

Blood-feeding success of the mosquito *Aedes aegypti* depends on the transmission route of its parasite *Edhazardia aedis*

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We examined the effect of the microsporidian parasite *Edhazardia aedis* on the blood-feeding success of its host, the yellow fever mosquito *Aedes aegypti*. To do so, we exposed mosquito larvae to parasite spores at four intensities and three ages. The amount of blood obtained by adult female mosquitoes increased with increasing age at exposure and with decreasing intensity of exposure. These associations were only partly explained by the direct effects of the parasite on blood-feeding behaviour. Much of the associations were due to the parasite causing indirect effects to the mosquito because of correlations among its life history traits. The parasite increased age at pupation and decreased wing length, both of which were associated with the size of blood meals. Thus, trade-offs implicit in the host's life history – the correlations between wing length, age at pupation and blood meal size – were crucial in determining the parasite's effect on the host's blood-feeding success. *E. aedis* has two types of spores, a uninucleate spore used for horizontal transmission and a binucleate spore used for vertical transmission. This allowed us to investigate the influence of different transmission routes on the host's blood-feeding behaviour. While blood-feeding success decreased as the number of uninucleate spores increased, it was not influenced by the number of binucleate spores. Thus the parasite decreases its impact on the host, i.e. its virulence, during the part of the life cycle when transmission is vertical.

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A parasite's transmission route is thought to be one of the major factors determining the evolutionary pressure on parasite virulence (Anderson and May 1981). Parasites that are transmitted purely vertically from infected adults to their offspring have a common interest with the host in the production of host offspring (Dawkins 1990); they should therefore minimise their effect on their host's fitness. When transmission is horizontal from one host to another, via direct contact or via the environment, the parasite's and host's reproductive interests diverge. If the parasite's transmission rate increases with the harm it imposes on its host, evolution can favour parasites that impose high costs to their

hosts (Anderson and May 1981). Direct tests of this general prediction – that vertical transmission should be associated with a lower level of parasite virulence than horizontal transmission – are rare. Selection for vertically transmitting phages led to a more benevolent relationship with their bacterial host than did selection for horizontal phage transmission (Bull et al. 1991); nematode species with a high frequency of vertical transmission are less virulent to their fig wasp hosts than predominantly horizontally transmitted ones (Herre 1993); among ectoparasites of rock doves, vertically transmitted lice are less virulent than horizontally transmitted mites (Clayton and Tompkins 1994).

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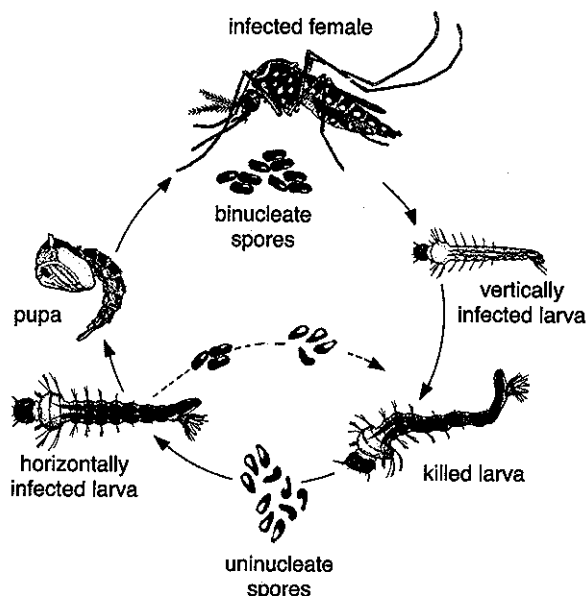


Fig. 1. Life cycle of *Edhazardia aedis*. Spore production by the parasite alternates between binucleate and uninucleate spores. Though this life-cycle of the parasite is fixed, the sequence of transmission routes is flexible. One route (solid line) alternates between vertical and horizontal transmission, while the other route (dashed line) bypasses vertical transmission and has repeated horizontal transmission.

We investigated whether parasites within an individual host modify their virulence according to their route of transmission. This was possible with the microsporidian parasite *Edhazardia aedis* (Kudo), which infects the yellow fever mosquito *Aedes aegypti* (L.) (Becnel et al. 1989), because it has both vertical and horizontal transmission. Mosquito larvae become horizontally infected by ingesting uninucleate spores from the aquatic environment (Fig. 1). After going through several developmental stages within an individual host, the parasite produces binucleate spores. In female mosquitoes, these transmit vertically by infecting their hosts' developing eggs. In the vertically infected larvae uninucleate spores are produced, which eventually kill the larva and then are released into the environment, initiating the cycle once more. Alternatively, the parasite may attempt to complete its whole life cycle – from horizontal infection through binucleate to uninucleate spores – within a single, horizontally infected larva, thus bypassing vertical transmission. However, the parasite may fail to kill the host as a larva, and the mosquito survives to emerge with uninucleate spores. These cannot be transmitted through the female's eggs (Hembree and Ryan 1982). Such unsuccessful attempts at repeated horizontal transmission offer the opportunity to investigate the harm caused by uninucleate spores, which need to kill their host as a larva in order to be transmitted horizontally, relative to binucleate

spores, which should do as little harm as possible to the mosquito to ensure efficient vertical transmission.

The harm caused by the parasite can be conveniently estimated with the mosquito's blood-feeding success, as blood-feeding is pivotal to a female mosquito's fitness. Not only is the protein that is contained in the blood directly converted into eggs, so that the amount of blood ingested by a female is positively correlated with her fecundity (Briegel 1990a, b), but blood is also used to maintain the female (Briegel 1990b).

Material and methods

We performed one experiment to investigate the effect of infection on the amount of blood ingested by females and another to investigate the effects of the two types of spores on blood-feeding success. In both experiments we used mosquitoes and parasites obtained from Dr. J. Becnel (United States Dept of Agriculture, Gainesville, FL) reared in a climate chamber maintained at $28 (\pm 0.5)^\circ\text{C}$ and $85 (\pm 5)\%$ relative humidity with a L:D 12:12 cycle.

Mosquitoes were hatched synchronously, added to 0.5 l of demineralised water in $10 \times 10 \times 10$ cm plastic pans and fed a standardised 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). Larvae were exposed to parasites for 24 h in 15 treatments: at age 1 day, 3 days or 5 days after hatching with concentrations of 0, 10, 10^2 , 10^3 or 10^4 spores/ml. Pans were checked daily and dead larvae were removed. Age at pupation was recorded to the nearest 24 h, and pupae were put into separate cages. Two days after adult females had emerged, we gave them the opportunity to feed on one of JCK's arms for four min and froze them at -20°C within 15 min of feeding.

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 1.52 (<http://rsb.info.nih.gov/nih-image/>). The mean length of the two wings was used for the analysis.

In the first experiment, we estimated the amount of blood ingested by an individual female with an established method (Briegel et al. 1979). Briefly, the abdomens of individual mosquitoes were ground in 1 ml of Drabkin's solution, and the amount of haemoglobin in the solution was estimated by the optical density measured in a spectrophotometer at 540 nm. From the optical density we calculated the volume of blood with a calibration curve obtained from the analysis of known amounts of blood.

The effect of infection on blood volume was analysed with a multiple regression of blood volume on age at

Table 1. Results of a multiple regression investigating the effects of intensity of infection and age at infection on the amount of blood obtained by a female *Aedes aegypti* offered to feed on a human arm during four min.

Effect	DF	SS	F	p	slope
Intensity of infection	1	16.021	6.871	0.009	-0.295
Age at infection	1	0.613	0.263	0.608	-0.039
Intensity × Age	1	6.409	2.749	0.098	0.052
Error	512	1193.810			

exposure and intensity of exposure to the parasites. The intensity of exposure was coded as 0 for controls and the logarithm (base 10) of the concentration for the treatments. Because exposure delayed age at pupation and decreased adult size, and both of these parameters influenced blood volume, we used a path analysis (Sokal and Rohlf 1995: chapter 16.3) to estimate the direct effects of exposure on blood volume and the indirect effects via wing length and age at pupation.

In the second experiment, we analysed only those mosquitoes harbouring spores. We counted uninucleate and binucleate spores in adult females by grinding the thorax and abdomen of mosquitoes in 0.5 ml of distilled water, homogenising the solution, and counting spores in a haemocytometer. We did not estimate blood volume, but evaluated blood-feeding success by the presence or absence of red colour in the homogenised solution.

Blood-feeding success was analysed with logistic regressions (Everitt 1977) of the presence of blood on the independent variable. The logarithm of the number of spores within an individual mosquito was used for all analyses.

Results

Experiment 1

Among the 516 females we investigated, the volume of blood ranged from 0 to 5.12 µl with a mean of 1.88 µl. As intensity of exposure increased, blood volume decreased (Table 1). Mosquitoes exposed to the highest concentration (10⁴ spores/ml) obtained on average only about half as much blood as unexposed mosquitoes or mosquitoes exposed to low concentrations (Fig. 2). The effect of exposure to 10³ spores/ml was noticeable only when the mosquitoes were exposed one day after hatching. This difference between ages led to a close to significant interaction of age at infection with intensity of infection (Table 1).

Age at exposure and intensity of exposure were associated not only with blood volume, but also with wing length and age at pupation (Table 2). A path analysis accounting for these correlations (Fig. 3) estimated the direct effect of exposure on the blood volume and its indirect effects via wing length and age at pupation (Table 3). Increasing the intensity of exposure de-

creased the amount of blood taken up by a mosquito indirectly by decreasing wing length and by increasing age at pupation. The two indirect effects were smaller than the direct effect. Increasing the age at exposure increased the blood volume through increases in wing length and decreases in age at pupation. The indirect effects were opposite to the direct effect: increasing age at exposure decreased the amount of blood taken up.

Experiment 2

Of a total of 161 females, 57% were blood-fed. Blood-feeding success ranged from 27% in the group of mosquitoes exposed on day 1 with 10³ spores/ml to 85% in the mosquitoes exposed on day 5 with 10 spores/ml. We found binucleate spores in 103 (64%) and uninucleate spores in 32 (20%) of the females. Most of the females with uninucleate spores also harboured binucleate spores (25 out of 32). Among the females

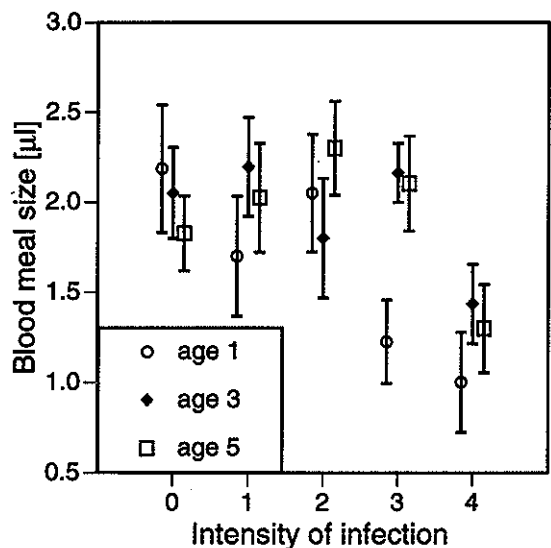


Fig. 2. Effect of intensity of infection on the amount of blood taken up by adult females. The points show the mean blood volume within a category (circles: mosquitoes infected 1 d after hatching, diamonds: 3 d after hatching, squares: 5 d after hatching). The vertical lines show the standard deviations. The intensity of infection is coded as 0 for controls and the logarithm (base 10) of the spore concentration for the treatments. The amount of blood obtained decreased as the intensity of infection increased and the effect of the parasite decreased as the age at infection increased.

Table 2. Correlations among the measured parameters. The correlation matrix shows the Pearson product-moment correlation coefficients.

Parameters measured	Intensity of infection	Age at infection	Wing length	Age at pupation	Blood volume
Intensity of infection	1	-0.043	-0.088	0.054	-0.107
Age at infection		1	0.362	-0.715	0.066
Wing length			1	-0.363	0.237
Age at pupation				1	-0.154
Blood volume					1

A correlation is significant at the 5% level if its absolute value >0.088; or >0.13 with the Dunn-Sidak-correction for multiple tests (Sokal and Rohlf 1995).

with uninucleate spores (Fig. 4a), the likelihood of obtaining blood decreased as the number of spores increased (logistic regression: slope = -0.159, $\chi^2 = 12.19$, $p = 0.0005$). In contrast, among the females with binucleate spores (Fig. 4b), no association between the likelihood of obtaining blood and the number of spores was apparent (logistic regression: slope = 0.005, $\chi^2 = 0.29$, $p = 0.59$).

Discussion

That *E. aedis* decreases blood-feeding success of its host is detrimental not only to its host but also to itself. Because, once in an adult female, it can only be transmitted through its host's eggs, its success depends on the mosquito's reproductive success (which is closely associated to blood-feeding success [Briegel 1990b]). One should therefore expect that the parasite has evolved to minimise the damage to its host (Dawkins 1990). This was indeed what we observed for binucleate spores, which are the ones responsible for vertical transmission: there was no apparent relationship between the number of binucleate spores and blood-feeding success. In contrast, blood-feeding success dropped considerably as the number of uninucleate spores increased. These cannot transmit vertically from adults to offspring; they can only transmit horizontally, if they develop within the larva, then kill the larva, and finally are released into the

environment. A possible explanation for the damage done by the uninucleate spores is that – as they can only transmit if the infected larva dies – the damage in the adults is a reflection of virulence in the larva.

Many other microsporidian parasites with vertical transmission routes are benign, e.g. *Thelohania benigna* in *Culex apicalis* (Kellen et al. 1965) and a microsporidian infecting the brine shrimp *Gammarus duebeni* (Smith and Dunn 1991). Other microsporidians, thought to be closely related to *Edhazardia*, kill males, from which transmission must be horizontal, but are relatively avirulent in females, from where transmission can be vertical (see Hurst [1991] for a review). Thus, microsporidia in general appear to have the potential to be benign. What is remarkable about *E. aedis* is that it appears to have linked the damage it inflicts to its developmental stage and its route of transmission. *E. aedis* contains its virulence when it shares a reproductive interest with its host, but does not when their reproductive interests diverge.

The parasite's detrimental effect on the adult mosquito appears to have a complex basis. Only part of the decrease in blood-feeding success was directly due to the infection; the other part was caused by the parasite's effects on the mosquito's life history traits and by the correlated changes in blood-feeding success. Specifically, the parasite increased the mosquito's age at pupation and decreased its wing length, while both of these changes were in turn associated with decreased

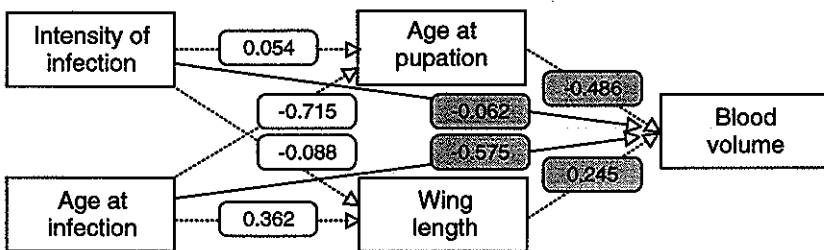


Fig. 3. Direct and indirect effects of infection on amount of blood taken up by adult females. The direct effects of intensity of infection and age at infection are represented by solid lines, the indirect effects via age at pupation and wing length are represented by dotted lines. The numbers drawn on the lines are the correlation coefficients taken from Table 2 (open boxes) or the estimated path coefficients (grey boxes).

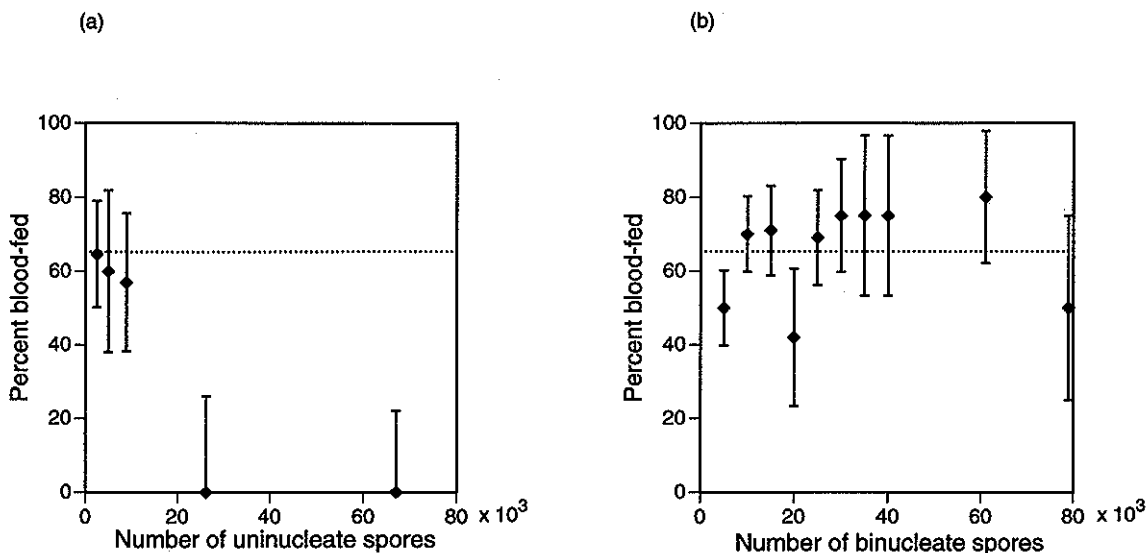


Fig. 4. Effect of the number of spores found in adult females on blood-feeding success. The diamonds show the percentage of females with blood within a group of females. The females were grouped according to the number of spores found so that at least four individuals were within each class. The x-axes show the mean number of spores within a class. The vertical bars show the standard error of the percentage. The dotted horizontal lines show the mean blood-feeding success. (a) Blood-feeding success decreased as the number of uninucleate spores increased. (b) There was no significant association between blood-feeding success and number of binucleate spores.

Table 3. Direct and indirect effects of intensity of infection and age at infection on the volume of blood taken up by female mosquitoes. The correlations are the products of the path coefficients along a given path in Fig. 2. We used the path analysis to estimate the magnitude and directions of interactions, rather than to test the statistical significance of individual coefficients (Sokal and Rohlf, 1995: 648).

	Effect	Correlation
Age at infection	Direct	-0.575
	Via wing length	0.089
	Via age at pupation	0.347
Intensity of infection	Direct	-0.062
	Via wing length	-0.022
	Via age at pupation	-0.026

blood-feeding success. Thus, correlations implicit in the host's life history – the correlations between wing length, age at pupation and blood meal size – were crucial in determining the parasite's effect on blood-feeding success and thus on reproductive success.

Previous studies (Hembree and Ryan 1982, Becnel et al. 1995) have shown that infection by *E. aedis* reduces the fecundity and longevity of female mosquitoes and suggested that vertical transmission of the parasite is inefficient. Neither study, however, attempted to separate the effects of uninucleate from those of binucleate spores. The intensity of exposure in both studies is sufficiently high to allow a proportion of adult females to harbour uninucleate spores. This suggests that the detriment may be due to the uninucleate spores rather than to the inefficient transmission of binucleate spores.

The mechanism reducing fecundity in mosquitoes infected with *E. aedis* is unknown. The parasite is tissue specific and does not destroy the ovaries (Becnel et al. 1989). Becnel et al. (1995) have suggested that the process of vertical infection by injecting sporoplasm into the oocytes damages them. Our results – that uninucleate spores decrease the blood-feeding success of the infected females – can jointly explain the reduced fecundity and longevity of infected females (Briegel 1990b). For this to occur, the parasite does not need to infect the reproductive organs themselves, but needs only to affect a trait correlated to blood-feeding success.

In conclusion, the virulence of *E. aedis* appears to be a complex trait, determined by a subtle interplay between the host and the parasite, by details of the parasite's life cycle and by trade-offs in the host's life history. It is essential to consider these interactions in order to gain a better understanding of the evolutionary pressure on the parasite's virulence (Ebert and Hamilton 1996, Ebert and Herre 1996).

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