Defence strategies against a parasitoid wasp in Drosophila: fight or flight?

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Hosts may defend themselves against parasitism through a wide variety of defence mechanisms, but due to finite resources, investment in one defence mechanism may trade-off with investment in another mechanism. We studied resistance strategies against the parasitoid wasp Leptopilina boulardi in two Drosophila species. We found that D. melanogaster had significantly lower physiological resistance against L. boulardi than D. simulans, and hypothesized that D. melanogaster might instead invest more heavily in other forms of defence, such as behavioural defence. We found that when given a choice between clean oviposition sites and sites infested with wasps, both D. melanogaster and D. simulans detected and avoided infested sites, which presumably limits later exposure of their offspring to infection. Unlike D. simulans, however, D. melanogaster laid significantly fewer eggs than controls in the forced presence of wasps. Our findings suggest that D. melanogaster relies more heavily on behavioural avoidance as defence against wasp parasitism than D. simulans, and that this may compensate for a lack of physiological defence.

Keywords: physiological and behavioural defences; Drosophila; parasitoid wasps; resistance; avoidance

1. INTRODUCTION

Since parasites decrease host fitness, natural selection should favour the evolution of host defences against parasitism. Besides physiological defences, hosts can use behavioural defences that prevent or reduce infection or attenuate parasite-induced fitness loss, including avoidance, grooming and self-medication [1]. Such defences may either protect the individual displaying the behaviour or its offspring [1,2]. Since defence mechanisms are often costly, it is expected that hosts will evolve or use only a subset of potential defence mechanisms [3].

Like many insects, Drosophila fruitflies are attacked by parasitoid wasps, which lay their eggs in fly larvae or pupae [4]. In some populations, over 50 per cent of hosts are infected, suggesting wasps exert strong selection pressure on young flies [5]. Fruitflies mount a physiological immune response (melanotic encapsulation) to kill wasp eggs, but female wasps inject venom with their egg to block this response, such that this defence is not always successful [6]. If the wasp egg survives, a wasp larva hatches and begins to consume the fly, leading to fly death.

We hypothesized that flies with weaker physiological defence against parasitoid wasps might show stronger behavioural defence, and conversely, that flies with more effective physiological defence would have reduced behavioural defence. Here, we studied the sister species Drosophila melanogaster and Drosophila simulans, which are thought to differ in their physiological defence against the figitid parasitoid wasp Leptopilina boulardi [7,8]. Both fly species have worldwide distributions and are attacked by L. boulardi in nature [9], and probably coevolved with L. boulardi in ancestral African habitats [7], suggesting they have had sufficient pressure and time to evolve defences against this parasitoid. We confirmed that D. melanogaster has lower physiological defence against this wasp than D. simulans. We then tested whether D. melanogaster compensates for this lack of physiological resistance by limiting oviposition in the presence of wasps, which would presumably reduce the number of offspring exposed to wasp attacks.

2. MATERIAL AND METHODS

(a) Insects

Large outbred populations of D. melanogaster and D. simulans were collected in Atlanta, Georgia (summer 2010) and maintained for several generations. Leptopilina boulardi wasp strain Lb17 was collected in winters, CA, USA (autumn 2002) [7], and has been maintained on the highly susceptible D. melanogaster strain Canton S. Flies aged 1–5 days and wasps aged 1–7 days post-eclosion were used for all experiments.

(b) Physiological defence

For each fly species, 40 early third instar larvae were placed in each of twenty 35 mm diameter Petri dishes containing 2 ml of Drosophila media, and the dishes were randomly assigned to the following treatments: (i) 16 dishes of fly larvae were exposed to five female Lb17 wasps for 2 h, and (ii) four control dishes of fly larvae had no wasps. The next day, six larvae from each wasp treatment dish were dissected to estimate the infection rate (because wasp eggs can be hard to locate, calculated infection rates are likely underestimates). Thirty larvae from each dish were then moved into standard Drosophila vials, and the numbers of flies and wasps that eclosed from these vials were counted two and four weeks later, respectively. Closed flies were externally examined for the presence of melanotically encapsulated wasp eggs in their abdomens.

(c) Behavioural defence: oviposition preference

Tests to determine whether flies prefer oviposition sites absent of wasps were conducted using six oviposition cages (0.6 m²), each containing two 60 mm diameter food dishes on opposite sides of the cage. In the middle of each food dish was placed a 25 mm diameter (12 cm³) clear plastic vial, either empty or holding 50 female wasps. The vials were pierced on the sides and covered on top with a fine mesh to allow for visual and olfactory contact between flies and wasps but preventing wasp escape. Approximately 300 female flies were released in each cage for 48 h and the number of eggs laid on each of the two food dishes was recorded. Drosophila simulans and D. melanogaster were tested simultaneously in three cages each per round, and each species was tested in each cage twice (12 replicates per species total) with control and wasp vials alternated to account for any side bias in oviposition preference.

(d) Behavioural defence: oviposition rate in the presence of wasps

To test whether fly species differ in egg-laying rate in the forced presence of wasps, groups of 25 female and five male flies (to ensure continued female access to sperm) were aspirated into 65 standard Drosophila food vials per fly species. After 1 day of acclimation, vials were randomly assigned to the following treatments: (i) 15 vials had no wasps, (ii) 12 vials had eight female wasps, (iii) 12 vials had eight male wasps, (iv) 13 vials had eight female wasps that were removed after 1 day, and (v) 13 vials had eight male wasps that were removed after 1 day. The number of eggs laid by the flies in each of the 130 vials was counted repeatedly over the lifespan of the female flies. At each count, flies and wasps (if present) were tapped into new vials,
dead male flies and wasps were replaced, and dead female flies were tabulated to generate survival curves by treatment. Counts were made at the following time-points: 4, 12, 24, 48 h, every 48 h until the 58th day and then every 72 h until the end of the experiment when all female flies had died on the 82nd day. Each vial was treated as a replicate.

(e) Statistical analysis
All analyses were performed in R v. 2.10.1. For the physiological resistance experiment, general linear models (GLMs) with binomial errors and logit link functions were used to examine the effect of fly species on: (i) the proportion of infected larvae (attack rate), (ii) the proportion of larvae that survived to adulthood, (iii) the proportion of eclosed flies with melanotically encapsulated wasp eggs, and a GLM with quasi-binomial errors was used to examine the proportion of flies from which wasps successfully eclosed. For the oviposition preference experiment, a GLM with quasi-binomial errors was used to investigate the effect of wasp presence and the effect of fly species on the proportion of eggs laid on the food dishes. For the oviposition rate experiment, a GLM with quasi-Poisson errors was used to investigate: (i) the effect of wasp presence on the average cumulative number of eggs laid by individual female flies of each species and (ii) the effect of fly species on the average cumulative number of eggs. A Cox proportional hazard survival model was used to study the effect of wasp presence on fruitfly survivorship, which was averaged across vials from the same treatment to avoid pseudoreplication.

3. RESULTS
(a) Physiological defence
*Drosophila melanogaster* and *D. simulans* control flies all successfully eclosed. The infection rate for flies exposed to wasps was similar between the two species (0.69 in *D. melanogaster* versus 0.70 in *D. simulans*; OR = 1.1, CI = (0.6–2), p = 0.760). However, there was a strong effect of fly species on survival after exposure to *L. boulardi*, with only 5.3 per cent of *D. melanogaster* larvae surviving to adulthood versus 24.4 per cent of *D. simulans* (figure 1a; OR = 5.73, CI = (3.64–9.02), p < 0.001). In addition, none of the surviving *D. melanogaster* flies contained melanized wasp eggs in their abdomens, while 32 per cent of *D. simulans* flies did (figure 1b; t = 6.2, d.f. = 15, p < 0.001; note that a Student’s t-test assuming unequal variance was used because logistic regression algorithm does not converge for p = 0). Finally, 64.3 per cent of *D. melanogaster* larvae developed into wasps compared with only 54.5 per cent of *D. simulans* (figure 1c; OR = 1.43, CI = (1.03–2.18), p = 0.04).

(b) Behavioural defence: oviposition preference
Overall, flies displayed a significant oviposition preference for wasp-free dishes (figure 1d; OR = 1.3, CI = (1.1–1.6), p = 0.006), but there was no effect of fly species (OR = 1.2, CI = (0.85–1.75), p = 0.300).

(c) Behavioural defence: oviposition rate in the presence of wasps
The presence of *L. boulardi* strongly affected *D. melanogaster* egg-laying; the average cumulative number of eggs laid per fly exposed to either female or male wasps was significantly lower than that of control...
flies (figure 2a; GLM, \( t = 5.98, p < 0.001 \)), and there was no oviposition difference among the four groups of wasp-exposed \( D. \) melanogaster females (figure 2a).

In contrast, there was no overall significant reduction in the number of eggs laid by \( D. \) simulans exposed to wasps compared with control flies (figure 2b; GLM, \( t = 1.6, p = 0.13 \)). The only significant difference in the average cumulative number of eggs laid by individual \( D. \) simulans females was observed between control flies and flies that were exposed to male wasps for a single day (figure 2b; \( t = 3.25, p = 0.003 \)). Furthermore, wasp presence differentially affected survivorship: wasp-exposed \( D. \) melanogaster had significantly shorter lifespan than control flies (figure 2c; \( z = 2, p = 0.047 \)), but there was no effect of wasp presence on \( D. \) simulans survival (figure 2d; \( z = 0.1, p = 0.900 \)).

4. DISCUSSION

As in previous work [7,8], we found that \( D. \) simulans larvae infected by parasitoid wasps are better able to melanotically encapsulate and kill the wasp eggs than \( D. \) melanogaster larvae. This heightened physiological defence may be related to the threefold greater number of constitutively produced haemocytes made by \( D. \) simulans compared with \( D. \) melanogaster [10] and suggests that \( D. \) simulans invests more in physiological immunity than \( D. \) melanogaster. We tested whether \( D. \) melanogaster relies on an alternative strategy of investing more heavily in behavioural defences.

We found that when \( D. \) melanogaster and \( D. \) simulans were given a choice between clean oviposition sites and sites with wasps, they laid a significantly greater proportion of their eggs on clean oviposition sites. Thus, flies sense wasps, presumably through visual and/or olfactory cues, and alter their reproductive behaviour in a manner that limits later exposure of their offspring to these parasites. In natural conditions, this strategy would probably impart a significant cost, as it would entail using energetic resources to leave known oviposition sites in search of wasp-free oviposition sites. We found no difference in oviposition site preference between fly species, demonstrating that both \( D. \) melanogaster and \( D. \) simulans engage in wasp avoidance behaviour.

However, in the forced presence of wasps, \( D. \) melanogaster showed a significant reduction in egg-laying, whereas \( D. \) simulans did not. Although only female wasps parasitize fruitfly larvae, both female
and male wasps reduced *D. melanogaster* egg-laying, suggesting that *D. melanogaster* uses cues expressed by both wasp sexes. At least two hypotheses may explain the reduced fecundity. First, *D. melanogaster* could be subject to a trade-off between offspring quantity and quality [11], such that when they could not avoid wasps they adjusted their oviposition to produce fewer, higher quality offspring that were better protected against wasp infection. Second, *D. melanogaster* may have a stronger avoidance and/or stress response to the presence of wasps than *D. simulans* that led them to expend more energy trying to escape forced co-habitation with wasps and less energy laying eggs. This difference in avoidance behaviour may not have been manifest in our laboratory preference trials when the flies were given a nearby alternative to wasp-infested oviposition sites. A greater amount of stress incurred to *D. melanogaster* by the forced presence of wasps could also explain their increased mortality, which is not easily explained by any potential trade-off between offspring quantity and quality. Surprisingly, lifetime *D. melanogaster* fecundity was reduced even when the flies were exposed to wasps for only 1 day, suggesting the stress of forced co-habitation with wasps has lasting effects.

Altogether, our data show that both *D. simulans* and *D. melanogaster* have evolved avoidance behaviours in response to the risk of infection of their offspring. However, when avoidance of wasps is impossible, *D. simulans* produce their normal complement of offspring, which have some chance of resisting wasp infection via physiological defence. On the other hand, *D. melanogaster*, whose offspring have little chance of mounting a successful physiological defence response, severely limit egg-laying in our potentially artificial trial conditions where avoidance of wasps is physically impossible. Our results are consistent with the idea that the differences in resistance strategies used by these two closely related fruitflies represent a trade-off between physiological and behavioural defences.

This work was funded by NIH grant AI081879 to T.A.S. and NSF grant DEB-1019746 to J.C.dR.