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Age and size at maturity of the mosquito *Culex pipiens* infected by the microsporidian parasite *Vavraia culicis*

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The costs of parasitism to a host's reproductive success (RS) often increase with time since infection. For hosts experiencing this type of infection, it is predicted that they will maximize their RS by bringing forward their schedule of reproduction. This is because the costs associated with such a response can be discounted against a reduced future RS due to parasitism. The microsporidian *Vavraia culicis* is a natural parasite of mosquitoes and one whose costs increase over time as its spores accumulate and damage host tissues. As larvae, male and female *Culex pipiens* mosquitoes behaved differently towards infection with *V. culicis*. Infected females pupated earlier than uninfected females and tended to emerge as smaller adults, indicating a cost to their fecundity. However, the age and size at maturity of infected male mosquitoes was no different from uninfected males. The results of this study support theoretical predictions and highlight the potential roles that host gender and density-dependent interactions may have in determining the response of host life-history traits to parasitism.

Keywords: host; life history; reproductive value

1. INTRODUCTION

Parasites can alter the age- and stage-specific schedules of reproduction and the mortality of their hosts. Consequently, there are good reasons to expect that, if possible, hosts should adjust their life-history traits to maximize their reproductive success (RS) in the presence of parasitism (Minchella 1985; Hochberg *et al*. 1992; Forbes 1993, 1996; Perrin *et al*. 1996). A general prediction arising from these studies is that parasites whose costs to the host increase with time will selectively favour hosts that bring forward their schedule of reproduction. This arises because parasitism reduces the future RS of infected hosts and, thus, the costs associated with an increased investment in current reproductive effort (RE) can be discounted against a lower expectation of future RS. An alternative way to express this is that the future costs of parasitism increase the relative reproductive value of early reproduction.

An example of a host life-history response to parasitism is provided by the snail *Biomphalaria glabrata*. If hosts are infected by the trematode *Schistosoma mansoni* (a parasite that eventually castrates its host) soon after maturity, they respond with an increase in their rate of egg production (Minchella & Loverde 1981). This increased RE is costly to a snail's RS if it is uninfected: snails exposed to infection that remain uninfected also show an increase in their RE but ultimately have a lower RS than control snails not exposed to infection (Minchella & Loverde 1981). However, the ability of hosts to respond to infection depends on the age at which they are infected: snails infected as juveniles are already compromised by the time they reach maturity and are unable to reproduce (Gérard & Théron 1997).

An interesting example of a host response to parasitism is the differential RE shown by male and female great tits (*Parus major*) to ectoparasitism in the nest. Only males increase their rate of provisioning offspring when nests are experimentally infested with the hen flea *Ceratophyllus gallinae* (Christe *et al*. 1996). These blood-sucking parasites have an increasingly detrimental effect with time on the probability that chicks will fledge and contribute towards a parent's RS, unless additional resources can compensate for those lost to the fleas. The differential response in RE by parents was suggested to occur because the RS of males is more dependent on the fledging success of their current offspring than is the case for females, whose potential future RS is higher (Christe *et al*. 1996). In a related study, the cost of an increased RE was demonstrated by experimentally manipulating the number of offspring for males to rear. Males who had more offspring to rear had a higher RE and were more likely to contract malaria (Richner *et al*. 1995). Thus, the cost of increasing RE in response to one type of parasite might be increased infection by another. The ability of males to increase their RE may, however, be constrained by the amount of resources available in the local environment (De Lope *et al*. 1993).

In the experiment described below, we investigated the life-history traits of the mosquito *Culex pipiens* (Wied.) when infected by the microsporidian parasite *Vavraia culicis*

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(Weiser). This is a naturally occurring host–parasite relationship and one in which the mounting costs of an infection reduce the longevity and RS of adult mosquitoes, but without causing any discernible increase in the mortality of host larvae or pupae (Reynolds 1970). Mosquito larvae were exposed to varying concentrations of the parasite's spores. We were particularly interested in the host's age and size at maturity—two key traits in a mosquito's life history. We predicted that, if the mosquitoes could respond to parasitism, they should do so by shortening their pre-reproductive lifespan and pupating earlier. Such a response was expected to increase with the host's intensity of exposure to infection. As earlier pupation reduces the period of larval growth, we also expected earlier ages at pupation to be negatively correlated with the size of adult mosquitoes. Mosquitoes were also reared in three environments of larval food availability. This was done as food availability is known to influence mosquito life-history traits and may have interacted with parasitism. Furthermore, by using a range of food conditions we could increase the generality of the results and reduce the potential for hosts being constrained in their ability to alter their life-history traits due to environmental resource availability.

A mosquito's age and size at maturity is known to be sensitive to density-dependent interactions between larvae (Clements 1992). To try and minimize such effects, we reared each larva in isolation after exposure to infection. As exposure to infection does not guarantee infection, this isolation was also done to guard against potential interactions among infected and uninfected larvae in the same treatment. For example, if infected and uninfected larvae differ in their competitiveness for limited resources, any observed differences in their ages and sizes at maturity could be attributable to interactions between hosts rather than due to interactions between the host and parasite themselves. An additional complication of rearing cohorts of larvae together is that the strength of density-dependent interactions between larvae is an uncontrolled variable that changes with time as individuals grow, die or pupate. Hence, the outcome of a host–parasite interaction may well depend on the context in which it occurs and, in this study, we specifically aimed to reduce the potentially confounding influence of density-dependent factors on the host's life-history traits.

2. THE HOST–PARASITE RELATIONSHIP

Culex pipiens is a vector of a number of disease agents, most notably St Louis encephalitis and avian malaria (Van Riper & Van Riper 1986). Larvae are typically found in natural or artificial sites containing dirty, stagnant water with abundant organic matter. We used the S-Lab strain of *C. pipiens* (Georghiou *et al.* 1966), which was derived from stocks maintained in Michel Raymond's laboratory at the University of Montpellier II, France.

Microsporidia are single-celled, intracellular parasites that are amongst the most common pathogens of mosquitoes (Castillo 1980). *Vavraia culicis* itself can infect a number of mosquito genera (Weiser & Coluzzi 1972). Following the ingestion of spores by larvae, an infection is initiated when spores germinate and inject their contents directly into the cytoplasm of cells lining the host's alimentary canal. Once in a host cell, the parasite proliferates as it undergoes a series of developmental stages before producing its spores. Infected cells subsequently burst and disseminate the parasite's spores. Most spores are released back into the gut lumen and are passed out with the host's faeces and become available for horizontal transmission. Some spores get released into the host's body cavity or germinate *in situ* and spread the infection to adjacent tissues.

It is known that *V. culicis* is costly to the fitness of *C. pipiens* and that this cost is dose dependent: groups of 250–350 larvae exposed to concentrations of 6×10^3 or 1.2×10^4 spores ml^{-1} had net reproductive rates (R_o) that were 13 and 24% lower, respectively, than a matching uninfected population (Reynolds 1970).

The direct infection of a female's developing oocytes has not been reported, although parent-to-offspring (vertical) transmission may occur from spores on the surface of eggs (Weiser & Coluzzi 1972). Adult-to-adult transmission, via spores deposited on feeding surfaces, can also occur (Fox & Weiser 1959), although this is probably of limited importance in natural conditions due to the limited longevity of adult mosquitoes. The spores used in this experiment were derived from a stock of *V. culicis* isolated from *Aedes albopictus* in Florida and were provided by Dr J. J. Becnel (United States Department of Agriculture, Gainesville, USA).

3. METHODS AND DATA COLLECTION

The experiment described below consisted of three fully randomized blocks, each containing 12 treatments (four concentrations of exposure to the parasite's spores and three larval food availabilities) with 30 individual mosquitoes per treatment.

Within 12 h of hatching, 36 groups of 40 *C. pipiens* larvae were transferred to Petri dishes (diameter 55 mm) containing 10 ml of deionized water and 1 mg of brewer's yeast (Gaylord Hauser, Superlevure). These dishes were then randomly assigned to one of three experimental blocks and subdivided into four groups of three, representing the food and dosage treatments.

The four dosage treatments involved exposure to *V. culicis* spores at 0, 10^4, 10^5 or 10^6 spores cm^{-2}. All spores were derived from a single solution that was divided into three equal parts, one for each block. Each solution was serially diluted to the appropriate concentration of spores for each dosage treatment within each block. Control dishes were given a matching volume of deionized water. After 48 h of exposure to infection, larvae were rinsed in deionized water and separated into their own individual tube (diameter 20 mm × 90 mm) containing 5 ml of deionized water. All tubes corresponding to a particular Petri dish were kept together in the same rack. Thirty of the original 40 larvae from each Petri dish were chosen at random to participate in the experiment.

The three food treatments were applied after larvae had been isolated in individual tubes. Each larva was provided with either 4, 5 or 6 mg of yeast in 1 ml of deionized water, according to the food treatment their Petri dish had been assigned to.

All tube racks were placed on a single shelf in a room maintained at 28–30 °C and high humidity (>70%) with a 12 L:12 D photoperiod. The racks were first placed on the shelf according to block and then treatment conditions were arranged to minimize spatial effects within each block.

Until larvae were six days old, each tube was checked daily for mortality; dead individuals were collected, placed in 1.5 ml

plastic vials and stored at −20 °C. Once larvae had reached six days of age, tubes were checked every 12 h for pupation or emergence. In the event of pupation, the pupa was temporarily removed while the tube was rinsed and refilled with 5 ml of deionized water. A toothpick was also added for adult mosquitoes to rest upon. Once the pupa had been returned, the top of the tube was covered with a fine nylon gauze. After adult mosquitoes had emerged, the water within the tube was emptied out.

No food was provided to adult mosquitoes but they had access to water from a pad of moist cotton wool placed on the tube's gauze covering; the pad was resoaked every 12 h. We collected adult mosquitoes only after they had died, placed them into individual vials that were placed in an 80 °C stove for a minimum of 12 h, and recorded their dry weight to an accuracy of 1 μg. By not providing food to adult mosquitoes we forced them to survive by metabolizing energetic reserves. This was done to reduce the contribution of expendable reserves towards an estimation of the weight that constituted the fixed mass of an adult mosquito and which was determined during pupation.

The spore content of dry adult mosquitoes was measured by adding 0.25 ml of water to each vial, homogenizing its contents and counting the number of spores with a haemocytometer and a phase-contrast light microscope. Two separate counts were made for each adult, where only individuals containing spores on each count were regarded as being infected. This was a conservative measure to minimize the probability of false-positive identifications due to spore-like fragments of cuticle, etc. In both counts, the treatment conditions experienced by the mosquito were unknown to the observer. For infected individuals, the mean number of spores from the two counts was used in analyses.

The surplus larvae from each Petri dish were reared in the same isolated conditions as the 30 individuals involved in the experiment. However, at nine days of age they were killed, smeared on a glass slide, giemsa stained and individually checked for developmental stages of the parasite. This was done to provide an estimate of infection success independently of spore presence.

4. INITIAL ANALYSES AND RESULTS: INFECTION AND MORTALITY

Our preliminary inspection of the data involved analyses of variance (ANOVA) to assess the role of treatment conditions on the percentage of mosquitoes surviving to adulthood from each treatment and their probability of being infected. Both variables were analysed after an arcsin square-root transformation. These tests were followed by analyses of covariance (ANCOVA) to assess the rate of pre-adult mortality (the number of larvae and pupae dying in a 24 h period/the number of larvae and pupae at the beginning of the 24 h period) over time and the numbers of spores produced by an infection before its host died. The rate of pre-adult mortality was only considered between days 3 and 10 to avoid missing cells from treatments where each individual had either emerged or died.

In each analysis, food and dose treatments were taken as nominal fixed factors, and block as a nominal random effect. Fully factorial models were reduced by removing non-significant interactions ($p > 0.1$) until only main effects remained. Type III sums of squares were used throughout and so these analyses are not dependent on the order in which effects are entered in a model.

Table 1. *ANOVA for the arcsin-transformed* $\sqrt{}$*(percentage of mosquitoes reaching adulthood)*

source	df	s.s.[a]	F	p
block	2	0.291	10.601	<0.001
food	2	0.019	0.710	0.500
dose	3	0.034	0.819	0.494
error	28	0.384	—	—

[a] Sums of squares.

Table 2. *ANCOVA for the rate of larval and pupal mortality between days 3 and 10 of the experiment*

source	d.f.	s.s.	F	p
block	2	0.090	21.540	0.643
food	2	0.001	0.064	0.939
dose	3	0.021	2.039	0.210
day	1	0.534	126.840	<0.001
block × food	4	0.033	0.869	0.510
block × dose	6	0.020	0.347	0.899
food × dose	6	0.059	1.014	0.461
block × food × dose	12	0.117	2.186	0.013
error	251	1.116	—	—

Of the 1080 larvae that began the experiment, 324 survived to adulthood. The number of adults emerging from each block varied, but was not affected by food or dosage conditions (table 1). There was heterogeneity among blocks and treatments for the rate at which larvae and pupae died (table 2) and a strong increase in the rate of mortality with time that was independent of treatment conditions (table 2). The rate of mortality increased steadily from 0.06 on day 3 to 0.20 by day 10.

The percentage of mosquitoes that were infected (s.e.) increased with the intensity of exposure to spores (table 3): 0 (0), 11.1 (4.7), 30.9 (5.0) and 80.6 (4.4) for the 0, 10^4, 10^5 and 10^6 dosage treatments, respectively. These results agreed well with those based on histological evidence: 0 (0), 9.1 (13.4), 36.7 (11.5) and 68.4 (14.5), respectively. Individuals that had been exposed to infection but which did not harbour spores were considered as uninfected.

In total, 82 adult mosquitoes were found to contain spores. The number of spores produced by an infection increased exponentially with time (table 4) and increased with exposure to infection, although not significantly. The $\log_{10}$(mean number of spores per mosquito) for the 10^4, 10^5 and 10^6 dosage treatments (s.e.) were 3.73 (0.25), 4.11 (0.17) and 4.42 (0.09), respectively, and the regression coefficient of the log-linear regression was 0.172.

5. SUBSEQUENT ANALYSES AND RESULTS: AGE AND SIZE AT MATURITY

Our subsequent analyses focused on the age at pupation and adult dry weight (age and size at maturity) of the mosquitoes. As these traits are correlated and both influence mosquito fitness, a multiple analysis of variance (MANOVA) approach was used to assess their joint

Table 3. *ANOVA for the arcsin-transformed* $\sqrt{}$*(probability of infection) in the treatments exposed to spores*

source	d.f.	s.s.	F	p
block	2	0.075	0.243	0.786
food	2	0.174	0.562	0.579
dose	2	3.374	10.895	<0.001
error	20	3.096	—	—

Table 4. *ANCOVA for the* $\log_{10}$ *(mean number of spores per mosquito) produced as a function of treatments and time*

source	d.f.	s.s.	F	p
block	2	0.789	0.703	0.527
food	2	0.331	0.310	0.749
dose	2	1.537	1.946	0.254
day	1	1.605	10.149	0.002
block × food	4	2.354	3.720	0.009
block × dose	4	1.619	2.560	0.047
error	66	10.435	—	—

Table 5. *Summary table for MANOVA statistics and the corresponding univariate tests for the effects of food and infection on the age and size at maturity of female and male mosquitoes*

	MANOVA[a]		ANOVA			
			ln(age at pupation)		dry adult weight	
source	F	p	F	p	F	p
females						
block	1.044	0.385	0.594	0.554	1.299	0.277
food	4.768	0.001	0.624	0.538	9.270	<0.001
infection	5.869	0.004	6.517	0.012	2.659	0.106
males						
block	3.128	0.015	2.650	0.274	0.086	0.921
food	4.692	0.001	1.748	0.178	6.801	0.002
infection	5.090	0.299	0.352	0.612	0.176	0.715
infection × block	2.583	0.038	2.210	0.114	3.860	0.023

[a] Tests are based on Wilks's lambda.

response to treatment effects. Fully factorial models were reduced as above. The MANOVA results are presented with the corresponding univariate analyses for each trait separately. As the MANOVA and ANOVA approaches overlap in the data being tested, we corrected the experimentwise error rate α to $\alpha' = 0.05/3 \approx 0.017$. The natural logarithm of ages at pupation was used in all analyses to normalize the distribution of error residuals.

Due to the extensive pre-adult mortality and the limited number of infected mosquitoes, there were not enough individuals of each sex in each dose treatment for a complete analysis. We decided to analyse each sex separately and to pool individuals across dosage treatments on the basis of whether they were infected or not. MANOVA and ANOVA tests (not reported) found that the life-history traits of control and exposed-but-uninfected mosquitoes were not different. Due to handling errors, full data (sex, age at pupation, dry weight and spore content) were only available for 256 out of the 324 individuals that emerged as adults.

(a) *Female mosquitoes*

The age and size at maturity of infected and uninfected female mosquitoes were different: infected female mosquitoes pupated earlier and tended to emerge as lighter adults (table 5 and figure 1*a*). When each trait was considered separately, the difference between infected and uninfected females was mainly due to infected females pupating earlier (table 5).

Larval food availability also altered the age and size at maturity of female mosquitoes; increasing food availability led to the emergence of heavier adults but had little effect on their ages at pupation (table 5 and figure 1*a*).

(b) *Male mosquitoes*

In contrast to female mosquitoes, the life-history traits of infected and uninfected male mosquitoes were not different (table 5 and figure 1*b*). Infection was not found

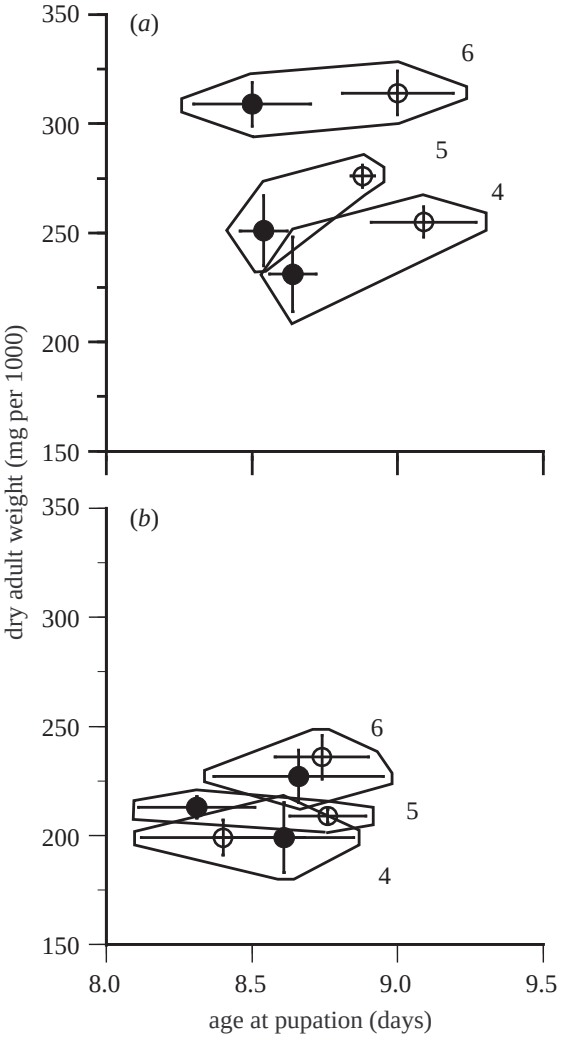

Figure 1. The age at pupation and dry adult weight of infected (filled symbols) and uninfected (open symbols) mosquitoes: (*a*) females and (*b*) males. The values shown are means (±s.e.) of each trait across blocks. Polygons enclosing pairs of points relate to larval food availability.

to influence either the age at which males pupated or their adult dry weight (table 5).

The life-history traits of male mosquitoes were influenced by the amount of food available to them as larvae (table 5 and figure 1*b*). As for the females, heavier male adults emerged as food availability increased but their ages at pupation were not affected (table 5).

6. DISCUSSION

The background mortality of larvae and pupae in the experiment was substantial (>70%) and could not be attributed to the treatment conditions we manipulated (table 1). Furthermore, there was a clear pattern that the rate of mortality was independent of treatment conditions and increased with time (table 2). Other studies involving mosquito hosts have also failed to find a significant effect of *V. culicis* on larval or pupal mortality (Bano 1958; Reynolds 1970). Subsequent studies carried out with a similar protocol in this laboratory and at the University of Montpellier II found this pattern of mortality to be replicable for the S-Lab strain of *C. pipiens* (Berticat 1998). If there is a causal factor responsible for this pattern, it is acting equally across treatment conditions and, therefore, does not unduly influence our analyses.

The prevalence of infection increased as larvae were exposed to higher concentrations of spores (table 3) and the number of spores produced by an infection increased exponentially with time (table 4). The latter point is of particular relevance as, providing spore production is costly, the negative consequences for hosts that do not bring forward their schedule of reproduction will accumulate rapidly (Hochberg *et al*. 1992). Our data contain no direct evidence that either an infection or its production of spores is costly to the host's future RS. However, we expect this to be the case, because of the extensive damage caused to host tissues upon spore production (Bano 1958) and because proteins co-opted for spore production will not be available for the production of a female's eggs.

When infected with *V. culicis* as larvae, female *C. pipiens* mosquitoes pupated earlier and emerged as smaller adults than uninfected females (table 5 and figure 1*a*). As a female mosquito's adult size is a key trait in determining her fecundity (Clements 1992), the earlier ages at pupation of infected females were associated with a cost to their potential RS. This result follows predictions of how a host should adjust its life-history traits in response to a parasite whose costs have a disproportional effect on its future RS (Hochberg *et al*. 1992). Alternatively, rather than being a phenotypic response by the host to parasitism, the observed pattern could be due to parasite manipulation of the host, not least because *V. culicis* can gain transmission from adult mosquitoes. Without a selection experiment to see whether female mosquitoes evolve earlier ages at pupation in response to repeated infection, it is difficult to distinguish between these possibilities. However, the advantages to *V. culicis* of inducing its host to pupate earlier are not obvious, particularly as field data suggest that its dispersion among larval sites by *C. pipiens* adults is negligible (Reynolds 1972).

In contrast to the relationship between *B. glabrata* and *S. mansoni* (Gérard & Théron 1997), the host studied here appears to have the ability to respond to parasitism when infected as a juvenile. This suggests that general predictions may need to be tailored to account for the dynamics of how particular hosts and parasites interact.

It is worth noting that the two infected populations of *C. pipiens* in Reynolds's (1970) experiment showed a higher rate of egg production in the first week of oviposition than the control population: high dose 98, low dose 163 and controls 76. This observation is suggestively analogous to an increase in early RE as shown by *B. glabrata* when infected with *S. mansoni* as young adults (Minchella & Loverde 1981). However, it has also been reported that 'the durations of the larval and pupal stages of the host were significantly ($P<0.1$) longer after infection' (Reynolds 1970, p. 342). Hence, if infected females were able to maintain their larval growth rates, they may have emerged as larger and more fecund adults than the control females.

Assuming that the infected female mosquitoes in Reynolds's (1970) experiment increased their early fecundity, both Reynolds's and our results would suggest that infected female *C. pipiens* shift their RE towards an earlier age. In Reynolds's (1970) study there was a greater investment in early egg production, while in our study females brought forward their age at pupation. As our experiment and that of Reynolds (1970) differ in methodology and strains of host and parasite used, no direct comparisons can be made between the experiments. However, our study, based on host–parasite interactions at the individual level and that of Reynolds (1970), based on host–parasite interactions at the population level, produced contrasting results for the age at pupation of infected female mosquitoes. This suggests that density-dependent factors may be of importance in mediating how host life-history traits respond to parasitism.

As in studies involving great tits (Richner *et al*. 1995; Christe *et al*. 1996), we found that males and females of the host responded differently to the same treatment: while the age and size at maturity of females was different for infected and uninfected female mosquitoes, the same was not true for males (table 5 and figure 1*b*). We suggest two reasons why this may be.

(i) Infected males do not pupate earlier because they cannot pupate earlier. Selection to reduce developmental time acts much more directly on male rather than female mosquitoes as adult size has little influence on male RS but correlates positively with a female's fecundity and longevity (Bradshaw *et al*. 1997). Consequently, male mosquitoes are likely to be at or near a limit to selection for their developmental time and constrained in their ability to respond to parasitism with earlier ages at pupation.

(ii) Males do not change their life-history traits because an infection by *V. culicis* has less effect on male RS than on female RS. Male mosquitoes are thought to invest the majority of their RE and RS in a single mating as soon as possible after emergence, with only few individuals mating more than once. Proportionately more females go on to reproduce more than once (Clements 1992) and so are more likely to experience time-associated costs of infection to their RS than will be the case for males.

In conclusion, the general prediction that hosts should bring forward their schedule of reproduction when infected by a parasite that increases the reproductive value of early reproduction was met by female *C. pipiens* mosquitoes parasitized by *V. culicis* and they did so at what would be a cost to their RS if uninfected. Infected male mosquitoes did not show the same behaviour and this may be related to different selection pressures acting on their age and size at maturity. These results were consistent across three environmental conditions of larval food availability and are unlikely to be confounded by density-dependent interactions between larvae.

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