A correlated response of a parasite's virulence and life cycle to selection on its host's life history

J. C. KOELLA* & P. AGNEW*†

*Department of Zoology, University of Aarhus, Universitetsparken B135, DK-8000 Århus C, Denmark
†Experimental Ecology, ETH Zürich, ETH Zentrum NW, CH-8092 Zürich, Switzerland

Keywords:

Aedes aegypti;
Edhazardia aedis;
life cycle;
life history;
virulence.

Abstract

We demonstrate a correlated response of the virulence and the mode of transmission of the microsporidian parasite *Edhazardia aedis* to selection on the age at pupation of its host, the mosquito *Aedes aegypti*. We selected three lines of mosquitoes each for early or late pupation and exposed the larvae after zero, two and four generations of selection to a low and a high concentration of the parasite's spores. Before selection the parasites induced a similar level of mortality in the six lines; after four generations of selection mortality was higher in the mosquitoes selected for late pupation than in those selected for early pupation. Overall, parasite-induced mortality was positively correlated with the mean age at pupation of the matching uninfected line. When they died, mosquitoes selected for early pupation harboured mostly binucleate spores, which are responsible for vertical transmission. Mosquitoes selected for late pupation were more likely to harbour uninucleate spores, which are responsible for horizontal transmission. The parasite enhanced this tendency for horizontal transmission by prolonging the larval period in the lines selected for late pupation, but not in the ones selected for early pupation. These results suggest that the genetic basis of the mosquito's age at pupation helps to determine the parasite's mode of transmission: parasites in rapidly developing mosquitoes are benign and transmit vertically, while parasites in slowly developing mosquitoes are virulent and transmit horizontally. Thus, as the host's life history evolves, the parasite's performance changes, because the host's evolution changes the environment in which the parasite develops.

Introduction

Since the observation that snails exposed to trematode parasites bring forward their maturity and increase their fecundity to lessen the parasite's impact on their reproductive success (Minchella & Loverde, 1981; Minchella, 1985), several studies have considered modifications of a host's life history as its adaptive response to parasite pressure (Michalakis & Hochberg, 1994). The marine snail *Cerithidea californica* matures at smaller sizes in populations where parasite prevalence is high (Lafferty, 1993); great tits, *Parus major*, increase their parental care and thus their reproductive effort, when nestlings are infected with the haematophagous flea *Ceratophyllus gallinae* (Perrin *et al*., 1996); the lizard *Lacerta vivipara* increases reproductive effort when infected with an ectoparasitic mite (Sorci & Clobert, 1995), which leads to offspring with higher quality from infected than from uninfected mothers. Such observations have been complemented with theoretical studies predicting the optimal evolutionary responses of age at maturity (Hochberg *et al*., 1992) or reproductive effort (Forbes, 1993; Perrin *et al*., 1996) to parasitism.

While the host's adaptive response to parasite exposure and infection has been investigated, the opposite influence – that of a host's life history on its parasite's transmission and life cycle – remains largely unexplored. There are, in principle, three ways in which a host's life history can influence its parasite's life cycle. First, many

Correspondence: Dr Jacob C. Koella. Present address: Laboratoire d'Ecologie, CC237, Université P. & M. Curie, 7 quai St. Bernard, 75252 Paris, France. E-mail: koella@biology.aau.dk

effects of the host's life history on the parasite's life cycle will be nonadaptive by-products of the host–parasite interaction; the parasite may be constrained by its host's life history. If, for example, the parasite's rate of transmission is associated with the host's size (as is the case in several mosquito-borne diseases such as malaria (Lyimo & Koella, 1992) and some viruses (Takahashi, 1976; Baqar *et al*., 1980; Grimstad *et al*., 1980)), an evolutionary change in body size will lead to an associated change in transmission rate. Second, the parasites may adapt to its host's life history by modifying the details of its life cycle, as shown in theoretical studies of the dynamics of parasites within their hosts (Koella & Antia, 1995; Sasaki & Iwasa, 1991). These studies emphasize the importance of the host's life history, in particular age-specific and parasite-induced mortality, in determining the life cycle of the parasite that achieves maximal transmission. Third, the parasite may actively manipulate its host and in particular modify its host's life history to enhance its transmission. That some parasites can modify their host's life histories is seen in the castration of snail hosts by their trematode parasites (Baudoin, 1975). The decreased reproductive effort by the host following castration gives rise to higher host survivorship and more rapid growth, which in turn gives rise to higher parasite transmission. The anther-smut fungus *Ustilago violacea*, which infects the dioecious herb *Silene alba*, not only castrates its host by causing females to produce a sterile, rudimentary ovary, but also changes the sex of the female flowers by inducing them to produce anthers (Alexander, 1989). In both males and females the anthers contain not pollen, but rather the fungal spores that are transmitted by pollinators.

The response of the parasite to the host's life history will depend on the complex interplay between many factors. In particular, the response may be constrained and influenced by the dynamics of the parasite within its host (Antia *et al*., 1993; Koella & Antia, 1995) and the genetic correlations among the parasite's life cycle traits (Bull, 1994; Ebert & Herre, 1996).

The present study illustrates these points by investigating the variability of the virulence and the mode of transmission of the microsporidian parasite *Edhazardia aedis* with relation to genetic variability of its host, the yellow fever mosquito *Aedis aegypti*. In particular, we selected the mosquito for early and late age at pupation and measured the correlated response in the parasite's virulence and mode of transmission. Furthermore, we investigated the modification of the mosquito's age at pupation by the parasite.

Materials and methods

The host

The yellow fever mosquito *Aedes aegypti* is ubiquitous in the tropics and subtropics. As a vector of yellow fever, dengue and other viruses, it has been the subject of extensive investigations (Christophers, 1960). We used the Rockefeller strain obtained from Dr W. Rudin (Swiss Tropical Institute, Basel, Switzerland).

The parasite

The microsporidian parasite *Edhazardia aedis* is specific to *Ae. aegypti* (Becnel, 1992). It has a complex life cycle (Fig. 1) involving vertical transmission with binucleate spores and horizontal transmission with uninucleate spores (Becnel *et al*., 1989). Mosquito larvae become horizontally infected by ingesting uninucleate spores from the aquatic environment. After going through several developmental stages, the parasite produces binucleate spores. If an infected mosquito has in the mean time emerged as an adult female, these spores infect her eggs for vertical transmission. After further development in the vertically infected larvae, uninucleate spores are produced. They eventually kill the larva and are then released into the environment, initiating the cycle once more. Alternatively, the parasite may complete its life cycle – from horizontal infection through binucleate to uninucleate spore production – within a single, horizontally infected larva, thus bypassing vertical transmission. For the purpose of this paper, the important aspect of the life cycle is that spore production follows a fixed sequence (after horizontal infection, binucleate spores precede uninucleate spores), while the sequence of transmission (horizontal or vertical) can vary.

We obtained *E. aedis* from Dr J. Becnel (United States Department of Agriculture, Gainesville, USA). This stock is derived from specimens collected in Thailand.

Experiments

General procedures

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, added them to 0.5 L of demineralized water in 10 × 10 × 10-cm plastic pans and fed them with a standardized amount of the fish food Tetramin™ (day 1: 0.06 mg per larva; day 2: 0.08 mg; day 3: 0.16 mg; day 4: 0.32 mg; days 5, 7, 9, …: 0.64 mg).

Selection procedure

Over four generations we selected three lines of mosquitoes for early and three lines for late age at pupation. In each generation, we reared 150 uninfected mosquitoes of each line. In the lines selected for rapid development the individuals pupating within 1 day of the first emergence were allowed to contribute to the next generation (this was generally about 50% of the mosquitoes); in the lines selected for slow development we took the individuals pupating at least 2 days after the first larva had pupated.

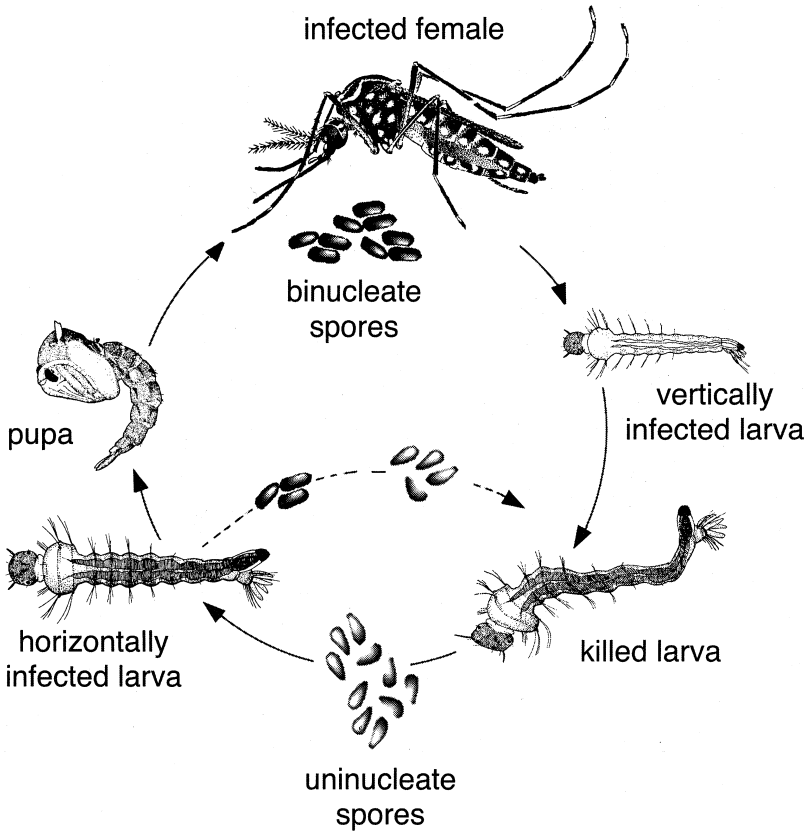

Fig. 1 Life cycle of the microsporidian parasite *Edhazardia aedis* infecting the yellow fever mosquito *Aedes aegypti*. The life cycle normally involves transovarial infection of eggs, the formation of uninucleate spores, oral infection of larvae and the formation of binucleate spores in the pupae and 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).

In contrast to the mosquito, the parasite experienced no selection due to our experiment, as the spores used for infections (see below) were derived directly from the USDA stock.

Infection

We investigated the effect of infection at three time points: before selection, and after two and after four generations of selection. We exposed 50 larvae of each line to uninucleate spores by adding the spores to the rearing pans 3 days after hatching. Two exposure levels were used: 500 spores mL^{-1} and 2000 spores mL^{-1}. Pans were checked daily and dead larvae were removed. Age at death and age at pupation were recorded to the nearest 24 h. In the exposed lines after four generations of selection, we estimated the numbers of binucleate and uninucleate spores in the dead larvae and pupae by homogenizing each dead mosquito in 1 mL of distilled water, adding 0.1 μL of the solution to a haemocytometer, counting the spores under a microscope at 400× magnification, and multiplying the number by 10^4. All spore numbers are given as $\log_{10}$(spores/mosquito).

We evaluated the influence of selecting mosquitoes for age at pupation on three traits related to the parasite's success at horizontal transmission: the probability that a mosquito died before it emerged, the number of uninucleate and binucleate spores harboured by the dead mosquitoes, and the prolongation of the larval period due to the parasite.

The probability of mortality before emergence was evaluated with a logistic analysis with the factors selection regime, intensity of exposure and line within selection regime.

Because of low sample sizes, the relationship between the probability that a dead individual harboured uninucleate or binucleate spores and the selection regime was evaluated with Fisher's exact test. The effect of selection on the number of spores in those mosquitoes harbouring at least one spore was evaluated with a Welch ANOVA, which accounts for different variances among groups. In both of these analyses low and high exposures were investigated separately, and the effect of line within selection regime was neglected.

The prolongation of the larval period is defined as the difference between an infected individual's age at death and the age at which it would have emerged, had it not been infected. We cannot, of course, estimate this on an individual mosquito; we can, however, estimate a mean for each group by comparing the distribution of age at emergence of the uninfected controls with the distribution of age at death of the infected larvae and pupae. Consider a treatment where a proportion p of the infected mosquitoes was killed by the parasite before

emerging and where the average age at their death was A_1, and where a proportion $1 - p$ emerged before death. We now assume that the proportion p corresponds to the most slowly developing individuals in the matching uninfected control line. We read from the observed distribution of pupation times in the uninfected controls the age A_2, by which the proportion p of the uninfected controls had not yet pupated. If A_1 is much larger than A_2, those mosquitoes that would have pupated between A_2 and A_1 (had they not been infected) are kept from pupating when they are infected. On the other hand, if the parasite had no effect on the mosquito's development or age at pupation, the two ages would be similar. We therefore defined prolongation as $A_1 - A_2$. Note that we did not consider the actual age at emergence of infected mosquitoes in estimating prolongation of the larval period. This age would be influenced by the proportion of mosquitoes killed by the parasite before emergence. As age at death was always later than average age of emergence, the estimates would be too low.

We investigated the effect of selection regime, intensity of exposure and line within selection on our measure of prolongation with an analysis of variance. Because each treatment results in one number for the prolongation, there were no replicates within treatment, so that the interaction between selection and intensity of exposure could not be investigated.

All statistical analysis were performed with the program JMP 3.1.6 by SAS Institute (http://www.sas.com/otherprods/jmp/Home.html). Nested effects were taken as random factors and are indented in the tables.

Results

Selection for early and late pupation

Four generations of selection in uninfected mosquitoes produced early lines with an average age at pupation of 6.9 days (means of lines: 6.8, 6.9, 7.0 days) and slow lines pupating at 7.9 days (means of lines: 7.7, 7.8, 8.3 days). The differences among lines were not significant. Males pupated about half a day earlier than females, with a tendency for the difference to vary among lines. The effect of selection was similar for the two sexes (Table 1).

Correlated response in parasite-induced mortality

Before selection, mortality of infected larvae ranged from 2 to 12% at the low intensity of exposure and from 34 to 50% at the high intensity (Fig. 2A). Two generations of selection led to clear differences in mortality among the selection regimes (Fig. 2B). These differences were enhanced after four generations of selection (Fig. 2C). After exposure to the low intensity of infection, 3.5% of the individuals in the early lines and 18% of the individuals in the late lines died, while after exposure to the high intensity, 19% and 55% died, respectively. That the correlated response to selection was indeed due to changes in age at pupation is suggested by the strong correlation between mortality and mean age at pupation of the corresponding uninfected line (Fig. 2D). The statistical analyses of these differences are shown in Table 2.

Table 1 Analysis of variance showing the effect of four generations of selection for early and for late pupation. Line within selection regime was considered a random effect; selection regime and sex were considered fixed effects.

Source	d.f.	Sum of squares	*F* ratio	*P*
Selection regime	1	156.0	17.7	0.014
Line [selection]	4	35.3	4.5	0.087
Sex	1	46.6	23.9	0.008
Sex ∗ selection	1	4.0	2.1	0.225
Sex ∗ line [selection]	4	7.8	2.4	0.047
Error	705	566.8		

Correlated response in the parasite's mode of transmission

We first evaluate the potential for horizontal transmission by considering the uninucleate spores in mosquitoes dying before emergence. After four generations of selection, after the low intensity of exposure, none of the mosquitoes selected for early pupation harboured uninucleate spores at their death, while 60% of the mosquitoes selected for late pupation did (Fig. 3A; two-tailed Fisher's exact test: $P = 0.042$). Among the mosquitoes harbouring spores, the mean number of $\log_{10}$(spores per mosquito) was 4.73 (SD of $\log_{10}$(spores): 0.56). After the high intensity of exposure, 35% of the larvae from the early lines and 42% of the mosquitoes from the late lines harboured uninucleate spores (Fig. 3B), but this difference was statistically insignificant (two-tailed Fisher's exact test: $P = 0.78$). The number of spores, however, was lower in the early lines (mean of $\log_{10}$(spores): 4.26, SD: 0.22) than in the late lines (mean: 4.74, SD: 0.58; Welch ANOVA allowing for unequal variances: $F[1,23.14] = 10.25$, $P = 0.004$).

The potential for vertical transmission depends on the presence of binucleate spores. In contrast to the uninucleate spores, their production did not depend on the selection regime. At both low and high exposures about 90% of the mosquitoes killed by the parasite harboured binucleate spores (low exposure: two-tailed Fisher's exact test: $P = 1$; high exposure: $P = 0.68$). After low exposure the mean $\log_{10}$-transformed numbers of spores were 4.77 (SD: 0.12) in the mosquitoes selected for early pupation and 4.83 (SD: 0.32) in those selected for late pupation (Welch ANOVA: $F[1,11.74] = 0.574$, $P = 0.464$). After high exposure, the means of $\log_{10}$(spores per mosquito) were 4.58 (SD: 0.48) and 4.62 (SD: 041) (Welch ANOVA: $F[1,23.00] = 0.072$, $P = 0.792$).

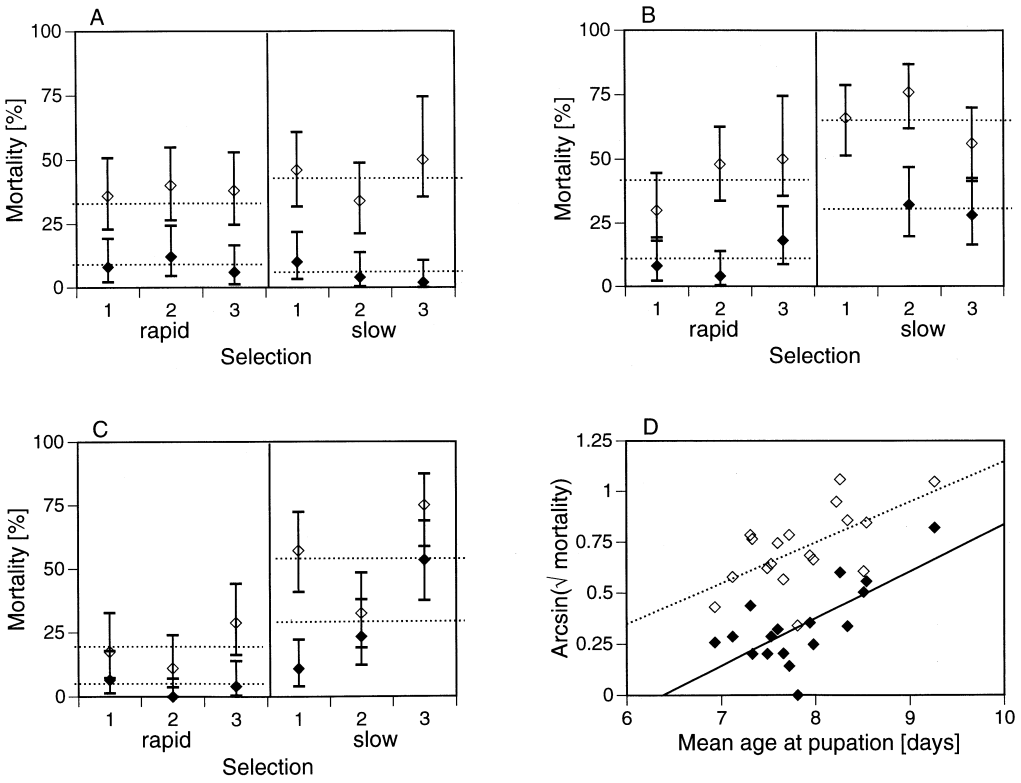

Fig. 2 Parasite-induced mortality (percentage dying before emergence) of three lines of mosquitoes selected for early pupation and three lines selected for late pupation. In each panel, the solid diamonds represent exposure to 500 spores mL^{-1}, the open diamonds exposure to 2000 spores mL^{-1}. In panels A–C the vertical lines represent the 95% confidence interval of the binomial distribution and the dotted horizontal lines represent mean mortality within selection regimes and intensities of infection. (A) Mortalities before selection did not differ among selection regimes or among lines within regimes. (B) Mortalities after two generations of selection were higher in the slowly than the rapidly developing mosquitoes after exposure to a high intensity of infection. Note that in line 1 of the mosquitoes selected for slow development, there were not enough larvae to infect at the low intensity. (C) Mortalities after four generations of selection differed among selection regimes after high exposure and among lines within selection regimes. (D) The pooled data from A–C, showing the mortality as a function of age at pupation. The *x*-axis shows the mean age at pupation of the corresponding uninfected lines; the *y*-axis shows the mortality of the infected line. Note that the lines are a visual aid and do not represent statistically valid regressions. The statistical analysis shown in Table 2 considers each generation separately.

Timing of events

Among the larvae and pupae killed by the parasite, the average age at death was 11.8 days. Mosquitoes selected during four generations for early pupation died earlier (mean: 9.7 days) than those selected for late pupation (mean: 12.4 days), while there was no apparent variation due to the line within selection regimes or due to the intensity of exposure (Table 3).

The observed response of uninucleate spore production to selection for age at pupation was mostly due to changes in the age at which the parasites killed the mosquitoes. When age at death was included in the analysis (Table 4), the proportion of dead larvae and pupae harbouring uninucleate spores and the number of uninucleate spores per dead individual increased with age at death (Fig. 4), but were not affected by the selection regime or the intensity of exposure. In contrast, the proportion of dead individuals with binucleate spores was independent of selection, intensity of exposure and age at death, while the number of binucleate spores per individual increased with the intensity of exposure.

Our measure of parasite-induced prolongation of the larval period was, on average, 1.23 days. The prolongation was more pronounced in the mosquitoes selected for late pupation (prolongation 2.57 days) than in those selected for early pupation (prolongation –0.38 days, i.e. the mosquitoes died before the average age of pupation of uninfected controls), but was not significantly affected by the intensity of exposure (low exposure: prolongation 1.20 days, high exposure: prolongation 1.26 days) (Table 5).

Discussion

The major result of our study is that genetic variability in the host's life-history traits was associated with variability in several aspects of the parasite's life cycle. First,

Table 2 Logistic analyses showing the effect of selection for early and for late pupation on the probability of dying before emergence. Note that in the analysis of the pooled data, the effects of age at pupation and intensity of exposure are nested within generation to avoid problems of repeated measures among generations.

Generation	Source	d.f.	χ^2	*P*
0	selection regime	1	0.39	0.530
	line [selection]	4	3.66	0.454
	intensity of exposure	1	74.09	<0.001
	intensity of exposure * selection	1	2.13	0.145
2	selection regime	1	18.07	<0.001
	line [selection]	4	17.76	0.001
	intensity of exposure	1	92.07	<0.001
	intensity of exposure * selection	1	0.23	0.633
4	selection regime	1	48.18	<0.001
	line [selection]	4	30.32	<0.001
	intensity of exposure	1	28.07	<0.001
	intensity of exposure * selection	1	1.29	0.256
Pooled	generation	2	10.11	0.006
	intensity of exposure [generation]	3	200.80	<0.001
	age at pupation [generation]	3	89.42	<0.001

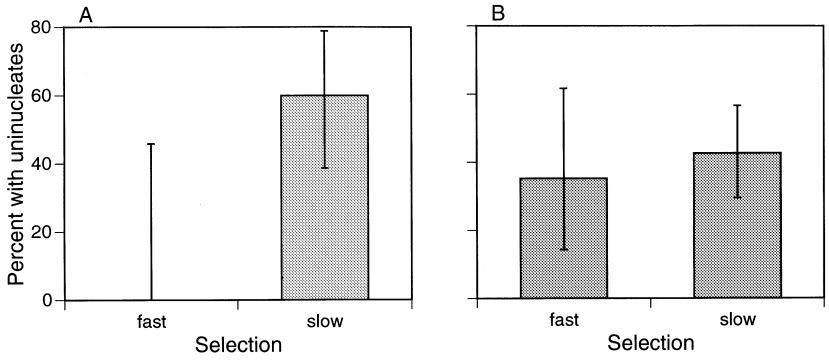

Fig. 3 Prevalence of horizontally transmitting uninucleate spores in dead larvae and pupae when the mosquitoes had been exposed to the parasite after four generations of selection. The bars represent the percentage of mosquitoes with uninucleate spores, the vertical lines show the 95% confidence interval of the estimated percentage in a binomial distribution. (A) After the low intensity of exposure uninucleate spores were found only in mosquitoes selected for slow development. (B) After the high intensity of exposure, uninucleate spores were prevalent in rapid and slow mosquitoes.

hosts selected for early pupation experienced less parasite-induced mortality than those selected for late pupation; thus age at pupation helped to determine the parasite's virulence (defined as the reduction in the host's fitness due to the parasite (Levin & Pimentel, 1981; Read, 1994)). Second, uninucleate spores, which are responsible for horizontal transmission, were mostly produced in mosquitoes selected for late pupation, while binucleate spores were produced in all mosquito lines. The two effects combined suggest that mosquitoes selected for early pupation survive infection to become adults capable of vertical transmission, while mosquitoes selected for late pupation die before emergence to allow horizontal transmission.

Selection on the host's life history – specifically, its age at pupation – thus brought with it a correlated response in the expression of parasite's life cycle. In other words, while the life cycle *per se* was constant (see Materials and methods and Fig. 1), its expression and the resulting

Table 3 Analysis of variance showing the effect of four generations of selection for early and for late pupation on time of death of the 103 mosquitoes killed by the parasite before they emerged.

Source	d.f.	Sum of squares	*P*
Selection regime	1	72.73	0.003
Line [selection]	4	5.67	0.585
Intensity of exposure	1	3.36	0.516
Intensity of exposure * selection	1	5.48	0.407
Error	95	750.07	

Table 4 The effect of age at death on the proportion of individuals harbouring spores (logistic analysis) and the $\log_{10}$-transformed number of spores per individual (analysis of variance). Larvae were infected after four generations of selection for early and late pupation. In the analyses of variance only those individuals with at least one spore were considered. The interactions could not be calculated in some cases due to missing cells, so we omitted them in all analyses. Due to missing data, the ANOVA table for the number of uninucleate spores is not complete; we could analyse only the effects of selection line, intensity of exposure and age at death within selection line.

		Proportion with spores			Number of spores		
Spore type	Source	d.f.	χ^2	*P*	d.f.	Sum of squares	*P*
uninucleate	selection regime	1	0.02	0.885			
	line [selection]	4	0.22	0.995	2	0.26	0.339
	intensity of exposure	1	0.17	0.684	1	0.03	0.598
	age [line, selection]	6	18.66	0.005	3	7.88	<0.001
	error				37	4.23	
binucleate	selection regime	1	0.00	0.991	1	0.05	0.583
	line [selection]	4	0.84	0.933	4	0.20	0.871
	intensity of exposure	1	2.02	0.156	1	0.71	0.037
	age [line, selection]	6	1.74	0.942	6	0.49	0.792
	error				76	11.97	

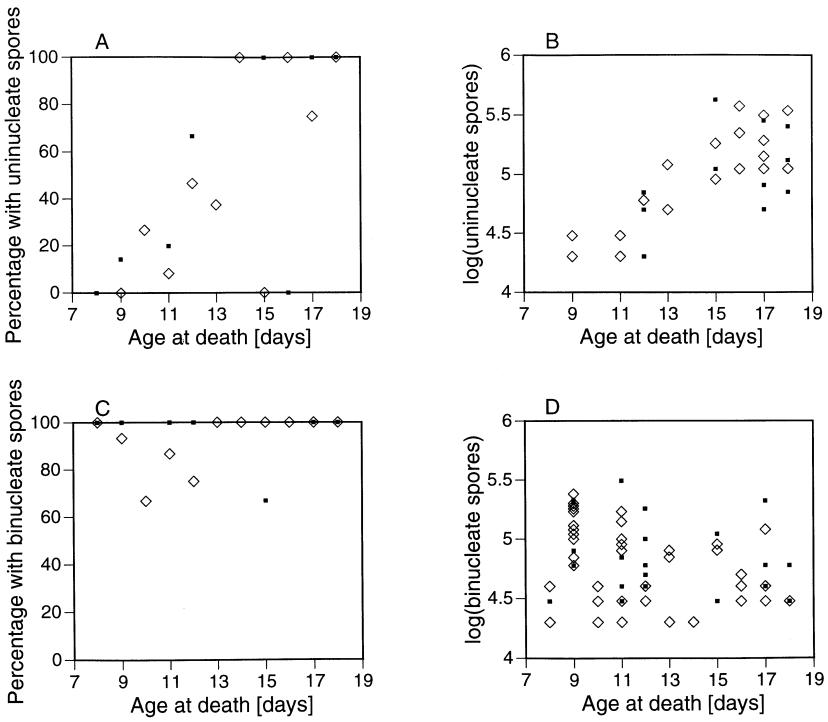

Fig. 4 Effects of age at death on spore production after four generations of selection for early and late pupation. In each panel the solid dots represent mosquitoes exposed to 500 spores mL^{-1}, the open diamonds exposure to 2000 spores mL^{-1}. Age at death was positively correlated with (A) the proportion of dead larvae and pupae harbouring uninucleate spores and (B) the number of uninucleate spores per dead individual. In contrast, age at death was not related to (C) the proportion of dead individuals harbouring binucleate spores or (D) the number of binucleate spores per dead individual. In panels A and C, the larvae dying on a given day were grouped to calculate the percentage with spores. In panels B (44 individuals) and D (89 individuals), only individuals harbouring at least one spore are shown and each larva is represented by an individual point. Many points, however, are overlaid and therefore hidden.

mode of transmission were determined by the parasite's environment, the host. Our results thus suggest that the genetics of the host contribute to the determination of the parasite's mode of transmission and virulence.

These results have implications for several evolutionary aspects of host–parasite interactions. First, they make clear that, in order to understand the virulence, one must consider coevolutionary processes (Ebert & Hamilton, 1996; Ebert & Herre, 1996) and in particular that the host has some control over the parasite. Though host-control, i.e. the effect of the host's genetic background on the parasite's transmission, has been acknowledged for vertically transmitted symbionts (Frank, 1996a), it has usually been neglected in theoretical (Bull, 1994; Frank, 1996b) and empirical (Read & Schrag, 1991) studies of the evolution of virulence. Perhaps the main reason for this neglect is that many parasites have much shorter generation times than their hosts and thus should have

Table 5 Analysis of variance showing the effect of four generations of selection for early and for late pupation on the prolongation of the juvenile period by the parasite. Due to the way we calculated the prolongation (see Material and methods), each treatment results in one number; therefore there were not enough data to include the interaction term.

Source	d.f.	Sum of squares	*P*
Selection regime	1	24.77	0.008
Intensity of exposure	1	0.68	0.455
Line [selection]	4	6.13	0.345
Error	4	4.01	

the potential to evolve at higher rates (Hafner *et al*., 1994). However, in many systems, e.g. the one described here, the host and parasite have similar generation times, so that the reciprocal effect of genetic changes in the host on the parasite cannot be neglected.

Understanding coevolutionary effects will also be important for applied issues of biological control. Some microsporidia, including *E. aedis*, are being considered as biological control agents of mosquitoes (Sweeney & Becnel, 1991). Our results suggest that the increased pressure by *E. aedis* will select for earlier pupation as a mechanism of resistance. This could lead to ineffective control within only a few generations: parasites would tend to cause less mortality in the larvae, have more vertical transmission and cause less damage in the adults. Such an evolutionary response of the host's life history to parasite pressure – that virulent parasites should select for hosts with earlier maturity – has been predicted with theoretical models (Hochberg *et al*., 1992) and observed in several systems. Thus, in the marine snail, *Cerithidea californica*, there is a negative correlation between the size at maturity, and thus age at maturity, within a site and prevalence of trematode parasites (Lafferty, 1993). Similar results have been found in other trematode–snail interactions (Minchella, 1985). The negative correlation has also been observed in other mosquito–parasite systems: resistance of *Ae. aegypti* to the malaria parasite *Plasmodium gallinaceum* was associated with a short developmental period and changes in other life history traits (Yan *et al*., 1997). In contrast, resistance of *Drosophila melanogaster* against one of its viruses is often associated with later age at pupation (Gomariz-Zilber & Thomas-Orillard, 1993). Our study also contrasts with another study showing a genetic correlation between age at pupation and resistance (Boots & Begon, 1993): selection of Indian meal moths, *Plodia interpunctella*, for resistance against a granulosis virus was associated with longer developmental time and increased pupal weight. These similarities and differences may be explained by the age-specificity of parasite infection (Michalakis & Hochberg, 1994).

Analogous results have been found in predator–prey interactions (Lafferty, 1993), where predators often induce defensive phenotypes in their prey. Tadpoles of the grey treefrog, for example, are more inactive when they are reared in ponds with predatory dragonfly larvae, and they develop large, brightly coloured tail fins (McCollum & van Buskirk, 1996). These characteristics decrease the mortality due to predation. In many other cases, the predator-induced defence is associated with the prey's life history traits. Strong predator pressure in juveniles is associated with early maturity in, for example, guppies (Reznick & Endler, 1982), daphnia (Edley & Law, 1988) and the dipteran *Chironomus tentans* (Ball & Baker, 1996). In some of these studies the association has a genetic basis (Reznick & Endler, 1982).

The mechanistic basis for the influence of the host's age at pupation on the parasite's life cycle can be easily understood. First, the variability in parasite-induced mortality associated with age at pupation necessarily affects the parasite's mode of transmission and life cycle, because vertical transmission relies on a host's survival to adulthood while horizontal transmission of *E. aedis* relies on larval or pupal death. Second, our results suggest more subtle interactions determining the parasite's mode of transmission, based on details of the parasite's life cycle. After horizontal infection binucleate spores must be produced before uninucleate spores (Becnel *et al*., 1989). This leads to a constraint in the timing of transmission events. If mosquitoes develop rapidly, the parasite will not have had time to produce uninucleate spores before the mosquito emerges and only vertical transmission is possible. On the other hand, in slowly developing mosquitoes the parasite has time to produce many uninucleate spores, so that horizontal transmission is possible. Even if slowly developing mosquitoes survived infection, there would only be little opportunity for vertical transmission, because the presence of uninucleate spores in adults decreases the mosquito's reproductive success (Agnew & Koella, 1997; Koella & Agnew, 1997), and thus the effectiveness of vertical transmission. These ideas show the importance of the parasite's dynamics within its host. Here we emphasize that the parasite's dynamics impose a constraint on the expression of the life cycle, while other studies have focused on the possibility that a parasite can modify the details of its growth to enhance its transmission success. These are mostly theoretical (Sasaki & Iwasa, 1991; Antia *et al*., 1994; Koella & Antia, 1995), but recent empirical work has shown that malaria parasites change their growth pattern to compensate for the impact of antimalarial drugs (Buckling *et al*., 1997).

Although we have emphasized that the host has considerable control over the parasite's life cycle, the parasite is able to maintain some control over its mode of transmission. When the host develops slowly, the parasite delays the host's pupation. Previous studies have already shown that the prolongation is strongest at low temperatures (Becnel & Undeen, 1992), i.e. at slow

growth. By prolonging the larval period, a parasite therefore has a longer opportunity to produce uninucleates before it has to kill the mosquito and transmit. On the other hand, when the mosquito develops rapidly, the parasite does not delay pupation. If it did, it would increase the chance that the mosquito would emerge with uninucleate spores and decrease vertical transmission (Agnew & Koella, 1997; Koella & Agnew, 1997). Of course, this difference might be a simple reflection of the age at pupation: in slowly developing mosquitoes the parasite can replicate up to high densities and might therefore have more control over the mosquito. However, one should then also expect that a high intensity of exposure, which leads to a high density of parasites, would also lead to more prolongation than low intensity, contrary to our observations.

In summary, the parasite's potential for horizontal transmission was influenced by genes controlling the mosquito's life history. In slowly developing mosquitoes the parasite was more likely than in rapidly developing ones to produce uninucleate spores, kill its host as a larva or pupa and thus transmit horizontally. That the host's life history can evolve to modify its parasite's life cycle and virulence has implications for several areas of evolutionary biology and epidemiology. First, in discussions about the evolution of virulence it is usually assumed that the host does not evolve and that the parasite evolves the level of virulence that maximizes its transmission. This approach has found support from several studies on the evolution of parasite virulence (Anderson & May, 1982; Bull *et al*., 1991; Herre, 1993). However, if the host can evolve to manipulate the parasite's virulence and life cycle, the evolutionary pressures on the parasite will be changed and the parasite may be kept from its optimal virulence (Ebert & Hamilton, 1996). Second, a widely accepted theory for the maintenance of sexual reproduction (Ladle, 1992) asserts that parasite pressure keeps asexual competitors from invading a sexual population. However, if the hosts can evolve to decrease the parasite's virulence, the pressure for sexual reproduction may decrease. Third, in programs using *E. aedis* as a biological control agent, an evolutionary response of the mosquito's life history, i.e. earlier pupation, may result in *E. aedis* using more vertical transmission and being less virulent. Such an effect would reduce the poential of this parasite as a biological control agent and could even be counterproductive.

Acknowledgments

J. Becnel and W. Rudin supplied us with parasites and mosquitoes. We thank M. Hochberg, Y. Michalakis, A. P. Møller, F. Vollrath and anonymous reviewers for comments on earlier versions of the manuscript. The project was supported by grants from the Swiss Nationalfonds and the danish Research Councils.

References

Agnew, P. & Koella, J.C. 1997. Virulence, parasite mode of transmission and host fluctuating asymmetry. *Proc. Roy. Soc. Lond. B* **264**: 9–15.

Alexander, H.M. 1989. An experimental field study of anther-smut disease of *Silene alba* caused by *Ustilago violacea*: genotypic variation and disease incidence. *Evolution* **43**: 835–847.

Anderson, R.M. & May, R.M. 1982. Coevolution of hosts and parasites. *Parasitology* **85**: 411–426.

Antia, R., Koella, J.C., Levin, B.R., Garnett, G.P. & Anderson, R.M. 1993. Parasite evolution in response to immunological defences. *Oxford Surveys Evol. Biol.* **9**: 383–405.

Antia, R., Levin, B.R. & May, R.M. 1994. Within-host population dynamics and the evolution and maintenance of microparasite virulence. *Am. Nat.* **144**: 457–472.

Ball, S.L. & Baker, R.L. 1996. Predator-induced life history changes: antipredator behavior costs or facultative life history shifts? *Ecology* **77**: 1116–1124.

Baqar, S., Hayes, C.G. & Ahmed, T. 1980. The effect of larval rearing conditions and adult age on the susceptibility of *Culex tritaeniorhynchus* to infection with West Nile virus. *Mosq. News* **40**: 165–171.

Baudoin, M. 1975. Host castration as a parasitic strategy. *Evolution* **29**: 335–352.

Becnel, J.J. 1992. Safety of *Edhazardia aedis* (Microspora: Amblyosporidae) for nontarget aquatic organisms. *J. Am. Mosq. Control. Assoc.* **8**: 256–260.

Becnel, J.J., Sprague, V., Fukuda, T. & Hazard, E.I. 1989. Development of *Edhazardia aedis* (Kudo, 1930) N.G., N. Comb. (Microsporida: Amblyosporidae) in the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *J. Protozool.* **36**: 119–130.

Becnel, J.J. & Undeen, A.H. 1992. Influence of temperature on developmental parameters of the parasite-host system *Edhazardia aedis* (Microsporida: Amblyosporidae) and *Aedes aegypti* (Diptera, Culicidae). *J. Invertebr. Pathol.* **60**: 299–303.

Boots, M. & Begon, M. 1993. Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Func. Ecol.* **7**: 528–534.

Buckling, A.G.J., Taylor, L.H., Carlton, J.M.R. & Read, A.F. 1997. Adaptive changes in *Plasmodium* transmission strategies following chloroquine chemotherapy. *Proc. Roy. Soc. Lond. B* **264**: 553–559.

Bull., J.J. 1994. Perspective: virulence. *Evolution* **48**: 1423–1437.

Bull., J.J., Molineux, I.J. & Rice, W.R. 1991. Selection of benevolence in a host–parasite system. *Evolution* **45**: 875–882.

Christophers, S.R. 1960. *Aëdes aegypti (L.). the Yellow Fever Mosquito. its Life History, Bionomics and Structure*. Cambridge University Press, Cambridge.

Ebert, D. & Hamilton, W.D. 1996. Sex against virulence: the coevolution of parasitic diseases. *Tree* **11**: 79–82.

Ebert, D. & Herre, E.A. 1996. The evolution of parasitic diseases. *Parasitol. Today* **12**: 96–101.

Edley, M.T. & Law, R. 1988. Evolution of life histories and yields in experimental populations of *Daphnia magna*. *Biol. J. Linn. Soc.* **34**: 309–326.

Forbes, M.R.L. 1993. Parasitism and host reproductive effort. *Oikos* **67**: 444–450.

Frank, S.A. 1996a. Host control of symbiont transmission – the separation of symbionts into germ and soma. *Am. Nat.* **148**: 1113–1124.

Frank, S.A. 1996b. Models of parasite virulence. *Quart. Rev. Biol.* **71**: 37–78.

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.

Grimstad, P.R.Q.E., Ross, and, G.B. & Craig, J.r. 1980. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. II. Modification of mosquito feeding behavior by virus infection. *J. Med. Entomol.* **17**: 1–7.

Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W. & Nadler, S.A. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science* **265**: 1087–1089.

Herre, E.A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259**: 1442–1445.

Hochberg, M.E., Michalakis, Y. & de Meeus, T. 1992. Parasitism as a constraint on the rate of life-history evolution. *J. Evol. Biol.* **5**: 491–504.

Koella, J.C. & Agnew, P. 1997. Blood-feeding success of the mosquito *Aedes aegypti* depends on the transmission route of its parasite *Edhazardia aedis*. *Oikos* **78**: 311–316.

Koella, J.C. & Antia, R. 1995. Optimal pattern of replication and transmission for parasites with two stages in their life cycle. *Theor. Pop. Biol.* **47**: 277–291.

Ladle, R.J. 1992. Parasites and sex: catching the Red Queen. *Tree* **7**: 405–408.

Lafferty, K.D. 1993. The marine snail, *Cerithidea californica*, matures at smaller sizes where parasitism is high. *Oikos* **68**: 3–11.

Levin, S. & Pimentel, D. 1981. Selection of intermediate rates of increase in parasite-host systems. *Am. Nat.* **117**: 308–315.

Lyimo, E.O. & Koella, J.C. 1992. Relationship between body size of adult *Anopheles gambiae s.l.* & infection with the malaria parasite *Plasmodium falciparum*. *Parasitology* **104**: 233–237.

McCollum, S.A. & van Buskirk, J. 1996. Costs and benefits of a predator-induced polyphenism in the grey treefrog *Hyla chryoscelis*. *Evolution* **50**: 583–593.

Michalakis, Y. & Hochberg, M.E. 1994. Parasitic effects on host life-history traits: a review of recent studies. *Parasite* **1**: 291–294.

Minchella, D.J. 1985. Host life-history variation in response to parasitism. *Parasitology* **90**: 205–216.

Minchella, D.J. & Loverde, P.T. 1981. A cost of increased early reproductive effort in the snail *Biomphalaria glabrata*. *Am. Nat.* **118**: 876–881.

Perrin, N., Christe, P. & Richner, H. 1996. On host life-history response to parasitism. *Oikos* **75**: 317–320.

Read, A.F. 1994. The evolution of virulence. *Trends Microbiol.* **2**: 73–76.

Read, A.F. & Schrag, S.J. 1991. The evolution of virulence: experimental evidence. *Parasitol. Today* **7**: 296–297.

Reznick, D. & Endler, J.A. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**: 160–177.

Sasaki, A. & Iwasa, Y. 1991. Optimal growth schedule of pathogens within a host: switching between lytic and latent cycles. *Theor. Pop. Biol.* **39**: 201–239.

Sorci, G. & Clobert, J. 1995. Effects of maternal parasite load on offspring life-history traits in the common lizard (*Lacerta vivipara*). *J. Evol. Biol.* **8**: 711–723.

Sweeney, A.W. & Becnel, J.J. 1991. Potential of microsporidia for the biological control of mosquitoes. *Parasitol. Today* **7**: 217–220.

Takahashi, M. 1976. The effects of environmental and physiological conditions of *Culex tritaeniorhynchus* on the pattern of transmission of Japanese encephalitis virus. *J. Med. Entomol.* **13**: 275–284.

Yan, G., Severson, D.W. & Christensen, B.M. 1997. Costs and benefits of mosquito refractoriness to malaria parasites: implications for genetic variability of mosquitoes and genetic control of malaria. *Evolution* **51**: 441–450.

Received 10 July 1997; revised 3 September 1997; accepted 24 September 1997