Constraints on the reproductive value of vertical transmission for a microsporidian parasite and its female-killing behaviour

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Summary

1. Several species of microsporidian parasites use a mixture of vertical and horizontal transmission among their respective mosquito host species. These relationships vary in how hosts are exploited for either vertical or horizontal transmission. An important factor is that only females can provide vertical transmission. Two types of relationship have particularly been contrasted; those where vertically infected larvae of both sexes are killed late in their development and contribute towards the parasite's horizontal transmission, and those where only male larvae are killed while females experience benign infections, become adults, and contribute towards the parasite's vertical transmission success. The selective killing of males in these latter relationships leads to female-biased adult sex ratios. These differences among relationships have been suggested to depend on the environmental conditions they experience and on the relative efficiency of vertical or horizontal transmission.

2. The transmission behaviour of the microsporidian parasite *Edhazardia aedis* (Becnel, Fukuda & Sprague) following vertical transmission was studied as a function of larval food availability to its host, the mosquito *Aedes aegypti* (L.). The number of vertically infected mosquitoes dying before reaching adulthood increased as larval food availability became less. However, proportionately more females died as food availability decreased and adult mosquito populations became increasingly male-biased.

3. The fate of vertically infected mosquitoes was closely related to their larval growth rate. As food availability decreased and larval growth rates slowed, the parasite's infection had more time to produce its spores and induce the host's mortality before emergence. This disproportionately affected female mosquitoes as they pupate later than males, especially as larval growth rates slow. As late ages at pupation are associated with smaller adult size ($\approx$ lower fecundity), the potential vertical transmission offered by slowly growing female larvae is limited and more success may be gained by exploiting them for horizontal transmission.

4. Our results indicate that the relative reproductive value of vertical or horizontal transmission altered with an ecological parameter (larval food availability). Constraints in the life-history traits of each organism helped to explain why the observed sex ratio was biased towards males rather than females.

Key-words: horizontal transmission, host, life-history traits, life cycles, mosquito.

Journal of Animal Ecology (1999) **68**, 1010–1019

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Introduction

The fitness of parasitic organisms is usually gauged by their transmission success (Anderson & May 1991). Many parasites gain fitness with only one form of transmission (normally horizontal), but several use a combination of vertical and horizontal transmission. Examples of such parasites are found in nematodes (Herre 1993), viruses (Fulhorst *et al.* 1994), helminths (Shoop 1991), phages (Bull, Molineux & Rice 1991) and fungi (Kover, Dolan & Clay 1997). As only females produce offspring and because of the nature of cytoplasmic inheritance, vertical transmission often only occurs via female hosts (Hurst 1993). This constraint leads to selection of maternally inherited parasites that increase their transmission success by biasing the sex ratio of their hosts towards females, for example, by feminizing males (Terry, Dunn & Smith 1997). Another way for parasites to bias host sex ratios towards females is selectively to kill males. In almost all of these cases, males are killed at an early stage in their development, either as embryos or soon after birth (Hurst & Majerus 1993). The main advantage to the parasite of killing males early is thought to be that it increases the resources available to their infected sisters and ultimately their reproductive success. A notable, and reportedly unique (Hurst 1991), exception to this early male-killing behaviour is the late male-killing behaviour shown by some microsporidian parasites towards their mosquito hosts (Kellen *et al.* 1965).

Kellen *et al.* (1965) categorized the development of 16 microsporidian parasites in their larval mosquito hosts following vertical transmission. Maternally infected males in several of these relationships die in their fourth larval instar as a result of the production and accumulation of horizontally transmitting spores. In contrast, female larvae do not experience infections that produce such spores. Instead, the females survive to become adults and subsequently transmit the parasite vertically to their offspring. Kellen *et al.* (1965) called this a Type I infection; it is this sex-specific development that results in the pattern of late male-killing peculiar to these protozoan parasites. In other relationships (Type II), females accounted for up to 25% of the mortality in the fourth larval instar as a result of spore production. Males and females were equally likely to be killed in some relationships (Type III), with a few females surviving to transmit the parasite vertically. A further relationship described an equal but limited amount of spore production in males and females that was not usually fatal to either sex (Type IV).

It was unknown for another 20 years that the life cycle of some of the microsporidia described by Kellen *et al.* (1965) involve an intermediate host (Andreadis 1985; Sweeney, Hazard & Graham 1985). In such cases, the spores released by dead male or female larvae are not directly infectious to mosquito larvae but to copepods. Further development in these secondary hosts results in the production of spores that are infectious to mosquito larvae. Armed with this information, Hurst (1991) interpreted the variation in mosquito–microsporidian relationships in terms of the relative efficiency of vertical or horizontal transmission for producing the next parasite generation; different conditions would dictate the extent to which female larvae should be exploited for horizontal transmission. In particular, Type I (late male-killing) relationships were predicted to occur in conditions where the probability of successfully infecting a female mosquito's eggs is high and where the availability of intermediate hosts is low or patchy. The opposite conditions, where a lower percentage of a female's eggs are infected and where intermediate hosts are abundant, were predicted to favour Type III relationships.

This study involves the mosquito *Aedes aegypti* and its microsporidian parasite *Edhazardia aedis*. Contrary to Hurst's prediction, this Type III relationship involves a microsporidian that is efficient at vertical transmission (Becnel, Garcia & Johnson 1995) and where no intermediate host is involved (Becnel *et al.* 1989). The probability of pre-adult mortality and the adult sex ratio of maternally infected larvae as a function of environmental food availability was investigated. Our results extend those discussed above and highlight the role of life-history parameters in host–parasite relationships and the utility of reproductive value (Fisher 1958) as a means to assess the relative fitness a parasite might gain by vertical or horizontal transmission (Frank 1996).

Materials and methods

THE HOST–PARASITE RELATIONSHIP

The yellow fever mosquito *Aedes aegypti* is found throughout the tropics and subtropics, particularly in association with human habitation (Christophers 1960). Larvae are typically found inhabiting a range of natural and artificial containers that hold clean freshwater, e.g. flower pots, bamboo shoots, water storage jars. These sites vary in a number of characteristics, such as their ephemerality, ecological complexity and food availability. This spatial and temporal heterogeneity is experienced both at the level of the population and family: females spread eggs from within a clutch over a number of sites (Apostol *et al.* 1994).

The ecological distribution of *Edhazardia aedis* is less known. A single infected *Ae. aegypti* larva from Puerto Rico provided the type specimen (Kudo 1930). The next record was from Thailand, where it was found to be one of the most common pathogens

infecting larvae of *Ae. aegypti* (Hembree 1979). Its complex life cycle involves the production of binucleate spores for vertical transmission and uninucleate spores for horizontal transmission. Each type of spore is produced at a different stage in the life cycle and is specific to its mode of transmission. For full details of the life cycle see, Becnel *et al.* (1989) and Johnson, Becnel & Undeen (1997).

Vertical transmission is via infected eggs; there is no paternal transmission (Hembree & Ryan 1982). As vertically infected larvae grow, *E. aedis* proliferates as it undergoes a number of soft-bodied developmental stages within host cells, particularly those of fat body tissue. This developmental sequence finishes with the production of uninucleate spores. The accumulation of these spores is associated with the mortality of male and female larvae at the fourth instar or pupal stage. Uninucleate spores are then released into the larval habitat and are directly infectious to *Ae. aegypti* larvae that ingest them (horizontal transmission). This sequence of vertical then horizontal transmission, however, does not always occur. Vertically infected larvae may survive to become adults and leave the larval habitat. While this precludes the opportunity for horizontal transmission, infected females can transmit the parasite vertically to their offspring. For this to happen, *E. aedis* needs to germinate its uninucleate spores within the host and undergo another series of soft-bodied developmental stages before producing its binucleate spores. These spores are responsible for vertical transmission when they germinate and inoculate a female's developing oocytes.

The Rockefeller strain of *Ae. aegypti* used in our experiments was provided by Dr W. Rudin at the Swiss Tropical Institute, Basel. Stocks of *E. aedis* were provided by Dr J.J. Becnel at the United States Department of Agriculture (Gainesville, Florida) and had been derived from specimens collected in Thailand.

EXPERIMENTAL PROTOCOL AND DATA COLLECTION

Two experiments are described that each involved two generations of the host. As outlined in greater detail below, the first generation of mosquitoes were horizontally infected as larvae and reared to adulthood. Eggs harvested from this parental generation were vertically infected and used in our experiments. Eggs harvested from a matching but uninfected generation were used as controls. Larvae from these infected and uninfected populations were reared in conditions of different food availability and various life-history parameters were recorded.

The parental generation of mosquitoes in each experiment was derived from the Rockefeller strain of *Ae. aegypti* and reared in standard conditions as described in Agnew & Koella (1997) and below. At 72 h post-hatch, two groups of 400 larvae were transferred to glass beakers containing 200 mL of water and some powdered fish food (Tetramin™). One beaker also contained uninucleate spores at a concentration of 10^3 mL^{-1}. Twenty-four hours later, larvae were removed from these beakers, rinsed, and returned to their original trays. These two populations are now referred to as the uninfected and infected treatments, respectively.

Adults emerging from the infected and uninfected treatments were kept in separate population cages (40 × 40 × 40 cm) held at 28 ($\pm$ 0·5) °C, 85 ($\pm$ 5)% humidity with a 12 h : 12 h (L : D) photoperiod. Female mosquitoes were presented with the opportunity to feed on a human arm for 5 min every third day. Eggs were collected from each cage 2 weeks after females first took blood meals.

In each experiment, eggs from the uninfected and infected cages were synchronously hatched under reduced atmospheric pressure. Within 6 h of hatching, eight groups of 100 larvae from each population were separated into plastic rearing trays (10 × 10 × 10 cm) containing 300 mL of tap water. Two treatments within each population were then assigned one of four food availabilities comprising: 25%, 50%, 75% and 100%, respectively, of a standard daily ration of ground fish food (Tetramin™). The standard ration per larva was: day 1, 0·06 mg; day 2, 0·08 mg; day 3, 0·16 mg; day 4, 0·32 mg; even-numbered days thereafter, 0·64 mg.

In Experiment I, larval and pupal mortality were recorded on a daily basis and cadavers were removed. Individuals pupating on the same day and in the same treatment were transferred together into pupation chambers (small beakers containing water under larger inverted plastic cups) and the sex of emerging adults was recorded.

In Experiment II, pupae were collected and transferred as above; larval mortality was not recorded. No food was provided for adults and 2 days after emergence each pupation chamber was frozen at −20 °C. Treatment, sex and day of pupation were recorded and individual mosquitoes were transferred to 1·5 mL plastic vials for storage at −20 °C until further investigation.

The spore content of individual mosquitoes in Experiment II was determined by adding 0·5 mL of deionized water to each vial, mechanically homogenizing the contents and counting the number and type of spores with a haemocytometer and a phase-contrast light microscope.

The left and right wings of adult female mosquitoes in Experiment II were measured. Length, from the distal end of the alula to the peripheral tip of vein R3, was measured with NIH Image (version 1·52) software to an accuracy of 0·01 mm via a video camera linked to a dissecting microscope. The

fluctuating asymmetry (FA) of these wings (left–right) was normally distributed (Shapiro–Wilk $W = 0.980$, $P = 0.094$) with a mean not significantly different from zero ($t = -0.099$, $P = 0.094$).

STATISTICAL ANALYSES

Two-way analyses of variance (ANOVA) with infection and larval food availability as fixed effects were used to assess the proportion and sex ratio of mosquitoes surviving to adulthood in each treatment; experiment and replicate within experiment were treated as blocks and therefore as random effects. The proportion of mosquitoes surviving to adulthood from each treatment was arcsine-transformed. This transformation was not used to analyse the proportion of either sex in the adult population, as it failed to give normally distributed error residuals. Instead, the sex ratio of surviving adults is presented. As the sensitivity of this measure is influenced by the number of individuals contributing towards each estimate, the analysis was weighted by the number of mosquitoes that contributed towards the calculation of each estimate. The relative proportion of mosquitoes from infected treatments that survived to adulthood from each experiment, replicate and food condition was analysed with a regression model against the median age at pupation of the matching uninfected treatment. The variance accounted for by the above analyses and the Shapiro–Wilk W-test for deviation from normality of error residuals are given at the foot of each table (see below).

Age at pupation of male and female mosquitoes differs considerably (Christophers 1960) and so was analysed separately for each sex. It was not possible to transform these data to normalize their distributions or to give heterogeneous variances. Consequently, they were analysed with nonparametric analyses of variance (Sokal & Rohlf 1995); this required each experiment and each replicate within the experiment to be analysed separately.

The number of binucleate and uninucleate spores that emerging mosquitoes in Experiment II harboured were analysed separately for each replicate with a Welsh-corrected one-way ANOVA; this parametric test allows for heterogeneity of variances among groups (Sokal & Rohlf 1995). To determine the underlying relationship between time and spore production, the treatment effects of food and replicate were removed from spore numbers and ages at pupation. Correlations among these residuals were assessed non-parametrically.

Treatments effects of food and replicate were also removed from the numbers of spores and the fluctuating asymmetry of female mosquitoes from the infected treatments of Experiment II. The relation-

ship between these residual spore numbers and residual FA was assessed by multiple regression.

The statistical package JMP (version 3·1.6) was used for all analyses (SAS Institute 1994)

Results

Most mosquitoes from the uninfected treatments survived to become adults in each food condition and in both experiments (Fig. 1). The sex ratio of these uninfected adults was biased towards males and varied little across food conditions (Fig. 2). The age at which uninfected mosquitoes became adults increased as larval food availability became less; females also pupated relatively later than males as food availability decreased (Fig. 3).

The number of mosquitoes surviving to adulthood in the infected treatments was less than for the uninfected, particularly as larval food availability decreased (Fig. 1, Table 1). The high average mortality of these mosquitoes in the lowest food condition (86%) indicates that most larvae were actually infected and that food availability altered their probability of surviving to adulthood. Of these surviving adults, there was a general bias towards males, which increased in the infected treatments as larval food availability decreased (Fig. 2, Table 2). As for their uninfected counterparts, ages at pupation became later for infected mosquitoes as food availability became less (Fig. 3). However, infected female mosquitoes pupated significantly earlier than uninfected females as larval food availability decreased (Fig. 3, Table 3). Infected male mosquitoes showed a similar but much weaker trend (Fig. 3, Table 3).

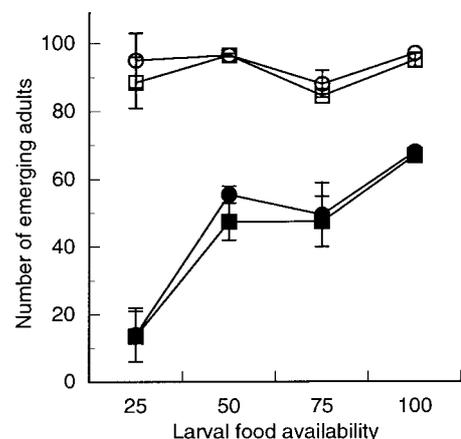The increasing contrast in mortality between

Fig. 1. Effect of larval food availability on mosquito survival to adulthood ($\pm$ SE) in the infected (closed symbols) and uninfected (open symbols) treatments. First and second experiments, respectively, shown in circles and squares.
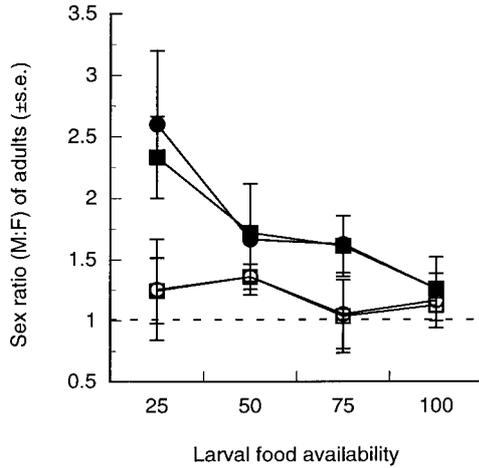
Fig. 2. Sex ratio (male : female) of adult mosquitoes ($\pm$ SE) as a function of larval food availability in the infected (closed symbols) and uninfected (open symbols) treatments. First and second experiments, respectively, shown in circles and squares.
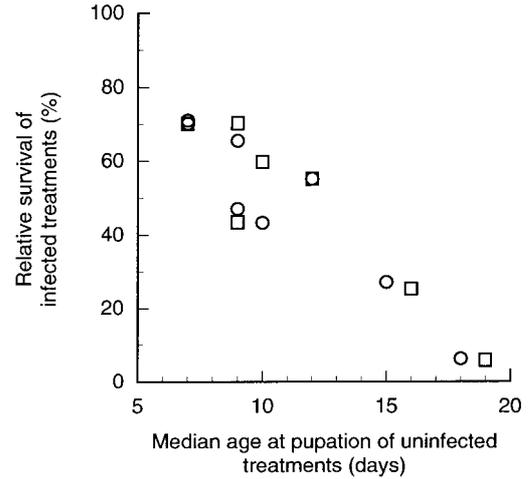

Fig. 4. Relative survival to adulthood of mosquitoes in the infected treatments as a function of the median age at pupation of mosquitoes in the matching uninfected treatment. First and second experiments, respectively, shown in circles and squares.

uninfected and infected mosquitoes as larval food availability decreased was reflected in their developmental traits; as median ages of pupation became later for uninfected mosquitoes, an increase in mortality of mosquitoes in the matching infected treatment was seen (Fig. 4, Table 4). The infected mosquitoes dying as larvae or pupae tended to do so after those that went on to become adults had already pupated (Fig. 5). This indicates that the number of adults emerging in the infected treatments did not arise as a consequence of parasite-induced mortality at an early stage in larval life.
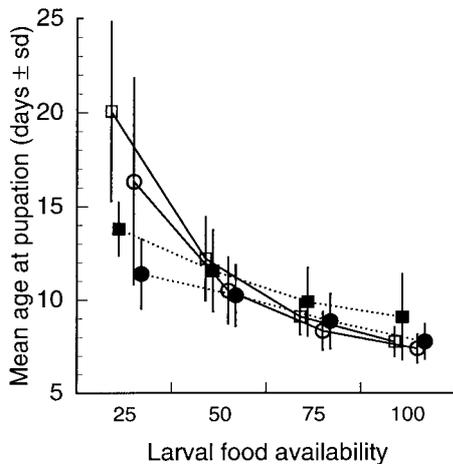

Fig. 3. Age at pupation of female (squares) and male (circles) mosquitoes ($\pm$ SD) as a function of larval food availability. Infected and uninfected treatments shown as open and closed symbols, respectively. Symbols are staggered along the *x*-axis to expose error bars.
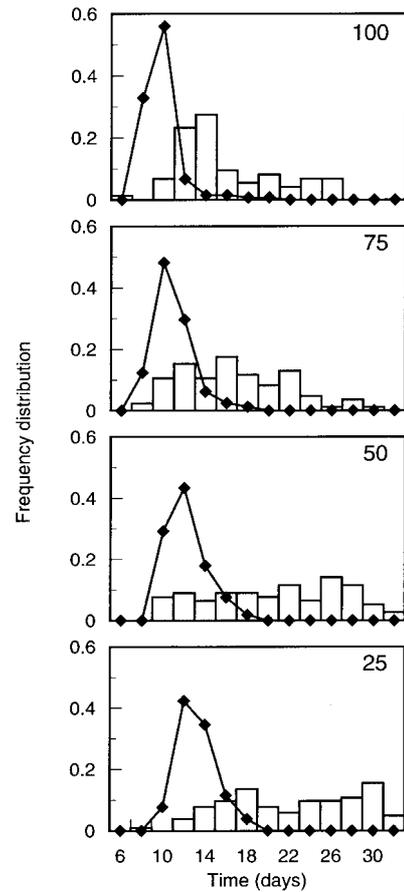

Fig. 5. Distribution of ages at pupation of mosquitoes in the infected treatments that survived to adulthood (lines) and those dying as larvae or pupae (columns) in the first experiment. Larval food availability indicated in the top right corner of each frame.

Table 1. ANOVA of treatment effects on arcsine-transformed percentage of mosquitoes surviving to adulthood

Source	d.f.	m.s.	F	P
Experiment	1	0·016	17·913	0·052
Replicate [Experiment]	2	0·001	0·087	0·917
Infection	1	1·805	172·720	< 0·001
Food	3	0·163	15·550	< 0·001
Infection × Food	3	0·127	12·123	< 0·001
Error	21	0·010		

$R^2 = 0.94$; $W = 0.982$; $P = 0.889$.

Table 2. ANOVA of treatment effects on weighted sex ratio of adult mosquitoes (males : females)

Source	d.f.	m.s.	F	P
Experiment	1	0·384	0·019	0·903
Replicate [Experiment]	2	20·001	3·245	0·059
Infection	1	111·603	18·100	< 0·001
Food	3	38·625	6·264	0·003
Infection × Food	3	24·967	4·049	0·020
Error	21	6·166		

$R^2 = 0.64$; $W = 0.944$; $P = 0.121$.

Infected mosquitoes surviving to adulthood in Experiment II harboured more uninucleate spores (replicate 1, $F_{3,87·920} = 3·202$, $P = 0·027$; replicate 2, $F_{3,96·238} = 3·468$, $P = 0·019$) and fewer binucleate spores (replicate 1, $F_{3,62·962} = 4·731$, $P = 0·005$; replicate 2, $F_{3,22·311} = 2·468$, $P = 0·088$. Combined test of probability, $\chi^2_4 = 15·489$, $P = 0·004$) as larval food availability increased. For individuals from the infected treatments, the effects of food and replicate were removed from ages at pupation and the number of spores per mosquito. Correlations between residual ages at pupation and the residual numbers of spores were both positive and stronger for uninucleate spores than binucleate spores (uninucleate spores, Spearman's $\rho = 0·273$, $P < 0·001$; binucleate spores, Spearman's $\rho = 0·101$, $P = 0·053$). The FA of female mosquito wings was an increasing function of uninucleate spore but not binucleate spore numbers or their interaction, once treatment effects had been removed from each parameter (Table 5).

Discussion

Varying the amount of food in the environment of vertically infected larvae had a pronounced effect on their probability of surviving to adulthood. Whereas almost all larvae survived to adulthood in each food condition of the uninfected treatments, the number doing so was less in the infected treatments, particularly as food availability decreased (Fig. 1, Table 1). Thus, environmental food availability is an important factor for the parasite's fitness, as it alters the probability that infected hosts will become adults. The emergence of adults essentially forces the para-site into using only female adults for vertical transmission rather than gaining horizontal transmission from males and females killed as larvae or pupae.

The role of food availability on mortality in the infected treatments was closely aligned to its effect on larval growth rates and their ages at pupation: the longer that uninfected mosquitoes took to reach pupation, the greater the probability that mosquitoes in the matching infected treatment would die before becoming adults (Fig. 4, Table 4). This arises because slower growth rates and later ages at pupation provide more time for the production and accumulation of uninucleate spores. Hosts with fast growth rates are able to reach adulthood before the mounting costs of their infection induce larval or pupal mortality. Figure 5 illustrates this, showing that the mortality of infected larvae and pupae occurred later than the age at pupation of infected mosquitoes surviving to adulthood.

Unlike previous studies on the late-killing behaviour of maternally inherited microsporidian infections, our study found the sex ratio of infected adults was biased towards males rather than females. Furthermore, males made a greater contribution to the adult population as larval food availability became less (Fig. 2, Table 2). This pattern probably emerges as a result of differences in the life-history traits of male and female mosquitoes. Male mosquitoes need only spend a minimal period as larvae because additional larval growth, to emerge as larger adults, has only a limited effect on their reproductive success (Benjamin & Bradshaw 1994). The size of adult female mosquitoes, however, is important to their reproductive success as it

Table 3. Non-parametric analyses of variance for age at pupation of mosquitoes from each experiment and replicate

| | Experiment I | | | | | | Experiment II | | | | | |
| | Replicate *i* | | | Replicate *ii* | | | Replicate *i* | | | Replicate *ii* | | |
Source	d.f.	H	P	d.f.	H	P	d.f.	H	P	d.f.	H	P
Females												
Infection	1	0·554	0·457	1	0·799	0·371	1	0·514	0·473	1	0·911	0·340
Food	3	135·990	<0·001	3	134·904	<0·001	3	144·159	<0·001	3	132·031	<0·001
Infection Food	3	8·634	0·035	3	8·465	0·037	3	8·177	0·043	3	8·528	0·036
Error	243			224			256			226		
Males												
Infection	1	0·322	0·570	1	0·536	0·464	1	0·854	0·355	1	0·587	0·444
Food	3	154·093	<0·001	3	207·907	<0·001	3	162·175	<0·001	3	204·730	<0·001
Infection Food	3	5·011	0·171	3	4·252	0·236	3	3·836	0·280	3	4·868	0·182
Error	273			316			289			322		

correlates closely with their fecundity (Christophers 1960). As larval growth rates slow, female mosquitoes have a greater incentive than males to remain as larvae and continue developing. A consequence of this is that, as average larval growth rates continue to slow, females are more likely to be killed as larvae or pupae than males in the presence of a mounting infection (developing equally in hosts of either gender) and a male-biased sex ratio will occur in the adults.

The interaction seen between infection and food for ages at pupation for female but not male mosquitoes can also be understood in light of the different patterns of growth shown by each gender (Fig. 3, Table 3). As food availability becomes less and the average growth rate slows, a larger proportion of the female population is likely to be killed before pupation. Correspondingly, the decreasing fraction of females that pupate as food availability becomes less will be those with the fastest growth rates. In this way, the increasing costs of infection have an effect akin to truncation selection: as the average growth rate slows, a smaller proportion of the faster growing individuals will reach the stage of pupation. Consequently, only the faster-growing females in infected treatments will contribute towards an estimation of age at pupation as the average growth rate slows. The same rationale applies equally to males, but as their general growth rate is faster, the truncating effect of infection on the distribution of their ages at pupation will be less. Thus, from life history differences, the interaction between infection and food conditions for age at pupation is expected to be stronger for females than for males; as was the case (Fig. 3, Table 3). We note that the earliest ages at pupation for infected females were not earlier than the fastest uninfected females; the same is true for males. This suggests that infected hosts did not, or could not, pupate any earlier than uninfected mosquitoes. Such a difference would indicate that hosts were prepared to sacrifice some of their size-dependent reproductive success against losses to their future reproductive success as a result of parasitism (Minchella & Loverde 1981; Michalakis & Hochberg 1994).

LIFE-HISTORY CONSTRAINTS

How does the behaviour of this Type III relationship fit in with those predicted by Hurst (1991)? Hurst's suggestion was that the variation observed in mosquito–microsporidian relationships is probably related to the relative efficiency of vertical or horizontal transmission for getting the parasite to the next generation. In particular, the efficacy of vertical transmission was taken to be the percentage of a female's eggs that become infected. However, this restricted definition misses an important factor; the

Table 4. Linear regression for mortality in the infected treatments against age at pupation in the matching uninfected treatment

Source	d.f.	m.s.	F	P
Experiment	1	0·007	0·640	0·509
Replicate [Experiment]	2	0·011	1·629	0·240
Time pupation uninfected	1	0·606	93·260	< 0·001
Error	11	0·006		

$R^2 = 0.90$; $W = 953$; $P = 0.527$.

vertical transmission success that can be gained from a female host is not just a function of the proportion of offspring infected, but also, of how many offspring are produced. There are at least three constraints that can influence the parasite's potential vertical transmission success.

HOST LIFE-HISTORY CONSTRAINTS

As mentioned above, a female mosquito's adult size strongly influences her fecundity, it also correlates positively with her longevity (Christophers 1960). However, the gains of spending longer in larval development must be balanced against the probability of dying before maturity and reproduction. The predicted, and observed, reaction norm for the age and size at maturity of female mosquitoes is for later pupation and the emergence of smaller adults as larval growth rates slow (Berrigan & Koella 1994). From the perspective of a maternally inherited parasite, the potential vertical transmission success offered by female mosquitoes will decrease as larval growth rates slow. If these females can otherwise be exploited for horizontal transmission, then their life-history traits may have a strong influence on the relative fitness to be gained by vertical or horizontal transmission.

The data presented above and elsewhere (Agnew & Koella 1999; Koella & Agnew 1999) suggest that, whether following vertical or horizontal transmission, *E. aedis* is more likely to kill its female hosts and exploit them for horizontal transmission when the reproductive value of vertical transmission is limited by a female mosquito's life-history traits.

(II) PARASITE LIFE-HISTORY CONSTRAINTS

The binucleate and uninucleate spores produced by *E. aedis* are specific to vertical and horizontal transmission, respectively. This specificity may increase the parasite's overall transmission success but imposes a constraint, in that two types of spores need to be produced where one may suffice, e.g. *Nosema* species gain horizontal and vertical (transovarial) transmission from a single type of spore (Sprague, Becnel & Hazard 1992).

The obligate sequence in which these two spore types are produced in the life cycle also imposes a constraint. Following vertical transmission, *E. aedis* must produce and germinate uninucleate spores before it can produce its binucleate spores. Thus, the time at which binucleate spores can first be produced and vertical transmission attempted becomes restricted. Evidence of this effect for *E. aedis*, can be seen in the decreased number of binucleate spores found as food availability increased (leading to earlier ages at pupation) and in the underlying increase in binucleate spore numbers with time. A consequence of this time-dependency in the parasite's life cycle, is that infected female mosquitoes with early emergence can gain the opportunity to produce uninfected offspring; Sweeney, Doggett & Gullick (1989) document an example of this time-dependency and its influence on vertical transmission success in the relationship between *Amblyospora dyxenoides* and *Culex annulirostris*.

As there is no paternal transmission (another constraint), the problem of producing binucleate spores in vertically infected male larvae is not important as males can only be exploited for horizontal transmis-

Table 5. Multiple regression of residual fluctuating asymmetry against residual spore numbers

Source	d.f.	s.s.	F	P
Binucleate spores	1	0·001	0·089	0·766
Uninucleate spores	1	0·028	7·294	0·008
Interaction	1	0·011	2·874	0·093
Error	114	0·438		

$R^2 = 0.08$; $W = 0.971$; $P = 0.115$.

sion. However, the number of male mosquitoes surviving to adulthood, particularly as food availability increased, suggests that they are able to grow at a rate that outpaces the ability of their infections to induce mortality. This leads to the suggestion that there is an upper constraint to the rate at which *E. aedis* can replicate within host cells.

(III) CONSTRAINTS ARISING FROM THE HOST–PARASITE INTERACTION

A further constraint on the vertical transmission success of *E. aedis* arising from the production of uninucleate spores is their negative effect on female mosquitoes. Following horizontal transmission, the probability of blood-feeding and the volume of blood taken is much smaller in the presence and with the number of uninucleate spores (Koella & Agnew 1997). Without this source of protein, a female's fecundity will be extremely limited (Christophers 1960). A negative indicator of fitness, the fluctuating asymmetry of wings, also increases with the number of uninucleate spores produced after horizontal transmission (Agnew & Koella 1997). The same effect also occurs with uninucleate spore production following vertical transmission (Table 5). These results all indicate that, even if *E. aedis* does produce binucleate spores in time to infect an emerging female's eggs, the costs associated with uninucleate spore production are likely severely to constrain her reproductive success and any vertical transmission success for the parasite. Consequently, whether a female mosquito's eggs are infected or not, is unlikely to accurately reflect the parasite's potential vertical transmission success.

In a link to other studies on the evolutionary ecology of host–parasite relationships, we would like to echo the sentiments of May (1991) that trade-offs involving a parasite's transmission success and its virulence will be constrained by the life histories of both host and parasite, and that Fisher's reproductive value is a useful tool with which to proceed (Frank 1996).

Conclusion

Interactions among the life cycles and life-history traits of host and parasite are important in determining the outcome of this relationship following vertical transmission. The parasite's exploitation of female hosts for horizontal transmission is probably less related to its chances of infecting a female's offspring than to the potential vertical transmission success, or reproductive value, these females offer. This latter effect is strongly delimited by the host's life-history traits and their sensitivity to parameters, such as, environmental food availability. Equally, the ability of the parasite to exploit male or female

hosts for either mode of transmission is limited by constraints arising from its own life cycle and life-history traits. Such constraints limit the parasite's ability to kill male larvae and can lead to the counterintuitive observation of hosts with male-biased adult sex ratios.

The variation seen in mosquito–microsporidian relationships is, as Hurst (1991) suggested, likely to be shaped by the environmental conditions encountered and their influence on vertical and horizontal transmission. However, rather than viewing the relative efficiency of the two modes of transmission, a more encompassing perspective is gained by considering the reproductive value of each mode of transmission in a life-history framework involving trade-offs and constraints for each organism in the conditions they encounter.

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