Mechanisms of damage prevention, signalling, and repair impact the ability of *Drosophila* to tolerate enteric bacterial infection

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Abstract

Many insects thrive on decomposing and decaying organic matter containing a large diversity of both commensal and pathogenic microorganisms. The insect gut is therefore frequently exposed to pathogenic threats and must be able not only to detect and clear these potential infections, but also be able to repair the resulting damage to gut tissues in order to tolerate relatively high microbe loads. In contrast to the mechanisms that eliminate pathogens, we currently know less about the mechanisms of disease tolerance, and most of this knowledge stems from systemic infections. Here we investigated how well-described mechanisms that either prevent, signal, control, or repair tissue damage during infection contribute to the phenotype of disease tolerance during gut infection. We orally infected adult *Drosophila melanogaster* flies with the bacterial pathogen *Pseudomonas entomophila* in several loss-of-function mutants lacking epithelial responses including damage preventing *dcy* (drosocrystallin - a major component of the peritrophic matrix), damage signalling *upd3* (unpaired protein, a cytokine-like molecule), damage controlling *irc* (immune-regulated catalase, a negative regulator of reactive oxygen species) and tissue damage repairing *egfr^l* (epidermal growth factor receptor). Overall, we detect effects of all these mechanisms on disease tolerance. The deterioration of the peritrophic matrix in *dcy* mutants resulted in the highest loss of tolerance, while loss of function of either *irc* or *upd3* also reduced tolerance in both sexes. The absence of tissue damage repair signalling (*egfr^l*) resulted in a severe loss in tolerance in male flies but had no substantial effect on the ability of female flies to tolerate *P. entomophila* infection, despite carrying greater microbe loads than males. Together, our findings provide empirical evidence for the role of damage limitation mechanisms in disease tolerance and highlight how sex differences in these mechanisms could generate sexual dimorphism in immunity.

Introduction

Animals have evolved diverse responses to infection, including behavioural avoidance of infection, physical barriers to pathogen entry and a variety of humoral and cellular immune responses (Buchon, Silverman, and Cherry 2014; Kuraishi, Hori, and Kurata 2013; Vale et al. 2018). These responses have been particularly well described in the fruit fly *Drosophila*, where signalling pathways such as *IMD* and *Toll* are recognised as major contributors to pathogen clearance during infection (Apidianakis and Rahme 2011; Buchon, Silverman, and Cherry 2014; Hoffmann 2003; Hultmark 2003; Kuraishi, Hori, and Kurata 2013; Lemaitre and Hoffmann 2007). In addition to mechanisms that clear or otherwise quantitatively reduce pathogen burdens, there is an increasing appreciation that mechanisms that promote disease tolerance are at least equally as important during infection and in recovery to a healthy state (Raberg, Sim, and Read 2007; Ayres and Schneider 2012; Medzhitov, Schneider, and Soares 2012; Soares, Teixeira, and Moita 2017; McCarville and Ayres 2018). Disease tolerance is defined as the ability of hosts to maintain health despite harbouring relatively high pathogen loads, a phenotype that has been observed in several species, including insects (Howick and Lazzaro 2014; Lissner and Schneider 2018; Oliveira, Bahia, and Vale 2020) rodents (Palaferri Schieber et al. 2015; Raberg, Sim, and Read 2007; Cumnock et al. 2018); birds (Adelman et al. 2013; Bonneaud et al. 2019); and humans (Regoes et al. 2014; Nahrendorf, Ivens, and Spence 2021; Soares, Teixeira, and Moita 2017).

The mechanisms of pathogen clearance are well-described in many animal species (Cooper 2018), but we currently know less about the mechanisms underlying disease tolerance. Given that tolerance reflects a distinct ability to maintain health independently of pathogen clearance, we might expect these mechanisms to be related to processes such as detoxification, reduction of inflammation, or tissue damage control and cellular renewal (Ayres and Schneider 2012; Soares, Teixeira, and Moita 2017; Soares, Gozzelino, and Weis 2014). Genome-wide association or transcriptomic studies in *Drosophila* have highlighted potential candidate genes underlying variation in disease tolerance, reviewed in (Lissner and Schneider 2018), but it remains unclear how these genes interact with known mechanisms of immunity and recovery.

Furthermore, almost all candidate genes for disease tolerance in *Drosophila* arise from systemic infections(Howick and Lazzaro 2017; Troha et al. 2018; Lissner and Schneider 2018), leaving a gap in our knowledge about disease tolerance during oral infections. Orally acquired infections are especially relevant given the pervasive nature of oral-faecal transmission routes, and in the context of the ecology of most insects, like *Drosophila*, that thrive on decomposing and decaying organic

matter containing a large diversity of microorganisms, both commensal and pathogenic (Chandler et al. 2011; Corby-Harris et al. 2007). For example, recent genomic analyses of *Drosophila* samples collected across Europe revealed sequences belonging to multiple species of bacteria, fungi and viruses, many of which had not been previously described (Kapun et al. 2020; Wallace et al. 2021). The insect gut is therefore frequently exposed to pathogenic threats and must be able not only to detect and clear these potential infections, but also be able to repair the resulting damage to gut tissues in order to tolerate relatively high numbers of ingested pathogens.

Here, we aimed to specifically test how known mechanisms that either prevent, reduce, or repair tissue damage in the fly gut contribute to the phenotype of disease tolerance. The *Drosophila* gut is a compartmentalized tubular organ which is structurally and functionally similar to the vertebrate intestinal tract (Buchon, Broderick, and Lemaitre 2013; Buchon and Osman 2015; Kuraishi, Hori, and Kurata 2013; Lemaitre and Miguel-Aliaga 2013). We can consider several stages comprising gut defence in *Drosophila*. The first involves the physical barrier of the gut epithelia and the peritrophic matrix (*PM*), which is a layer of chitin and glycoproteins that lines the insect midgut lumen. The *PM* is functionally analogous to mammalian mucus membrane in the digestive tract and acts as the first line of defence against invading pathogens (Hegedus et al. 2009; Kuraishi, Hori, and Kurata 2013). A major component of the *PM* is *drosocrystallin* (*dcr*). Loss-of-function mutations in *dcr* increase the width of the peritrophic matrix, increasing its permeability to larger molecules and to leakage of microbial cells, including pathogens, in to the haemolymph (Kuraishi, Hori, and Kurata 2013). *Dcr* deficient flies therefore exhibit increased susceptibility to oral bacterial infections.

As second mode of defence during gut infections is the production of reactive oxygen species (ROS) by the gut epithelia. For example, oral infection with *P. entomophila* in *D. melanogaster* causes massive destruction of the gut epithelium (Vodovar et al. 2005; Dieppois et al. 2015). In response to ingested *P. entomophila*, ROS production is induced by two NADPH enzymes- *nox* (NADPH oxidase) and *duox* (dual oxidase), while *irc* (immune-reactive catalase) negatively regulates ROS production once the infection threat is controlled, which otherwise, would lead to cytotoxic effects (Buchon et al. 2013; 2009; Kuraishi, Hori, and Kurata 2013). ROS production not only targets pathogens directly, but also plays additional roles in triggering signalling pathways that lead to the production of *IMD* or *Toll* responsive antimicrobial peptides (Buchon et al. 2009; Lemaitre and Hoffmann 2007; Myllymäki, Valanne, and Rämet 2014; Myllymäki and Rämet 2014; Valanne, Wang, and Rämet 2011).

The final stage in gut defence is to repair the tissue damage caused during the infection. Damage-signalling cytokine-like molecules *upd3* are released from damaged cells which trigger the *Jak/Stat* pathway, stimulating the proliferation of intestinal stem cells (*ISCs*) and their differentiation into enterocytes (*ECs*) via *egfr*^l (epidermal growth factor receptor) signalling (Buchon et al. 2010; Chakrabarti et al. 2016; Jiang et al. 2011; Weng et al. 2018). Flies lacking *Jak/Stat* or *Egfr* are therefore highly susceptible to bacterial infections due to their inability to repair and renew damaged tissue (Buchon et al. 2010; Jiang et al. 2011; 2009; Marianes and Spradling 2013; Myllymäki and Rämet 2014).

To investigate how these mechanisms of damage prevention (*dcy*), signalling (*upd3*) control (*irc*) and renewal (*egfr*^l) contribute to disease tolerance during gut infections we employed oral infections in *Drosophila* lines carrying loss-of-function mutations in each of these genes on a common genetic background (*w¹¹¹⁸*). We orally challenged these flies with a range of infection doses of *Pseudomonas entomophila* and then quantified their effects on survival, pathogen loads and disease tolerance responses during period of peak infection burden.

Figure 1: Schematic representation of *Drosophila* epithelial immune response following enteric infection. Several stages of tissue damage prevention and repair epithelial response in the *Drosophila* can be found. **Stage-I:** involves the physical barrier the peritrophic matrix (PM - layer of chitin and

glycoproteins) that lines the *Drosophila* midgut lumen. A major component of the PM is *dcy* (*drosocrystallin*) and loss-of-function mutations in *dcy* increase the width of the PM, increasing permeability to pathogens. **II**: production of reactive oxygen species (ROS) by the gut epithelia - enteric infections cause massive destruction of the gut epithelium and in response to ingested pathogens, ROS production is induced by *nox* (NADPH oxidase) and *duox* (dual oxidase), while *irc* (immune-reactive catalase) negatively regulates $\rightarrow$ ROS production by suppressing the cytotoxic effects of ROS once the infection threat is lessened, avoiding immunopathology. In addition, ROS production is known to trigger signalling pathways the IMD or *Toll* that leads to the production of antimicrobial peptides (*AMPs*) $\rightarrow$ critical for pathogen clearance in the gut. **III**: tissue renewal process after infection-induced damage - The final stage in gut defence is to repair the damage caused during enteric infections. Damage-signalling cytokine-like molecules *upd3*, released from damaged cells trigger the *Jak/Stat*-pathway, stimulating the proliferation of intestinal stem cells (*ISCs*) and their differentiation into enterocytes (*ECs*) via *egfr¹* (epidermal growth factor receptor) signalling which are indispensable for maintaining homeostasis and renewing the damaged cells/tissues. Figure created using Biorender.

Materials and methods

Fly strains: The following fly stocks were obtained from VDRC (Vienna *Drosophila* Resource Centre) and Bloomington Stock Centre, Indiana: *dcy* (BL26106), *irc* (BL29191), *egfr¹* (BL2079), *upd3* (BL19355). All the mutants were subsequently backcrossed into the wild type *w¹¹¹⁸* (VDRC stock# 60000) for at least 10 generations. All fly lines were maintained in plastic vials (12 ml) on a standard cornmeal diet (Lewis's medium) at a constant temperature of 25°C ($\pm$ 2°C).

Bacterial culture preparation: To test the impact of bacterial infection on fly survival we used the gram-negative bacteria *Pseudomonas entomophila*, that commonly infects a broad range of insects and other invertebrates. In flies, *P. entomophila* infection mainly occurs in the intestinal epithelium and eventually causes death (Buchon et al. 2009; Vodovar et al. 2005). To obtain bacterial cultures for oral exposure, we inoculated frozen bacterial stock cultures stored at -80°C onto fresh 15ml LB broth (media composition) and incubated overnight at 37°C with shaking at 120rpm (revolutions per minute). The overnight cultures were diluted 1: 100 into 500ml of fresh LB broth and incubated again at 30°C with shaking at 120rpm. At the mid-log phase ($OD_{600}=0.75$), we harvested the bacterial cells by centrifugation at 5000rpm for 15 min and re-suspended the bacterial pellet in 5% sucrose (Siva-Jothy et al. 2018). The final inoculum was adjusted to three different bacterial concentrations or infection dose $OD_{600}=10$ (low dose), $OD_{600}=25$ (medium dose) and $OD_{600}=45$ (high dose).

Oral infection assay: Before infecting flies we initially prepared infection vials by pipetting around 350µl of standard agar (1L triple distilled H₂O, 20g agar, 84g brown sugar, 7ml Tegosept anti-fungal agent) onto lid of a 7ml tubes (bijou vials) and allowed it to dry. Simultaneously, we starved

the experimental flies on 12ml agar vials for 4-5 hours. Once the agar in the bijou lids dried, we placed a filter disc (Whattmann-10) in the lid and pipetted 80µl of bacterial culture directly onto the filter disc. For control (mock) infections, we replaced bacterial culture with 5% sucrose solution. We then orally exposed flies inside the bijou vials for about 18-hours and then transferred the flies onto fresh vials containing standard cornmeal (Siva-Jothy et al. 2018).

Experimental design: To test how mechanisms of damage repair contribute to disease tolerance we employed a split vial design (see **Fig. 2**). Here, after oral bacterial exposure each vial containing about 25 flies of each infection treatment, sex and fly line combination were divided into 2 vials for measuring (1) survival following infection (15 flies/combination) and (2) internal bacterial load (10 flies/combination) see **Fig. 2**. With this split-vial design we were able to use replicate- matched data for both survival and bacterial load in order to estimate (3) disease tolerance for each fly line that is, the linear relationship between fly survival and internal bacterial load.

Bacterial load measurement: To test whether variation in mortality of experimental flies after *P. entomophila* infection is explained by the ability to clear infection, we measured bacterial load at three timepoints (immediately after oral exposure 0-15 minutes, 24-hours, and 96-hours) by exposing 3–5-day old flies with $OD_{600}=25$ *P. entomophila* cells. To confirm oral bacterial infection, we thoroughly surface-sterilised flies (group of 3) with 70% ethanol for 30-60 seconds and then rinsed twice with sterile distilled water. We plated the second wash on LB agar plates and incubated overnight at 30°C to confirm that surface bacteria were successfully removed after alcohol sterilization. We transferred flies onto 1.5ml micro centrifuge tubes and homogenized using a motorized pestle for approximately 30-60 seconds in 100µl LB broth (n=30 homogenates/sex/infection treatment/fly line). We performed serial dilution of each homogenate up to 10^{-6} -fold and added 4µl aliquot on a LB agar plate. After this, we incubated the plate overnight for about 18-hours at 30°C and counted the resultant bacterial colonies manually. We note that mock-infected control fly homogenates did not produce any colonies on LB agar plates (Siva-Jothy et al. 2018). We also measured bacterial load at 24-hour following oral *P. entomophila* infection for low dose ($OD_{600}=10$) and high ($OD_{600}=45$) infection dose (See Supplementary Information **Figs. S1** and **S2**).

Statistics

1. **Survival following oral infection:** We analysed survival data using a mixed effects Cox model using the R package ‘coxme’ (Therneau 2015). We specified the model as: survival ~ fly

line * treatment * sex * (1 | vials/block), with ‘fly line’, ‘treatment’ and ‘sex’ and their interactions as fixed effects, and ‘vials’ nested in ‘block’ as a random effect for wild type w^{1118} and mutant flies.

2. Internal bacterial load: We found that residuals of bacterial load data were non-normally distributed when tested using Shapiro–Wilks’s test. Hence, we first log-transformed the data and then confirmed that the log-transformed residuals were still non-normally distributed. Subsequently, we used a non-parametric one-way ANOVA (Kruskal-Wallis test) to test the effects of each fly line, that is, wild-type w^{1118} and mutant flies on males and females separately following oral *P. entomophila* infection.

3. Measuring disease tolerance: Finally, to understand how tissue damage signalling and repair mechanisms affect disease tolerance in males and females during oral *P. entomophila* infection, we analysed the linear relationship between fly survival against bacterial load by fitting linear models (Raberg, Sim, and Read 2007; Schneider and Ayres 2008; Medzhitov, Schneider, and Soares 2012; Louie et al. 2016; Gupta and Vale 2017; Oliveira, Bahia, and Vale 2020). We assessed differences in disease tolerance (fly survival with increasing bacterial load) using ANCOVA by fitting ‘fly line’ and ‘sex’ as categorical fixed effects, ‘average bacterial load’ as a continuous covariate and their interactions as fixed effects. Significant interaction effects between fly line and bacterial load would indicate that the slope of the relationship between fly survival and load varies between fly lines, that is, the tolerance response differs between lines. Because our interest was to find out to what extent loss-of damage prevention and tissue repair mechanisms differed from wildtype w^{1118} in tolerating enteric bacterial infections we compared the estimates of slope for wild type w^{1118} line with each of the mutant line for both males and females separately using a pairwise comparison (f-test).

Figure 2: Split-vial experimental design to assay (1) survival (~15 flies/combination in a vial) and (2) internal bacterial load (~10 flies/combination in a vial) following oral bacterial infection with *Pseudomonas entomophila* to test how epithelial immune response including damage prevention and tissue repair mechanisms in *Drosophila* gut contribute to (3). disease tolerance. For the split-vial design, after oral exposure with each vial containing about 25 flies (of each infection treatment, sex, fly line combination) were divided into 2 vials, each for survival (around 15 flies) and bacterial load (around 10 flies). Each point in disease tolerance panel (3) represents replicate-matched data [n=30 vials/infection treatment/sex/fly line – with each vial containing around 25 flies] from survival (1) and bacterial load (2).

Results

1. Flies lacking damage-preventing *dcy* are more susceptible to oral *P. entomophila* infections than those lacking components that minimise, signal or repair tissue damage

Following oral infection with three different doses of *Pseudomonas entomophila*, we found that flies lacking major components of gut immunity such as tissue damage - preventing *dcy*, signalling *upd3*, repairing *egfr^d* and controlling *irc*, were all significantly more susceptible to oral *P. entomophila* infections than the wild type w^{1118} flies (**Fig. 3B** and **1A**, **Table 1**; infection dose $OD_{600}=25$; see Supplementary **Figs. S1 and S2**, **Table S1** for low $OD_{600}=10$ and high $OD_{600}=45$ infection doses). Among these mutants, *dcy* mutants were particularly susceptible to infection **Fig. 3B** and **1A** for survival, **Table 1** for hazard ratio; infection dose $OD_{600}=25$). The effect of each mutation on the survival of flies following infection was similar in males and females (fly line x sex x treatment interaction = non-significant, **Table S1**), and was consistent at both lower ($OD_{600}=10$) and higher infection ($OD_{600}=45$) doses (see Supplementary **Figs. S1 and S2**, **Table S1** for low $OD_{600}=10$ and high $OD_{600}=45$ infection dose).

Figure 3. Survival curves for (A) females and (B) males of wildtype *w¹¹¹⁸* and mutant flies exposed to oral *P. entomophila* of $OD_{600}=25$ (medium dose, n=30 vials of ~30 flies/treatment/sex/fly line). (C) Estimated hazard ratios for males and female flies (wild type *w¹¹¹⁸* and mutant flies) calculated from the survival curves. A greater hazard ratio (>1) indicates higher susceptibility to infection. [* indicates that the mutant flies are significantly different from wild type *w¹¹¹⁸* flies for males and females respectively]. See **Figs. S1** and **S2** for similar results at lower dose ($OD_{600}=10$) and higher dose ($OD_{600}=45$).

Fly line	Sex	Estimate	p	lower 95%	upper 95%
<i>dcy</i>	Female	1.520	<0.01	1.32	1.74
	Male	1.516	<0.01	1.32	1.73
<i>Egfr¹</i>	Female	1.370	<0.01	1.20	1.55
	Male	1.374	<0.01	1.21	1.55
<i>irc</i>	Female	1.284	<0.01	1.13	1.45
	Male	1.314	<0.01	1.16	1.48
<i>upd3</i>	Female	1.307	<0.01	1.15	1.48
	Male	1.261	<0.01	1.11	1.42

Table 1: Summary of estimated hazard ratio from the cox proportional model. A greater hazard ratio (>1) indicates that mutant flies are more susceptible to oral *P. entomophila* infection than *w¹¹¹⁸* wildtype flies.

2. Both wild-type and flies with disrupted tissue damage prevention and repair mechanisms show sex differences in bacterial load during oral infections

The higher susceptibility of mutants to oral bacterial exposure could either be caused by their inability to suppress the bacterial growth or due to their inability to tolerate the damage inflicted during oral infection. To distinguish between these mechanisms, we first quantified internal bacterial loads across several time points, that is, 0-15 minutes, 24-hours and 96-hours following oral exposure to *P. entomophila*. All fly lines showed sex-differences at 24-hours peak load, though by 96-hours this sex difference was no longer present in flies lacking damage sensing (*upd3*) and tissue renewal mechanisms (*egfr*) (**Fig. 4, Table S2**). Flies lacking the negative regulator of ROS *irc* always exhibited lower levels of bacterial load compared to wild type flies (**Fig. 4B, Table S2**).

Figure 4. (A) Bacterial load measured as CFUs- colony forming units using infection dose (medium $OD_{600}=25$) after different time points 0-15 minutes (immediately after the oral bacterial exposure), 24-hours and 96-hours following infection with *P. entomophila* for wildtype *w¹¹¹⁸* flies and mutants [n=30 vials of (~30 flies/treatment/sex/fly line)]. **(B)** bacterial load at 24-hours following infection [significantly different fly lines are connected with different alphabets using Tukey's HSD as a post hoc analysis of pairwise comparisons]. See **Figs. S1** and **S2** for similar results at lower dose ($OD_{600}=10$) and higher dose ($OD_{600}=45$).

3. Tissue damage repair mechanisms in gut-epithelia mediate sex differences in disease tolerance to oral bacterial *P. entomophila* infections

While some of the variation in survival between mutants (**Fig. 3**) may be explained by variation in resistance [that is, their ability to clear infection (**Fig. 4**)], some of that variation may also arise due to differences in tolerance. We were therefore interested in measuring disease tolerance in these lines using the reaction norm of survival relative to bacterial loads, where the slope of the linear relationship reflects the degree of tolerance: steep negative slopes indicate a rapid mortality with increases in pathogen loads (low tolerance), while less steep or flat slopes reflect relatively more tolerant host (Raberg, Sim, and Read 2007; Schneider and Ayres 2008; Louie et al. 2016; Kutzer and Armitage 2016). In statistical terms, the differences in tolerance between lines are indicated by a significant interaction between the bacterial load and the fly line for survival, which reflects the overall rate at which fly health (survival) changes with bacterial load (tolerance) between fly lines (Howick and Lazzaro 2014; Kutzer and Armitage 2016; Louie et al. 2016).

Overall, we found that all mutant lines lacking major components of tissue damage limitation showed reduced tolerance to oral bacterial *P. entomophila* infections compared to wild type *w¹¹¹⁸* flies (**Fig. 5, Table 3**). Here, the differences in tolerance between wild type *w¹¹¹⁸* and mutant flies are indicated by a significant interaction between the fly line bacterial load for survival, which reflects the overall rate at which fly health (survival) changes with bacterial load (tolerance) between fly lines in both males and females (**Fig. 5, Table 2**). Males lacking *egfr^J* show lower tolerance to bacterial infection than females (**Fig. 5, Table 2**), suggesting sexual dimorphism in gut cell renewal. However, both males and females lacking major component of the peritrophic matrix that is, *dcy* showed significantly reduced tolerance (**Fig. 5**), indicating the general importance of tissue damage preventing epithelial barrier in defence against oral infections (Kuraishi, Hori, and Kurata 2013).

One notable finding was that *egfr^J* flies, lacking tissue repair and renewal signalling, showed sex-differences in tolerance during oral *P. entomophila* bacterial infections (**Fig. 5, Table 2 and 3**). Previous studies suggest that *D. melanogaster* males are more susceptible to intestinal infections possibly due to males having fewer *ISCs* (intestinal stem cells, a major part of *egfr*-mediated tissue repair process) (Regan et al. 2016; Rera, Clark, and Walker 2012). Males also have lower basal rate of division, overall, making them more susceptible to intestinal oral infection, compared to females. Instead, we found that loss of function of tissue renewal mechanisms makes females more susceptible to *P. entomophila* oral infections across wide range of infection doses (see *Supplementary Figs. S1 and S2, Table S1* for low $OD_{600}=10$ and high dose $OD_{600}=45$ survival data).

Figure 5. (A). The relationship between fly survival (measured as average lifespan) and bacterial load (as mean CFUs – colony forming units), analysed using linear models for female and male flies (*w¹¹¹⁸* and mutant flies). Each point shows data for average lifespan and mean bacterial load (CFUs) of 30 vials (with each vial containing about 25 individual flies/fly line/sex combination) after 24-hours post oral bacterial exposure. The data shown here are for the infection doses (medium $OD_{600}=25$). **(B).** shows the slope of the linear reaction norm extracted from the linear models. [* indicates significant difference in tolerance between males and females in tolerance to bacterial infections (interaction between the bacterial load and the sex for each fly line measured using ANCOVA, Table 2),* indicates that mutant fly lines are significantly different from wild type *w¹¹¹⁸*, analysed using pairwise f-test from linear norm estimates see Table 3].

Sex	Source	df	Sum of Sq.	F ratio	p
Female	Fly line	4	66.33	71.6	<0.01
	Bact. load	1	19.27	83.3	<0.01
	Fly line x Bact. load	4	10.02	10.8	<0.01
Male	Fly line	4	84.60	62.8	<0.01
	Bact. load	1	28.39	84.3	<0.01
	Fly line x Bact. load	4	14.88	11.0	<0.01

Table 2. Summary of ANCOVA. To assess differences in infection tolerance (fly survival with increasing bacterial burden) following oral *P. entomophila* infection with $OD_{600}=25$ infection dose, after 24-hours. We analysed ANCOVA and fitted ‘sex’ as categorical fixed effects, ‘average bacterial load’ as a continuous covariate and their interactions as fixed effects for each of the fly lines (*w¹¹¹⁸* and mutants).

<i>Sex</i>	<i>Fly line</i>	<i>SSE</i>	<i>F ratio</i>	<i>p</i>
Female	<i>dcy</i> vs. <i>w¹¹¹⁸</i>	7.32	4.85	0.03
	<i>egfr^d</i> vs. <i>w¹¹¹⁸</i>	18.32	1.88	0.17
	<i>irc</i> vs. <i>w¹¹¹⁸</i>	17.75	28.00	<0.01
	<i>upd3</i> vs. <i>w¹¹¹⁸</i>	18.33	29.79	<0.01
Male	<i>dcy</i> vs. <i>w¹¹¹⁸</i>	10.66	2.17	0.14
	<i>egfr^d</i> vs. <i>w¹¹¹⁸</i>	29.07	21.41	<0.01
	<i>irc</i> vs. <i>w¹¹¹⁸</i>	16.06	18.72	<0.01
	<i>upd3</i> vs. <i>w¹¹¹⁸</i>	24.87	27.01	<0.01

Table 3. Summary of pairwise comparisons (f-test) of linear slope estimates from linear reaction norm for wildtype *w¹¹¹⁸* flies and mutants.

Discussion

In the present work we tested how mechanisms of tissue damage prevention (*dcy*), signalling (*upd3*) control (*irc*) and renewal (*egfr^d*) contribute to disease tolerance during enteric infection. We present evidence that all of these mechanisms contribute to disease tolerance, and that some of these effects are sexually dimorphic. Previous studies have identified several candidate genes associated with disease tolerance, including - *CrebA*, *grainyhead* and *debris buster*, *dFOXO* (Troha et al. 2018; Howick and Lazzaro 2017; Dionne et al. 2006; Lissner and Schneider 2018). However, these genes arising from transcriptomic, genome-wide association (GWAS) or global profiling of gene expression (microarrays) studies have been screened from flies that are systemically infected to bacterial pathogens and do not seem to be associated with the well described damage prevention and tissue repair processes in the *Drosophila* gut, or with any canonical immune response. Here, we took a more targeted approach to specifically investigate how some of the mechanisms that prevent, sense, control and repair tissue damage affect disease tolerance during enteric bacterial infections.

Though repairing infection-damage is crucial to fly survival, we found that mutant flies with disrupted tissue damage-preventing (*dcy*) are particularly susceptible to oral infections compared to those lacking components that minimise, signal or repair tissue damage. In other words, preventing tissue damage would appear to be better than repairing from the perspective of fly survival. This result is consistent with previously described *dcy* knockout phenotypes. The loss-of-function in *dcy* increases the peritrophic matrix width by half and increases its permeability to larger molecules, including pathogens (Kuraishi, Hori, and Kurata 2013), making the gut leaky and compromising gut barrier function during oral bacterial infections (Opota et al. 2011; Kuraishi et al. 2011; Chakrabarti et al. 2012; Blemont et al. 2013). We also found increased bacterial loads measured after 24-hours following infection in both male and female *dcy* mutants, This is likely because of

the combination of leaky gut and pore-forming toxin produced by *P. entomophila* (Kuraishi, Hori, and Kurata 2013) resulted in higher bacterial growth in the fly haemolymph.

In the case of *upd3*-deficient flies, we found reduced survival and higher bacterial loads compared to wild type *w¹¹¹⁸* flies. Previous work has shown that in response to *P. entomophila* infections, excessive ROS (reactive oxygen species) produced by host cells destroy the gut epithelia and block the gut repair process (Han and Ulevitch 2005; Lambeth 2007; Chakrabarti et al. 2012). The *JNK* and *Hippo* pathways are activated in damaged *ECs* (enterocytes), which produce *upd3*, in turn activating the *Jak/Stat* pathway in *ISCs* (intestinal stem cells). Meanwhile, *upd3* also triggers the activation of *EGFR* signalling in *ISCs* to promote their proliferation and differentiation to renew the damaged cells/tissues (Chakrabarti et al. 2012; Kuraishi, Hori, and Kurata 2013; Buchon, Broderick, and Lemaitre 2013). We also found that loss-of-function in *irc* (immune-regulated catalase – negative regulator of ROS), results in lower bacterial loads, and this is likely because ROS is higher leading to greater clearance.

Regarding the effects of these damage limitation mechanisms on disease tolerance, overall, we found that both male and female wild type *w¹¹¹⁸* flies were quite tolerant of enteric bacterial infections (reflected in their relatively flat tolerance slopes, **Fig. 5. Table 3**), while disrupting any damage prevention and tissue repair mechanism lowered disease tolerance (steep decline in slopes for all the mutant lines). While we found reduced tolerance in all mutants, disrupting some components of damage limitation had particularly severe effects on disease tolerance. Significant reductions in disease tolerance were observed in flies with loss-of-function in *irc* and *upd3*, and in these mutants the effect was comparable in both sexes. *Irc*-deficient flies are unable to regulate ROS levels which would lead to increased cytotoxic effects (Buchon et al. 2009; Kuraishi, Hori, and Kurata 2013) while *upd3* mutants are unable to trigger the activation of the *Jak/Stat* pathway (Jiang et al. 2009; Buchon et al. 2010). Our results suggest that the absence of these mechanisms affects fly health independently of pathogen load, thereby resulting in changes in disease tolerance.

We observed, the fastest decline in tolerance in male flies lacking *egfr^J*, but the disease tolerance of female *egfr^J* mutants appeared unaffected. This sex difference in tolerance may arise as the result of sex differences in gut physiology and repair. Recent work has demonstrated that during oral *Ecc15* infection, males showed significantly lower gut *ISCs* in response to infection, while female had higher *ISCs* and were resistant to infection and other stress (Regan et al. 2016). The differentiation and proliferation of *ISCs* (intestinal stem cells) via *Jak/Stat* signalling into *ECs* (enterocytes) via

egfr is indispensable for tissue renewal. Loss of *egfr*^J signalling might therefore be felt more severely in males than in females, explaining why male but not female *egfr*^J mutants showed severe a decrease in disease tolerance. To date, only a small proportion of studies have compared sex-differences in intestinal immunity, with the majority of work focusing on one particular sex, usually females (Ayyaz, Li, and Jasper 2015; Chakrabarti et al. 2016; Regan et al. 2016; Belmonte et al. 2020).

Another possibility for the observed sex-difference in tissue renewal process, could relate to sexual dimorphism in gut-plasticity and remodelling. For instance, females of mammals such as mice extensively remodel their guts, increasing both digestive and absorptive capacity depending on the nutritional demands of lactation (Speakman 2008). The remodelling of the gut might be one of the possible driving factors for dimorphism in gut immunity, since males and females differ in their nutritional needs (Belmonte et al. 2020). Studies using *Drosophila* have shown that males and females can make different diet or nutritional choices in accordance with their reproductive role and demand (Camus, Piper, and Reuter 2019) and that the *Drosophila* midgut plastically resizes in response to changes in dietary sugar and yeast (Bonfini et al. 2021). Whether gut remodelling and nutritional choice-demand causes sex-differences in tissue damage repair process during disease tolerance remains a question for future research.

Although host mechanisms of immune-mediated clearance are key for pathogen defence and elimination, there is an increasing appreciation that additional defence mechanisms which prevent, signal, repair or renew the extent of tissue damage are also key to infection outcomes by promoting disease tolerance (Soares, Gozzelino, and Weis 2014; Vale, Fenton, and Brown 2014). Tissue repair mechanisms that promote disease tolerance are interesting from a therapeutic perspective (Ayres and Schneider 2012; Medzhitov, Schneider, and Soares 2012; Vale et al. 2016). For instance, in mice, mechanisms that prevent damage or repair tissues have been shown to confer disease tolerance during malarial *Plasmodium* infection and also during co-infections by pneumonia causing bacteria (*Legionella pneumophila*) and influenza virus (Ferreira et al. 2011; Jamieson et al. 2013). Understanding how tissue damage prevention and repair mechanisms contribute to disease tolerance may also help explain how other arthropods are able to vector bacterial and viral infections without substantial health loss (Taracena et al. 2018; Oliveira, Bahia, and Vale 2020; Lambrechts and Saleh 2019). In summary, our results show that the absence of tissue repair processes resulted in severe loss of disease tolerance, and highlight how sex differences in some tissue damage repair mechanisms could generate sexual dimorphism in gut immunity.

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Authors' contribution

A.P and P.F.V conceived and designed the experiments; A.P carried out experiments with assistance from K.M.M; A.P analysed the data; A.P and P.F.V wrote the manuscript.

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Competing interests

None.

References

- Adelman, James S., Laila Kirkpatrick, Jessica L. Grodio, and Dana M. Hawley. 2013. "House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of *Mycoplasma Gallisepticum* Infection." *The American Naturalist* 181 (5): 674–89. <https://doi.org/10.1086/670024>.
- Apidianakis, Yiorgos, and Laurence G. Rahme. 2011. "Drosophila Melanogaster as a Model for Human Intestinal Infection and Pathology." *Disease Models & Mechanisms* 4 (1): 21–30. <https://doi.org/10.1242/dmm.003970>.
- Ayres, Janelle S, and David S Schneider. 2012. "Tolerance of Infections." *Annual Review of Immunology* 30: 271–94. <https://doi.org/10.1146/annurev-immunol-020711-075030>.
- Ayyaz, Arshad, Hongjie Li, and Heinrich Jasper. 2015. "Haemocytes Control Stem Cell Activity in the Drosophila Intestine." *Nature Cell Biology* 17 (6): 736–48. <https://doi.org/10.1038/ncb3174>.
- Belmonte, Rebecca L., Mary-Kate Corbally, David F. Duneau, and Jennifer C. Regan. 2020. "Sexual Dimorphisms in Innate Immunity and Responses to Infection in Drosophila Melanogaster." *Frontiers in Immunology* 10 (January): 3075. <https://doi.org/10.3389/fimmu.2019.03075>.

- Blemont, M., R. Vincentelli, C. Kellenberger, O. Opota, B. Lemaitre, A. Roussel, and P. Leone. 2013. "Crystallization and Preliminary X-Ray Analysis of Monalysin, a Novel β -Pore-Forming Toxin from the Entomopathogen *Pseudomonas Entomophila*." *Acta Crystallographica Section F: Structural Biology and Crystallization Communications* 69 (8): 930–33. <https://doi.org/10.1107/S174430911301885X>.
- Bonfini, Alessandro, Adam J Dobson, David Duneau, Jonathan Revah, Xi Liu, Philip Houtz, and Nicolas Buchon. 2021. "Multiscale Analysis Reveals That Diet-Dependent Midgut Plasticity Emerges from Alterations in Both Stem Cell Niche Coupling and Enterocyte Size." Edited by Lucy Erin O'Brien. *ELife* 10 (September): e64125. <https://doi.org/10.7554/eLife.64125>.
- Bonneaud, Camille, Luc Tardy, Mathieu Giraudeau, Geoffrey E. Hill, Kevin J. McGraw, and Alastair J. Wilson. 2019. "Evolution of Both Host Resistance and Tolerance to an Emerging Bacterial Pathogen." *Evolution Letters* 3 (5): 544–54. <https://doi.org/10.1002/evl3.133>.
- Buchon, Nicolas, Nichole A. Broderick, Takayuki Kuraishi, and Bruno Lemaitre. 2010. "Drosophila EGFR Pathway Coordinates Stem Cell Proliferation and Gut Remodeling Following Infection." *BMC Biology* 8 (1): 152. <https://doi.org/10.1186/1741-7007-8-152>.
- Buchon, Nicolas, Nichole A. Broderick, and Bruno Lemaitre. 2013. "Gut Homeostasis in a Microbial World: Insights from *Drosophila Melanogaster*." *Nature Reviews Microbiology* 11 (9): 615–26. <https://doi.org/10.1038/nrmicro3074>.
- Buchon, Nicolas, Nichole A. Broderick, Mickael Poidevin, Sylvain Pradervand, and Bruno Lemaitre. 2009. "Drosophila Intestinal Response to Bacterial Infection: Activation of Host Defense and Stem Cell Proliferation." *Cell Host & Microbe* 5 (2): 200–211. <https://doi.org/10.1016/j.chom.2009.01.003>.
- Buchon, Nicolas, and Dani Osman. 2015. "All for One and One for All: Regionalization of the Drosophila Intestine | Elsevier Enhanced Reader." *Insect Biochemistry and Molecular Biology*. <https://doi.org/10.1016/j.ibmb.2015.05.015>.
- Buchon, Nicolas, Dani Osman, Fabrice P. A. David, Hsiao Yu Fang, Jean-Philippe Boquete, Bart Deplancke, and Bruno Lemaitre. 2013. "Morphological and Molecular Characterization of Adult Midgut Compartmentalization in *Drosophila*." *Cell Reports* 3 (5): 1725–38. <https://doi.org/10.1016/j.celrep.2013.04.001>.
- Buchon, Nicolas, Neal Silverman, and Sara Cherry. 2014. "Immunity in *Drosophila Melanogaster* — from Microbial Recognition to Whole-Organism Physiology." *Nature Reviews Immunology* 14 (12): 796–810. <https://doi.org/10.1038/nri3763>.
- Camus, M Florencia, Matthew DW Piper, and Max Reuter. 2019. "Sex-Specific Transcriptomic Responses to Changes in the Nutritional Environment." Edited by Laurent Keller, Michael B Eisen, and Felix Zajitschek. *ELife* 8 (August): e47262. <https://doi.org/10.7554/eLife.47262>.
- Chakrabarti, Sveta, Jan Paul Dudzic, Xiaoxue Li, Esther Jeanne Collas, Jean-Phillipe Boquete, and Bruno Lemaitre. 2016. "Remote Control of Intestinal Stem Cell Activity by Haemocytes in *Drosophila*." Edited by Utpal Banerjee. *PLOS Genetics* 12 (5): e1006089. <https://doi.org/10.1371/journal.pgen.1006089>.
- Chakrabarti, Sveta, Peter Liehl, Nicolas Buchon, and Bruno Lemaitre. 2012. "Infection-Induced Host Translational Blockage Inhibits Immune Responses and Epithelial Renewal in the *Drosophila* Gut." *Cell Host & Microbe* 12 (1). <https://doi.org/10.1016/j.chom.2012.06.001>.
- Chandler, James Angus, Jenna Morgan Lang, Srijak Bhatnagar, Jonathan A. Eisen, and Artyom Kopp. 2011. "Bacterial Communities of Diverse *Drosophila* Species:

- Ecological Context of a Host–Microbe Model System.” *PLOS Genetics* 7 (9): e1002272. <https://doi.org/10.1371/journal.pgen.1002272>.
- Cooper, Edwin L., ed. 2018. *Advances in Comparative Immunology*. Springer International Publishing. <https://doi.org/10.1007/978-3-319-76768-0>.
- Corby-Harris, Vanessa, Ana Clara Pontaroli, Lawrence J. Shimkets, Jeffrey L. Bennetzen, Kristin E. Habel, and Daniel E. L. Promislow. 2007. “Geographical Distribution and Diversity of Bacteria Associated with Natural Populations of *Drosophila Melanogaster*.” *Applied and Environmental Microbiology* 73 (11): 3470–79. <https://doi.org/10.1128/AEM.02120-06>.
- Cumnock, Katherine, Avni S. Gupta, Michelle Lissner, Victoria Chevee, Nicole M. Davis, and David S. Schneider. 2018. “Host Energy Source Is Important for Disease Tolerance to Malaria.” *Current Biology* 28 (10): 1635–1642.e3. <https://doi.org/10.1016/j.cub.2018.04.009>.
- Dieppois, Guennaëlle, Onya Opota, Jorge Lalucat, and Bruno Lemaitre. 2015. “*Pseudomonas Entomophila*: A Versatile Bacterium with Entomopathogenic Properties.” In *Pseudomonas*, edited by Juan-Luis Ramos, Johanna B. Goldberg, and Alain Filloux, 25–49. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-017-9555-5_2.
- Dionne, Marc S., Linh N. Pham, Mimi Shirasu-Hiza, and David S. Schneider. 2006. “Akt and Foxo Dysregulation Contribute to Infection-Induced Wasting in *Drosophila*.” *Current Biology* 16 (20): 1977–85. <https://doi.org/10.1016/j.cub.2006.08.052>.
- Ferreira, Ana, Ivo Marguti, Ingo Bechmann, Viktória Jeney, Ângelo Chora, Nuno R. Palha, Sofia Rebelo, Annie Henri, Yves Beuzard, and Miguel P. Soares. 2011. “Sickle Hemoglobin Confers Tolerance to Plasmodium Infection.” *Cell* 145 (3): 398–409. <https://doi.org/10.1016/j.cell.2011.03.049>.
- Gupta, Vanika, and Pedro F. Vale. 2017. “Nonlinear Disease Tolerance Curves Reveal Distinct Components of Host Responses to Viral Infection.” *Royal Society Open Science* 4 (7): 170342. <https://doi.org/10.1098/rsos.170342>.
- Han, Jiahuai, and Richard J Ulevitch. 2005. “Limiting Inflammatory Responses during Activation of Innate Immunity.” *Nature Immunology* 6 (12): 1198–1205. <https://doi.org/10.1038/ni1274>.
- Hegedus, Dwayne, Martin Erlandson, Cedric Gillott, and Umut Toprak. 2009. “New Insights into Peritrophic Matrix Synthesis, Architecture, and Function.” *Annual Review of Entomology* 54 (1): 285–302. <https://doi.org/10.1146/annurev.ento.54.110807.090559>.
- Hoffmann, Jules A. 2003. “The Immune Response of *Drosophila*.” *Nature* 426 (6962): 33–38. <https://doi.org/10.1038/nature02021>.
- Howick, Virginia M., and Brian P. Lazzaro. 2014. “Genotype and Diet Shape Resistance and Tolerance across Distinct Phases of Bacterial Infection.” *BMC Evolutionary Biology* 14 (1): 56. <https://doi.org/10.1186/1471-2148-14-56>.
- Howick, Virginia M., and Brian P. Lazzaro. 2017. “The Genetic Architecture of Defence as Resistance to and Tolerance of Bacterial Infection in *Drosophila Melanogaster*.” *Molecular Ecology* 26 (6): 1533–46. <https://doi.org/10.1111/mec.14017>.
- Hultmark, Dan. 2003. “*Drosophila* Immunity: Paths and Patterns.” *Current Opinion in Immunology* 15 (1): 12–19. [https://doi.org/10.1016/S0952-7915\(02\)00005-5](https://doi.org/10.1016/S0952-7915(02)00005-5).
- Jamieson, Amanda M., Lesley Pasman, Shuang Yu, Pia Gamradt, Robert J. Homer, Thomas Decker, and Ruslan Medzhitov. 2013. “Role of Tissue Protection in Lethal Respiratory Viral-Bacterial Coinfection.” *Science* 340 (6137): 1230–34. <https://doi.org/10.1126/science.1233632>.

- Jiang, Huaqi, Marc O. Grenley, Maria-Jose Bravo, Rachel Z. Blumhagen, and Bruce A. Edgar. 2011. "EGFR/Ras/MAPK Signaling Mediates Adult Midgut Epithelial Homeostasis and Regeneration in *Drosophila*." *Cell Stem Cell* 8 (1): 84–95. <https://doi.org/10.1016/j.stem.2010.11.026>.
- Jiang, Huaqi, Parthiv H. Patel, Alexander Kohlmaier, Marc O. Grenley, Donald G. McEwen, and Bruce A. Edgar. 2009. "Cytokine/Jak/Stat Signaling Mediates Regeneration and Homeostasis in the *Drosophila* Midgut." *Cell* 137 (7): 1343–55. <https://doi.org/10.1016/j.cell.2009.05.014>.
- Kapun, Martin, Maite G Barrón, Fabian Staubach, Darren J Obbard, R Axel W Wiberg, Jorge Vieira, Clément Goubert, et al. 2020. "Genomic Analysis of European *Drosophila* *Melanogaster* Populations Reveals Longitudinal Structure, Continent-Wide Selection, and Previously Unknown DNA Viruses." *Molecular Biology and Evolution* 37 (9): 2661–78. <https://doi.org/10.1093/molbev/msaa120>.
- Kuraishi, Takayuki, Olivier Binggeli, Onya Opota, Nicolas Buchon, and Bruno Lemaitre. 2011. "Genetic Evidence for a Protective Role of the Peritrophic Matrix against Intestinal Bacterial Infection in *Drosophila* *Melanogaster*." *Proceedings of the National Academy of Sciences* 108 (38): 15966–71. <https://doi.org/10.1073/pnas.1105994108>.
- Kuraishi, Takayuki, Aki Hori, and Shoichiro Kurata. 2013. "Host-Microbe Interactions in the Gut of *Drosophila* *Melanogaster*." *Frontiers in Physiology* 4. <https://doi.org/10.3389/fphys.2013.00375>.
- Kutzer, Megan A.M., and Sophie A.O. Armitage. 2016. "Maximising Fitness in the Face of Parasites: A Review of Host Tolerance." *Zoology* 119 (4): 281–89. <https://doi.org/10.1016/j.zool.2016.05.011>.
- Lambeth, J. David. 2007. "Nox Enzymes, ROS, and Chronic Disease: An Example of Antagonistic Pleiotropy." *Free Radical Biology and Medicine* 43 (3): 332–47. <https://doi.org/10.1016/j.freeradbiomed.2007.03.027>.
- Lambrechts, Louis, and Maria-Carla Saleh. 2019. "Manipulating Mosquito Tolerance for Arbovirus Control." *Cell Host & Microbe* 26 (3): 309–13. <https://doi.org/10.1016/j.chom.2019.08.005>.
- Lemaitre, Bruno, and Jules Hoffmann. 2007. "The Host Defense of *Drosophila* *Melanogaster*." *Annual Review of Immunology* 25 (1): 697–743. <https://doi.org/10.1146/annurev.immunol.25.022106.141615>.
- Lemaitre, Bruno, and Irene Miguel-Aliaga. 2013. "The Digestive Tract of *Drosophila* *Melanogaster*." *Annual Review of Genetics* 47 (1): 377–404. <https://doi.org/10.1146/annurev-genet-111212-133343>.
- Lissner, Michelle M, and David S Schneider. 2018. "The Physiological Basis of Disease Tolerance in Insects." *Current Opinion in Insect Science*, Global change biology * Molecular physiology, 29 (October): 133–36. <https://doi.org/10.1016/j.cois.2018.09.004>.
- Louie, Alexander, Kyung Han Song, Alejandra Hotson, Ann Thomas Tate, and David S. Schneider. 2016. "How Many Parameters Does It Take to Describe Disease Tolerance?" Edited by Andy P. Dobson. *PLOS Biology* 14 (4): e1002435. <https://doi.org/10.1371/journal.pbio.1002435>.
- Marianes, Alexis, and Allan C Spradling. 2013. "Physiological and Stem Cell Compartmentalization within the *Drosophila* Midgut." Edited by Andrea Brand. *ELife* 2 (August): e00886. <https://doi.org/10.7554/eLife.00886>.
- McCarville, JL, and JS Ayres. 2018. "Disease Tolerance: Concept and Mechanisms." *Current Opinion in Immunology*, Innate immunity 2018, 50 (February): 88–93. <https://doi.org/10.1016/j.coi.2017.12.003>.

- Medzhitov, Ruslan, David S. Schneider, and Miguel P. Soares. 2012. "Disease Tolerance as a Defense Strategy." *Science* 335 (6071): 936–41. <https://doi.org/10.1126/science.1214935>.
- Myllymäki, Henna, and Mika Rämet. 2014. "JAK/STAT Pathway in Drosophila Immunity." *Scandinavian Journal of Immunology* 79 (6): 377–85. <https://doi.org/10.1111/sji.12170>.
- Myllymäki, Henna, Susanna Valanne, and Mika Rämet. 2014. "The Drosophila Imd Signaling Pathway." *The Journal of Immunology* 192 (8): 3455–62. <https://doi.org/10.4049/jimmunol.1303309>.
- Nahrendorf, Wiebke, Alasdair Ivens, and Philip J Spence. 2021. "Inducible Mechanisms of Disease Tolerance Provide an Alternative Strategy of Acquired Immunity to Malaria." Edited by Florent Ginhoux and Satyajit Rath. *ELife* 10 (March): e63838. <https://doi.org/10.7554/eLife.63838>.
- Oliveira, José Henrique, Ana Cristina Bahia, and Pedro F. Vale. 2020. "How Are Arbovirus Vectors Able to Tolerate Infection?" *Developmental & Comparative Immunology* 103 (February): 103514. <https://doi.org/10.1016/j.dci.2019.103514>.
- Opota, Onya, Isabelle Vallet-Gély, Renaud Vincentelli, Christine Kellenberger, Ioan Iacovache, Manuel Rodrigo Gonzalez, Alain Roussel, Françoise-Gisou van der Goot, and Bruno Lemaitre. 2011. "Monalysin, a Novel β -Pore-Forming Toxin from the Drosophila Pathogen Pseudomonas Entomophila, Contributes to Host Intestinal Damage and Lethality." *PLOS Pathogens* 7 (9): e1002259. <https://doi.org/10.1371/journal.ppat.1002259>.
- Palaferrri Schieber, Alexandria M., Yujung Michelle Lee, Max W. Chang, Mathias Leblanc, Brett Collins, Michael Downes, Ronald M. Evans, and Janelle S. Ayres. 2015. "Disease Tolerance Mediated by Microbiome E. Coli Involves Inflammasome and IGF-1 Signaling." *Science* VOL 350 (ISSUE 6260). <https://www.science.org/doi/full/10.1126/science.aac6468>.
- Raberg, L., D. Sim, and A. F. Read. 2007. "Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals." *Science* 318 (5851): 812–14. <https://doi.org/10.1126/science.1148526>.
- Regan, Jennifer C, Mobina Khericha, Adam J Dobson, Ekin Bolukbasi, Nattaphong Rattanavirotkul, and Linda Partridge. 2016. "Sex Difference in Pathology of the Ageing Gut Mediates the Greater Response of Female Lifespan to Dietary Restriction." *ELife* 5 (February): e10956. <https://doi.org/10.7554/eLife.10956>.
- Regoes, Roland R., Paul J. McLaren, Manuel Battegay, Enos Bernasconi, Alexandra Calmy, Huldrych F. Günthard, Matthias Hoffmann, et al. 2014. "Disentangling Human Tolerance and Resistance Against HIV." Edited by David S. Schneider. *PLoS Biology* 12 (9): e1001951. <https://doi.org/10.1371/journal.pbio.1001951>.
- Rera, Michael, Rebecca I. Clark, and David W. Walker. 2012. "Intestinal Barrier Dysfunction Links Metabolic and Inflammatory Markers of Aging to Death in Drosophila." *Proceedings of the National Academy of Sciences* 109 (52): 21528–33. <https://doi.org/10.1073/pnas.1215849110>.
- Schneider, David S., and Janelle S. Ayres. 2008. "Two Ways to Survive Infection: What Resistance and Tolerance Can Teach Us about Treating Infectious Diseases." *Nature Reviews Immunology* 8 (11): 889–95. <https://doi.org/10.1038/nri2432>.
- Siva-Jothy, Jonathon A., Arun Prakash, Radhakrishnan B. Vasanthakrishnan, Katy M. Monteith, and Pedro F. Vale. 2018. "Oral Bacterial Infection and Shedding in Drosophila Melanogaster." *Journal of Visualized Experiments*, no. 135 (May): 57676. <https://doi.org/10.3791/57676>.

- Soares, Miguel P., Raffaella Gozzelino, and Sebastian Weis. 2014. "Tissue Damage Control in Disease Tolerance." *Trends in Immunology* 35 (10): 483–94. <https://doi.org/10.1016/j.it.2014.08.001>.
- Soares, Miguel P., Luis Teixeira, and Luis F. Moita. 2017. "Disease Tolerance and Immunity in Host Protection against Infection." *Nature Reviews Immunology* 17 (2): 83–96. <https://doi.org/10.1038/nri.2016.136>.
- Speakman, John R. 2008. "The Physiological Costs of Reproduction in Small Mammals." *Philosophical Transactions of the Royal Society B: Biological Sciences* 363 (1490): 375–98. <https://doi.org/10.1098/rstb.2007.2145>.
- Taracena, Mabel L., Vanessa Bottino-Rojas, Octavio A. C. Talyuli, Ana Beatriz Walter-Nuno, José Henrique M. Oliveira, Yessenia I. Angleró-Rodriguez, Michael B. Wells, George Dimopoulos, Pedro L. Oliveira, and Gabriela O. Paiva-Silva. 2018. "Regulation of Midgut Cell Proliferation Impacts Aedes Aegypti Susceptibility to Dengue Virus." *PLOS Neglected Tropical Diseases* 12 (5): e0006498. <https://doi.org/10.1371/journal.pntd.0006498>.
- Therneau, Terry. 2015. "Mixed Effects Cox Models." In *Mixed Effects Cox Models*. CRAN repository. <https://repo.bppt.go.id/cran/web/packages/coxme/vignettes/coxme.pdf>.
- Troha, Katia, Joo Hyun Im, Jonathan Revah, Brian P. Lazzaro, and Nicolas Buchon. 2018. "Comparative Transcriptomics Reveals CrebA as a Novel Regulator of Infection Tolerance in D. Melanogaster." *PLOS Pathogens* 14 (2): e1006847. <https://doi.org/10.1371/journal.ppat.1006847>.
- Valanne, Susanna, Jing-Huan Wang, and Mika Rämet. 2011. "The Drosophila Toll Signaling Pathway." *The Journal of Immunology* 186 (2): 649–56. <https://doi.org/10.4049/jimmunol.1002302>.
- Vale, Pedro F., Andy Fenton, and Sam P. Brown. 2014. "Limiting Damage during Infection: Lessons from Infection Tolerance for Novel Therapeutics." *PLOS Biology* 12 (1): e1001769. <https://doi.org/10.1371/journal.pbio.1001769>.
- Vale, Pedro F., Luke McNally, Andrea Doeschl-Wilson, Kayla C. King, Roman Popat, Maria R. Domingo-Sananes, Judith E. Allen, Miguel P. Soares, and Rolf Kümmerli. 2016. "Beyond Killing: Can We Find New Ways to Manage Infection?" *Evolution, Medicine, and Public Health* 2016 (1): 148–57. <https://doi.org/10.1093/emph/eow012>.
- Vale, Pedro F., Jonathon Siva-Jothy, Andre Morrill, and Mark Forbes. 2018. "The Influence of Parasites on Insect Behavior." In *Insect Behavior: From Mechanisms to Ecological and Evolutionary Consequences*, 274–91. OUP Oxford. <https://drive.google.com/file/d/1aQfAPN47IUILHmCu6d144vEYSOSEbcuM/view?usp=sharing>.
- Vodovar, N., M. Vinals, P. Liehl, A. Basset, J. Degrouard, P. Spellman, F. Boccard, and B. Lemaitre. 2005. "Drosophila Host Defense after Oral Infection by an Entomopathogenic Pseudomonas Species." *Proceedings of the National Academy of Sciences* 102 (32): 11414–19. <https://doi.org/10.1073/pnas.0502240102>.
- Wallace, Megan A, Kelsey A Coffman, Clément Gilbert, Sanjana Ravindran, Gregory F Albery, Jessica Abbott, Eliza Argyridou, et al. 2021. "The Discovery, Distribution, and Diversity of DNA Viruses Associated with Drosophila Melanogaster in Europe." *Virus Evolution* 7 (1). <https://doi.org/10.1093/ve/veab031>.
- Weng, Meng-Shih, Jer-Hwa Chang, Wen-Yueh Hung, Yi-Chieh Yang, and Ming-Hsien Chien. 2018. "The Interplay of Reactive Oxygen Species and the Epidermal Growth Factor Receptor in Tumor Progression and Drug Resistance." *Journal of Experimental & Clinical Cancer Research* 37 (1): 61. <https://doi.org/10.1186/s13046-018-0728-0>.

Supplementary information:

Figure S1. (A) Survival curves for male and female flies of wild-type w^{1118} and mutants with disrupted tissue damage prevention and repair process in adult *Drosophila*, exposed to $OD_{600}=10$ (low dose) of oral *Pseudomonas entomophila* infection **(B)** Bacterial load measured at 24-hours after $OD_{600}=10$ of oral *Pseudomonas entomophila* infection for male and female flies of wild-type w^{1118} and mutant flies. Significantly different fly lines are connected with different alphabets using Tukey's HSD as a post hoc analysis of pairwise comparisons in panel B.

Figure S2. (A) Survival curves of male and female flies of wild-type *w¹¹¹⁸* and mutant flies exposed to OD₆₀₀=45 (high dose) of oral *Pseudomonas entomophila* infection **(B)** Bacterial load measured at 24-hours after OD₆₀₀=45 of oral *Pseudomonas entomophila* infection for male and female flies of wild-type *w¹¹¹⁸* and mutant flies. Significantly different fly lines are connected with different alphabets using Tukey's HSD as a post hoc analysis of pairwise comparisons in panel B.

Table S1. Summary of mixed effects Cox model, for male and female wildtype *m¹¹⁸* and mutant flies orally infected with 3 different doses of *P. entomophila*. We used 3-5-day old adult males and females of each fly lines (wildtype and mutants) and specified the model as: Survival ~ Treatment x Sex x Fly line (1 | vial/block), with ‘Treatment’, ‘Sex’ and ‘Fly line’ as fixed effects, and ‘vials’ nested in ‘block’ as a random effect. The table shows model output (ANOVA) for survival post oral infection for wildtype *m¹¹⁸* flies and mutant flies.

<i>Infection dose</i>	<i>Source</i>	<i>Loglik</i>	<i>Chisq</i>	<i>df</i>	<i>p</i>
<i>OD₆₀₀=10</i>	Treatment	-12880	968.61	1	< 0.001
	Sex	-12872	17.367	1	< 0.001
	Fly line	-12669	404.70	4	< 0.001
	Treatment x sex	-12669	0.9114	1	0.33
	Treatment x fly line	-12652	33.944	4	< 0.001
	Sex x fly line	-12650	3.9894	4	0.40
	Treatment x sex x fly line	-12647	6.4149	4	0.17
	<i>Random effect vial/ block</i>	<i>Std dev 8.88E-04</i>			
<i>OD₆₀₀=25</i>	Treatment	-37499	3058.87	1	< 0.001
	Sex	-37487	25.046	1	< 0.001
	Fly line	-37257	459.75	4	< 0.001
	Treatment x sex	-37250	14.685	1	< 0.001
	Treatment x fly line	-37240	18.773	4	< 0.001
	Sex x fly line	-37236	7.8494	4	0.09
	Treatment x sex x fly line	-37235	2.9252	4	0.57
	<i>Random effect vial/ block</i>	<i>Std dev 1.92E-03</i>			
<i>OD₆₀₀=45</i>	Treatment	21144	3256.2	1	< 0.001
	Sex	21141	5.5753	1	< 0.001
	Fly line	21101	80.198	4	< 0.001
	Treatment x sex	21101	1.0625	1	0.30
	Treatment x fly line	21098	5.7909	4	0.21
	Sex x fly line	21094	6.6203	4	0.15
	Treatment x sex x fly line	21094	1.8525	4	0.76
	<i>Random effect vial/ block</i>	<i>Std dev 0.019</i>			

Table S2. Summary of log₁₀ transformed bacterial load data after exposure to OD₆₀₀=25 of oral *P. entomophila* infection for male and female wildtype *w¹¹¹⁸* and mutant flies, analysed using non-parametric one-way ANOVA (Kruskal–Wallis test) at 3 different timepoints (0-15 minutes, 24-hours and 96-hours following oral infection)

	<i>Sex</i>	<i>Fly line</i>		<i>Mean diff</i>	<i>Z</i>	<i>p</i>
<i>0-15 min</i>	<i>Female</i>	<i>egfr1</i>	<i>w¹¹¹⁸</i>	3.50	0.77	0.43
		<i>irc</i>	<i>w¹¹¹⁸</i>	-7.83	-1.73	0.08
		<i>upd-3</i>	<i>w¹¹¹⁸</i>	-11.03	-2.44	0.01
		<i>dcy</i>	<i>w¹¹¹⁸</i>	-11.16	-2.47	0.01
	<i>Male</i>	<i>egfr1</i>	<i>w¹¹¹⁸</i>	29.56	6.55	< 0.001
		<i>dcy</i>	<i>w¹¹¹⁸</i>	28.96	6.423	< 0.001
		<i>upd-3</i>	<i>w¹¹¹⁸</i>	28.96	6.42	< 0.001
		<i>irc</i>	<i>w¹¹¹⁸</i>	28.033	6.21	< 0.001

	<i>Sex</i>	<i>Fly line</i>		<i>Mean diff</i>	<i>Z</i>	<i>p</i>
<i>24-hours</i>	<i>Female</i>	<i>dcy</i>	<i>w¹¹¹⁸</i>	24.30	5.388	<0.001
		<i>upd-3</i>	<i>w¹¹¹⁸</i>	22.23	4.930	<0.001
		<i>egfr1</i>	<i>w¹¹¹⁸</i>	-7.233	-1.604	0.108
		<i>irc</i>	<i>w¹¹¹⁸</i>	-18.96	-4.206	<0.001
	<i>Male</i>	<i>upd-3</i>	<i>w¹¹¹⁸</i>	28.70	6.364	<0.001
		<i>dcy</i>	<i>w¹¹¹⁸</i>	25.90	5.743	<0.001
		<i>egfr1</i>	<i>w¹¹¹⁸</i>	-7.233	-1.604	0.10
		<i>irc</i>	<i>w¹¹¹⁸</i>	-14.16	-3.141	0.001

	<i>Sex</i>	<i>Fly line</i>		<i>Mean diff</i>	<i>Z</i>	<i>p</i>
<i>96-hours</i>	<i>Female</i>	<i>dcy</i>	<i>w¹¹¹⁸</i>	26.90	5.965	< 0.001
		<i>upd-3</i>	<i>w¹¹¹⁸</i>	6.100	1.352	0.17
		<i>irc</i>	<i>w¹¹¹⁸</i>	-5.166	-1.145	0.25
		<i>egfr1</i>	<i>w¹¹¹⁸</i>	-8.566	-1.899	0.057
	<i>Male</i>	<i>dcy</i>	<i>w¹¹¹⁸</i>	26.50	5.876	< 0.001
		<i>egfr1</i>	<i>w¹¹¹⁸</i>	22.73	5.041	< 0.001
		<i>upd-3</i>	<i>w¹¹¹⁸</i>	20.36	4.516	< 0.001
		<i>irc</i>	<i>w¹¹¹⁸</i>	17.56	3.895	< 0.001