Current Research in Insect Science 6 (2024) 100098

Contents lists available at ScienceDirect

Current Research in Insect Science

journal homepage: www.elsevier.com/locate/cris

# Idiosyncratic effects of bacterial infection on female fecundity in *Drosophila melanogaster*

Aabeer Basu [*], Vandana Gupta [#], Kimaya Tekade [##], Nagaraj Guru Prasad [*]

*Department of Biological Sciences, Indian Institute of Science Education and Research Mohali, Sector 81, SAS Nagar, PO Manauli, Punjab, 140306, India*

ARTICLE INFO

*Keywords:*
Terminal investment
Life-history theory
Cost of immunity
Fecundity compensation
Pathogenic bacterial infection
Inter-individual variation

ABSTRACT

Existing theories make different predictions regarding the effect of a pathogenic infection on the host capacity to reproduce. Terminal investment theory suggests that due to the increased risk of mortality, and the associated risk of losing future opportunity to reproduce, infected individuals would increase their investment towards reproduction. Life-history theory posits that due to energetic and resource costs associated with mounting an immune defense, hosts would decrease their investment towards reproduction, and reallocate resources towards defense and survival. Additionally, Somatic damage incurred by the host due to the infection is also expected to compromise the host capacity to reproduce. We explored these possibilities in *Drosophila melanogaster* females experimentally infected with pathogenic bacteria. We tested if the effect of infection on female fecundity is pathogen specific, determined by infection outcome, and variable between individual infected females. We observed that the mean, population level change in post-infection female fecundity was pathogen specific, but not correlated with mortality risk. Furthermore, infection outcome, i.e., if the infected female died or survived the infection, had no effect on fecundity at this level. At individual resolution, females that died after infection exhibited greater variation in fecundity compared to ones that survived the infection. This increased variation was bidirectional, with some females reproducing in excess while others reproducing less compared to the controls. Altogether, our results suggest that post-infection female fecundity is unlikely to be driven by risk of mortality and is probably determined by the precise physiological changes that an infected female undergoes when infected by a specific pathogen.

## 1. Introduction

The response of an infected host to a pathogenic infection is not limited to mounting an immune response in order to eliminate the invading pathogen and improving survival. Responses to infection often include a variety of physiological and behavioral changes that are not considered part of a canonical immune response (Parker et al., 2011). One such extra-immunological response is modulation of host investment towards reproduction, which manifests in the form of a change in reproductive output (Minchella 1985). Mounting an immune response requires investment of vast amounts of resources, and such investment often comes at the cost of other organismal functions (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Schmid-Hempel 2005). Reallocation of resources from reproduction towards immune function is common under such a scenario, which leads to reduction in host reproductive output (Ordovas-Montanes et al., 2022). Alternatively, faced with imminent death due to the pathogenic infection, hosts may increase investment towards immediate reproduction, mounting a terminal investment like response and thereby increasing reproductive output, to compensate for the reduced lifespan (Minchella and Loverde 1981; Pike et al., 2019; Schulz et al., 2023). Additionally, reproductive output of the host can be compromised if the infection directly (via virulence factors produced by the pathogen; Hurd 2001, Frank and Schmid-Hempel 2008) or indirectly (via immunopathology; Sadd and Siva-Jothy 2006) leads to damage of the host soma, including the reproductive organs. Therefore, the effect of pathogenic infection on host reproduction can vary, and depend on various factors, both intrinsic and extrinsic to the host (Duffield et al., 2017).

* Corresponding authors.
*E-mail addresses:* aabeerkbasu@gmail.com (A. Basu), prasad@iisermohali.ac.in (N.G. Prasad).
# Present address: Institute of Evolutionary Sciences, University of Montpellier, Place Eugène Bataillon, 34,095, Montpellier, Cedex 05, France
## Present address: Institute of Immune Medicine, University of Regensburg, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, 93,053, Regensburg, Germany

https://doi.org/10.1016/j.cris.2024.100098
Received 31 December 2023; Received in revised form 16 August 2024; Accepted 18 September 2024
Available online 27 September 2024

Previous studies exploring the effect of infection on host reproduction in *Drosophila melanogaster* flies have demonstrated that such effects can be determined by a variety of factors, including but not limited to, the type of pathogen used for infection. Parasitoid infection during larval period reduces fecundity of adult females (Carton and David 1983; Fellowes et al., 1999). Viral infection in adults can both increase or suppress reproductive output, contingent upon host genotype and route of infection (Gupta et al., 2017). Across different studies, bacterial infections have been demonstrated to increase (Hudson et al., 2020), decrease (Brandt and Schneider 2007; Linder and Promislow 2009), or have no effect on female fecundity (Kutzer and Armitage 2016; Kutzer et al., 2018; Kutzer et al., 2019). The diversity of experimental results is driven by various factors, including host susceptibility to the infecting pathogen (Stephenson 2019), route of infection (Martins et al., 2013; Behrens et al., 2014), host genotypic differences and their potential interactions with environmental factors (McKean et al., 2008; Vale and Little 2012). Host diet is another major factor that determines the effect of infection on fecundity in female flies (Kutzer and Armitage 2016; Kutzer et al., 2018; Hudson et al., 2020).

Pathogens differ from one another in terms of pathogenicity and virulence: both in terms of the amount of damage inflicted on the host physiology and the mechanistic basis of this damage (Dionne and Schneider 2008; Vallet-Gely et al., 2008; Buchon et al., 2014; Troha and Buchon 2019). Beyond physiological consequences, each pathogen also entails a different risk of mortality on part of that host, which can influence the host decision to invest into reproduction, in accordance with classical life-history theory (Minchella 1985). When the risk of mortality due to an infection is low, hosts are expected to prioritize defense and suppress reproduction, while when the risk of mortality is high beyond a threshold, hosts are expected to prioritize investment towards current reproduction leading to increased reproductive output (Duffield et al., 2017). A pathogen-specific effect of infection on host reproduction is therefore expected, at least at the level of population-mean response.

At the level of individual hosts, infection outcome might influence host reproductive output. On one hand, hosts that succumb to infection and perish lose all future opportunity to reproduce, and therefore are expected to prioritize investment towards current reproduction, compared to hosts that survive and recover from the infection. On the other hand, hosts that perish are expected to have suffered much greater somatic damage compared to hosts that are able to survive the infection, and therefore their reproductive output should suffer greater reduction. Irrespective of which of these scenarios is at play, these two groups of hosts, ones that survive and ones that die, are expected to have different reproductive outputs, compared to one another, and compared to the uninfected hosts. Therefore, both pathogen identity and infection outcome should be important determinants which, individually or interactively, determine post-infection reproductive output of a host.

In this study, therefore, we explore the effects of pathogenic bacterial infections on female fecundity in *Drosophila melanogaster*, focusing on if (a) pathogen identity, and (b) infection outcome (i.e., whether the infected female dies or survives the infection) dictate the effect of infection on female fecundity. Our results suggest that post-infection change in population-mean fecundity is pathogen specific and is independent of both risk of mortality imposed on the females and the infection outcome, suggesting a possible role of the precise mechanism of host-pathogen interaction in determining the effects of infection on female fecundity. Additionally, we observe an increase in inter-individual variability in fecundity in infected females, independent of the identity of the infecting pathogen, but only in case of individuals that succumb to infection. We speculate that this variation may be potentially explained by inter-individual variation in physiological state or genetic variability within our host population.

## 2. Materials and methods

### 2.1. Host population and general handling

Flies from BRB2 population - a large, lab adapted, outbred *Drosophila melanogaster* population - were used for the experiments. The Blue Ridge Baseline (BRB) population was originally established by hybridizing 19 wild-caught isofemale lines (Singh et al., 2015), and has been maintained since then as an outbred population on a 14-day discrete generation cycle with census size of about 2800 adults in each generation. Every generation, eggs are collected from population cages (plexiglass cages: 25 cm length × 20 cm width × 15 cm height) and dispensed into vials (25 mm diameter × 90 mm height) with 8 ml banana-jaggery-yeast food medium, at a density of 70 eggs per vial. 40 such vials are set up; the day of egg collection is demarcated as day 1. The vials are incubated at 25 °C, 50–60% RH, 12:12 hour LD cycle; under these conditions the egg-to-adult development time for these flies is about 9–10 days. On day 12 post egg collection all adults are transferred to population cage and provided with fresh food plates (banana-jaggery-yeast food medium in a 90 mm Petri plate) supplemented with ad libitum live yeast paste. On day 14, the cage is provided with fresh food plate, and 18 hours later eggs are collected from this plate to begin the next generation.

### 2.2. Pathogen handling and infection protocol

Four bacterial pathogens were used in this study for infecting the flies: *Bacillus thuringiensis* (*Bt*; obtained from DSMZ, Germany, catalogue number: DSM2046), *Pseudomonas aeruginosa* (*Pa*; obtained from MTCC, India, catalogue number: 4999), *Serratia marcescens* (*Sm*; Martins et al., 2013), and *Enterococcus faecalis* (*Ef*; Lazzaro et al., 2006). All four pathogens used for infection are natural pathogens of insects and are known to cause lethal infection in *D. melanogaster* flies following systemic infection (Shirasu-Hiza and Schneider 2007; Dionne and Schneider 2008). Two of the pathogens, *Pa* and *Sm*, are Gram-negative bacteria, while the rest of the two pathogens, *Bt* and *Ef*, are Gram-positive bacteria. Bacteria representing both Gram-character were used in the experiments, since in *D. melanogaster*, Gram-negative and Gram-positive bacterial pathogens are detected by separate immune surveillance components and are defended against by separate immune mechanisms, with some overlap and crosstalk between the elicited defense mechanisms (Lemaitre and Hoffman 2007; Dionne and Schneider 2008; Vallet-Gely et al., 2008; Buchon et al., 2014). Previous work has demonstrated that oral infection with *Pa* increases female fecundity in *D. melanogaster* (Hudson et al., 2020) while infection via septic injury to the thorax with *Pa* decreases female fecundity in a genotype specific manner (Linder and Promislow 2009). Infection via septic injury to the abdomen with *Sm* has also been shown to compromise female fecundity in *D. melanogaster* (Brandt and Schneider 2007).

All pathogens are maintained in the lab as glycerol stocks, and are cultured in Luria Bertani broth (Himedia, M1245); cultures are incubated at 30 °C for *Bt*, and 37 °C for *Pa, Sm* and *Ef*. Overnight culture of bacteria grown from glycerol stocks was diluted (1:100) in fresh LB medium and incubated till confluency (optical density $OD_{600}$ = 1.0–1.2). The bacterial cells were pelleted down by centrifugation and re-suspended in sterile 10 mM $MgSO_4$ buffer at $OD_{600}$ = 1.0. OD600 = 1.0 for *Bt* corresponds to $10^4$ cells/ml, for *Pa* corresponds to $10^8$ cells/ml, for *Sm* corresponds to $10^6$ cells /ml, and for *Ef* corresponds to $10^7$ cells/ml. Flies were infected via septic injury, by pricking them at the dorsolateral side of the thorax with a fine needle (Minutien pin, 0.1 mm, Fine Science Tools, CA, item no. 26,001–10) dipped in bacterial suspension under light $CO_2$ anesthesia. Flies for sham-infections were similarly treated but pricked with needle dipped in sterile 10 mM $MgSO_4$ buffer. Uninfected control flies were only subjected to $CO_2$ anesthesia.

### 2.3. Generation of experimental flies

Eggs were collected from BRB2 population cages and distributed into food vials with 8 ml of standard food medium at a density of 70 eggs per vial. These vials were incubated as per the general maintenance regime. Twelve days post egg-laying flies were flipped into fresh food vials and hosted for two more days before experimentation. This ensured all focal females were 4–5 day old, sexually mature and inseminated, at the time of infections. Flies were again flipped into fresh food vials 6 hours before being subjected to experimental treatments (as described below).

### 2.4. Experimental design

2.4.a. Experiment 1. Focal females were randomly distributed into five treatments: (a) infected with *Bacillus thuringiensis* (*Bt*), (b) infected with *Pseudomonas aeruginosa* (*Pa*), (c) infected with *Serratia marcescens* (*Sm*), (d) sham-infected controls, and (e) uninfected controls. The entire experiment was independently replicated three times. Flies were placed in fresh food vials after being subjected to respective treatments. For each treatment 10 vials were set up, each with 8 females for oviposition; each vial was used as a unit of replication. The vials were monitored every 2 hours to record any mortality, for 24 hours post-infection, divided into two consecutive 12-hour windows. Flies alive at the end of first 12-hour window were flipped into fresh food vials (one-to-one mapping of vial identity), and flies alive at the end of 24 hours were discarded (censored). The number of eggs in each vial was counted at the end of respective 12-hour windows. The vials were then incubated under standard maintenance conditions for the eggs to develop into adults, and 12 days after the oviposition period, all adult progeny were counted under light $CO_2$ anesthesia and transferred to fresh food vials.

2.4.b. Experiment 2. Focal females were randomly distributed into two treatments: (a) infected with bacteria, and (b) sham-infected controls. Four pathogens were used in this experiment: the three used for experiment 1 and *Enterococcus faecalis* (*Ef*). For infected treatment, 120 females were individually hosted in vials for oviposition, while for sham-infected controls 40 females were hosted individually. The experiment was replicated thrice with each pathogen. (Due to a handling accident, one replicate with Sm had sample size of 60 and 30 females for infected and sham-infected treatments, respectively.) The vials were monitored every 2 hours for any mortality, for 48 hours post-infection, after which the alive flies were discarded. The vials were then incubated under standard maintenance conditions for the eggs to develop into adults, and 12 days later the number of adult progeny was counted for each individual female.

### 2.5. Statistical analysis

All analyses were carried out using R statistical software, version 4.1.0 (R Core Team, 2021).

2.5.a. Experiment 1. Survival data was analyzed using mixed-effects Cox proportional hazards model, with 'infection treatment' as a fixed factor and 'replicate' as a random factor. Female fecundity in each vial was normalized before analysis as follows:

$$\textit{Female fecundity}\ (\textit{egges per female per hour}) = \frac{\textit{Total number of eggs in the vial}}{\textit{Summation of lifespan of eight females in the vial}},$$

and

$$\textit{Female fecundity}\ (\textit{progeny per female per hour}) = \frac{\textit{Total number of projeny in the vial}}{\textit{Summation of lifespan of eight females in the vial}}.$$

Egg-to-adult viability for eggs laid by females in each vial was calculated as follows:

$$\textit{Viability} = \frac{\textit{Total number of progeny in the vial}}{\textit{Total number of eggs in the vial}}.$$

Female fecundity and egg-to-adult viability were analyzed using type-III analysis of variance (ANOVA), with 'infection treatment' as a fixed factor and 'replicate' as a random factor. Post-hoc pairwise comparisons were carried out using Tukey's HSD.

Significance tests for random effects are tabulated in supplementary table S5.

**2.5.b. Experiment 2.** Fecundity of individual females was normalized as follows:

$$\textit{Female fecundity}\ (\textit{progeny per hour}) = \frac{\textit{Number of eggs in the vial}}{\textit{Lifespan of the individual female in the vial}}.$$

To test for the effect of infection in general, female fecundity was analyzed using type-III ANOVA, with 'infection treatment' as a fixed factor and 'replicate' as a random factor. To test for the effect of infection outcome, infected females were categorized as *infected-alive* and *infected-dead*, and a new factor 'category' was created with three levels: sham-infected, infected-alive, and infected-dead. Thereafter, female fecundity was analyzed using type-III ANOVA, with 'category' as a fixed factor and 'replicate' as a random factor. Post-hoc pairwise comparisons were carried out using Tukey's HSD. Pairwise comparison of variances between 'category' was carried out using Levene's test after pooling data from all three replicates for each pathogen. Effect of female lifespan on female fecundity was tested using type-III ANOVA with 'time of death' as a continuous, fixed factor and 'replicate' as a random factor.

Significance tests for random effects are tabulated in supplementary table S5.

## 3. Results

### 3.1. Effect of infection treatment on female fecundity

In this experiment we tested the effects of bacterial infection, and the identity of the infecting pathogen, on female fecundity and egg-to-adult viability of the offspring thus produced. The infected (with *Bacillus thuringiensis, Pseudomonas aeruginosa*, and *Serratia marcescens*) and control (uninfected and sham-infected) females were housed in food vials in groups of eight. The survival of these females was monitored every two hours, for twenty-four hours, and the number of eggs (and progenies) produced by females in each vial was counted. The reproductive output of females in each vial was calculated by dividing the total number of eggs (or progeny) produced by the summation of how long each female survived in that vial. This normalization was done to account for the fact that females across different infection treatments die at different rates (Fig. 1.a), and a female might have greater absolute reproductive output by virtue of simply living longer than another female that died early.

Infection treatment significantly increased female mortality, in a pathogen-specific manner. All females infected with *S. marcescens* (hereafter *Sm*) and *P. aeruginosa* (hereafter *Pa*) died after being infected within the observation window, while about half of the females infected with *B. thuringiensis* (hereafter *Bt*) died after being infected (Fig. 1.a, supplementary table S1). Mortality of sham-infected females did not differ significantly from that of uninfected females.

Infection treatment had a significant effect on female reproductive output, at both the egg ($F_{4,147}$: 58.778, $p < 2.2\ e^{-16}$; Fig. 1.b) and progeny ($F_{4,147}$: 62.565, $p < 2.2\ e^{-16}$; Fig. 1.c) stages, with the change in reproductive output being pathogen specific. *Sm*-infected females laid a significantly greater number of eggs, and produced a greater number of progeny, per female per hour compared to females from all other treatments (supplementary tables S2.a and S2.b). *Bt*-infected and *Pa*-infected females produced similar number of eggs and progeny

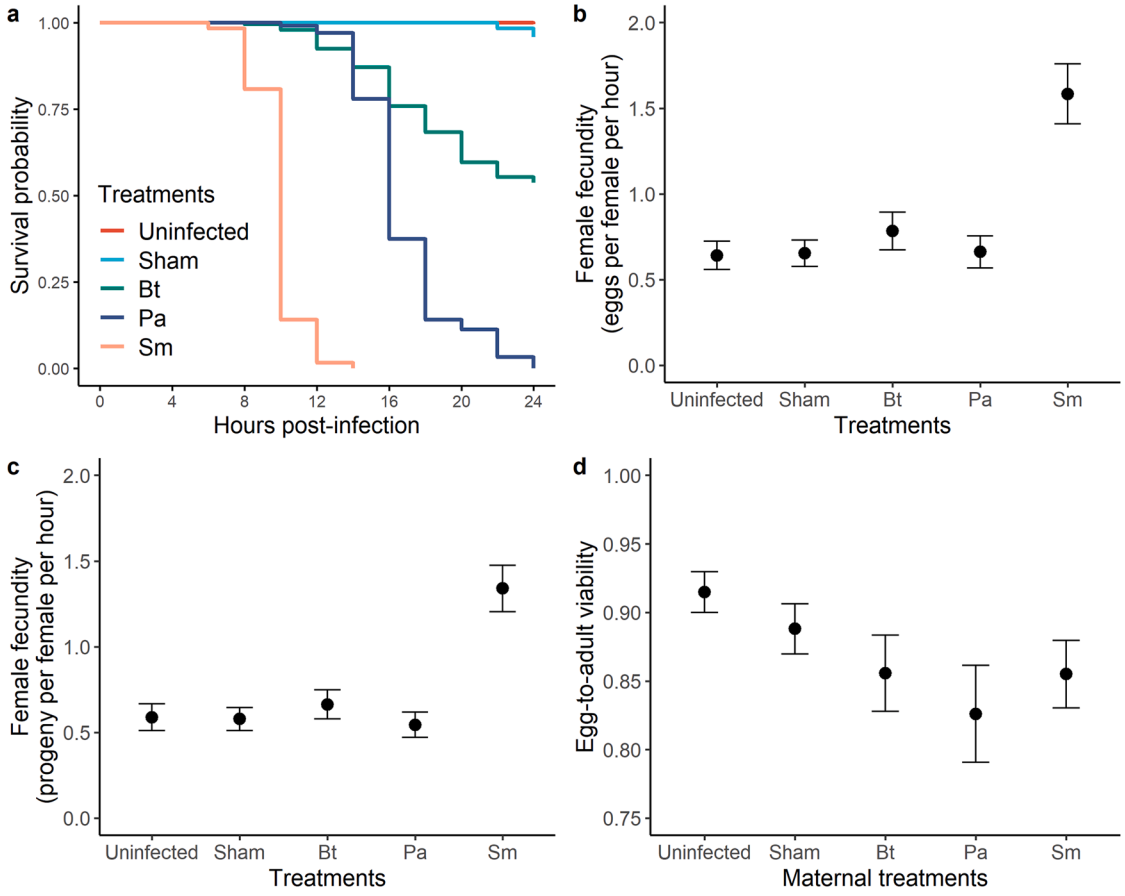


**Fig. 1.** Pathogen specific effects of bacterial infections on survival and reproductive output of *Drosophila melanogaster* females. (a) Post-infection survival of infected and control females. (b) Post-infection female fecundity in terms of eggs produced (mean ± 95% CI). (c) Post-infection female fecundity in terms of progeny produced (mean ± 95% CI). (d) Egg-to-adult viability of eggs laid by infected females (mean ± 95% CI).

compared to females from both control treatments (supplementary table S2.a and S2.b).

Infection treatment of the mother had a significant effect on egg-to-adult viability ($F_{4,150}$: 7.985, $p = 7.304\ e^{-06;}$ Fig. 1.d). Viability of eggs laid by uninfected females and sham-infected females did not differ from one another (supplementary table S2.c). Eggs laid by all infected females had lower viability compared to eggs of uninfected females, with eggs from *Pa*-infected females exhibiting the greatest reduction in viability, significantly lower than eggs from both uninfected and sham-infected females (supplementary table S2.c).

### 3.2. *Effect of infection outcome on female fecundity*

In this experiment we tested if the reproductive output of individual infected females differs depending upon whether they succumb to infection or not. Infected and control females were housed in food vials individually: the survival of these females was monitored every hour, for forty-eight hours, and the number of progenies produced by these females were counted. The reproductive output of the individual female in each vial was normalized by dividing the total number of progenies produced by how long the female in that vial survived. This normalization accounted for the fact that, both across and within each infection treatment, females varied in terms of how long they survived post infection. Females that survive extra can have greater absolute reproductive output simply by the virtue of living longer. In this experiment females were subjected to infection with four bacterial pathogens: *B. thuringiensis, E. faecalis* (hereafter *Ef*), *P. aeruginosa*, and *S. marcescens*.

Post-infection survival was significantly affected by the infection treatment and depended on the identity of the infecting pathogen (supplementary table S3). About half of *Bt*-infected females (Fig. 2.a) and *Ef*-infected females (Fig. 2.c) died after being infected. All *Pa*-infected (Fig. 2.e) and *Sm*-infected (Fig. 2.g) females died from infection.

Infection treatment had a significant effect on female reproductive output (progeny produced per hour) in case of three out of four pathogens. *Bt*-infected females did not differ significantly in terms of fecundity compared to sham-infected control females ($F_{1,475}$: 2.687, $p = 0.102$; Fig. 2.b). Analyzed separately, *infected-dead* females (females that died after being infected) and *infected-alive* females (females that survived the infection) had similar progeny output, which were comparable to progeny output of sham-infected females (supplementary table S4). Fecundity of *Ef*-infected females was overall less compared to sham-infected females ($F_{1,348}$: 27.085, $p = 3.287\ e^{-07}$; Fig. 2.d). Analyzed separately, both *infected-dead* and *infected-alive* females have reduced fecundity compared to sham-infected females but did not differ between themselves (supplementary table S4). Fecundity of *Pa*-infected females was significantly less than that of sham-infected females ($F_{1,474}$: 4.373, $p = 0.037$; Fig. 2.f, supplementary table S4). Fecundity of *Sm*-infected females was significantly greater than that of sham-infected females ($F_{1,408}$: 25.5, $p = 6.684\ e^{-07}$; Fig. 2.h, supplementary table S4).

In case of pathogens for which lethality of infected females were not hundred percent, infection outcome had a significant effect on variance of post-infection reproductive output. For *Bt*-infected females (Fig. 2.b), *infected-dead* females exhibited greater variance in fecundity compared to both *infected-alive* (Levene's test: $F_{1,356}$: 22.06, $p = 3.78\ e^{-06}$) and sham-infected females (Levene's test: $F_{1,333}$: 21.847, $p = 4.295\ e^{-06}$), which do not differ in variance from one another (Levene's test: $F_{1,261}$: 0.78, $p = 0.378$). For *Ef*-infected females (Fig. 2.d), *infected-dead* females exhibited greater variance in fecundity compared to both *infected-alive*

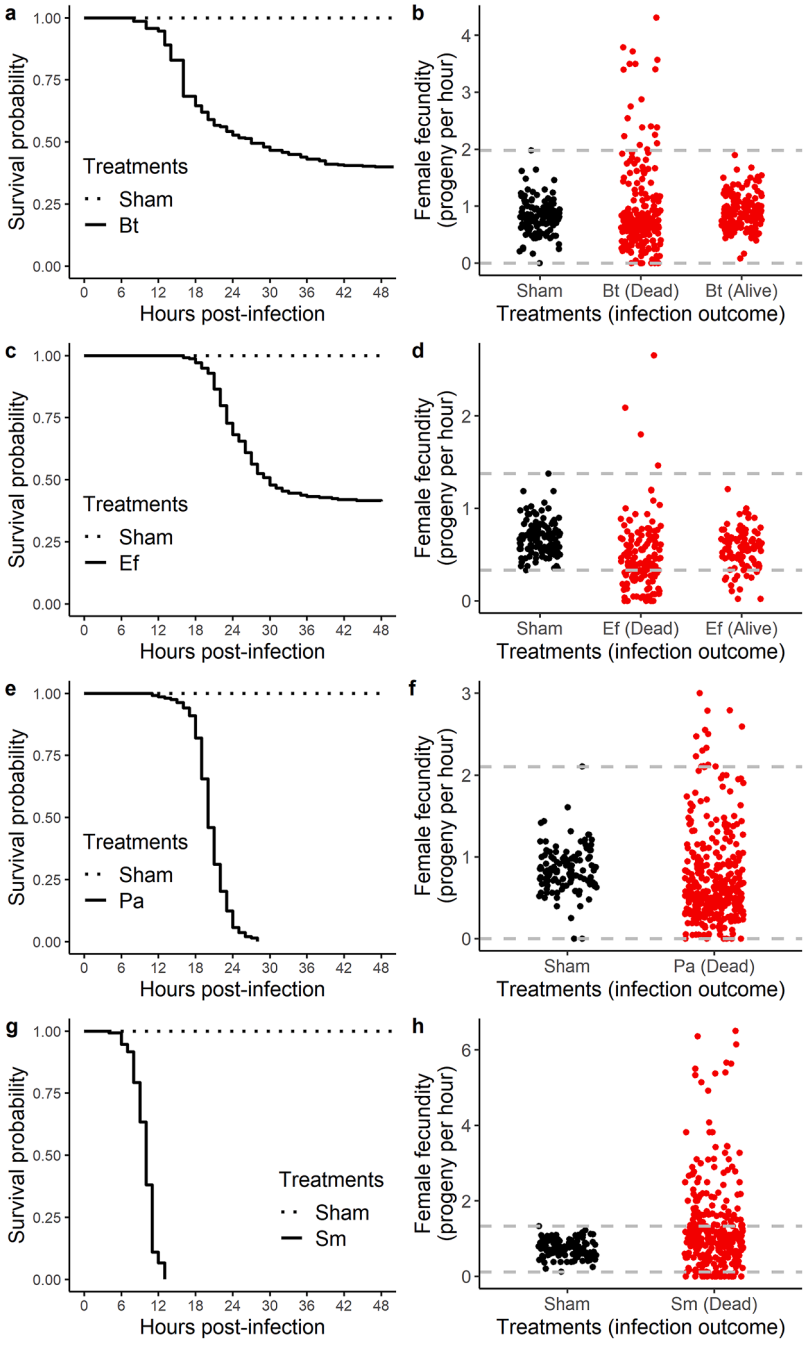


**Fig. 2.** Effect of infection outcome on post-infection fecundity of *D. melanogaster* females infected with bacterial pathogens. (a) Survival of females infected with *B. thuringiensis*. (b) Fecundity of females infected with *B. thuringiensis*. (c) Survival of females infected with *E. faecalis*. (d) Fecundity of females infected with *E. faecalis*. (e) Survival of females infected with *P. aeruginosa*. (f) Fecundity of females infected with *P. aeruginoas*. (g) Survival of females infected with *S. marcescens*. (h) Fecundity of females infected with *S. marcescens*. Dashed lines in panels b, d, f, and h represent the range of fecundity for sham-infected control females.

(Levene's test: $F_{1,236}$: 9.244, $p = 2.63\ e^{-03}$) and sham-infected females (Levene's test: $F_{1,258}$: 14.92, $p = 1.419\ e^{-04}$), which do not differ in variance from one another (Levene's test: $F_{1,216}$: 0.609, $p = 0.436$). For the pathogens with hundred percent mortality, fecundity of *infected-dead* females exhibited greater variance compared to fecundity of sham-infected females, for both *Pa*-infected (Levene's test: $F_{1,475}$: 19.795, $p = 1.075\ e^{-05}$; Fig. 2.f) and *Sm*-infected females (Levene's test: $F_{1,408}$: 40.875, $p = 4.444\ e^{-10}$; Fig. 2.h).

Among the *infected-dead* females, progeny output was negatively correlated with female lifespan for *Bt*-infected females with a small effect size ($F_{1,210}$: 6.323, $p = 0.012$; partial eta-square, 95% CI: 0.03, 0.0–1.0). For *Ef*, progeny output of *infected-dead* females was positively correlated with female lifespan with a small effect size ($F_{1,140}$: 5.925, $p = 0.016$; partial eta-square, 95% CI: 0.04, 0.0–1.0). Progeny output was not correlated with lifespan for both *Pa*-infected ($F_{1,357}$: 0.5711, $p = 0.45$) and *Sm*-infected ($F_{1,290}$: 0.151, $p = 0.698$) females.

## 4. Discussion

Pathogenic infections can have varied effects on host reproduction. When infection affects reproduction, there are two expected outcomes – a post-infection increase or a decrease in reproductive output – but the observed effects do not always agree with the theoretical expectations for a myriad of reasons (Hurd 2001; Abbate et al., 2015; Duffield et al.,

2017), with certain studies reporting no effect of infection on host reproduction. In this study we explored if the effect of pathogenic bacterial infection on female fecundity in *Drosophila melanogaster* changes depending upon the identity of the infecting bacterial pathogen, and upon whether the host dies or survives the pathogenic infection.

To account for the possibility that any fly by virtue of having survived a few extra hours might be able to produce a few extra eggs, instead of comparing the absolute number of eggs or progeny produced by flies across different treatments, we normalized the number of eggs (or progeny) produced by the number of hours survived by the females and compared this *normalized* reproductive output across the treatments. This is especially important since the period of post-infection survival of females differ considerably in our experiments, both across different treatments and within each infection treatment. The process of normalization is described in detail in Section 2.5.

Before beginning the study, we had the following expectations, assuming that the patterns of post-infection host reproductive output is primarily driven by the risk of mortality imposed upon the host:

(a) Females infected with a pathogen that imposes greater lethality would have greater fecundity compared to females infected with a pathogen that is not always lethal.
(b) Females that die after being infected (susceptible females) would have greater fecundity compared to females that survive the infection (resilient females).
(c) Among females that die after being infected, there will be a negative correlation between fecundity and post-infection lifespan.

One would expect to see different, if not opposite, results if the patterns of post-infection reproductive output is majorly determined by the costs of mounting a defense response and the amount of damage incurred by the infected hosts, and assuming that lethality is proportional to damage incurred. One caveat of our experimental design is that the different pathogens, in addition to representing different risks of mortality, represent different mechanisms of pathogenesis, and therefore, our results might be confounded by these two factors.

Results from our experiments suggest that the effect of infection on mean female fecundity is pathogen specific, although the change in mean fecundity is not proportional to risk of mortality imposed on the infected females. In our experiments, infection with both *Serratia marcescens* (*Sm*) and *Pseudomonas aeruginosa* (*Pa*) was completely lethal at the dose we used for experimentation, while infection with *Bacillus thuringiensis* (*Bt*) and *Enterococcus faecalis* (*Ef*) was partially lethal, with a considerable portion of infected females surviving past the acute phase of infection (Figs. 1.a and 2.a, c, e, g). Therefore, we expected that both *Sm*- and *Pa*-infected females would exhibit increased post-infection fecundity compared to controls, and this increase would be greater than that what is exhibited by *Bt*- and *Ef*-infected females. We observed that *Sm*-infected females exhibited a net increase in fecundity (Figs. 1.b, c and 2.h), *Ef*-infected females exhibited a net decrease in fecundity (Fig. 2.d), while *Bt*-infected females did not show any change (Figs. 1.b, c and 2.h). *Pa*-infected females exhibited either no change (experiment 1, Fig. 1.b and c) or a mild decrease (experiment 2, Fig. 2.f) in fecundity. Our results therefore disagreed with our expectations. It has been previously theorized that post-infection increases in fecundity, as posited by terminal investment theory, would not be observed if infection compromises progeny quality (Perrin et al., 1996). This can potentially explain why *Pa*-infected females do not exhibit increased fecundity, even though infection with *Pa* is always lethal. Offspring of *Pa*-infected females were least viable amongst the all the treatments, an observation that has also been previously reported in case of systemic *Pa* infection (Ye et al., 2009).

Furthermore, the effect of infection on mean fecundity was independent of infection outcome: susceptible (*infected-dead*) females had similar fecundity compared to resilient (*infected-alive*) females. *Bt*-infected females, independent of their infection outcome, did not show any mean change in fecundity (Fig. 2.b), while *Ef*-females exhibited compromised mean fecundity irrespective of their infection outcomes (Fig. 2.d). This, in addition to the above results, suggests that the effect of infection on female fecundity is determined by the physiological consequences that accompany an infection, and not simply by risk of mortality and loss of potential future opportunity of reproduction, as hypothesized by terminal investment (Minchella 1985) and fecundity compensation (Parker et al., 2011) theories. Our results partially agree with predictions from Forbes (1993) that post-infection reproductive output of the host should be determined by an interaction between whether infection compromises future reproduction (due to host death or sterility) and whether infection compromises present reproduction (due to host physiological changes). Many physiological changes simultaneously occur in an infected host, some of which are caused by the pathogens while others are consequences of the hosts' response to infection, including but not limited to mounting an immune defense (Shirasu-Hiza and Schneider 2007; Dionne and Schneider 2008; Schmid-Hempel 2009). Further studies are required to decipher which physiological change is the major determining factor in governing reproductive output of an infected host. It may be speculated that host fecundity is likelier to be compromised if the pathogen localizes in and/or damages the host reproductive tissue directly (Brandt and Schneider 2007). Pathogens can also alter host fecundity by affecting overall metabolism of the host (Arnold et al., 2013; Vincent et al., 2020) or by eliciting an immunopathologic immune response (Sadd and Siva-Jothy 2006) that leads to damage to either reproductive tissues or tissues responsible to resource storage (viz. fat bodies).

Across all pathogens used in our experiments, susceptible (*infected-dead*) females exhibit greater inter-individual variability in fecundity compared to both resilient (*infected-alive*) females and sham-infected control females (Fig. 2.b, d, f, h). This increased variability was observed irrespective of how lethal the infection was. We tested if this variability was correlated with post-infection lifespan of the females but did not find consistent evidence for it. To our knowledge, post-infection increases in variability in fecundity, or any other fitness determining traits, has not been reported before in *D. melanogaster*.

The source of heterogeneity in disease outcomes is an active area of research. Previous studies have reported heterogeneous response to infection in *D. melanogaster* flies for various traits, including disease resistance, disease tolerance, pathogen transmission capacity, fecundity, and behavioural traits such as locomotor activity, aggregation behaviour, etc. (Kutzer and Armitage 2016; Kutzer et al., 2019; Siva-Jothy and Vale 2019; White et al., 2020; Siva-Jothy and Vale 2021; Hidalgo et al., 2022; Hidalgo and Armitage 2022; Romano et al., 2022; Kutzer et al., 2023). This heterogeneity in response to infection is often determined by the host genotype, but inter-individual variation independent of genetic variation is also commonly observed. Our results demonstrate that infected females exhibit greater variability in fecundity, especially when they are susceptible to the infection. We have no definite explanation for this observed variability, since it is not explained by any factors tested in our experiments, and hence can only speculate about its potential underlying cause. One, this variability can be purely stochastic without any evolutionary consequences, as is sometimes observed in case of measurements of life-history traits (Steiner and Toljapurkar 2012). Two, given that we obtained our experimental females from a large, outbred fly population, the observed variability may reflect standing genotypic variation for response to infection within the host population. Genotypic differences among host, in terms of both disease resistance and tolerance, is a common factor known to determine disease outcomes, including post-infection reproductive output (Råberg et al., 2007; Vale and Little 2012; Parker et al., 2014; Kutzer et al., 2018; Kutzer et al., 2019). And three, the observed heterogeneity might reflect variation in the quality (Wilson and Nussey 2010) and physiological state (McNamara and Houston 1996) of individual females. The physiological state of an individual is a potent predictor of both its future capacity to

reproduce and to expend resources towards present needs, and therefore has been theorized to influence host post-infection responses, including reproductive output (Duffield et al., 2017).

Post-infection change in host fecundity is often interpreted in terms of costs of mounting an immune response (Lochmiller and Deerenberg 2000; Schmid-Hempel, 2003; Schmid-Hempel 2005; McKean et al., 2008). Costlier the mounted defense, in terms of energy and resources expended, greater is the expected reduction in fecundity. Our results clearly demonstrate that infection does not guarantee an alteration of fecundity, and mean fecundity of infected females can both increase and decrease. In addition to that, change in mean fecundity is not always corroborated by change in fecundity of individual infected females. This is best demonstrated in case of *Sm*-infected (Fig. 2.h) and *Ef*-infected (Fig. 2.d) females in our second experiment, where although there is a directional net change in mean fecundity (an increase in case of *Sm*- and a decrease in case of *Ef*-infected females), certain individual infected females exhibit fecundity beyond the range of fecundity of control females in both directions. This also holds for *Bt*-infected (Fig. 2.b) and *Pa*-infected (Fig. 2.f) females where there is no net change in mean fecundity. Therefore, among infected females, some individuals exhibit apparent increase in fecundity (suggesting terminal response), some individuals exhibit apparent decrease in fecundity (suggesting presence of costs), while others exhibit no alteration of fecundity. Therefore, we recommend that, *one*, interpreting post-infection change in fecundity in terms of costs of immune defense is too simplistic and insufficient, and *two*, it is important to study response to infection at the level of individual hosts, as population mean responses provide incomplete information.

In summary, in our study we measured the effect of pathogenic infection on *D. melanogaster* female fecundity across various pathogens, and across infection outcomes (death vs. survival). Our results suggest that although pathogen identity is a key predictor of post-infection female fecundity at the level of population mean response, risk of mortality is apparently not the force driving post-infection fecundity change. We therefore postulate that infection-accompanied alteration in host physiology might be the primary factor determining post-infection host fecundity. Since different pathogens have different mechanisms of pathogenesis, future studies can explore if females infected with different doses of the same bacteria respond differently. This would help tease apart the true role of mortality risk from that of pathogenesis, assuming that mortality risk co-varies with infection dose. Future studies can also test if the within-host site of pathogen localization alters the effect of infection on host fecundity. Our results also demonstrated that susceptible females dying of infection exhibit great variability in fecundity, which was absent in both resilient and control females. We speculate that this may be driven by variation in various factors, including host genetics, amount of damage incurred and mitigated by the host, and host physiological state. Further studies are necessary to elucidate what determines this variability in response to pathogenic infections at the level of individual hosts.

## CRediT author statement

Aabeer Basu: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review & editing, visualization

Vandana Gupta: investigation, writing – review & editing

Kimaya Tekade: investigation, writing – review & editing

Nagaraj Guru Prasad: writing – review & editing, supervision, funding acquisition

## Funding

The study was funded by intra-mural funding from IISER Mohali, India, to NGP. AB was supported by Senior Research Fellowship for PhD students from CSIR, Govt. of India. VG was supported by INSPIRE fellowship for undergraduate studies from DST, Govt. of India. KT was supported by KVPY fellowship for undergraduate studies from DST, Govt. of India. The funding organizations had no role in design and execution of the study or in interpretation of the results.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data for this paper are available in the Dryad Digital Repository at https://doi.org/10.5061/dryad.pnvx0k6qt.

## Acknowledgements

The authors thank Paresh Nath Das for logistical support during execution of the experiments reported here. The authors also thank Dr. Aparajita Singh and Dr. Rochishnu Dutta for their constructive comments on earlier drafts of the manuscript. The authors thank Dr. Elio Sucena and Tania Paulo (Instituto Gulbenkian Ciencia, Portugal) for providing the *Serratia marcescens* isolate, and Prof. Brian Lazzaro (Cornell University, USA) for providing the *Enterococcus faecalis* isolate used in the experiments.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2024.100098.

## References

Abbate, J.L., Kada, S., Lion, S., 2015. Beyond mortality: sterility as a neglected component of parasite virulence. Edited by Glenn F Rall. PLoS Pathog. 11 (12), e1005229. https://doi.org/10.1371/journal.ppat.1005229. December 3.

Arnold, P.A., Johnson, K.N., White, C.R., 2013. Physiological and metabolic consequences of viral infection in *Drosophila melanogaster*. J. Exper. Biol. Jeb, 088138. https://doi.org/10.1242/jeb.088138.

Behrens, S., Peuß, R., Milutinović, B., Eggert, H., Esser, D., Rosenstiel, P., Schulenburg, H., Bornberg-Bauer, E., Kurtz, J., 2014. Infection routes matter in population-specific responses of the red flour beetle to the entomopathogen bacillus thuringiensis. BMC Genom. 15 (1), 445. https://doi.org/10.1186/1471-2164-15-445. June 7.

Brandt, S.M., Schneider, D.S., 2007. Bacterial infection of fly ovaries reduces egg production and induces local hemocyte activation. Develop. Compar. Immunol. 31 (11), 1121. https://doi.org/10.1016/j.dci.2007.02.003. January 1–30.

Buchon, N., Silverman, N., Cherry, S., 2014. Immunity in drosophila melanogaster — from microbial recognition to whole-organism physiology. Nat. Rev. Immunol. 14 (12), 796–810. https://doi.org/10.1038/nri3763. December.

Carton, Y., David, J.R., 1983. Reduction of fitness inDrosophila adults surviving parasitization by a cynipid wasp. Experientia 39 (2), 231. https://doi.org/10.1007/BF01958917. February 1–33.

Dionne, M.S., Schneider, D.S., 2008. Models of infectious diseases in the fruit fly drosophila melanogaster. Dis. Model. Mech. 1 (1), 43–49. https://doi.org/10.1242/dmm.000307. July 1.

Duffield, K.R., Bowers, E.K., Sakaluk, S.K., Sadd, B.M., 2017. A Dynamic Threshold Model for Terminal Investment. Behav. Ecol. Sociobiol. (Print) 71 (12), 185. https://doi.org/10.1007/s00265-017-2416-z. December 3.

Fellowes, K., Godfray, 1999. The relative fitness of drosophila melanogaster (Diptera, Drosophilidae) that have successfully defended themselves against the parasitoid asobara tabida (Hymenoptera, Braconidae). J. Evol. Biol. 12 (1), 123. https://doi.org/10.1046/j.1420-9101.1999.00018.x. –28.

Forbes, Mark, R.L., 1993. Parasitism and host reproductive effort. Oikos 67 (3), 444–450. https://doi.org/10.2307/3545356.

Frank, S.A., Schmid-Hempel, P., 2008. Mechanisms of pathogenesis and the evolution of parasite virulence. J. Evol. Biol. 21 (2), 396–404. https://doi.org/10.1111/j.1420-9101.2007.01480.x. March.

Gupta, V., Stewart, C.O., Rund, S.S.C., Monteith, K., Vale, P.F., 2017. Costs and benefits of sublethal drosophila C virus infection. J. Evol. Biol. 30 (7), 1325–1335. https://doi.org/10.1111/jeb.13096. July.

Hidalgo, B., Armitage, S.A.O., 2022. Host resistance to bacterial infection varies over time, but is not affected by a previous exposure to the same pathogen. Front. Physiol. 13. https://doi.org/10.3389/fphys.2022.860875.
Hidalgo, B.A., Silva, L.M., Franz, M., Regoes, R.R., Armitage, S.A.O., 2022. Decomposing virulence to understand bacterial clearance in persistent infections. Nat. Commun. 13, 5023. https://doi.org/10.1038/s41467-022-32118-1.
Hudson, A.L., Moatt, J.P., Vale, P.F., 2020. Terminal investment strategies following infection are dependent on diet. J. Evol. Biol. 33 (3), 309–317. https://doi.org/10.1111/jeb.13566.
Hurd, H.., 2001. Host fecundity reduction: a strategy for damage limitation? Trend. Parasitol. 17 (8), 363. https://doi.org/10.1016/S1471-4922(01)01927-4. August 1–68.
Kutzer, M.a.M., Kurtz, J., Armitage, S.a.O., 2018. Genotype and diet affect resistance, survival, and fecundity but not fecundity tolerance. J. Evol. Biol. 31 (1), 159. https://doi.org/10.1111/jeb.13211. –71.
Kutzer, M.A.M., Armitage, S.A.O., 2016. The effect of diet and time after bacterial infection on fecundity, resistance, and tolerance in drosophila melanogaster. Ecol. Evol. 6 (13), 4229. https://doi.org/10.1002/ece3.2185. –42.
Kutzer, M.A.M., Kurtz, J., Armitage, S.A.O., 2019. A multi-faceted approach testing the effects of previous bacterial exposure on resistance and tolerance. J. Anim. Ecol. 88 (4), 566. https://doi.org/10.1111/1365-2656.12953. –78.
Kutzer, M.A.M., Gupta, V., Neophytou, K., Doublet, V., Monteith, K.M., Vale, P.F., 2023. Intraspecific genetic variation in host vigour, viral load and disease tolerance during Drosophila C virus infection. Open Biol. 13, 230025. https://doi.org/10.1098/rsob.230025.
Lazzaro, B.P., Sackton, T.B., Clark, A.G., 2006. Genetic variation in drosophila melanogaster resistance to infection: a comparison across bacteria. Genetics 174 (3), 1539. https://doi.org/10.1534/genetics.105.054593. November–54.
Lemaitre, B., Hoffmann, J., 2007. The host defense of drosophila melanogaster. Annu. Rev. Immunol. 25, 697–743. https://doi.org/10.1146/annurev.immunol.25.022106.141615.
Linder, J.E., Promislow, D.E.L., 2009. Cross-generational fitness effects of infection in drosophila melanogaster. Fly (Austin) 3 (2), 143. https://doi.org/10.4161/fly.8051. April 15–50.
Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88 (1), 87–98. https://doi.org/10.1034/j.1600-0706.2000.880110.x.
Martins, N.E., Faria, V.G., Teixeira, L., Magalhães, S., Sucena, É., 2013. Host adaptation is contingent upon the infection route taken by pathogens. PLoS Pathog. 9 (9), e1003601. https://doi.org/10.1371/journal.ppat.1003601. September 26.
McKean, K.A., Yourth, C.P., Lazzaro, B.P., Clark, A.G., 2008. The evolutionary costs of immunological maintenance and deployment. BMC Evol. Biol. 8 (1), 76. https://doi.org/10.1186/1471-2148-8-76. March 3.
McNamara, J.M., Houston, A.I., 1996. State-dependent life histories. Nature 380 (6571), 215. https://doi.org/10.1038/380215a0. March–21.
Minchella, D.J., 1985. Host life-history variation in response to parasitism. Parasitology 90 (1), 205. https://doi.org/10.1017/S0031182000049143. February–16.
Minchella, D.J., Loverde, P.T., 1981. A cost of increased early reproductive effort in the snail biomphalaria glabrata. Am. Nat. 118 (6), 876. –81.
Ordovás-Montañés, M., Preston, G.M., Drew, G.C., Rafaluk-Mohr, C., King, K.C., 2022. Reproductive consequences of transient pathogen exposure across host genotypes and generations. Ecol. Evol. 12, e8720. https://doi.org/10.1002/ece3.8720.
Parker, B.J., Barribeau, S.M., Laughton, A.M., Roode, J.C.de, Gerardo, N.M., 2011. Non-immunological defense in an evolutionary framework. Trend. Ecol. Evol. (Amst.) 26 (5), 242. https://doi.org/10.1016/j.tree.2011.02.005. May 1–48.
Parker, B.J., Garcia, J.R., Gerardo, N.M., 2014. Genetic variation in resistance and fecundity tolerance in a natural host-pathogen interactions: brief communication. Evolut. (N.Y.). https://doi.org/10.1111/evo.12418. Mayn/a-n/a.
Perrin, N., Christe, P., Richner, H., 1996. On host life-history response to parasitism. Oikos 75 (2), 317. https://doi.org/10.2307/3546256. –20.
Pike, V.L., Ford, S.A., King, K.C., Rafaluk-Mohr, C., 2019. Fecundity compensation is dependent on the generalized stress response in a nematode host. Ecol. Evol. 9, 11957–11961. https://doi.org/10.1002/ece3.5704.
R Core Team, 2021. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.
Råberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318 (5851), 812. https://doi.org/10.1126/science.1148526. November 2–14.
Romano, V., Lussiana, A., Monteith, K.M., MacIntosh, A.J.J., Vale, P.F., 2022. Host genetics and pathogen species modulate infection-induced changes in social aggregation behaviour. Biol. Lett. 18, 20220233. https://doi.org/10.1098/rsbl.2022.0233.
Sadd, B.M, Siva-Jothy, M.T, 2006. Self-harm caused by an insect's innate immunity. In: Proceedings of the Royal Society B: Biological Sciences, 273, p. 2571. https://doi.org/10.1098/rspb.2006.3574. August–74.
Schmid-Hempel, P., 2005. Evolutionary ecology of insect immune defenses. Annu. Rev. Entomol. 50 (1), 529–551. https://doi.org/10.1146/annurev.ento.50.071803.130420.
Schmid-Hempel, P., 2009. Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. Philosoph. Transact. Roy. Soc. B: Biolog. Sci. 364 (1513), 85–98. https://doi.org/10.1098/rstb.2008.0157. January 12.
Schmid-Hempel, P., 2003. Variation in immune defence as a question of evolutionary ecology. In: Proceedings of the Royal Society of London. Series B: Biological Sciences, 270, p. 357. https://doi.org/10.1098/rspb.2002.2265. February 22–66.
Schulz, N.K.E., Stewart, C.M., Tate, A.T., 2023. Female investment in terminal reproduction or somatic maintenance depends on infection dose. Ecol. Entomol. 48, 714–724. https://doi.org/10.1111/een.13268.
Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trend. Ecol. Evol. (Amst.) 11 (8), 317. https://doi.org/10.1016/0169-5347(96)10039-2. August 1–21.
Shirasu-Hiza, M.M., Schneider, D.S., 2007. Confronting physiology: how do infected flies die? Cell. Microbiol. 9 (12), 2775. https://doi.org/10.1111/j.1462-5822.2007.01042.x. –83.
Singh, K., Kochar, E., Prasad, N.G., 2015. Egg viability, mating frequency and male mating ability evolve in populations of drosophila melanogaster selected for resistance to cold shock. PLoS ONE 10 (6), e0129992. https://doi.org/10.1371/journal.pone.0129992. June 11.
Siva-Jothy, J.A., Vale, P.F., 2021. Dissecting genetic and sex-specific sources of host heterogeneity in pathogen shedding and spread. PLoS Pathog. 17, e1009196. https://doi.org/10.1371/journal.ppat.1009196.
Siva-Jothy, J.A., Vale, P.F., 2019. Viral infection causes sex-specific changes in fruit fly social aggregation behaviour. Biol. Lett. 15, 20190344. https://doi.org/10.1098/rsbl.2019.0344.
Steiner, U.K., Tuljapurkar, S., 2012. Neutral theory for life histories and individual variability in fitness components. Proceed. Natl. Acad. Sci. 109 (12), 4684. https://doi.org/10.1073/pnas.1018096109. March 20–89.
Stephenson, J.F., 2019. Parasite-induced plasticity in host social behaviour depends on sex and susceptibility. Biol. Lett. 15 (11), 20190557. https://doi.org/10.1098/rsbl.2019.0557. November 29.
Troha, K., Buchon, N., 2019. Methods for the Study of Innate Immunity in *Drosophila Melanogaster*. WIREs Developmental Biology 8 (5), e344. https://doi.org/10.1002/wdev.344. September.
Vale, P.F., Little, T.J., 2012. Fecundity compensation and tolerance to a sterilizing pathogen in daphnia. J. Evol. Biol. 25 (9), 1888. https://doi.org/10.1111/j.1420-9101.2012.02579.x. –96.
Vallet-Gely, I., Lemaitre, B., Boccard, F., 2008. Bacterial strategies to overcome insect defences. Nat. Rev. Microbiol. 6, 302–313. https://doi.org/10.1038/nrmicro1870.
Vincent, C.M., Simoes da Silva, C.J., Wadhawan, A., Dionne, M.S., 2020. Origins of metabolic pathology in francisella-infected drosophila. Front. Immunol. 11.
Wilson, A.J., Nussey, D.H., 2010. What is individual quality? An evolutionary perspective. Trend. Ecol. Evol. (Amst.) 25 (4), 207–214. https://doi.org/10.1016/j.tree.2009.10.002. April 1.
White, L.A., Siva-Jothy, J.A., Craft, M.E., Vale, P.F., 2020. Genotype and sex-based host variation in behaviour and susceptibility drives population disease dynamics. Proceed. Roy. Soc. B: Biolog. Sci. 287, 20201653. https://doi.org/10.1098/rspb.2020.1653.
Ye, Y.H., Chenoweth, S.F., McGraw, E.A., 2009. Effective but costly, evolved mechanisms of defense against a virulent opportunistic pathogen in drosophila melanogaster. PLoS Pathog. 5 (4), e1000385. https://doi.org/10.1371/journal.ppat.1000385. April 17.