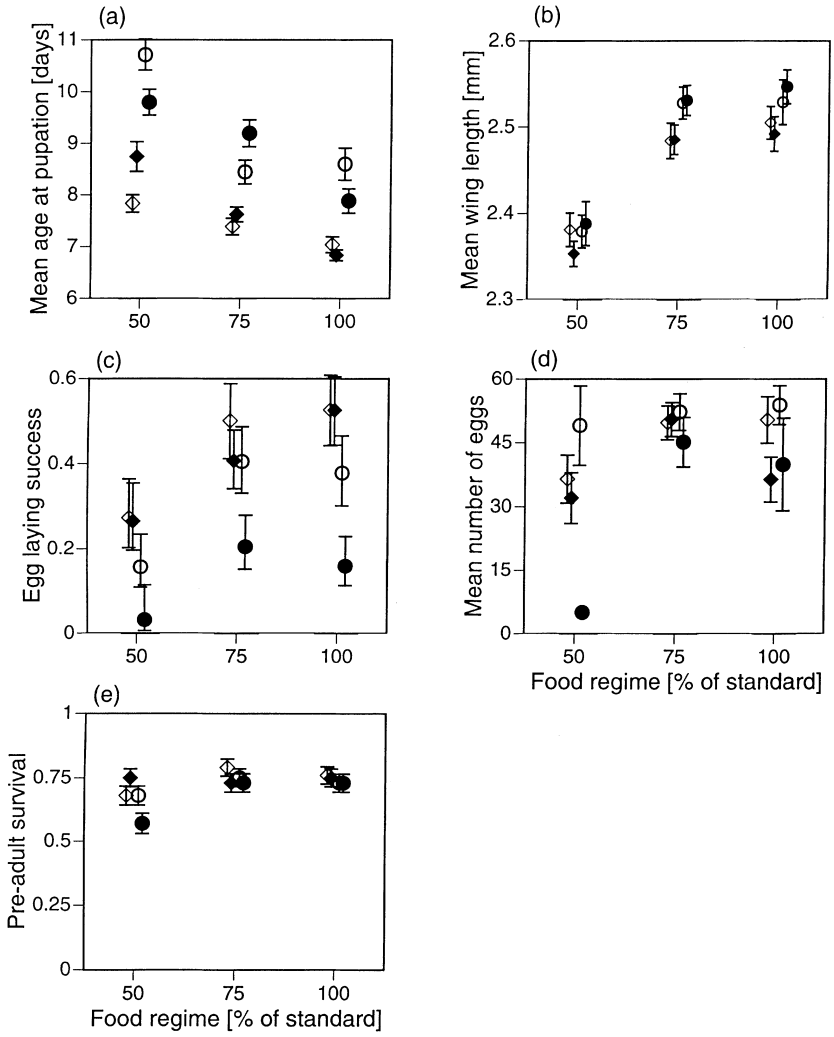

Fig. 2 Effects of larval food conditions and parasite infection on selected life history traits of *Ae. aegypti* selected for early or late pupation. (a) Age at pupation. (b) Adult size of females, measured as wing length. (c) Likelihood that a female laid at least one egg. (d) Number of eggs laid in first clutch. (e) Pre-adult survival. In all graphs, symbols show the mean value, and vertical bars show the standard errors of the mean. Circles represent mosquitoes selected for late pupation, diamonds represent mosquitoes selected for early pupation. Solid symbols represent infected, open symbols represent uninfected mosquitoes.

and if larvae were infected (uninfected: 38%; infected: 28%). It was also influenced by the selection regime, with mosquitoes selected for early pupation being more likely to lay eggs (early pupation: 42%; late pupation: 23%). A similar trend was observed for the number of eggs laid in the first clutch (Fig. 2d). Under the best conditions, a female laid about 50 eggs, but the number dropped to about five for infected females selected for late pupation and reared at the lowest food availability.

Pre-adult survival was generally about 70%, with little effect of either food level or infection (Fig. 2e). Only at the lowest food level did infection decrease survival.

Correlated responses

Wing length was generally greater in the lines selected for late pupation (Fig. 3a–c), though the slopes were close to 0 under 50% food. We found no difference between infected and uninfected mosquitoes except at 75% food, where uninfected mosquitoes had a stronger positive correlation than infected ones.

The proportion of mosquitoes that laid at least one egg tended to be lower in the lines selected for late pupation (Fig. 3d–f). The apparent differences in the correlated responses among food and infection levels were not statistically significant, owing to large standard errors of the estimated slopes. In contrast, the correlated responses of the number of eggs differed among treatments (Fig. 3g–i). While food level had only a small effect on the response of uninfected mosquitoes, the response of infected ones decreased from a positive slope at 100% food to a very negative one at 50% food.

There was no apparent response to selection for age at pupation in the proportion of mosquitoes surviving to adulthood (Fig. 3j–l).

These results are summarized in Fig. 4, which shows the slopes of the correlated responses calculated with the

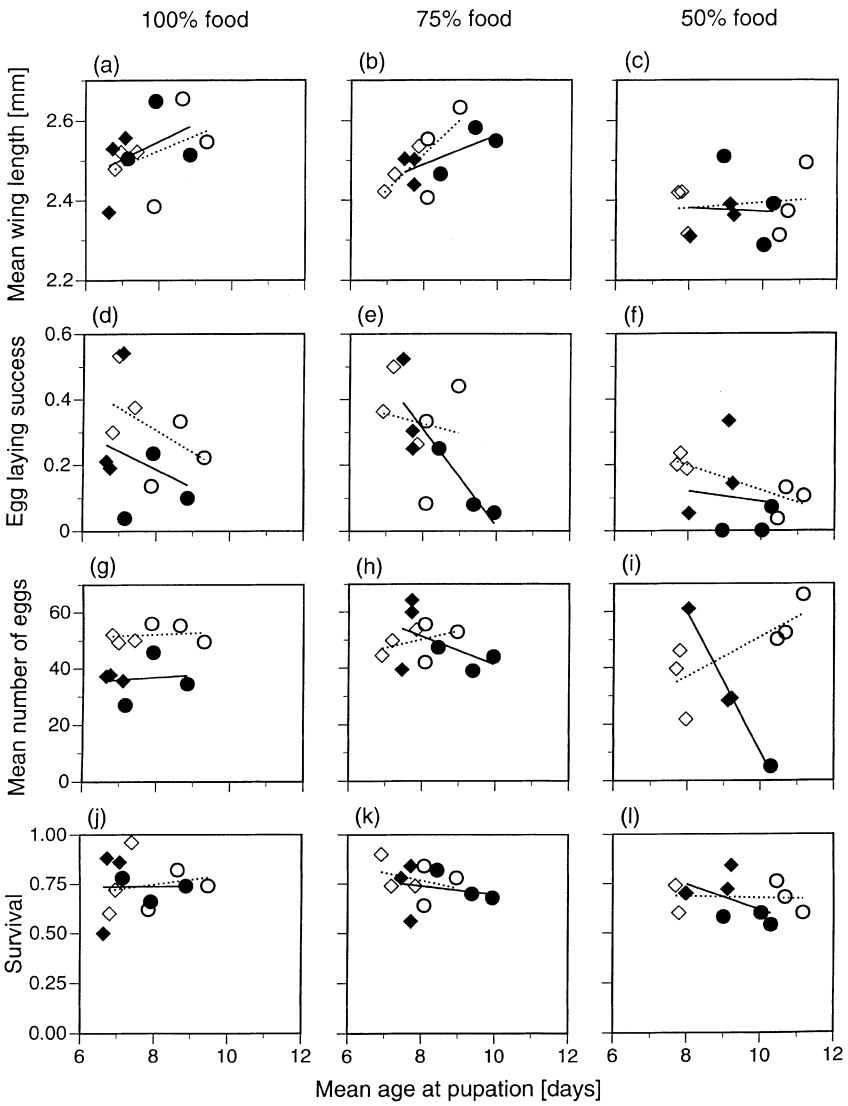

Fig. 3 Correlated life history responses to selection on age at pupation of *Ae. aegypti*. The symbols represent the means within selection lines, the lines show the regressions of the means. Open symbols and dotted lines represent uninfected, closed symbols and solid lines represent infected mosquitoes; diamonds represent mosquitoes selected for early pupation, circles represent those selected for late pupation. The graphs on the left (a, d, g, j) show the correlated responses for mosquitoes reared at 100% food, the graphs in the middle (b, e, h, k) show mosquitoes reared at 75% food and the mosquitoes on the right (c, f, i, l) show the mosquitoes reared at 50% food. Graphs (a), (b) and (c) show the correlated response of adult size, measured as wing length; (d), (e) and (f) show the response of the likelihood that a female laid at least one egg; (g), (h) and (i) show the response of the number of eggs laid during the first clutch; (j), (k) and (l) show the response of pre-adult survival.

nested analysis of covariance, and in Table 2, which shows the results of the analyses of covariance.

The association between mean wing length within a selection line and the two measures of fecundity were also influenced by the food level and infection (Table 3). Among uninfected mosquitoes, there was a positive correlation between mean age at pupation and fecundity at all food levels (Fig. 5d–f). Infection generally lowered fecundity. Furthermore, among infected mosquitoes the correlation between wing length and number of eggs was positive for well-fed individuals, but tended to become negative at worse food conditions (Fig. 5e,f). The proportion of mosquitoes that laid at least one egg tended to respond similarly as the number of eggs laid (Fig. 5a–c), though only the effects of food and infection, but not that of wing length, were statistically significant.

Discussion

The results show that infection by the parasite *E. aedis* can modify the correlated response of the life history traits of its host *Ae. aegypti* to selection for age at pupation. Thus, the parasite will not only impose selection pressure on its host, but will also influence the genetic structure of the host's life history and thus its evolutionary possibilities.

Before discussing these coevolutionary aspects in more detail, we briefly discuss how environmental variability influenced the mosquito's life history, compare the results of our experiments with other work on mosquitoes

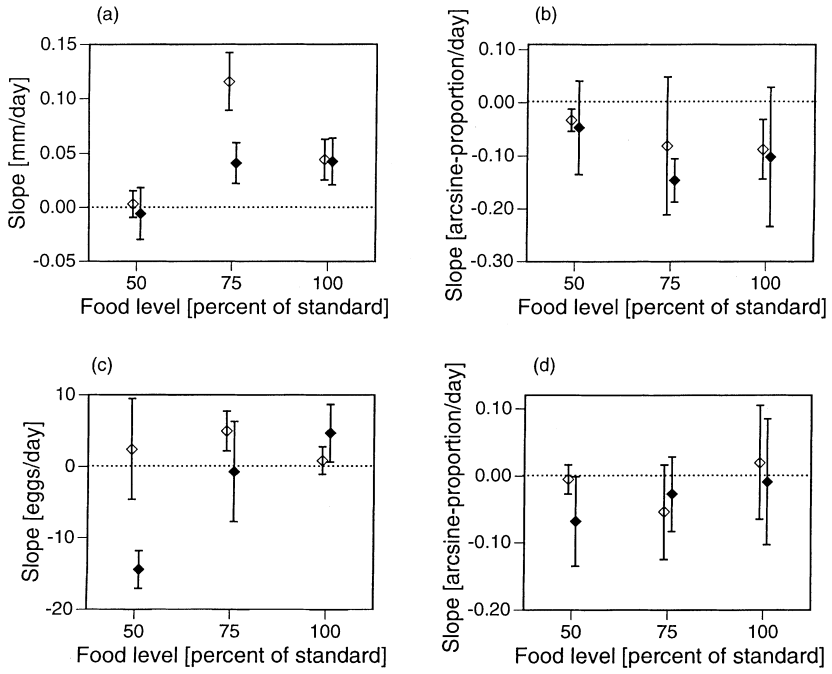

Fig. 4 Slopes of correlated responses to selection for age at pupation, as a function of larval food availability and parasite infection. The slopes were calculated with a nested analysis of covariance. Open symbols and dotted lines represent uninfected, closed symbols and solid lines represent infected mosquitoes; diamonds represent mosquitoes selected for early pupation, circles represent those selected for late pupation. The vertical lines show the standard errors of the slopes. (a) Correlated response of wing length. (b) Correlated response of the likelihood that a female laid at least one egg. (c) Correlated response of the number of eggs laid in the first clutch. (d) Correlated response of pre-adult survival.

and discuss the influence of environmental variability on the correlated responses.

Environmental variability

The responses of the mosquitoes to environmental variation were similar to what has been observed in other studies. Thus, lowering the amount of food available to the larvae delayed pupation (Marcovitch, 1960; Moore & Whitacre, 1972; Suleman, 1982), decreased the adult size (Nayar, 1969) and decreased fecundity (Table 1). The latter response is usually thought to be an indirect response to food, owing to the positive correlation generally observed between fecundity and adult size (Steinwascher, 1982; Nasci, 1986; Packer & Corbet, 1989; Briegel, 1990). Indeed, after we had controlled for the effects of wing length and infection, the effect of food on the number of eggs was not significant (Table 3); the effect of food, however, on the likelihood that a mosquito laid at least one egg was significant (ANOVA nested within selection lines: $F_{6,127} = 2.34$; $P = 0.03$). Thus, larval food availability appeared to have an indirect effect on fecundity, but a direct effect on egg laying success.

The amount of food also had a strong effect on age at pupation and adult size (wing length). The effects of infection with the microsporidian parasite on most of the life-history traits we measured were generally low, and

Table 2 Analysis of covariance showing the effects of food level and infection on the correlated responses of the measured life history traits on selection for age at pupation. All analyses were done with the mean values within food level, infection status and selection line; the proportions of pre-adult mortality and of mosquitoes that laid at least one egg were arc-sine transformed. The table shows the interaction terms only if they had been included by a step-wise analysis.

	Wing length			Number of eggs			Egg laying success			Pre-adult mortality		
Source	d.f.	SS	*F* ratio	d.f.	SS	*F* ratio	d.f.	SS	*F* ratio	d.f.	SS	*F* ratio
Food level	2	0.150	14.08***	2	543	1.94	2	0.142	2.08	2	0.027	0.82
Infection	1	<0.001	0.02	1	822	5.86*	1	0.238	6.99*	1	0.006	0.37
Age at pupation	1	0.021	3.94†	1	334	2.38	1	0.247	7.25*	1	0.009	0.54
Food * infection				2	1069	3.81*						
Food * age				2	632	2.25						
Infection * age				1	827	5.89*						
Food * infection * age				2	893	3.18†						
Error	31	0.166		22	3087		31	1.055		31	0.513	

†$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 Analysis of covariance showing the effects of food level and infection on the association between the wing length and the two fecundity measures, the number of eggs and the proportion of mosquitoes laying at least one egg. The analyses were done with the mean values within food level, infection status and selection line; the proportion of mosquitoes that laid at least one egg was arc-sine transformed. The table shows the interaction terms only if they were significant.

	Number of eggs			Egg laying success		
Source	d.f.	SS	*F* ratio	d.f.	SS	*F* ratio
Food level	2	292	2.37	2	0.318	3.79*
Infection	1	2211	35.88***	1	0.248	5.90*
Wing length	1	272	4.41*	1	<0.001	0.02
Food * infection	2	2353	19.10***			
Food * wing	2	312	2.54			
Infection * wing	1	2308	37.45***			
Food * infection * wing	2	2365	19.19***			
Error	22	1356		31	1.301	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

were strongest in conditions of low food availability. As in previous studies, infection with *E. aedis* did not influence adult size (Becnel *et al.*, 1995; Nasci *et al.*, 1992). It did, however, reduce blood-feeding success (Koella & Agnew, 1997) and thus fecundity (Becnel *et al.*, 1995), in particular when mosquitoes developed slowly (Koella & Agnew, 1997). It also delayed pupation of the mosquito lines selected for late pupation and reared with a low amount of food (Koella & Agnew, 1998).

Correlated responses

In our stock of *Aedes aegypti*, adult size and fecundity responded to selection on age at pupation in directions that depended on the rearing conditions of the larval mosquitoes. Mosquitoes that had obtained a full diet and had not been infected by *E. aedis* developed into larger adults, if they had been selected for later pupation. As their fecundity was not significantly affected by selection for age at pupation, the increase of adult survival associated with larger body size in many mosquitoes (Hawley, 1985; Nasci, 1986; Packer & Corbet, 1989), including *Ae. aegypti* (Steinwascher, 1982), suggests that the genetic covariance structure would constrain the evolution towards earlier pupation. This conclusion does not change when the mosquitoes are infected, as the correlated responses are similar for infected and uninfected mosquitoes.

A similar genetic structure has been found in the pitcher-plant mosquito, *Wyeomyia smithii*, where selection on age at pupation led to no apparent response in fecundity or pre-adult survival. Unfortunately, no data on adult size are given. However, in *Drosophila* flies that had been reared

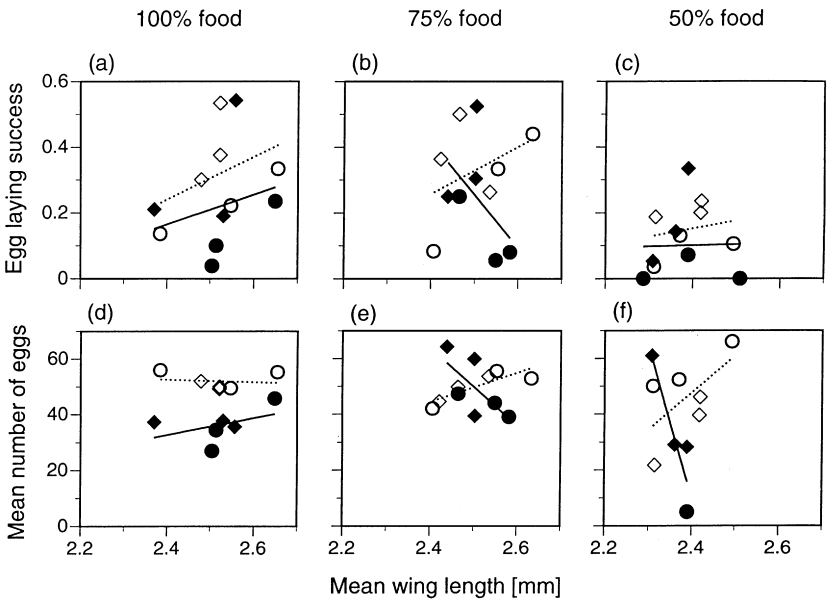

Fig. 5 Correlations, based on mean values within selection lines, between wing length and two measures of fecundity after selecting *Ae. aegypti* for age at pupation. The symbols represent the means within selection lines, the lines show the regressions of the means. Open diamonds represent uninfected, closed diamonds represent infected mosquitoes. The graphs on the left (a, d) show the slopes of the correlated responses for mosquitoes reared at 100% food, the graphs in the middle (b, e) show mosquitoes reared at 75% food, and the graphs on the right (c, f) show the mosquitoes reared at 50% food. Graphs (a), (b) and (c) show the correlation between wing length and the likelihood that a female laid at least one egg; (d), (e) and (f) show the correlation with the number of eggs laid during the first clutch.

in a good environment, there was a positive genetic correlation between adult size and age at pupation (Gebhardt & Stearns, 1988; Nunney, 1996), similar to what we had observed for the mosquitoes in this experiment.

If the same stocks of flies were reared under poor food conditions, the genetic correlation between age at pupation and adult size switched from a positive to a negative value (Gebhardt & Stearns, 1988). We observed a similar change of the correlated response, when we varied environmental conditions. If mosquitoes had obtained less food, the adult size no longer responded to selection for age at pupation; if, additionally, the mosquitoes had been infected, fecundity showed a strongly negative response to selection for age at pupation. Thus, under these conditions, the genetic covariance structure would tend to favour the evolution towards earlier pupation.

The correlated responses to selection for age at pupation are reinforced by the association between wing length and fecundity. Under good conditions, mosquitoes that had responded to selection with larger wings had higher fecundity that those with short wings. Such a positive correlation between wing length and fecundity is generally observed in mosquitoes (Steinwascher, 1982; Nasci, 1986; Packer & Corbet, 1989; Briegel, 1990). On the other hand, if larval food conditions were bad and mosquitoes were infected, larger mosquitoes tended to have lower fecundity than small ones. Such a change in the relationship between wing length and fecundity confirms a previous observation in this host–parasite system (Becnel *et al.*, 1995).

Coevolutionary possibilities

These results suggest that the mosquito's evolutionary response to parasite pressure would depend on the environmental conditions it is reared in. Under good conditions, the genetic correlation structure suggests that any selection for earlier pupation imposed by the parasite would be counteracted by the balance of selection pressures due to the correlated responses of other life history traits.

Under good conditions, the genetic correlations would tend to favour delayed pupation; under bad conditions, the genetic correlations would favour earlier pupation.

To gain a better understanding of the selection pressures and their evolutionary consequences, one must take account of some details of the host–parasite interaction. In particular, the detrimental effect of *E. aedis* increases as the developmental period of the mosquito increases. Not only are slowly developing mosquitoes more likely to be killed by the parasite (Koella & Agnew, 1998), but the mosquitoes that survive the infection are more likely to suffer as adults, if they have developed slowly, by blood-feeding less effectively (Koella & Agnew, 1997) and by having more asymmetric wings (Agnew & Koella, 1997). Thus, the parasite's main selection pressure is exerted on mosquitoes living in conditions that delay pupation, e.g. mosquitoes developing under low food levels. It is under such conditions that selection for earlier pupation will bring with it a correlated increase of fecundity. Thus, the selection pressure by the parasite will be reinforced, rather than constrained, by the genetic structure of the host's life history.

The details of the parasite's effect on its host also suggest a mechanism leading to the switch in the correlated response of fecundity from a positive value at high food levels to a negative value at low food levels. High food levels not only lead to rapid growth and early pupation on average, but also to low variability in age at pupation, while low food levels retard growth and increase the variability in age at pupation (Agnew & Koella, 1998). A combination of genes and the environment, both leading to slow development, will increase the proportion of individuals that pupate late enough for the parasite to have a severe effect on the adult's blood-feeding efficiency and fecundity.

Summary

Any prediction or understanding of the parasite's or the host's evolution will have to consider that the genetic correlations underlying the host's evolutionary response may depend on the environment in which the host is raised. In particular, the parasite itself may modify the genetic correlations, and thus influence the possible evolutionary trajectories of its host.

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