Title: Disparate regulation of *imd* drives sex differences in infection tolerance in *Drosophila melanogaster*

Crystal M. Vincent and Marc S. Dionne

MRC Centre for Molecular Bacteriology and Infection and Department of Life Sciences, Imperial College London, London SW7 2AZ, United Kingdom

Correspondence: crystal.vincent@imperial.ac.uk and m.dionne@imperial.ac.uk

Lead Contact: crystal.vincent@imperial.ac.uk

Abstract

Male and female animals exhibit differences in infection outcomes. One possible source of sexually dimorphic immunity is sex-specific costs of immune activity or pathology, but little is known about the independent effects of immune-induced versus microbe-induced pathology, and whether these may differ for the sexes. Here, through measuring metabolic and physiological outputs in wild-type and immune-compromised *Drosophila melanogaster*, we test whether the sexes are differentially impacted by these various sources of pathology. We find that in the fruit fly, the sexes exhibit differential immune activity but similar bacteria-derived metabolic pathology. We show that female-specific immune-inducible expression of *PGRP-LB*, a negative regulator of the Imd pathway, enables females to reduce immune activity in response to reductions in bacterial numbers. In the absence of this regulator, females are more resistant of infection, indicating that female-biased immune restriction comes at a cost. These data suggest that male and female flies exhibit broadly similar abilities to resist infection, but females exhibit greater tolerance of infection as a result of increased immune regulation.

Keywords: *Drosophila melanogaster*, *imd*, *PGRP-LB*, Infection, Sexual dimorphism

Introduction

Across multiple taxa, the sexes differ in their responses to infection, including differing incidences of infection, pathogen loads, pathogen-derived virulence, and efficacy of the immune response (D. F. Duneau et al., 2017; Klein and Flanagan, 2016; Lotter and Altfeld, 2019; Nunn et al., 2009; Taylor and Kimbrell, 2007; vom Steeg and Klein, 2016). Sex-specific costs of immune activity or pathology could come in the form of pathogen-induced harm (virulence), immunopathology, trade-offs between immunity and other important traits, or a combination of the three. The ability to lessen immune-induced pathology (disease tolerance) is an essential part of an effective immune response (Medzhitov et al., 2012; Soares et al., 2017); given this, the often observed difference in immune activity between the sexes (Klein and Flanagan, 2016) suggests differential immunopathology as a possible source of sex-specific infection outcomes.

That immunity results in trade-offs in longevity, reproduction, competitive ability and development, has been well demonstrated (Adamo et al., 2001; Jacot et al., 2004; Kraaijeveld and Godfray, 1997; McKean and Nunney, 2005; Moret and Schmid-Hempel, 2000; Schwenke et al., 2016; Simmons et al., 2010; Ye et al., 2009). In contrast, little is known about the independent effects of immune-induced versus microbe-induced pathology, and whether these may differ for the sexes. In order to distinguish these effects, we consider the response of wild-type and immunocompromised male and female flies to infection with *Escherichia coli*; this infection is efficiently controlled by the wild-type immune response but cannot be controlled in immunocompromised mutants. We sought to distinguish between pathology resulting from the immune response and pathology resulting from the microbe. Our metabolic and physiological assays allowed us to test whether the sexes are differentially impacted by these various sources of pathology. We show differential immune regulation

between the sexes, such that females limit immune activity at the cost of bacterial clearance but to the benefit of reproductive fitness.

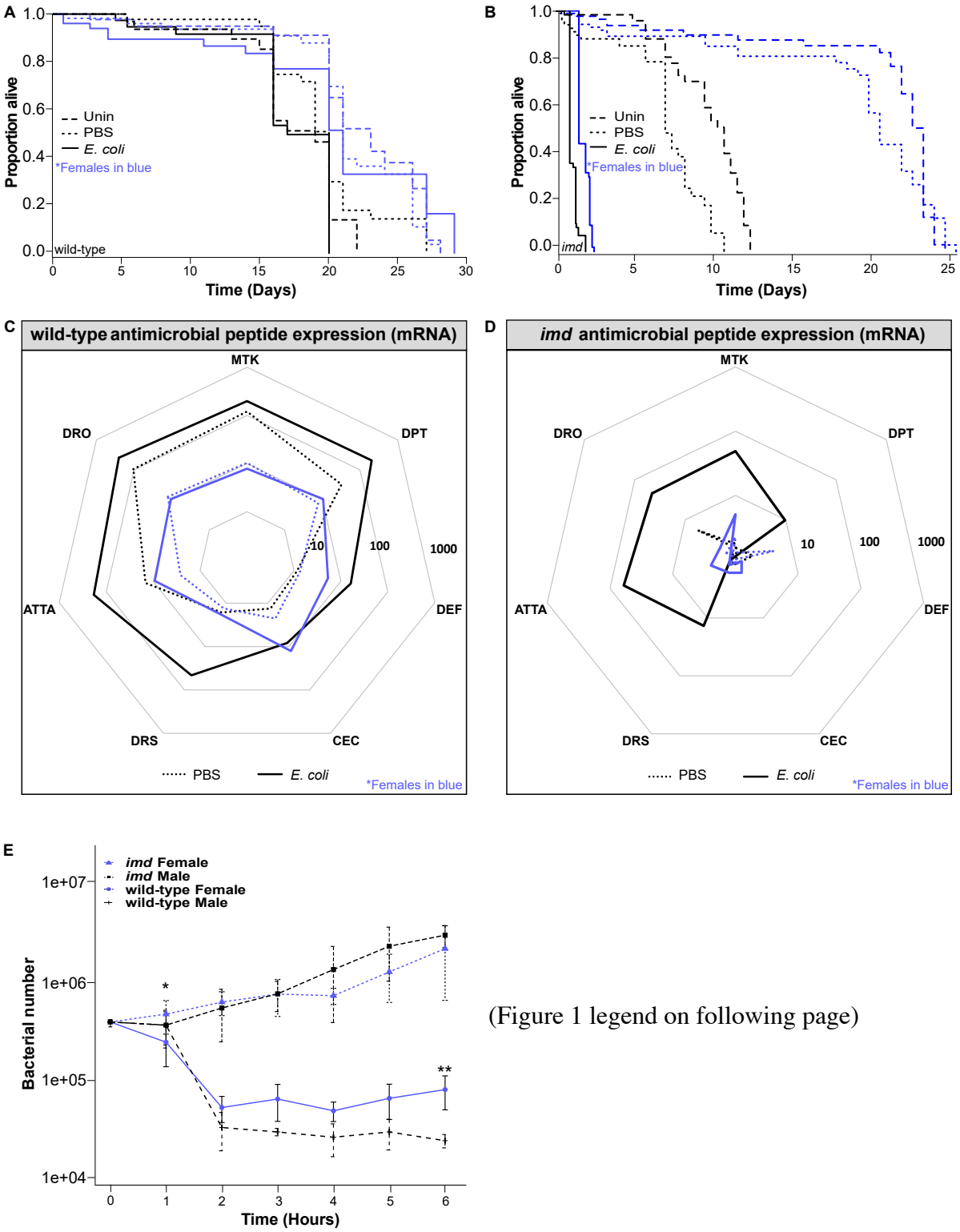
Results

imd is critical for survival during *E. coli* infection

Work in our lab as well as others has found that *D. melanogaster* infected with *E. coli* either eliminate the bacteria or maintain them at low levels at no obvious cost to the host (D. Duneau et al., 2017; Khalil et al., 2015). Independent of infection status, females lived longer than males. As expected, we did not find a strong effect of infection with live or dead (heat-killed) *E. coli* on the lifespan of wild-type flies (Fig. 1A, Figure 1 – figure supplement 1A). These findings confirm that *E. coli* infection is non-lethal in flies with an intact immune system and indicate that neither prior activation of the immune response, nor *E coli* virulence, leads to a sex difference in survival (Fig. 1A).

imd mutants have a defect in the signalling pathway required to activate production of the antimicrobial peptides (AMPs) responsible for clearing Gram-negative bacteria, including *E. coli* (Hoffmann and Reichhart, 2002; Lemaitre et al., 1995). We infected *imd* mutants with *E. coli*; we also injected a subset of these flies with latex beads to inhibit their ability to phagocytose bacteria (Elrod-Erickson et al., 2000), resulting in flies with both phagocytosis and antimicrobial peptide activity inhibited. *imd* flies of both sexes had significantly reduced survival when infected with *E. coli* compared to their PBS and uninfected controls (Fig. 1B). In males, PBS-injection alone led to a shorter lifespan. Whilst *imd* females showed a reduced lifespan when injected with PBS, this was not significantly different from uninfected controls. Inhibiting the phagocytic response with latex beads did not affect survival in either sex (Figure 1 – figure supplement 1B), further supporting the idea that AMP activity plays the primary role in this infection. As in wild-type flies, independent of infection status, *imd*

females lived longer than males. Together with the wild-type findings, these experiments confirm that the Imd pathway is critical for surviving *E. coli* infection for both sexes.



(Figure 1 legend on following page)

Figure 1. Sex-specific outcomes of *E. coli* infection in wild-type and *imd* flies. Representation in all plots: males - black; females - blue.
(A and B) Survival of *E. coli* infected flies. *Escherichia coli* infected flies are indicated by solid lines. Uninfected and PBS controls are indicated by long and short dashed lines, respectively. Wild-type flies (A) had an average median survival across all treatments of 21.5d and 18.5d for females and males, respectively (Coxph: df = 7, n = 396, Wald test = 43.75, p = 2e-07). There was no effect of treatment on survival in either sex. Median survival of *E. coli* infected *imd* flies (B) was 41h and 27h for females and males, respectively (Coxph: df = 9, n = 255, Wald test = 126.2, p = <2e-16). Both PBS and infection reduced male survival whilst female survival was only affected by infection. Survivals were performed 2 or 3 times, each repeat included 2 or 3 biological replicates/ treatment consisting of 20-40 flies each.
(C and D) Antimicrobial peptide transcript levels 6h post infection in wild-type (C) and *imd* (D) flies. Expression is shown relative to uninfected flies of the same genotype/sex. Solid lines represent infection with *E. coli* whilst dotted are PBS injected. The area contained within the innermost heptagon represents induction levels falling between one and ten times that of the uninfected controls. The middle and outer heptagons represent 100 and 1000-fold induction, respectively. These data are also shown, represented differently, in Figure 1 – figure supplement 2. AMP assays were performed 2 - 4 times, each repeat included 3 or 4 biological replicates/treatment consisting of 3 flies each.
(E) Bacterial quantification in wild-type and *imd* flies of both sexes. *imd* flies exhibited no difference in bacterial number. Wild-type females had fewer bacteria 1h after injection (t-test = -2.495, p = 2.03e-02, n = 26) but more bacteria at 6h (t-test = 5.397, p = 1.3e-03, n = 25). No difference in bacterial load in wild-type flies was observed at any other time. Markers indicate means and bars represent SE. Statistical significance: * p<0.05; ** p<0.01. Quantifications were performed twice, each repeat included 8-16 biological replicates consisting of 1 fly each.

Sex-specific AMP induction and bacterial load is observed in wild-type but not *imd* flies

The primary described role of the Imd pathway is to drive expression of antimicrobial peptides in response to infection with an array of bacteria, including *E. coli* (De Gregorio et al., 2002). Having confirmed that this pathway is critical for defence against *E. coli* (Figure 1A, B), we measured AMP expression in wild-type flies 6h after infection (the reported peak of relevant AMP production in males (Lemaitre et al., 1997)) to see if induction differed between the sexes. We found that infected males, on average, had AMP transcript levels 16x greater than females (Fig. 1C, Figure 1 – figure supplement 2A). This magnitude of difference was not found in PBS injected animals, confirming that differences in wound-induced expression were not the cause. As expected, AMP expression was markedly reduced in *imd* mutants; however, we did observe some AMP induction in these flies, possibly

attributable to partial retention of *imd* activity (Pham et al., 2007) or Toll pathway activation (Fig. 1D, Figure 1 – figure supplement 2B). This residual AMP expression was particularly prominent in male flies.

Greater AMP expression observed in male flies could result from males harbouring more bacteria. Indeed, one potential cause of sex differences in survival during infection could be that bacterial proliferation differs (Yamamoto et al., 1991), causing one sex to experience more pathology due to greater bacterial loads. We only considered the first 6h of infection as bacterial numbers are significantly reduced from the initial inoculum within this period, becoming undetectable at 24h. Even with the observed difference in AMP induction, we found no significant difference between the sexes in *E. coli* numbers throughout most of the assayed time in wild-type flies (Fig. 1E), with the exception of one hour after injection when females had significantly fewer bacteria, and six hours after injection when females had significantly more.

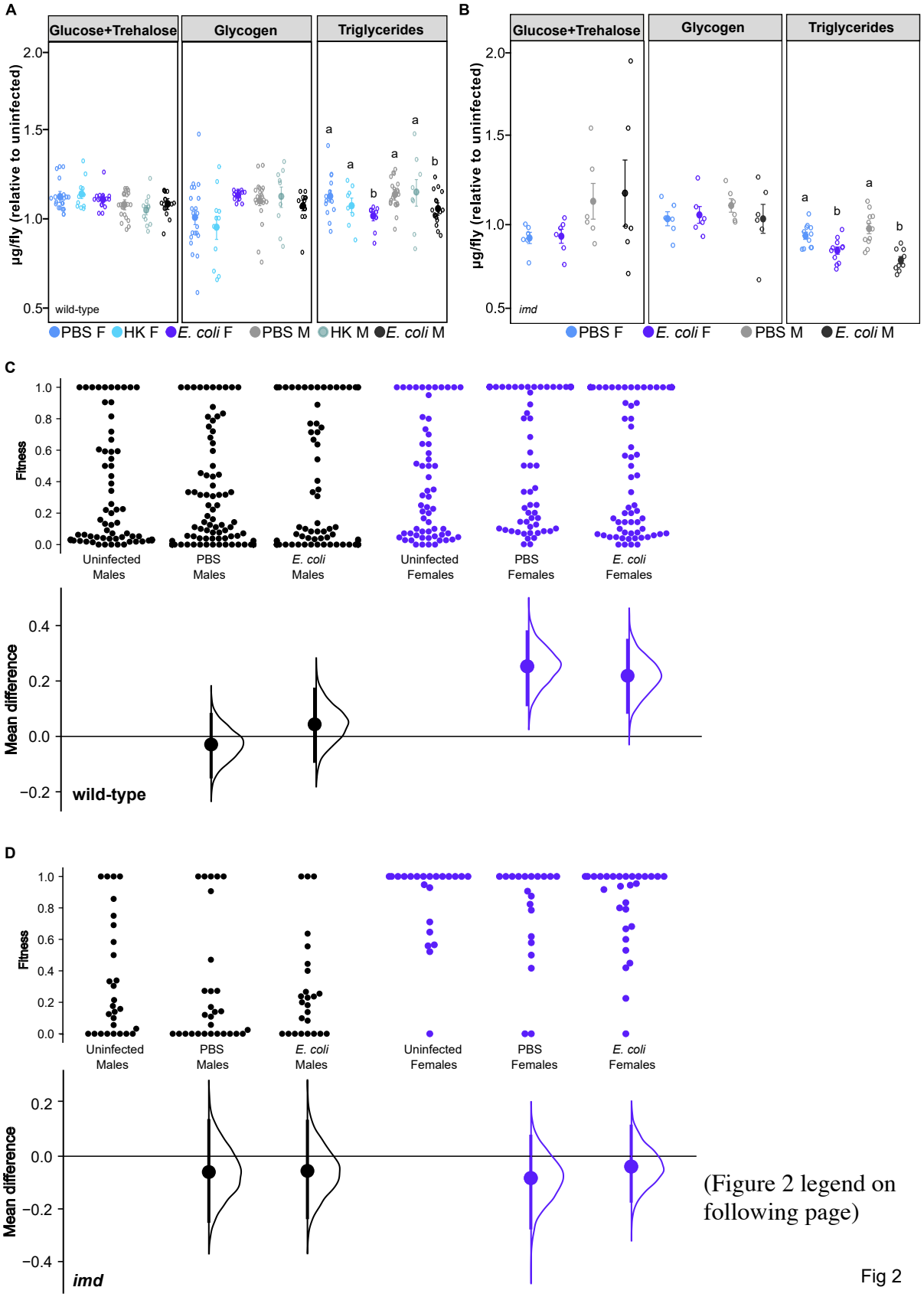
Having observed that *imd* mutants were susceptible to *E. coli* infection, and the large disparity between the sexes in AMP induction, we tested whether the effectively AMP-deficient *imd* females had higher bacterial loads than males, but observed no significant difference in bacterial numbers between the sexes (Fig. 1E). Furthermore, pre-injection of latex beads to block phaogcytosis did not generate a difference in bacterial numbers between male and female *imd* mutants, though bead-injected animals had roughly 6x more colony forming units (CFUs, Figure 1 – figure supplement 1C). These results suggest that *imd* flies were overwhelmed by the number of bacteria to the point that a further inability to clear bacteria via phagocytosis did not affect the lethality of the infection.

Infection with *E. coli* leads to depletion of triglycerides

Because resources are finite, individuals must manage investments in multiple biological processes. The ability to draw on metabolic reserves of triglyceride or glycogen allows animals to run temporary metabolic deficits in response to unexpected costs (e.g. immunity). Conversely, when reserves are depleted in animals fed *ad libitum*, this likely reflects the presence of a cost that cannot be met by redirecting energy away from other processes. We hypothesised that the sexes might differ in immune activity if they experience different infection-induced metabolic burdens, or have differing abilities to meet these burdens. To test this, we assayed metabolic changes during infection with and without an intact AMP response (wild-type and *imd* flies, respectively). Previous studies in *D. melanogaster* found that lethal bacterial infections can lead to hyperglycaemia, as well as a reduction in triglyceride and glycogen stores (Chambers et al., 2012; Dionne et al., 2006; Vincent et al., 2020). Therefore, we looked at levels of free sugar, nutrient stores (triglyceride and glycogen), and respiration during *E. coli* infection in wild-type flies (which survive the infection) and *imd* mutants (which succumb to the infection). We found that 6h post infection with *E. coli*, flies had significantly less stored triglyceride than their PBS controls; this effect was independent of both genotype and sex (Fig. 2A, B, Figure 2 – table supplement 1). Importantly, infection with heat-killed *E. coli* did not deplete triglyceride, indicating that this effect is pathogen-driven. Free sugar and glycogen levels as well as respiration were unaffected by infection (Fig. 2A, B, Figure 2 – figure supplement 1).

Because animals spend significant energy on reproduction and reproductive effort is likely to restrict or trade-off with immunity (Lawniczak et al., 2007), changes to reproductive activity during infection have the potential to elucidate shifting allocation of resources. We assayed reproductive fitness during infection. We placed infected flies in tubes with flies of the opposite sex and 'competitors' of the same sex, but of a different genotype (*Dh44*[3xP3-DsRed]). We allowed flies to mate for 10h and then discarded adults. Offspring resulting

from matings with competitors were easily identifiable by their red-fluorescent eyes. Fitness was calculated as the ratio of focal offspring to total offspring (Vincent and Sharp, 2014).



(Figure 2 legend on following page)

Fig 2

Figure 2. No sex difference in pathology of *E. coli* infection in wild-type and *imd* flies. Representation in all plots: males (black); females (blue).
(A and B) Triglyceride and carbohydrate levels in *E. coli*-infected flies. In wild-type flies (A) no effect of sex nor infection status was found on circulating sugar (glucose + trehalose) and glycogen levels. In contrast, there was an effect of both sex and infection status on triglyceride; males had more triglyceride than females (AOV: df = 1, n = 40, F = 0.0319, p = 4.7e-02) and overall, *E. coli* infection led to triglyceride depletion (AOV: df = 1, n = 40, F = 0.183, p = 1.8e-05). There was a significant interaction between sex and infection such that *E. coli* infection led to significant triglyceride depletion in males relative to their PBS controls (p-adjusted = 2.9e-04). *imd* flies (B) exhibited parallel metabolic shifts to wild-type; no effect of sex nor infection status was found on circulating sugar (glucose + trehalose) and glycogen levels. There was no effect of sex on triglyceride levels, but there was an overall effect of both treatment (AOV: df = 1, n = 49, F = 44.971, p = 2.8e-08) and the interaction between sex and treatment on triglycerides; *E. coli* infection led to triglyceride depletion in both sexes, relative to their PBS controls (AOV: df = 1, n = 49, F = 7.417, p = 9.2e-03; M PBS-M *E. coli*, p-adjusted = 4.0e-07; F PBS-F *E. coli* p-adjusted = 1.01e-02). Bars indicate SE. Letters indicate statistical groupings. Full statistics including non-significant results can be found in Figure 2 – table supplement 1. All assays were performed 2 or 3 times, each repeat included 4 biological replicates/treatment consisting of 3 (carbohydrates) or 8 (triglycerides) flies each.
(C and D) Reproductive fitness in wild-type (C) and *imd* (D) flies. Cumming estimation plots showing the mean difference between each treatment and the uninfected controls. The upper axes show the distribution of the raw data; mean differences are shown on the lower axes as a bootstrap sampling distribution. Each dot represents one sample (fitness of 1 group of focal flies) and error bars represent 95% confidence intervals. *E. coli* infected and PBS injected wild-type females produced more offspring than uninfected females (PBS: Mann-U = 1448, p = 1.3e-04, n = 137; *E. coli*: Mann-U = 1849.5, p = 1.6e-03, n = 148), and *E. coli* infected females had greater reproductive success than *E. coli* infected males (Kruskal-Wallis = 32.19, p = 5.4e-06, posthoc-Dunn's = 0.04). Unlike females, male fitness was unaffected by infection (PBS: Mann-U = 3421, p = 0.35, n = 160; *E. coli*: Mann-U = 2723, p = 0.49, n = 143). There was no effect of infection on reproduction for *imd* mutants (Males: PBS: Mann-U = 464, p = 0.35, n = 58; *E. coli*: Mann-U = 369, p = 0.74, n = 53; Females: PBS: Mann-U = 255, p = 0.34, n = 42; *E. coli*: Mann-U = 362, p = 0.37, n = 51). Fitness assays were repeated 2 or 3 times, with 15 biological replicates/treatment consisting of 3 flies each.

Wild-type females injected with either *E. coli* or sterile PBS had significantly greater reproductive success compared to their uninfected controls; in contrast, male reproduction was unaffected by infection (Fig. 2C). After normalizing the data to uninfected controls, we observed a significant difference in fitness between *E. coli* infected males and females, with females exhibiting greater reproductive output. This increased reproduction by females could be facilitated by the redirection of resources that would otherwise be directed toward AMP

production (Fig. 1C); this possibility suggests that females trade off bacterial clearance with reproductive fitness. Infection had no effect on the fitness of *imd* mutants (Fig. 2D).

imd activity is differentially regulated by the sexes

We anticipate adaptive metabolic shifts in wild-type animals during infection to be reflected in the efficient resolution of infection-induced gene expression once a threat has been cleared. Our measurements of AMPs focused on six hours after infection because this is the observed peak of *Diptericin* induction in wild-type animals (Lemaitre et al., 1997). However, our data indicate that bacterial numbers are controlled earlier than this, suggesting that by six hours, an efficient immune response would have reduced AMP expression. Previous work demonstrated that persistent activation of the Imd pathway is responsible for pathology during *Erwinia carotovora carotovora* infection in *D. melanogaster* (Charroux et al., 2018). These findings, combined with our observation that infected females have greater reproductive fitness and decreased AMP expression, suggested that females might appear to have lower immune activation because they were better able to shut down the immune response once bacterial numbers were successfully reduced. We tested this by assaying antimicrobial peptide expression three hours after infection, during the period when bacterial killing was observed; at this timepoint, we found that female AMP induction was equivalent to (or greater than) that of males (Fig. 3A). Additionally, AMP levels of infected animals at 3h were notably greater in females than at 6h (Fig. 3B), whilst male levels were unchanged (Fig. 3C), suggesting that females were responsive to bacterial killing as a cue to shut down immune activity.

Several negative regulators of Imd pathway activity have been described (Aparicio et al., 2013; Fernando et al., 2014; Kleino et al., 2008; Zaidman-Rémy et al., 2006). To identify mechanisms underlying the greater ability of females to turn off immune activation in

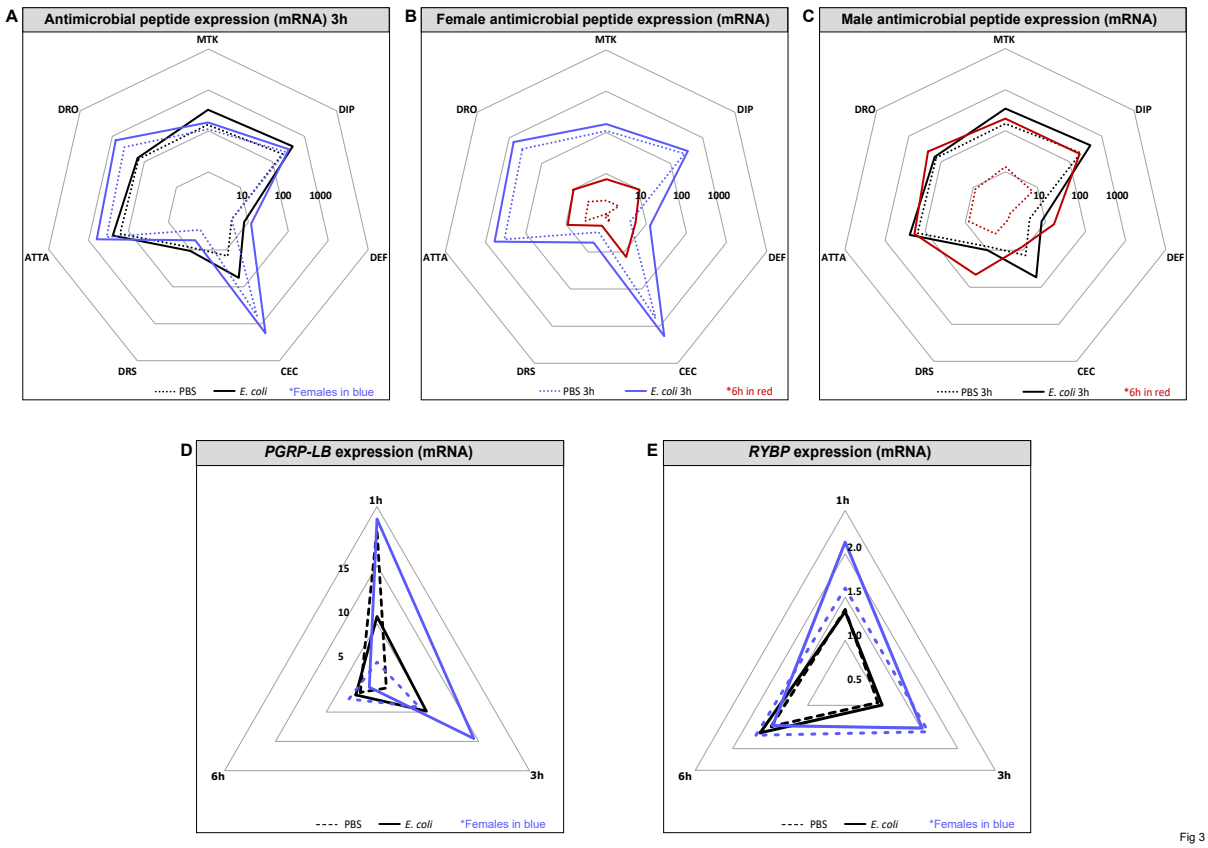


Fig 3

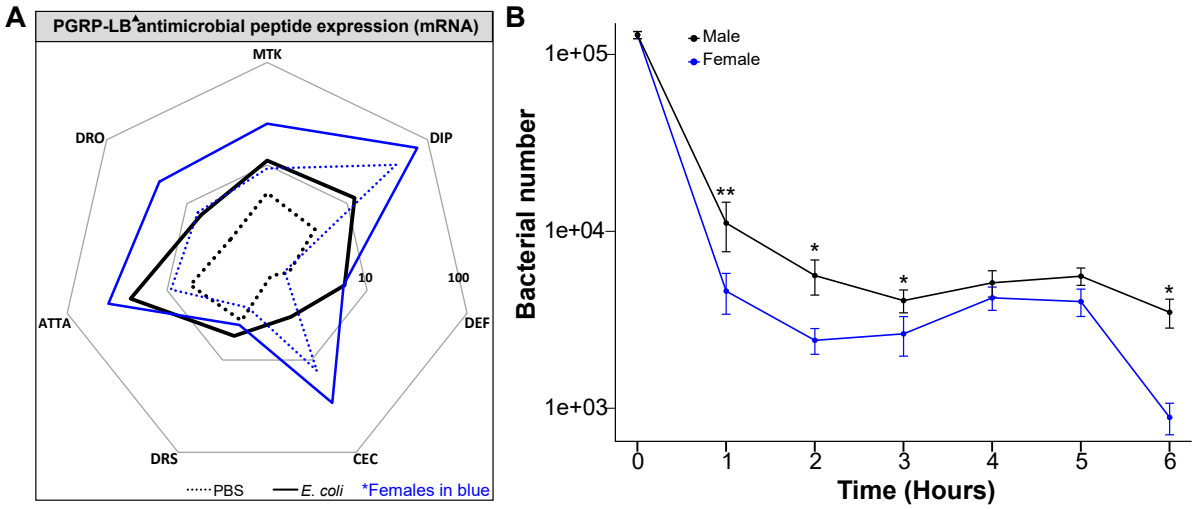
Figure 3. Sex-specific temporal regulation of *imd* during *E. coli* infection wild-type flies. Representation in all plots: males - black; females - blue.
(A-C) Antimicrobial peptide transcript levels in males and females 3h after infection (A), females (B) and males (C) 3h and 6h after infection. Expression is shown relative to uninfected flies of the same genotype/sex. Solid lines represent infection with *E. coli* whilst dotted are PBS injected. Time points are as indicated on plots. The area contained within the innermost heptagon represents induction levels falling between one and ten times that of the uninfected controls (downregulation was not observed in any of the tested genes). The middle and outer heptagons represent 100 and 1000-fold induction, respectively.
(D and E) Expression of (D) *PGRP-LB* and (E) *RYBP* 1, 3, and 6 hours after infection in male and female flies. Plotted values are relative to the uninfected controls. Solid lines represent infection with *E. coli* whilst dotted lines represent PBS injection. AMP assays were performed 2 - 4 times, each repeat included 3 or 4 biological replicates/treatment consisting of 3 flies each.

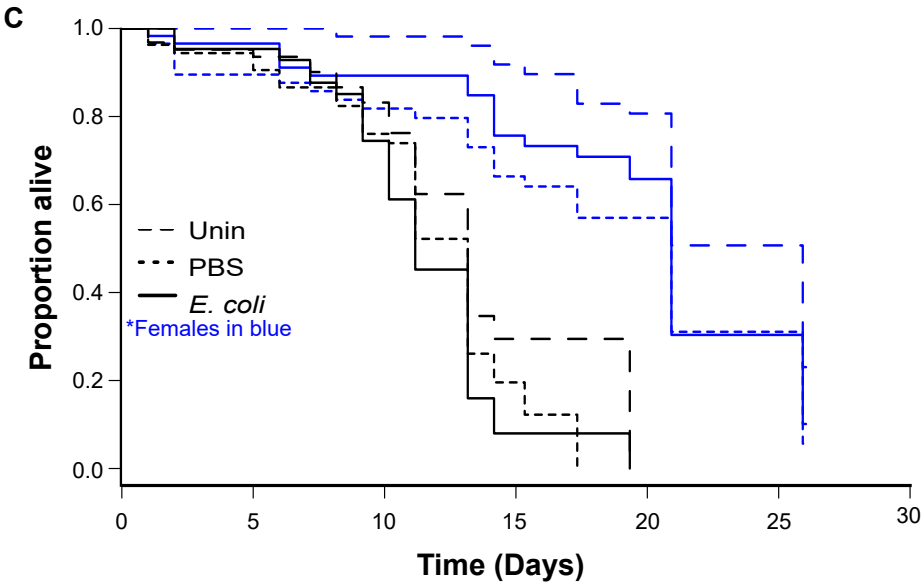
response to successful bacterial killing, we assayed these regulators for sex-biased expression. Two negative regulators – *PGRP-LB* and *RYBP* – were expressed at higher levels specifically in *E. coli* infected females 3 hours post-infection (Fig. 3D, E). *PGRP-LB* is an amidase that degrades the DAP-type peptidoglycan of Gram-negative bacteria, dampening activation of the Imd pathway by degrading the activating ligand (Zaidman-Rémy et al., 2006). In contrast, *RYBP* inhibits Imd pathway activity by promoting proteasomal

degradation of the pathway's NF-κB transcription factor, *Relish* (Aparicio et al., 2013). *PGRP-LB* was of particular interest because it reduces pathway activity by degrading free peptidoglycan – that is, it will reduce pathway activity when the immune response has been effective. To test whether *PGRP-LB* activity was responsible for the sex difference in AMP activity observed, we infected male and female *PGRP-LB* null mutants with *E. coli* and measured AMP production. In the absence of *PGRP-LB*, the male-biased AMP expression observed six hours following infection with *E. coli* was abolished (Fig. 4A). Moreover, these mutants had a strong and sex-specific effect on bacterial load. As in wild-type flies, *PGRP-LB*$^{\Delta}$ mutants drastically reduced bacterial load within the first 2 hours post-infection, at which time bacterial numbers effectively plateaued. However, in contrast to what we saw in wild-type flies, *PGRP-LB*$^{\Delta}$ males had significantly more bacteria than females throughout most of the 6h period assayed (Fig. 4B), confirming our supposition that wild-type females downregulate AMP activity at a cost of resistance, and indicating that sex-specific *PGRP-LB* induction has important functional consequences for the realised immune response.

PGRP-LB$^{\Delta}$ males and females exhibit parallel metabolic shifts during infection

We next aimed to identify the effects of *PGRP-LB* on the physiological consequences of immune activation. Similar to what we observed in wild-type flies, uninfected *PGRP-LB*$^{\Delta}$ females lived longer than males (Fig. 4C). Wounding had a significant impact on survival in both sexes, with both PBS and *E. coli* injected animals having reduced survival (though the two treatments did not differ from each other). Wounding induces AMPs (Lemaitre et al., 1997); that wounding alone was able to reduce survival in *PGRP-LB*$^{\Delta}$ flies – which have a reduced ability to downregulate *imd* – indicates that unfettered *imd* activity has survival costs for both sexes, and suggests that *PGRP-LB* may have chronic, non-sex-specific effects on





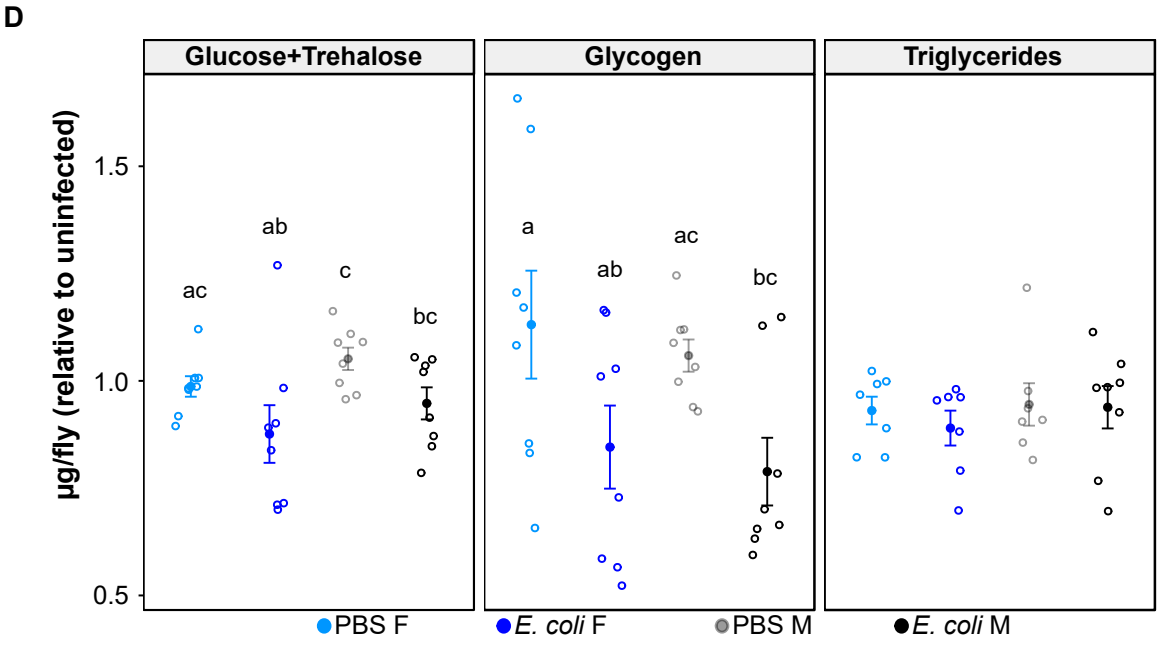


Figure 4. *PGRP-LB$^{\Delta}$* males and females exhibit parallel metabolic shifts during infection.

Representation in all plots: males - black; females - blue.

(A) AMP expression is shown relative to uninfected flies of the same genotype/sex. Solid lines represent infection with *E. coli* whilst dotted are PBS injected. The area contained within the innermost heptagon represents induction levels falling between one and ten times that of the uninfected controls. The outer heptagon represents 100-fold induction. Assays were performed twice, each repeat included 4 biological replicates/treatment consisting of 3 flies each.

(B) Bacterial load observed over the first 6h of infection. Males had significantly higher bacterial loads throughout most of the observation period (1h: Mann-U = 21, p = 6.1e-03, n = 23; 2h Mann-U = 48, p = 0.039, n = 27; 3h: Mann-U = 52, p = 0.022, n = 29; 4h Mann-U = 75, p = 0.45, n = 27; 5h: t-test = -1.49, p = 0.15, n = 24; 6h Mann-U = 1.5, p = 0.012, n =12). Markers indicate means and bars represent SE. Statistical significance: * p<0.05; ** p<0.01. Quantifications were performed twice, each repeat included 8-16 biological replicates consisting of 1 fly each.

(C) Survival of flies infected with *E. coli* indicated by solid lines. Uninfected and PBS controls are indicated by long and short dashed lines, respectively. *E. coli*-infected females had a median survival 86% greater than that of males (Female = 20.9d, Male = 11.2d; Coxph: df = 5, n = 344, Wald test = 125.5, p = 1.0e-16). Survivals were repeated twice, each repeat included 2 biological replicates/treatment consisting of 20-40 flies each.

(D). The amount of circulating sugars in *E. coli*-infected animals was lower than in PBS controls (AOV: df = 1, n = 30, F = 6.44, p = 1.7e-02); whereas sex had no effect on circulating sugars, nor was there a significant interaction between the two. Similarly, *E. coli*-infection led to marked reduction in stored glycogen (AOV: df = 1, n = 30, F = 9.41, p = 4.8e-03), with no effect of sex, nor a significant interaction between sex and treatment. Neither infection status nor sex effected triglyceride levels. Large filled markers indicate means while smaller circles represent individual data points. Letters indicate statistical groupings. Bars indicate SE. All assays were performed twice, each repeat included 4 biological replicates/treatment consisting of 3 (carbohydrates) or 8 (triglycerides) flies each. Full statistics including non-significant results can be found in Figure 4 - table supplement 1.

Imd pathway activity as well as the sex-specific effect stemming from acute transcriptional induction we have reported here.

Finally, we used the *PGRP-LB$^{\Delta}$* mutant to explore the extent to which the metabolic consequences of acute infection are driven by host or pathogen-derived activities. We predicted that if triglyceride loss during *E. coli* infection in wild-type and *imd* flies is driven entirely by pathogen-derived costs, that the nearly 10-fold reduction of bacterial load observed in infected *PGRP-LB$^{\Delta}$* flies might be sufficient to abrogate triglyceride loss; conversely, if triglyceride loss were driven by Imd pathway activity, the prolonged Imd

pathway activation observed in *PGRP-LB* mutants should result in greater loss of triglyceride than in wild-type animals. We found that triglyceride levels were unaffected by *E. coli* infection, confirming that Imd pathway activity was not the cause of triglyceride depletion in this infection. Infected flies had lower levels of circulating sugars and glycogen, independent of sex (Fig. 4D, Figure 4 – table supplement 1). Bacteria have been shown to make use of host resources during infection (Chen et al., 2017; Gyuranecz et al., 2010; Radlinski et al., 2018) and while this would be surprising in these infections as bacterial numbers are declining in wild-type and *PGRP-LB*$^{\Delta}$ flies, it remains a possibility. Indeed, the depletion of circulating sugars and glycogen in *PGRP-LB*$^{\Delta}$ flies supports a model of pathogen-derived glycogenolysis (Gyuranecz et al., 2010).

Discussion

Differences between males and females in immune activity and infection outcomes are pervasive throughout the animal kingdom. Here, we have explored the differences between male and female *Drosophila* in their response to a non-pathogenic Gram-negative bacterial infection. Though both males and females could control this infection at the cost only of transient metabolic depletion, our analysis revealed that females maintained much stricter control of their own immune response; this was achieved by female-specific transcriptional induction of a peptidoglycan amidase that degrades peptidoglycan fragments liberated from bacteria after they are killed, effectively enabling the female immune response to monitor its own effectiveness and to shut down when no longer needed. Elimination of this mechanism improved bacterial killing by the female immune response.

Stricter regulation of the Imd pathway by females suggests that immune activity may come at a greater burden to them. Uninfected wild-type females had a median survival 9.6% greater than females injected with: PBS, heat-killed *E. coli* and live *E. coli* (Figure 1 – table

supplement 1). In contrast, only injection with live *E. coli* affected male survival (down 11.7% from uninfected). Because heat-killed *E. coli* are able to activate the immune response without causing mortality (shown here and (Eleftherianos et al., 2014)), these findings indicate that immune activation comes at a greater cost to females. The same trend can be seen in *PGRP-LB$^{\Delta}$* flies where male survival was only affected by injection with live *E. coli* (down 17.9% from uninfected), whereas female survival was negatively impacted equally by PBS and *E.* coli injection (down 23.9% from uninfected) (Figure 4 – table supplement 2). Together these data support the idea that the Imd response is costly (*PGRP-LB$^{\Delta}$* flies were more negatively impacted than wild-type) and that its activity poses a greater burden to females

While females appear to pay a greater cost of immune activity, this cost does not appear to be additive as the sexes experience similar metabolic shifts during infection. The possibility that wild-type females trade-off bacterial clearance with reproductive activity is an interesting one and raises the question as to whether females would downregulate AMP activity if bacterial load were not decreasing. Previous work has shown that lethal infection with *P. aeruginosa* can lead to increased reproductive output in both male and female *D. melanogaster* (Vincent and Sharp, 2014). That we only observed greater fitness in females may indicate that such 'terminal investment' is triggered by different mechanisms in the sexes.

Unsurprisingly, *PGRP-LB$^{\Delta}$* flies had nearly 1/10 the bacterial load of wild-type over the first 6h post-infection (Fig. 1E, Fig. 4D); one possibility is bacterial loads in *PGRP-LB$^{\Delta}$* flies were maintained at levels low enough to avoid triglyceride wasting. Recent work has shown that during infection with the Gram-negative pathogen *Francisella novicida*, flies where bacterial numbers were kept low due to antibiotic treatment failed to exhibit metabolic shifts (including triglyceride loss) during infection. In contrast, when bacterial numbers

increased (still in the presence of antibiotic treatment), metabolic shifts during infection were again observed, suggesting that these changes were associated with bacterial load rather than a direct effect of the antibiotics on metabolism (Vincent et al., 2020). This idea of a 'tipping point' for infection pathology is likely to be a fruitful area for future inquiry.

The immune response, as we normally envision it, includes responses to infection that protect the host by killing pathogens or restricting their growth (resistance). In contrast, tolerance is defined as the ability to maintain health during infection. Experimentally, a more tolerant host is one that remains healthy longer at a given pathogen load (Ayres and Schneider, 2008; Medzhitov et al., 2012). Recent years have seen increasing interest in tolerance, driven in part by the idea of improving tolerance as a therapeutic approach to infection. However, despite the large body of theory surrounding tolerance, the ability to detect tolerant phenotypes (Vincent and Sharp, 2014), and the identification of tolerance-associated genes (Ayres and Schneider, 2008; Dionne et al., 2006; Howick and Lazzaro, 2017), we still know very little about the fundamental mechanisms of tolerance. It has previously been shown that *PGRP-LB* contributes to infection tolerance (Zaidman-Rémy et al., 2006); we show that this activity is in fact sexually dimorphic. We show that phenomenological differences in tolerance between the sexes can be used to identify fundamental mechanisms of infection tolerance and that the sex-specific regulation of inhibitors of immune signalling can underlie strong, complex differences in immune dynamics between the sexes.

Methods

General experimental procedures

w^{1118} flies and w^{1118};imd^{10191} were used as wild-type and Imd pathway mutants (Pham et al., 2007), respectively. *PGRP-LB*$^{\Delta}$ mutant lines used were obtained from the Bloomington Stock Center and have been previously described (Paredes et al., 2011). For all experiments, flies were collected with 24h following eclosion and kept in same-sex vials for 5 - 7 days in groups of 20. Thus, all experiments were conducted on flies between 5 and 8 days old. Flies were maintained on a sugar-yeast diet (10% w/v yeast, 8% fructose, 2% polenta, 0.8% agar, supplemented with propionic acid and nipagin) at 25°C. Injections were carried out using a pulled-glass capillary needle and a Picospritzer injector system (Parker, New Hampshire, US). Following injection flies were kept at 29°C. Bacteria were grown from single colonies overnight at 37°C shaking. Each fly was injected with 50 nanolitres of *E. coli* suspended in PBS (OD_{600} = 1.0 ~100,000 bacteria). Following re-suspension in PBS, a subset of bacteria designated for the 'heat-killed' treatment was incubated for 1h at 65°C. Sterile PBS was used as a wounding control. A subset of *imd* flies were pre-injected with either PBS or 0.2μm latex beads, FluoSpheres, Carboxylate-Modified Microspheres (Invitrogen) to inhibit phagocytosis as previously described (Elrod-Erickson et al., 2000; Pham et al., 2007). Briefly, beads were washed 3x in sterile PBS and resuspended in PBS at one fourth of the original volume of the bead stock. Flies were injected with 50nL of bead-PBS solution or PBS alone, left for 16h, and then injected with PBS or *E. coli*.

Survival assays

Survival experiments were performed at 29°C with 15-25 flies/vial. Survival was monitored daily and flies were tipped into fresh vials every 4 days.

Bacterial quantification

For each sample, 1 fly was homogenised in 100μl of sterile ddH_2O. Homogenates were serially diluted and plated onto LB agar plates where they incubated for 16-18h. Following incubation, the number of individual bacterial colonies observed on each plate was quantified and back-calculated to determine the number of CFUs present in each fly.

Gene expression – Quantitative reverse transcription PCR

For each sample, 3 flies were homogenised in 100μl of the single-step RNA isolation reagent TRI Reagent (Sigma), followed by a chloroform extraction and precipitation in isopropanol. The resultant pellet was then washed with 70% ethanol. Pellets were resuspended and subject to DNase treatment. Revertaid M-MuLV reverse transcriptase and random hexamers (Thermo Scientific) were used to carry out cDNA synthesis. 5μl of each cDNA sample was put into a 'neat' standards tube; this tube was later used to generate standards which were used to generate a standard curve for each gene. Each cDNA sample was diluted and this diluted sample used for analysis.

We used Sensimix with SYBR Green no-ROX (Bioline) for qRT-PCR. The cycling conditions were as follows: Hold 95°C for 10 min, then 45 cycles of 95°C for 15s, 59°C for 30s, 72°C for 30s, followed by a melting curve. Gene expression was calculated based on the standard curve generated during each run, normalized to the value of our housekeeping gene, *Rpl1*. Samples from PBS and infected treatments were then divided by the mean value of their uninfected controls to generate expression values relative to uninfected flies.

All gene expression experiments were performed at least twice, with three or more biological replicates per experiment.

Table 1. Primer sequences used for qRT-PCR

Gene	***Forward***	***Reverse***
AttA	5'- CACAATGTGGTGGGTCAGG -3'	5'- GGCACCATGACCAGCATT -3'
CecA1	5'- TCTTCGTTTTCGTCGCTCTC -3'	5'- CTTGTTGAGCGATTCCCAGT -3'
Def	5'- TTCTCGTGGCTATCGCTTTT -3'	5'- GGAGAGTAGGTCGCATGTGG -3'
Dpt	5'- ACCGCAGTACCCACTCAATC -3'	5'- CCCAAGTGCTGTCCATATCC -3'
Dro	5'- CCATCGAGGATCACCTGACT -3'	5'- CTTTAGGCGGGCAGAATG -3'
Drs	5'- GTACTTGTTCGCCCTCTTCG -3'	5'- CTTGCACACACGACGACAG -3'
Mtk	5'- TCTTGGAGCGATTTTTCTGG -3'	5'- TCTGCCAGCACTGATGTAGC -3'
Rpl1	5'- TCCACCTTGAAGAAGGGCTA -3'	5'- TTGCGGATCTCCTCAGACTT -3'
PGRP-LB	5'- TGATCGGAGATTGGAGAACC -3'	5'- AAGGCGATCAGGTTCTTGG -3'
RYBP	5'- GCGAAGGTGATCGAGGAG -3'	5'- GAGTTCAGGCGTGGCTTTC -3'

Measurement of triglycerides

Triglycerides were measured using thin layer chromatography (TLC) assays as described elsewhere (Al-Anzi et al., 2009). Briefly, each sample consisted of 10 flies; flies were placed

in microcentrifuge tubes and stored at -80°C until the time of analysis. To perform the TLC assay, samples were removed from the -80°C freezer and spun down (3 min at 13,000 rpm at 4°C) in 100μl of a 3:1 (v/v) mix of chloroform and methanol. Flies were then homogenised and subject to a further 'quick spin'. Standards were generated using lard dissolved in the same chloroform : methanol solution. We loaded 2μl of each standard and 20μl of each sample onto a silica gel glass plate (Millipore). Plates were then placed into a chamber pre-loaded with solvent (a 4:1 (v/v) mix of hexane and ethyl ether) and left to run until the solvent reached a point 1cm short of the edge of the plate. Plates were then removed from the chamber, allowed to dry, and stained with CAM solution (Al-Anzi et al., 2009). Plates were baked at 80°C for 15-25min and imaged using a scanner. Triglyceride was quantified in Image J using the Gel Analysis tool.

Measurement of carbohydrates (glucose + trehalose and glycogen)

Each sample consisted of 3 flies that were homogenised in 75µl of TE + 0.1% Triton X-100 (Sigma Aldrich). Samples were incubated for 20 min at 75°C and stored at –80°C. Prior to the assay, samples were incubated for 5 min at 65°C. Following incubation, 10µl from each sample was loaded into 4 wells of a 96-well plate. Each well was designated to serve as a measurement for either: control (10µl sample + 190µl H_20), glucose (10µl sample + 190µl glucose reagent (Sentinel Diagnostics)), trehalose (10µl sample + 190µl glucose reagent + trehalase (Sigma Aldrich)), or glycogen (10µl sample + 190µl glucose reagent + amyloglucosidase (Sigma Aldrich)). A standard curve was generated by serially diluting a glucose sample of known concentration and adding 190µl of glucose reagent to 10µl of each standard. Standards were always run at the same time and in the same plate as samples. Plates were incubated for 1.5h -3h at 37°C following which the absorbance for each well at 492 nm was determined using a plate reader.

Respirometry

Respiration in flies was measured using a stop-flow gas-exchange system (Q-Box RP1LP Low Range Respirometer, Qubit Systems, Ontario, Canada, K7M 3L5). Eight flies from each treatment were put into an airtight glass tube and supplied with our standard fly food via a modified pipette tip. Each tube was provided with CO_2-free air while the 'spent' air was concurrently flushed through the system and analysed for its CO_2 and O_2 content. In this way, evolved CO_2 and consumed O_2 were measured for each tube every ~ 44 min (the time required to go through each of the 7 vials in sequence). For most replicates of the respirometry assay, there were 2 uninfected, 2 PBS and 3 infected vials.

Fitness assay

Flies were collected within 7 hours of eclosion to ensure virginity. To assess fitness, immediately following injection with either PBS or *E. coli*, flies were placed into vials with uninfected competitors of the same sex, and potential mates of the opposite sex. Competitor flies expressed DsRed marker eyes, this marker allowed for easy identification of offspring resulting from focal flies - any DsRed eyed offspring were the progeny of competitor flies. Flies were allowed to mate for 10h as this interval exceeds the time required for flies to significantly reduce the number of *E. coli*, thus, allowing us to observe fitness throughout the infection. In one block, *E. coli* fitness assays were left for 24h, we have included these data as fitness values are normalized within each replicate and therefore independent of assay duration. After the mating period, flies were discarded and vials were left for 14 days to allow resultant offspring time to develop and eclose. Offspring of focal and competitor flies were scored by eye colour.

Statistical analysis

Data were analysed in R Studio with R version 3.5.1 (RStudio Team, 2015). Survival data were analysed using Cox proportional hazards models and Log-Rank tests for pairwise comparisons. For all other assays, we first tested for normality of data which dictated whether an ANOVA, t-test, Kruskal – Wallis analysis of variance, or Mann-Whitney U test was used to calculate differences between treatments with sex and infection status as factors. When appropriate, we performed post-hoc Tukey or Dunn analyses to identify specific differences between treatments. All assays consist of 2-4 replicates. Fitness data were both analysed and plotted using the estimation statistics tool provided at "estimationstats.com" (see also reference (Ho et al., 2019)).

Acknowledgements

Members of the Dionne lab provided useful feedback on the manuscript. This work was supported by MRC Research Grant (MR/R00997X/1) and Wellcome Trust Investigator Award 207467/Z/17/Z.

Competing interests

No competing interests.

References

Adamo SA, Jensen M, Younger M. 2001. Changes in lifetime immunocompetence in male and female Gryllus texensis (formerly G. integer): trade-offs between immunity and reproduction. *Anim Behav* **62**:417–425. doi:10.1006/anbe.2001.1786

Al-Anzi B, Sapin V, Waters C, Zinn K, Wyman RJ, Benzer S. 2009. Obesity-blocking neurons in Drosophila. *Neuron* **63**:329–341. doi:10.1016/j.neuron.2009.07.021

Aparicio R, Neyen C, Lemaitre B, Busturia A. 2013. dRYBP contributes to the negative regulation of the Drosophila Imd pathway. *PLOS ONE* **8**:e62052. doi:10.1371/journal.pone.0062052

Ayres JS, Schneider DS. 2008. A signaling protease required for melanization in Drosophila affects resistance and tolerance of infections. *PLoS Biol* **6**:e305. doi:10.1371/journal.pbio.0060305

Chambers MC, Song KH, Schneider DS. 2012. Listeria monocytogenes infection causes metabolic shifts in Drosophila melanogaster. *PloS One* **7**:e50679. doi:10.1371/journal.pone.0050679

Charroux B, Capo F, Kurz CL, Peslier S, Chaduli D, Viallat-Lieutaud A, Royet J. 2018. Cytosolic and secreted peptidoglycan-degrading enzymes in Drosophila respectively control local and systemic immune responses to microbiota. *Cell Host Microbe* **23**:215-228.e4. doi:10.1016/j.chom.2017.12.007

Chen F, Rydzewski K, Kutzner E, Häuslein I, Schunder E, Wang X, Meighen-Berger K, Grunow R, Eisenreich W, Heuner K. 2017. Differential Substrate Usage and Metabolic Fluxes in Francisella tularensis Subspecies holarctica and Francisella novicida. *Front Cell Infect Microbiol* **7**. doi:10.3389/fcimb.2017.00275

De Gregorio E, Spellman PT, Tzou P, Rubin GM, Lemaitre B. 2002. The Toll and Imd pathways are the major regulators of the immune response in Drosophila. *EMBO J* **21**:2568–2579. doi:10.1093/emboj/21.11.2568

Dionne MS, Pham LN, Shirasu-Hiza M, Schneider DS. 2006. Akt and FOXO dysregulation contribute to infection-induced wasting in Drosophila. *Curr Biol CB* **16**:1977–1985. doi:10.1016/j.cub.2006.08.052

Duneau D, Ferdy J-B, Revah J, Kondolf H, Ortiz GA, Lazzaro BP, Buchon N. 2017. Stochastic variation in the initial phase of bacterial infection predicts the probability of survival in D. melanogaster. *eLife* **6**:e28298. doi:10.7554/eLife.28298

Duneau DF, Kondolf HC, Im JH, Ortiz GA, Chow C, Fox MA, Eugénio AT, Revah J, Buchon N, Lazzaro BP. 2017. The Toll pathway underlies host sexual dimorphism in resistance to both Gram-negative and Gram-positive bacteria in mated Drosophila. *BMC Biol* **15**:124. doi:10.1186/s12915-017-0466-3

Eleftherianos I, More K, Spivack S, Paulin E, Khojandi A, Shukla S. 2014. Nitric Oxide Levels Regulate the Immune Response of Drosophila melanogaster Reference Laboratory Strains to Bacterial Infections. *Infect Immun* **82**:4169–4181. doi:10.1128/IAI.02318-14

Elrod-Erickson M, Mishra S, Schneider D. 2000. Interactions between the cellular and humoral immune responses in Drosophila. *Curr Biol* **10**:781–784. doi:10.1016/S0960-9822(00)00569-8

Fernando MDA, Kounatidis I, Ligoxygakis P. 2014. Loss of Trabid, a new negative regulator of the Drosophila Immune-Deficiency pathway at the level of TAK1, reduces life span. *PLOS Genet* **10**:e1004117. doi:10.1371/journal.pgen.1004117

Gyuranecz M, Erdélyi K, Fodor L, Jánosi K, Szépe B, Füleki M, Szőke I, Dénes B, Makrai L. 2010. Characterization of Francisella tularensis Strains, Comparing Their Carbon Source Utilization. *Zoonoses Public Health* **57**:417–422. doi:10.1111/j.1863-2378.2009.01238.x

Ho J, Tumkaya T, Aryal S, Choi H, Claridge-Chang A. 2019. Moving beyond P values: data analysis with estimation graphics. *Nat Methods* **16**:565–566. doi:10.1038/s41592-019-0470-3

Hoffmann JA, Reichhart J-M. 2002. Drosophila innate immunity: an evolutionary perspective. *Nat Immunol* **3**:121–126. doi:10.1038/ni0202-121

Howick VM, Lazzaro BP. 2017. The genetic architecture of defence as resistance to and tolerance of bacterial infection in Drosophila melanogaster. *Mol Ecol* **26**:1533–1546. doi:10.1111/mec.14017

Jacot A, Scheuber H, Brinkhof MWG. 2004. Costs of an induced immune response on sexual display and longevity in field crickets. *Evolution* **58**:2280. doi:10.1554/03-660

Khalil S, Jacobson E, Chambers MC, Lazzaro BP. 2015. Systemic bacterial infection and immune defense phenotypes in Drosophila Melanogaster. *J Vis Exp* e52613. doi:10.3791/52613

Klein SL, Flanagan KL. 2016. Sex differences in immune responses. *Nat Rev Immunol* **16**:626–638. doi:10.1038/nri.2016.90

Kleino A, Myllymäki H, Kallio J, Vanha-aho L-M, Oksanen K, Ulvila J, Hultmark D, Valanne S, Rämet M. 2008. Pirk is a negative regulator of the Drosophila Imd pathway. *J Immunol Baltim Md 1950* **180**:5413–5422. doi:10.4049/jimmunol.180.8.5413

Kraaijeveld AR, Godfray HC. 1997. Trade-off between parasitoid resistance and larval competitive ability in Drosophila melanogaster. *Nature* **389**:278–280. doi:10.1038/38483

Lawniczak MKN, Barnes AI, Linklater JR, Boone JM, Wigby S, Chapman T. 2007. Mating and immunity in invertebrates. *Trends Ecol Evol* **22**:48–55. doi:10.1016/j.tree.2006.09.012

Lemaitre B, Kromer-Metzger E, Michaut L, Nicolas E, Meister M, Georgel P, Reichhart JM, Hoffmann JA. 1995. A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the Drosophila host defense. *Proc Natl Acad Sci U S A* **92**:9465–9469. doi:10.1073/pnas.92.21.9465

Lemaitre B, Reichhart JM, Hoffmann JA. 1997. Drosophila host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc Natl Acad Sci U S A* **94**:14614–14619. doi:10.1073/pnas.94.26.14614

Lotter H, Altfeld M. 2019. Sex differences in immunity. *Semin Immunopathol* **41**:133–135. doi:10.1007/s00281-018-00728-x

McKean KA, Nunney L. 2005. Bateman's principle and immunity: phenotypically plastic reproductive strategies predict changes in immunological sex differences. *Evolution* **59**:1510. doi:10.1554/04-657

Medzhitov R, Schneider DS, Soares MP. 2012. Disease Tolerance as a Defense Strategy. *Science* **335**:936–941. doi:10.1126/science.1214935

Moret Y, Schmid-Hempel P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**:1166–1168.

Nunn CL, Lindenfors P, Pursall ER, Rolff J. 2009. On sexual dimorphism in immune function. *Philos Trans R Soc B Biol Sci* **364**:61–69. doi:10.1098/rstb.2008.0148

Paredes JC, Welchman DP, Poidevin M, Lemaitre B. 2011. Negative regulation by amidase PGRPs shapes the Drosophila antibacterial response and protects the fly from innocuous infection. *Immunity* **35**:770–779. doi:10.1016/j.immuni.2011.09.018

Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS. 2007. A specific primed immune response in Drosophila is dependent on phagocytes. *PLoS Pathog* **3**. doi:10.1371/journal.ppat.0030026

Radlinski LC, Brunton J, Steele S, Taft-Benz S, Kawula TH. 2018. Defining the Metabolic Pathways and Host-Derived Carbon Substrates Required for Francisella tularensis Intracellular Growth. *mBio* **9**. doi:10.1128/mBio.01471-18

RStudio Team. 2015. RStudio: Integrated Development for R. RSudio, Inc. Boston, MA.

Schwenke RA, Lazzaro BP, Wolfner MF. 2016. Reproduction-immunity trade-offs in insects. *Annu Rev Entomol* **61**:239–256. doi:10.1146/annurev-ento-010715-023924

Simmons LW, Tinghitella RM, Zuk M. 2010. Quantitative genetic variation in courtship song and its covariation with immune function and sperm quality in the field cricket Teleogryllus oceanicus. *Behav Ecol* **21**:1330–1336. doi:10.1093/beheco/arq154

Soares MP, Teixeira L, Moita LF. 2017. Disease tolerance and immunity in host protection against infection. *Nat Rev Immunol* **17**:83–96. doi:10.1038/nri.2016.136

Taylor K, Kimbrell D. 2007. Host Immune Response and Differential Survival of the Sexes in Drosophila. *Fly (Austin)* **1**:197–204. doi:10.4161/fly.5082

Vincent CM, Sharp NP. 2014. Sexual antagonism for resistance and tolerance to infection in Drosophila melanogaster. *Proc R Soc B Biol Sci* **281**:20140987. doi:10.1098/rspb.2014.0987

Vincent CM, Simoes da Silva CJ, Wadhawan A, Dionne MS. 2020. Origins of Metabolic Pathology in Francisella-Infected Drosophila. *Front Immunol* **11**. doi:10.3389/fimmu.2020.01419

vom Steeg LG, Klein SL. 2016. SeXX Matters in Infectious Disease Pathogenesis. *PLoS Pathog* **12**:e1005374. doi:10.1371/journal.ppat.1005374

Yamamoto Y, Saito H, Setogawa T, Tomioka H. 1991. Sex differences in host resistance to Mycobacterium marinum infection in mice. *Infect Immun* **59**:4089–4096.

Ye YH, Chenoweth SF, McGraw EA. 2009. Effective but costly, evolved mechanisms of defense against a virulent opportunistic pathogen in Drosophila melanogaster. *PLoS Pathog* **5**:e1000385. doi:10.1371/journal.ppat.1000385

Zaidman-Rémy A, Hervé M, Poidevin M, Pili-Floury S, Kim M-S, Blanot D, Oh B-H, Ueda R, Mengin-Lecreulx D, Lemaitre B. 2006. The Drosophila amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity* **24**:463–473. doi:10.1016/j.immuni.2006.02.012

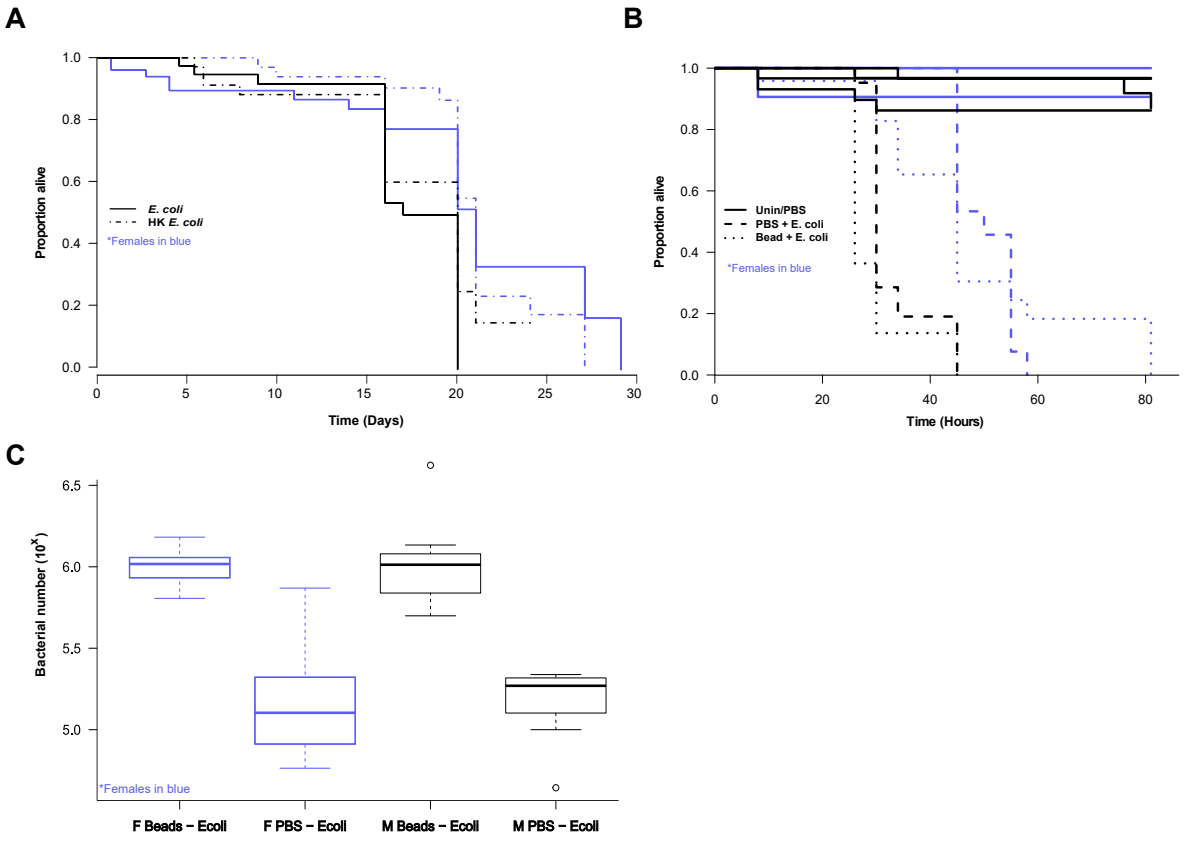


Figure 1 - figure supplement 1. (A) Survival of wild-type flies infected with live and heat-killed (HK) *E. coli*. Males and females are represented by black and blue tracings, respectively. HK bacteria were incubated for 1h at 65 C. Live *E. coli* data are replotted from figure 1a. (B) Survival and (C) bacterial load 6h p.i. of imd mutants pre-injected with either beads or PBS prior to *E. coli*. In boxplot, median value is indicated by horizontal bars, top and bottom of boxes represent upper and lower quartiles (respectively). Whiskers indicate maximum and minimum values. Dots indicate outliers.

Figure 1 supplementary table 1: wild-type median survival (days)

Treatment	Median survival	upper CI	lower CI	% change
Uninfected M	18.8	19.8	15.8	-
PBS M	19.8	20.8	18.8	+5.3
HK *E. coli* M	19.8	20.8	15.8	+5.3
E. coli M	16.8	NA	15.8	-11.7
Uninfected F	22.8	26.8	19.8	-
PBS F	20.8	25.8	20.8	-9.6
HK *E. coli F*	20.8	20.8	19.8	-9.6
E. coli F	20.8	NA	19.8	-9.6

*HK - heat-killed; M - males; F - females;
% change from uninfected control

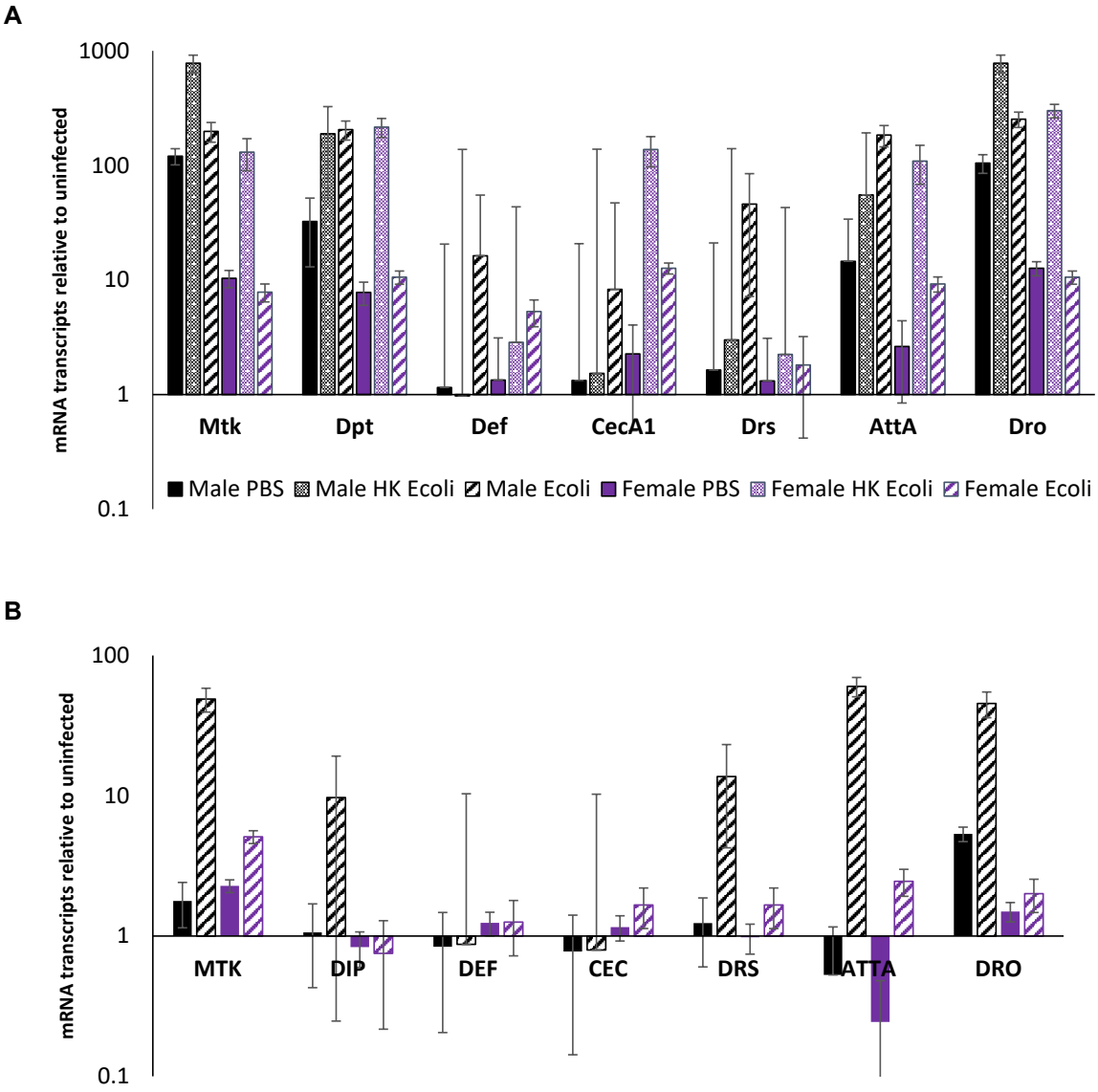


Figure 1 – figure supplement 2. Antimicrobial peptide expression data 6h following *E. coli* injection in (A) w^{1118} and (B) imd^{10191} flies. All genes were standardized to the housekeeping gene Ribosomal protein 1. Data presented are mean values relative to expression in uninfected controls. Whiskers represent standard error.

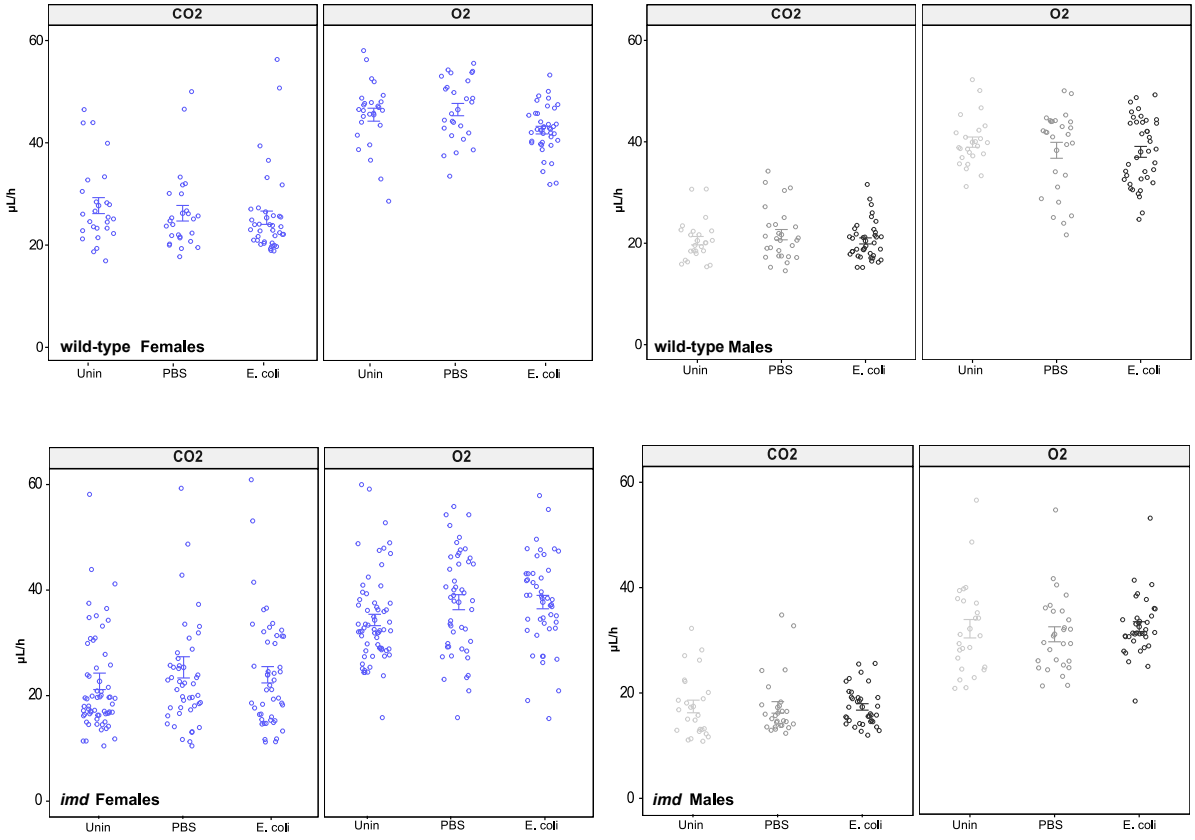


Figure 2 - figure supplement 1. Respiration of infected males and females. Respiration was measured for six hours following infection. Markers represent one vial consisting of 8 flies. Bars indicate SE. All assays were repeated 3 or 4 times with 2 or 3 samples/treatment.

Figure 2 supplementary table 1: Metabolic statistics

wild-type Glucose

Variable	Df	Sum Sq	F	p
Sex	1	0.00259	0.474	0.499
Infection status	1	0.00181	0.331	0.572
Sex*Infection status	1	0.00037	0.068	0.797

wild-type Glycogen

Variable	Df	Sum Sq	F	p
Sex	1	0.0062	0.392	0.538
Infection status	1	0.0031	0.194	0.664
Sex*Infection status	1	0.0043	0.268	0.611

wild-type Triglyceride

Variable	Df	Sum Sq	F	p
Sex	1	0.03191	4.246	0.0466*
Infection status	1	0.1832	24.374	1.8e-05*
Sex*Infection status	1	0.00548	0.729	0.398

***imd* Glucose**

Variable	Df	Sum Sq	F	p
Sex	1	0.327	4.302	0.0512
Infection status	1	0.0049	0.065	0.801
Sex*Infection status	1	0.0021	0.028	0.869

***imd* Glycogen**

Variable	Df	Sum Sq	F	p
Sex	1	0.004204	0.22	0.644
Infection status	1	0.004572	0.239	0.630
Sex*Infection status	1	0.015096	0.79	0.385

***imd* Triglyceride**

Variable	Df	Sum Sq	F	p
Sex	1	0	0	0.999
Infection status	1	0.3487	44.971	2.8e-08*
Sex*Infection status	1	0.0575	7.417	9.2e-03*

Figure 4 supplementary table 1: Metabolic statistics

PGRP-LB Glucose

Variable	Df	Sum Sq	F	p
Sex	1	0.0369	2.576	0.12
Infection status	1	0.0921	6.438	0.017*
Sex*Infection status	1	0.0001	0.007	0.935

PGRP-LB Glycogen

Variable	Df	Sum Sq	F	p
Sex	1	0.0334	0.509	0.4813
Infection status	1	0.6176	9.412	4.8e-03*
Sex*Infection status	1	0.0004	0.007	0.93468

PGRP-LB Triglyceride

Variable	Df	Sum Sq	F	p
Sex	1	0.007	0.499	0.487
Infection status	1	0.0039	0.275	0.605
Sex*Infection status	1	0.0021	0.151	0.701

Figure 4 supplementary table 2: *PGRP-LB* median survival (days)

Treatment	Median survival	upper CI	lower CI	% change
Uninfected M	13.2	13.2	13.2	-
PBS M	13.2	13.2	11.2	0
E. coli M	11.2	13.2	10.2	-17.9
Uninfected F	25.9	25.9	20.9	-
PBS F	20.9	20.9	17.3	-23.9
E. coli F	20.9	20.9	20.9	-23.9

*M - males; F - females; % change from uninfected control