Mechanisms of damage prevention, signalling, and repair impact disease tolerance

Arun Prakash[1,2]*, Katy M. Monteith[1], Pedro F. Vale[1]*

[1]Institute of Evolutionary Biology, School of Biological Sciences
University of Edinburgh, EH9 3FL, United Kingdom.

[2]Current address: Vanderbilt University, 465 21st Ave S, MRB-III
Biological Sciences, Nashville, TN 37212, USA

*Correspondence
arunpadmaprakash@gmail.com
pedro.vale@ed.ac.uk

Keywords: Disease tolerance, Gut-epithelial immunity, Tissue damage repair, Oral bacterial infection, enteric infection, infection dose

Abstract

The insect gut is frequently exposed to pathogenic threats and must not only clear these potential infections, but also tolerate relatively high microbe loads. In contrast to the mechanisms that eliminate pathogens, we currently know less about the mechanisms of disease tolerance. We investigated how well-described mechanisms that either prevent, signal, control, or repair damage during infection contribute to the phenotype of disease tolerance. We established enteric infections with the bacterial pathogen *Pseudomonas entomophila* in transgenic lines of *Drosophila melanogaster* fruit flies affecting *dcy* (a major component of the peritrophic matrix), *upd3* (a cytokine-like molecule), *irc* (a negative regulator of reactive oxygen species) and $egfr^{1}$ (epithelial growth factor receptor). Flies lacking *dcy* experienced the highest susceptibility, while loss of function of either *irc* or *upd3* reduced tolerance in both sexes. The disruption of $egfr^{1}$ 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 evidence for the role of damage limitation mechanisms in disease tolerance and highlight how sex differences in these mechanisms could generate sexual dimorphism in disease tolerance.

Introduction

Many insects thrive on decomposing and decaying organic matter containing a diversity of commensal and pathogenic microorganisms. Like most animals, insects 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 [1–3]. 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 [1,2,4–7]. In addition to mechanisms that reduce pathogen burdens, it is increasingly recognised that mechanisms promoting disease tolerance are equally important during recovery to a healthy state [8–12]. 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 [13–17] rodents [18,8,19]; birds [20,21]; and humans [22,23,11].

The mechanisms of pathogen clearance are well-described in many animal species [24], but we currently know less about the mechanisms underlying disease tolerance. Given that tolerance reflects the ability to maintain health independently of pathogen clearance, we might expect tolerance mechanisms to be related to processes such as detoxification, reduction of inflammation, or tissue damage control and cellular renewal [9,11,25]. Genome-wide association or transcriptomic studies in *Drosophila* have highlighted potential candidate genes underlying phenotypic variation in disease tolerance [14], but it remains unclear how many of these genes interact with known mechanisms of immunity and recovery.

Furthermore, almost all candidate genes for disease tolerance in *Drosophila* arise from systemic infections [14,26,27], leaving a gap in our knowledge about disease tolerance during orally acquired infections, which are especially relevant in the context of the ecology of most insects, that thrive on decomposing and decaying organic matter containing a diversity of both commensal and pathogenic microorganisms [28–30]. 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 well-established mechanisms that either prevent, reduce, or repair tissue damage 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 [2,31–33]. We can consider several stages comprising

79 gut defence in *Drosophila* (***Fig.*** *SI-1)*. The first involves the physical barrier of the gut epithelia
80 and the peritrophic matrix (*PM*), which is a layer of chitin and glycoproteins that lines the insect
81 midgut lumen. The *PM* is functionally analogous to mammalian mucus membrane in the
82 digestive tract and acts as the first line of defence against invading pathogens [2,34]. A major
83 component of the *PM* is *drosocrystallin* (*dcy*). Loss-of-function mutations in *dcy* increase the
84 permeability of the peritrophic matrix to larger molecules and allow leakage of microbial cells,
85 including pathogens, into the haemolymph. *Dcy* deficient flies therefore exhibit increased
86 susceptibility to oral bacterial infections [2].
87
88 Another mode of defence during gut infections is the production of reactive oxygen species
89 (*ROS*) by the gut epithelia. For example, in response to ingested *Pseudomonas entomophila*,
90 *ROS* production is induced by two NADPH enzymes- *nox* (NADPH oxidase) and *duox* (dual
91 oxidase), while *irc* (immune-reactive catalase) negatively regulates *ROS* production once the
92 infection threat is controlled, which otherwise, would lead to cytotoxic effects [2,35,36]. *ROS*
93 production not only targets pathogens directly, but also plays additional roles in triggering
94 signalling pathways that lead to the production of *IMD*- or *Toll*-responsive antimicrobial
95 peptides [36–39].
96
97 The final stage in gut defence is to repair the damage caused during the infection. Damage-
98 signalling cytokine-like molecules *upd3* are released from damaged cells which trigger the
99 *Jak/Stat*-pathway, stimulating the proliferation of intestinal stem cells (*ISCs*) and their
100 differentiation into enterocytes (*ECs*) via *egfr*[1] (epidermal growth factor receptor) signalling
101 [40–42]. Flies lacking *Jak/Stat* or *Egfr* are therefore highly susceptible to bacterial infections
102 due to their inability to repair and renew damaged tissue [40,42,43].
103
104 To investigate how these mechanisms of damage prevention (*dcy*), signalling (*upd3*) control
105 (*irc*) and renewal (*egfr*) contribute to disease tolerance during gut infections we employed oral
106 infections in *Drosophila* transgenic lines with loss-of-function in each of these genes on a
107 common genetic background (w^{1118}). We orally challenged these flies with three infection
108 doses of *Pseudomonas entomophila* and then quantified their effects on survival, pathogen
109 loads and disease tolerance responses during period of peak infection burden.
110
111
112 **Materials and methods**

113 *Fly strains:* The following fly stocks were obtained from the Bloomington Stock Centre, Indiana:
114 *dcy* (w^{1118}; Mi{ET1}^{CrysMB08319}; FB#*26106*) [44], *irc* (w^{1118}; Mi{ET1}^{IrcMB11278}; *FB#29191*) [45],

115 (*#2079*), *upd3* (*$w^{1118;}$* P{XP}^{upd3d11639}*; FB#19355*) [46]. These lines were subsequently
116 isogenised by backcrossing onto the same *w^{1118}* background (*VDRC stock# 60000*) for at least
117 10 generations. The egfr^{t1} mutant was a kind gift from Carla Saleh (Pasteur Institute, Paris)
118 and previously isogenised to *w^{1118}* first by replacing the chromosomes not containing the
119 mutation using balancer chromosomes and then by backcrossing at least ten times
120 to *w^{1118}* line [47]. All fly lines were maintained in plastic vials (12 ml) on a standard sugar-
121 cornmeal medium at a constant temperature of 25°C (±2°C).
122
123 <u>*Bacterial culture preparation:*</u> To test the impact of bacterial infection on fly survival we used
124 the gram-negative bacteria *Pseudomonas entomophila*, that commonly infects a broad range
125 of insects and other invertebrates. In flies, *P. entomophila* infection mainly occurs in the
126 intestinal epithelium and eventually causes death [35]. To obtain bacterial cultures for oral
127 exposure, we inoculated frozen isogenic bacterial stock cultures stored at -80℃ onto fresh
128 15ml LB broth (media composition) and incubated overnight at 37°C with shaking at 120rpm
129 (revolutions per minute). The overnight cultures were diluted 1:100 into 500 ml of fresh LB
130 broth and incubated again at 30°C with shaking at 120rpm. At the mid-log phase (OD_{600}=0.75),
131 we harvested the bacterial cells by centrifugation at 5000rpm for 15 min and re-suspended
132 the bacterial pellet in 5% sucrose [48]. The final inoculum was adjusted to three different
133 bacterial concentrations or infection dose OD_{600}=10 (low dose), OD_{600}=25 (medium dose) and
134 OD_{600}=45 (high dose).
135
136 <u>*Experimental design*</u>: Measuring tolerance as a linear reaction norm requires collecting
137 matching data on survival and pathogen loads, ideally from the same individual. However, this
138 is challenging in the fly model because quantifying microbe loads requires destructive
139 sampling. Instead, we considered the vial as the unit of replication, and employed a split vial
140 design (***Fig***. *SI*-2). In total, we set up 500 infection vials, split across 2 experimental blocks
141 (n=10 vials for OD_{600}=10 and OD_{600}=45; n=30 vials for OD_{600}=25, for each combination of sex
142 (2) /fly line (5) – with each vial containing 27-30 flies for all doses). Following oral bacterial
143 exposure (see below) each vial containing 25 flies of each infection treatment, sex and fly line
144 combination were split into two vials for measuring (1) survival following infection (15
145 flies/combination) and (2) internal bacterial load (10 flies/combination) (***Fig***. *SI-2)*. This split-
146 vial design allowed us to use replicate-matched data for both the proportion of flies surviving
147 and the average bacterial load for each replicate vial to estimate the linear relationship
148 between fly survival and internal bacterial load for each fly line.
149
150 <u>*Oral infection assay:*</u> Before infecting flies we prepared infection vials by pipetting 350µl of
151 standard agar (1L triple distilled H_2O, 20g agar, 84g brown sugar, 7ml Tegosept anti-fungal

agent) onto the lids of 7ml tubes (bijou vials) and allowed it to dry. Simultaneously, we starved the experimental flies in 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 a 5% sucrose solution. We then orally exposed flies inside the bijou vials for 18-hours and then transferred the flies onto fresh vials containing standard sugar-cornmeal medium [48].

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 using 3–5-day old flies (w^{1118} and transgenic flies) following three different doses of *P. entomophila* enteric infection. We used either OD_{600}=10 (low dose), OD_{600}=25 (medium dose) or OD_{600}=45 (high dose). For OD_{600}=25 (medium dose), we measured bacterial load at three timepoints (immediately after oral exposure 0-15 minutes, 24-hours, and 96-hours) following *P. entomophila* infection. 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 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 [48].

Statistics

Survival following oral infection: We analysed survival data using a mixed effects Cox model using the R package 'coxme' [49]. 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 w^{1118} and flies deficient of damage prevention and repair mechanisms in gut-epithelia.

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 Kruskal-Wallis test to test the effects of each fly line, that is, control w^{1118} and transgenic flies for males and females separately following oral *P. entomophila* infection.

188 *Measuring disease tolerance:* Finally, to understand how damage signalling and repair
189 mechanisms affect disease tolerance in males and females during oral *P. entomophila*
190 infection, we analysed the linear relationship between fly survival against bacterial load by
191 fitting linear models [8,50,10,16,51,15]. We assessed differences in disease tolerance (fly
192 survival with increasing bacterial load) by fitting 'fly line' and 'sex' as categorical fixed effects,
193 'average bacterial load (log_{10})' as a continuous covariate, and their interactions as fixed
194 effects. Significant interaction effects between fly line and bacterial load would indicate that
195 the slope of the relationship between fly survival and load varies between fly lines, that is, the
196 tolerance response differs between lines. Because our interest was to quantify the effect of
197 damage prevention and repair mechanisms on disease tolerance, we compared the slope
198 estimates of each of the transgenic lines with the slope of w^{1118} line using a pairwise
199 comparison (f-test).

200

201

202 **Results**

203 1. <u>Flies lacking *dcy* are more susceptible to oral *P. entomophila* infections than those</u>
204 <u>lacking components that minimise, signal or repair damage</u>

205 Following oral infection with three different doses of *Pseudomonas entomophila*, flies with
206 disrupted components of damage prevention (*dcy)*, signalling (*upd3)*, renewal ($egfr^1$)and
207 regulation (*irc)*, were all significantly more susceptible to oral *P. entomophila* infections
208 compared to w^{1118} flies (*Fig. 1, Table SI-1*; *Fig. 1A* for infection dose OD_{600}*=25; Fig. 1B* for
209 infection dose OD_{600}*=10; Fig. 1C* for infection dose OD_{600}*=45*). Among these lines, *dcy*
210 knockouts were particularly susceptible to infection *Fig.1* and *Fig. SI-3* for survival*, Fig. 1,*
211 *Table SI-2* for hazard ratio. The effect of each gene disruption on the survival of flies following
212 infection was similar in males and females (fly line x sex x treatment interaction = non-
213 significant, *Table SI-1*), compared to control w^{1118} flies at both lower (OD_{600}*=10*) and higher
214 infection (OD_{600}*=45*) doses (*Fig. 1, Table SI-1*).

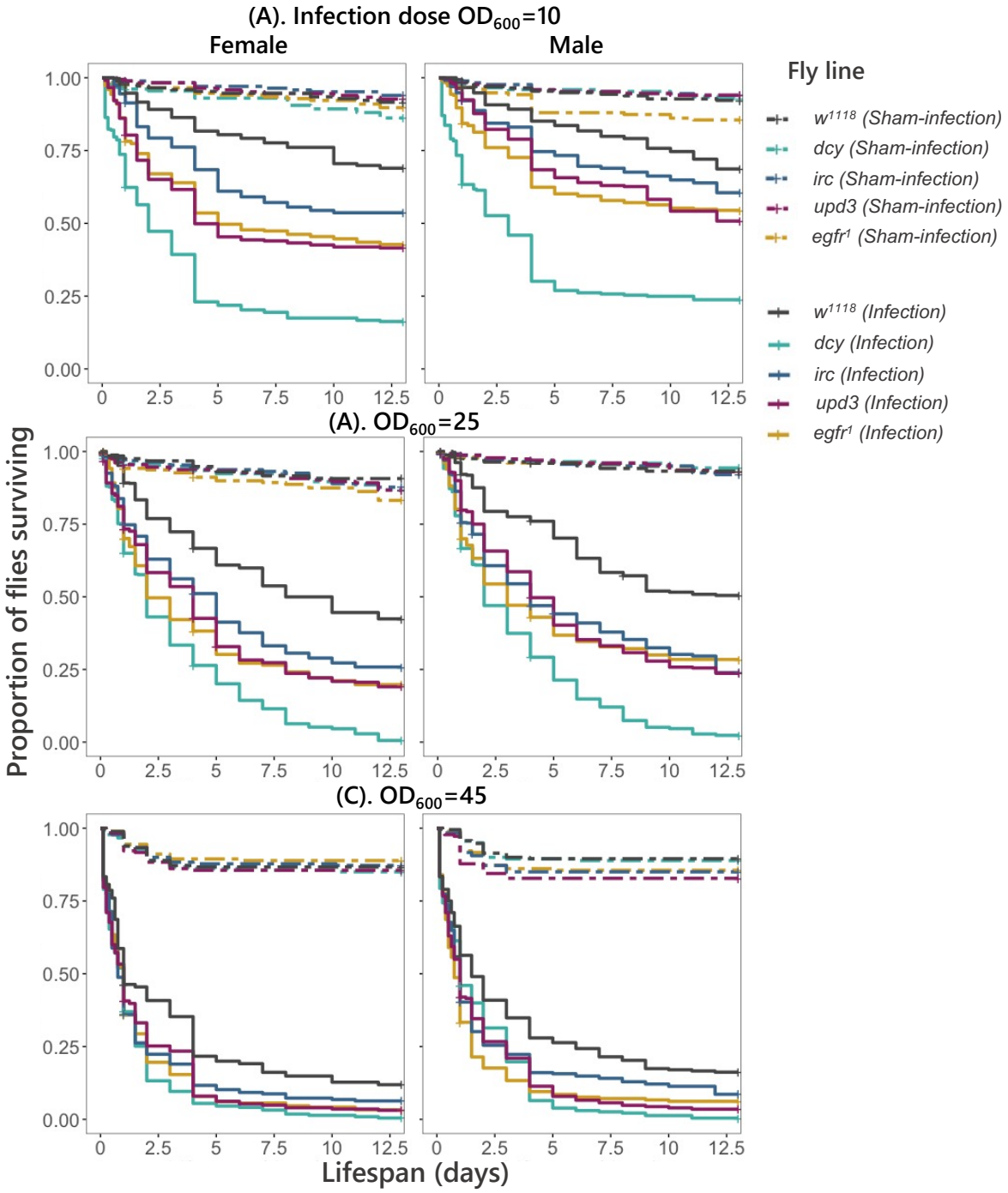
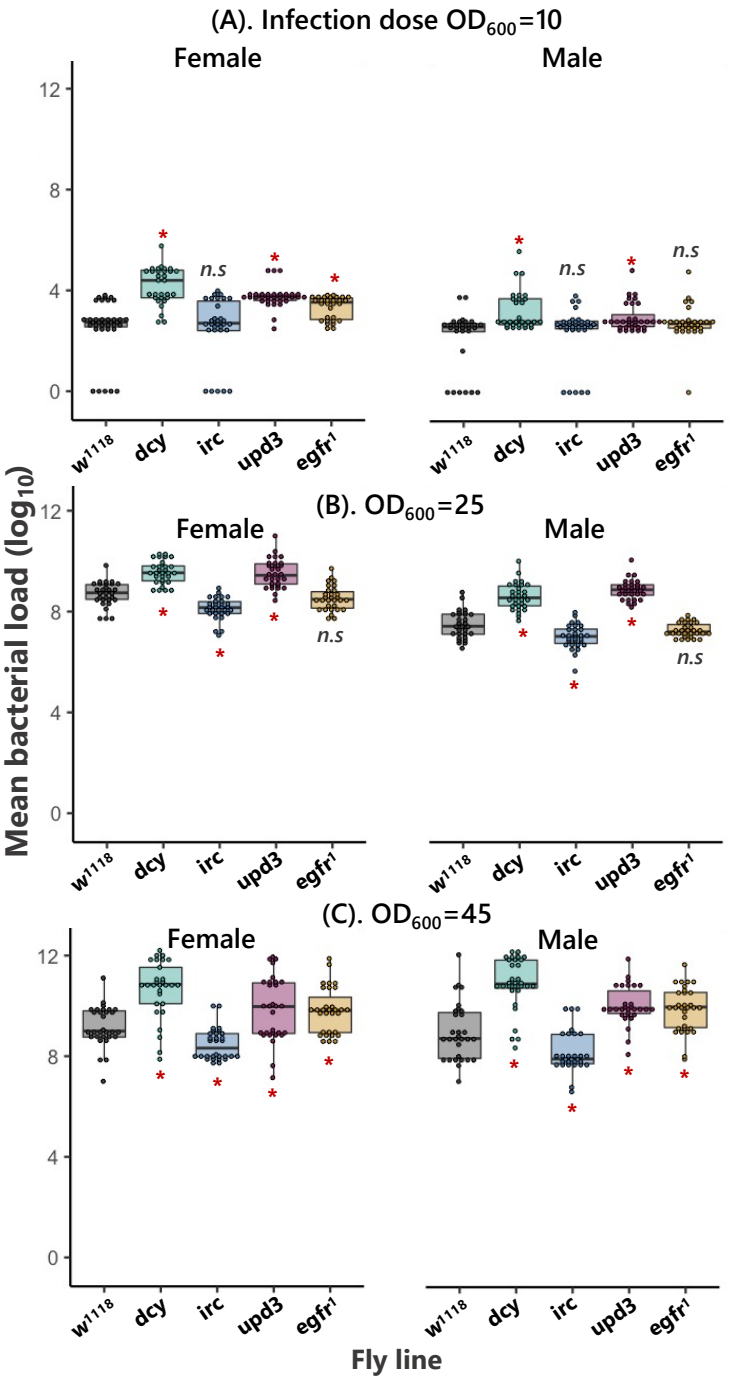


Figure 1. Kaplan Meier curves for females and males of w^{1118} and transgenic flies with loss-of-function in gut-epithelial response, exposed to oral *P. entomophila* of infection dose ***(A).*** $OD_{600}=10$, n=10 vials per treatment/sex/fly line **(*B*).** $OD_{600}=25$, n=30 vials per reatment/sex/fly line ***(C).*** $OD_{600}=45$. n=10 vials per treatment/sex/fly line. For all doses, 15-17 flies per replicate vial).

2. Both w^{1118} and flies with disrupted tissue damage prevention and repair mechanisms show sex differences in bacterial load during oral infections

226 The higher susceptibility of all transgenic flies to oral bacterial exposure could either be caused
227 by their inability to supress the bacterial growth or due to their inability to tolerate the damage
228 inflicted during oral infection. To distinguish between these mechanisms, following the
229 overnight oral exposure to *P. entomophila*, we first quantified internal bacterial loads at 15
230 minutes, 24-hours and 96-hours. We observed a peak in microbe loads at 24h post infection
231 in all fly lines (see ***Fig.*** *SI-4, Table SI-3)*. All fly lines showed sex-differences at 24-hours peak
232 load, though by 96-hours following oral infection this sex difference was no longer present in
233 flies lacking *upd3* or *egfr* expression (***Fig****. SI-4,* ***Table*** *SI-3*). Flies lacking *irc* expression always
234 exhibited levels of bacterial load similar to the w^{1118} flies across all infection doses (***Fig****. 2,*
235 ***Table*** *SI-3*).



236

237 Figure 2. Bacterial load measured as CFUs- colony forming units using infection dose ***(A).***
238 *OD_{600}= 10 (low dose)* ***(B).*** *OD_{600}=25 (medium dose)* and ***(C).*** *OD_{600}=45 (high dose)* after
239 24-hours following oral bacterial exposure with *P. entomophila* for w^{1118} flies and transgenic
240 lines [n=30 vials of which 12-15 flies/treatment/sex/fly line for bacterial load measurement].
241 Significantly different transgenic lines from control w^{1118} flies are denoted with '*' analysed
242 using pairwise comparisons between w^{1118} and for males and females respectively.
243
244

245 3. Damage repair mechanisms mediate sex differences in disease tolerance during oral
246 bacterial *P. entomophila* infections

While some of the variation in survival between fly lines (***Fig**. 1*) may be explained by variation in resistance [that is, their ability to clear infection (***Fig**. 2*)], 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 [8,50,16,52]. We focused on flies infected with the intermediate dose ($OD_{600}=25$), as we had 30 replicate-paired measurements of survival and microbe loads. We found that the disruption of damage prevention, signalling and repair genes showed reduced tolerance, measured as the rate at which fly survival changed with bacterial load relative to w^{1118} flies (***Fig**. 3, **Table** 2*). Both males and females lacking the major component of the peritrophic matrix *dcy* showed significantly reduced survival (***Figs**. 1 and 3*), indicating the general importance of damage preventing epithelial barrier in defence against oral infections [2]. Flies lacking the damage renewal mechanisms $egfr^1$ showed sex-differences in disease tolerance (***Fig**. 3, **Table** 1 and 2*). We found that the disruption of $egfr^1$ resulted in males, but not females becoming less tolerant of *P.entomophila,* showing a much steeper decline in survival with increasing microbe loads compared to females (**Fig**. 3). Notably, $egfr^1$ males are less tolerant than w1118 despite harbouring comparable microbe loads (Fig. 2).

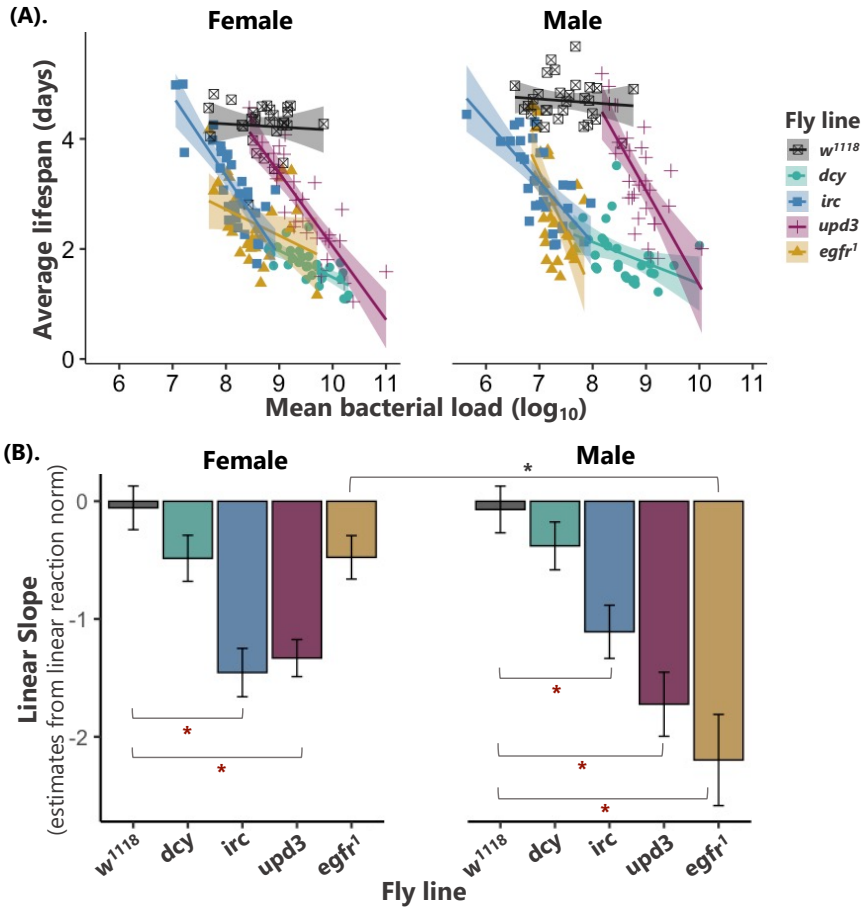


Figure 3. **(A).** The relationship between fly survival (measured as average lifespan) and bacterial load (as mean CFUs – colony forming units), analysed using linear models for female and male flies (w^{1118} and flies lacking damage prevention and repair mechanisms in gut-epithelia). Each point shows data for average lifespan and mean bacterial load (CFUs) of 30 vials (with each vial containing 25 individual flies/fly line/sex combination) after 24-hours post oral bacterial exposure. The data shown here are for the medium infection dose OD_{600}=25. **(B).** shows the slope of the linear reaction norm extracted from the linear models. [grey '*' 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 1), maroon '*' indicates that fly lines (flies lacking damage prevention and repair mechanisms in gut-epithelia) are significantly different from w^{1118}, analysed using pairwise f-test from linear norm estimates see Table 2].

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 1. 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 (w^{1118} and flies lacking damage prevention and repair mechanisms in gut-epithelia).

Sex	Fly line	SSE	F ratio	p
Female	*dcy* vs. w^{1118}	7.32	4.85	0.03
	$egfr^1$ vs. w^{1118}	18.32	1.88	0.17
	irc vs. w^{1118}	17.75	28.00	<0.01
	upd3 vs. w^{1118}	18.33	29.79	<0.01
Male	*dcy* vs. w^{1118}	10.66	2.17	0.14
	$egfr^1$ vs. w^{1118}	29.07	21.41	<0.01
	irc vs. w^{1118}	16.06	18.72	<0.01
	upd3 vs. w^{1118}	24.87	27.01	<0.01

Table 2. Summary of pairwise comparisons (f-test) of linear slope estimates from linear reaction norm for w^{1118} flies and flies lacking damage prevention and repair mechanisms in gut-epithelia.

305 **Discussion**

306 In the present work we tested how mechanisms of damage prevention (*dcy*), signalling (*upd3*)
307 control (*irc*) and renewal (*egfr*) contribute to disease tolerance during enteric infection. We
308 present evidence that all these mechanisms contribute to disease tolerance during bacterial
309 gut infection, and that some of these effects are sexually dimorphic. Previous transcriptomic,
310 genome-wide association (GWAS) or microarrays studies have identified several candidate
311 genes associated with disease tolerance, including - *CrebA, grainyhead and debris buster,*
312 *dFOXO* [14,26,27,53]. However, all this work has focused on flies infected systemically by
313 directly injecting bacteria into the fly. Here, we investigated tolerance during the natural oral
314 route of infection, and we took a more targeted approach to specifically investigate how some
315 of the well-described tissue damage prevention and repair mechanisms affect disease
316 tolerance during enteric bacterial infections.
317
318 Though repairing infection-damage is crucial to fly survival, we found that flies lacking
319 damage-preventing (*dcy*) are particularly susceptible to oral infections compared to those
320 lacking components that minimise, signal or repair damage. In other words, preventing
321 damage is clearly preferable to repairing damage from the perspective of fly survival. This
322 result is consistent with previous work showing that loss-of-function in *dcy* increases the
323 peritrophic matrix width making the gut leaky and compromising gut barrier function during
324 oral bacterial infections [54,44,55,56,2]. We also found increased bacterial loads relative to
325 the w^{1118} control line, measured after 24-hours following infection in both male and female *dcy*
326 knockouts. This is likely because of the combination of leaky gut and pore-forming toxin
327 produced by *P. entomophila* [2] resulting in higher bacterial growth in the fly haemolymph.
328
329 In the case of *upd3*-knockout flies, we found reduced survival and higher bacterial loads
330 compared to w^{1118} flies. Previous work has shown that in response to *P. entomophila*
331 infections, excessive reactive oxygen species (*ROS*) produced by host cells destroy the gut
332 epithelia and block the gut repair process [57,58,55]. The *JNK* and *Hippo* pathways are
333 activated in damaged enterocytes (*ECs*), which produce *upd3*, in turn activating the *Jak/Stat*
334 pathway in intestinal stem cells (*ISCs*). We found that both male and female *upd3* knockout
335 flies showed reduced tolerance and this is likely because in the absence of *upd*3 released
336 from damaged cells the *Jak/Stat*-pathway activation is reduced, which is further necessary for
337 *ISCs* proliferation and differentiation into enterocytes (*ECs*), together renew the damaged
338 tissues [55,2,31]. We also found that functional disruption of *irc* results in lower bacterial loads,
339 and this is expected because loss of this negative regulator of ROS results in higher *ROS*
340 levels [2], leading to improved bacterial clearance.

341

342 Regarding the effects of these damage limitation mechanisms on disease tolerance, overall,
343 we found that both male and female *w*^{1118} flies were quite tolerant of enteric bacterial infections
344 (reflected in their relatively flat tolerance slopes, ***Fig*** *3.* ***Table*** *2*), while disrupting most damage
345 prevention and repair mechanism lowered disease tolerance (decline in slopes relative to
346 w^{1118}). While we found reduced tolerance in all knockout lines, disrupting some components
347 of damage limitation had particularly severe effects on disease tolerance. Significant
348 reductions in disease tolerance were observed in flies with disrupted *irc* and *upd3*, and in these
349 fly lines the effect was comparable in both sexes. *Irc*-deficient flies are unable to regulate *ROS*
350 levels which would lead to increased cytotoxic effects [2,36] while *upd3* cytokine molecules
351 are important for the activation of the *Jak/Stat* pathway [43,40]. In case of *dcy*-knockout flies,
352 health did not deteriorate much further, possibly because it was already too poor to worsen
353 further (***Fig***. 3).
354

355 We observed the fastest decline in tolerance in male flies lacking *egfr*^{1}, but the disease
356 tolerance of female *egfr*^{1} knockouts appeared unaffected. This sex difference in tolerance may
357 arise as the result of sex differences in gut physiology and repair. Recent work has
358 demonstrated that during oral *Ecc15* infection, males showed significantly lower gut *ISCs* in
359 response to infection, while female had higher *ISCs* and were resistant to infection and other
360 stress [59]. The differentiation and proliferation of *ISCs* (intestinal stem cells) via *Jak/Stat*
361 signalling into *ECs* (enterocytes) via *egfr* is indispensable for tissue damage renewal. Loss of
362 *egfr*^{1} signalling might therefore be felt more severely in males than in females, explaining why
363 male but not female *egfr*^{1} knockouts showed severe a decrease in disease tolerance. To date,
364 only a small proportion of studies have compared sex-differences in intestinal immunity, with
365 the majority of work focusing on one particular sex, usually females [60,41,59,61].
366

367 Another possibility for the observed sex-difference in damage repair process, might relate to
368 gut-plasticity such as gut-remodelling. For instance, females of mammals such as mice
369 extensively remodel their guts, increasing both digestive and absorptive capacity depending
370 on the nutritional demands of lactation [62]. The remodelling of the gut might be one of the
371 possible driving factors for dimorphism in gut immunity, since males and females differ in their
372 nutritional needs [61]. Studies using *Drosophila* have shown that males and females can make
373 different diet or nutritional choices in accordance with their reproduction role and demand [63]
374 and the *Drosophila* midgut plastically resizes in response to changes in dietary sugar and
375 yeast [64]. Whether gut remodelling and nutritional choice-demand causes sex-differences in
376 damage repair process during disease tolerance remains a question for future research.

Concluding remarks

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 [25,65]. Tissue damage repair mechanisms that promote disease tolerance are interesting from a therapeutic perspective [9,10,66]. For instance, in mice, mechanisms that prevent or repair damage 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 [67,68]. 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 [15,69,70]. In summary, our results show that the disruption of tissue damage repair processes resulted in severe loss of disease tolerance and highlight how sex differences in some damage repair mechanisms could generate sexual dimorphism in gut immunity.

Data accessibility

All the raw data and analysis code can be accessed under a Creative Commons 4.0 License at https://doi.org/10.5281/zenodo.6510215 [71]. An earlier preprint is available from the biological preprint server *bioRxiv* at: https://www.biorxiv.org/content/10.1101/2021.10.03.462916v1 [72].

Acknowledgements

We thank V. Mongelli and CM. Saleh for sharing the *egfr* line and FM. Waldron for backcrossing *irc, upd3, dcy* fly lines in w^{1118} background. V. Doublet and E. Robertshaw for laboratory help. JC. Regan, AB. Pedersen, DH. Nussey, DJ. Obbard, TS. Salminen and Ashworth fly group members for helpful discussion. Finally, we thank A. Reid, A. Fulton, L. Rowe and J. King for help with media preparation. *Figs. SI-1 and SI-2* were created using Biorender.

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.

Funding

We acknowledge funding and support to P.F.V from Branco Weiss fellowship and Chancellor's Fellowship; A.P was supported by the Darwin Trust PhD studentship. We thank School of Biological Sciences, University of Edinburgh.

Competing interests

The authors declare no competing interests.

References

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

2. Kuraishi T, Hori A, Kurata S. 2013 Host-microbe interactions in the gut of Drosophila melanogaster. *Front. Physiol.* **4**. (doi:10.3389/fphys.2013.00375)

3. Vale PF, Siva-Jothy J, Morrill A, Forbes M. 2018 The influence of parasites on insect behavior. In *Insect Behavior: from mechanisms to ecological and evolutionary consequences*, pp. 274–291. OUP Oxford.

4. Apidianakis Y, Rahme LG. 2011 Drosophila melanogaster as a model for human intestinal infection and pathology. *Dis. Model. Mech.* **4**, 21–30. (doi:10.1242/dmm.003970)

5. Hoffmann JA. 2003 The immune response of Drosophila. *Nature* **426**, 33–38. (doi:10.1038/nature02021)

6. Hultmark D. 2003 Drosophila immunity: paths and patterns. *Curr. Opin. Immunol.* **15**, 12–19. (doi:10.1016/S0952-7915(02)00005-5)

7. Lemaitre B, Hoffmann J. 2007 The Host Defense of Drosophila melanogaster. *Annu. Rev. Immunol.* **25**, 697–743. (doi:10.1146/annurev.immunol.25.022106.141615)

8. Raberg L, Sim D, Read AF. 2007 Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. *Science* **318**, 812–814. (doi:10.1126/science.1148526)

9. Ayres JS, Schneider DS. 2012 Tolerance of Infections. *Annu. Rev. Immunol.* **30**, 271–94. (doi:https://doi.org/10.1146/annurev-immunol-020711-075030)

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

11. 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)

450 12. McCarville J, Ayres J. 2018 Disease tolerance: concept and mechanisms. *Curr. Opin.*
451 *Immunol.* **50**, 88–93. (doi:10.1016/j.coi.2017.12.003)

452 13. Howick VM, Lazzaro BP. 2014 Genotype and diet shape resistance and tolerance across
453 distinct phases of bacterial infection. *BMC Evol. Biol.* **14**, 56. (doi:10.1186/1471-2148-
454 14-56)

455 14. Lissner MM, Schneider DS. 2018 The physiological basis of disease tolerance in insects.
456 *Curr. Opin. Insect Sci.* **29**, 133–136. (doi:10.1016/j.cois.2018.09.004)

457 15. Oliveira JH, Bahia AC, Vale PF. 2020 How are arbovirus vectors able to tolerate
458 infection? *Dev. Comp. Immunol.* **103**, 103514. (doi:10.1016/j.dci.2019.103514)

459 16. Louie A, Song KH, Hotson A, Thomas Tate A, Schneider DS. 2016 How Many
460 Parameters Does It Take to Describe Disease Tolerance? *PLOS Biol.* **14**, e1002435.
461 (doi:10.1371/journal.pbio.1002435)

462 17. Ayres JS, Schneider DS. 2009 The Role of Anorexia in Resistance and Tolerance to
463 Infections in Drosophila. *PLoS Biol.* **7**, e1000150. (doi:10.1371/journal.pbio.1000150)

464 18. Palaferri Schieber AM, Lee YM, Chang MW, Leblanc M, Collins B, Downes M, Evans
465 RM, Ayres JS. 2015 Disease tolerance mediated by microbiome E. coli involves
466 inflammasome and IGF-1 signaling. *Science* **VOL 350**.

467 19. Cumnock K, Gupta AS, Lissner M, Chevee V, Davis NM, Schneider DS. 2018 Host
468 Energy Source Is Important for Disease Tolerance to Malaria. *Curr. Biol.* **28**, 1635-
469 1642.e3. (doi:10.1016/j.cub.2018.04.009)

470 20. Adelman JS, Kirkpatrick L, Grodio JL, Hawley DM. 2013 House Finch Populations
471 Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of
472 *Mycoplasma gallisepticum* Infection. *Am. Nat.* **181**, 674–689. (doi:10.1086/670024)

473 21. Bonneaud C, Tardy L, Giraudeau M, Hill GE, McGraw KJ, Wilson AJ. 2019 Evolution
474 of both host resistance and tolerance to an emerging bacterial pathogen. *Evol. Lett.* **3**,
475 544–554. (doi:10.1002/evl3.133)

476 22. Regoes RR *et al.* 2014 Disentangling Human Tolerance and Resistance Against HIV.
477 *PLoS Biol.* **12**, e1001951. (doi:10.1371/journal.pbio.1001951)

478 23. Nahrendorf W, Ivens A, Spence PJ. 2021 Inducible mechanisms of disease tolerance
479 provide an alternative strategy of acquired immunity to malaria. *eLife* **10**, e63838.
480 (doi:10.7554/eLife.63838)

481 24. Cooper EL, editor. 2018 *Advances in Comparative Immunology*. Springer International
482 Publishing. (doi:10.1007/978-3-319-76768-0)

483 25. Soares MP, Gozzelino R, Weis S. 2014 Tissue damage control in disease tolerance.
484 *Trends Immunol.* **35**, 483–494. (doi:10.1016/j.it.2014.08.001)

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

488 27. Troha K, Im JH, Revah J, Lazzaro BP, Buchon N. 2018 Comparative transcriptomics
489 reveals CrebA as a novel regulator of infection tolerance in D. melanogaster. *PLOS*
490 *Pathog.* **14**, e1006847. (doi:10.1371/journal.ppat.1006847)

491 28. Kapun M *et al.* 2020 Genomic Analysis of European Drosophila melanogaster
492 Populations Reveals Longitudinal Structure, Continent-Wide Selection, and Previously
493 Unknown DNA Viruses. *Mol. Biol. Evol.* **37**, 2661–2678. (doi:10.1093/molbev/msaa120)

494 29. Wallace MA *et al.* 2021 The discovery, distribution, and diversity of DNA viruses
495 associated with Drosophila melanogaster in Europe. *Virus Evol.* **7**.
496 (doi:10.1093/ve/veab031)

497 30. Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A. 2011 Bacterial Communities of
498 Diverse Drosophila Species: Ecological Context of a Host–Microbe Model System.
499 *PLOS Genet.* **7**, e1002272. (doi:10.1371/journal.pgen.1002272)

500 31. Buchon N, Broderick NA, Lemaitre B. 2013 Gut homeostasis in a microbial world:
501 insights from Drosophila melanogaster. *Nat. Rev. Microbiol.* **11**, 615–626.
502 (doi:10.1038/nrmicro3074)

503 32. Buchon N, Osman D. 2015 All for one and one for all: Regionalization of the Drosophila
504 intestine | Elsevier Enhanced Reader. *Insect Biochem. Mol. Biol.*
505 (doi:10.1016/j.ibmb.2015.05.015)

506 33. Lemaitre B, Miguel-Aliaga I. 2013 The Digestive Tract of Drosophila melanogaster.
507 *Annu. Rev. Genet.* **47**, 377–404. (doi:10.1146/annurev-genet-111212-133343)

508 34. Hegedus D, Erlandson M, Gillott C, Toprak U. 2009 New Insights into Peritrophic
509 Matrix Synthesis, Architecture, and Function. *Annu. Rev. Entomol.* **54**, 285–302.
510 (doi:10.1146/annurev.ento.54.110807.090559)

511 35. Vodovar N, Vinals M, Liehl P, Basset A, Degrouard J, Spellman P, Boccard F, Lemaitre
512 B. 2005 Drosophila host defense after oral infection by an entomopathogenic
513 Pseudomonas species. *Proc. Natl. Acad. Sci.* **102**, 11414–11419.
514 (doi:10.1073/pnas.0502240102)

515 36. Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B. 2009 Drosophila
516 Intestinal Response to Bacterial Infection: Activation of Host Defense and Stem Cell
517 Proliferation. *Cell Host Microbe* **5**, 200–211. (doi:10.1016/j.chom.2009.01.003)

518 37. Myllymäki H, Valanne S, Rämet M. 2014 The Drosophila Imd Signaling Pathway. *J.*
519 *Immunol.* **192**, 3455–3462. (doi:10.4049/jimmunol.1303309)

520 38. Myllymäki H, Rämet M. 2014 JAK/STAT Pathway in Drosophila Immunity. *Scand. J.*
521 *Immunol.* **79**, 377–385. (doi:10.1111/sji.12170)

522 39. Valanne S, Wang J-H, Rämet M. 2011 The Drosophila Toll Signaling Pathway. *J.*
523 *Immunol.* **186**, 649–656. (doi:10.4049/jimmunol.1002302)

524 40. Buchon N, Broderick NA, Kuraishi T, Lemaitre B. 2010 DrosophilaEGFR pathway
525 coordinates stem cell proliferation and gut remodeling following infection. *BMC Biol.* **8**,
526 152. (doi:10.1186/1741-7007-8-152)

527 41. Chakrabarti S, Dudzic JP, Li X, Collas EJ, Boquete J-P, Lemaitre B. 2016 Remote
528 Control of Intestinal Stem Cell Activity by Haemocytes in Drosophila. *PLOS Genet.* **12**,
529 e1006089. (doi:10.1371/journal.pgen.1006089)

530 42. Jiang H, Grenley MO, Bravo M-J, Blumhagen RZ, Edgar BA. 2011 EGFR/Ras/MAPK
531 Signaling Mediates Adult Midgut Epithelial Homeostasis and Regeneration in
532 Drosophila. *Cell Stem Cell* **8**, 84–95. (doi:10.1016/j.stem.2010.11.026)

533 43. Jiang H, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, Edgar BA. 2009
534 Cytokine/Jak/Stat Signaling Mediates Regeneration and Homeostasis in the Drosophila
535 Midgut. *Cell* **137**, 1343–1355. (doi:10.1016/j.cell.2009.05.014)

536 44. Kuraishi T, Binggeli O, Opota O, Buchon N, Lemaitre B. 2011 Genetic evidence for a
537 protective role of the peritrophic matrix against intestinal bacterial infection in
538 Drosophila melanogaster. *Proc. Natl. Acad. Sci.* **108**, 15966–15971.
539 (doi:10.1073/pnas.1105994108)

540 45. Ha E-M, Oh C-T, Ryu J-H, Bae Y-S, Kang S-W, Jang I, Brey PT, Lee W-J. 2005 An
541 Antioxidant System Required for Host Protection against Gut Infection in Drosophila.
542 *Dev. Cell* **8**, 125–132. (doi:10.1016/j.devcel.2004.11.007)

543 46. Wang L, Sexton TR, Venard C, Giedt M, Guo Q, Chen Q, Harrison DA. 2014 Pleiotropy
544 of the Drosophila JAK pathway cytokine Unpaired 3 in development and aging. *Dev.*
545 *Biol.* **395**, 218–231. (doi:10.1016/j.ydbio.2014.09.015)

546 47. Mongelli V, Lequime S, Kousathanas A, Gausson V, Blanc H, Nigg J, Quintana-Murci L,
547 Elena SF, Saleh M-C. 2022 Innate immune pathways act synergistically to constrain
548 RNA virus evolution in Drosophila melanogaster. *Nat. Ecol. Evol.* , 1–14.
549 (doi:10.1038/s41559-022-01697-z)

550 48. Siva-Jothy JA, Prakash A, Vasanthakrishnan RB, Monteith KM, Vale PF. 2018 Oral
551 Bacterial Infection and Shedding in Drosophila melanogaster. *J. Vis. Exp.* , 57676.
552 (doi:10.3791/57676)

553 49. Therneau T. 2015 Mixed Effects Cox Models. In *Mixed Effects Cox Models*, CRAN
554 repository.

555 50. Schneider DS, Ayres JS. 2008 Two ways to survive infection: what resistance and
556 tolerance can teach us about treating infectious diseases. *Nat. Rev. Immunol.* **8**, 889–895.
557 (doi:10.1038/nri2432)

558 51. Gupta V, Vale PF. 2017 Nonlinear disease tolerance curves reveal distinct components of
559 host responses to viral infection. *R. Soc. Open Sci.* **4**, 170342. (doi:10.1098/rsos.170342)

560 52. Kutzer MAM, Armitage SAO. 2016 Maximising fitness in the face of parasites: a review
561 of host tolerance. *Zoology* **119**, 281–289. (doi:10.1016/j.zool.2016.05.011)

562 53. Dionne MS, Pham LN, Shirasu-Hiza M, Schneider DS. 2006 Akt and foxo Dysregulation
563 Contribute to Infection-Induced Wasting in Drosophila. *Curr. Biol.* **16**, 1977–1985.
564 (doi:10.1016/j.cub.2006.08.052)

565 54. Opota O, Vallet-Gély I, Vincentelli R, Kellenberger C, Iacovache I, Gonzalez MR,
566 Roussel A, Goot F-G van der, Lemaitre B. 2011 Monalysin, a Novel ß-Pore-Forming
567 Toxin from the Drosophila Pathogen Pseudomonas entomophila, Contributes to Host
568 Intestinal Damage and Lethality. *PLOS Pathog.* **7**, e1002259.
569 (doi:10.1371/journal.ppat.1002259)

570 55. Chakrabarti S, Liehl P, Buchon N, Lemaitre B. 2012 Infection-Induced Host
571 Translational Blockage Inhibits Immune Responses and Epithelial Renewal in the
572 Drosophila Gut. *Cell Host Microbe* **12**. (doi:10.1016/j.chom.2012.06.001)

573 56. Blemont M, Vincentelli R, Kellenberger C, Opota O, Lemaitre B, Roussel A, Leone P.
574 2013 Crystallization and preliminary X-ray analysis of monalysin, a novel β-pore-
575 forming toxin from the entomopathogen Pseudomonas entomophila. *Acta*
576 *Crystallograph. Sect. F Struct. Biol. Cryst. Commun.* **69**, 930–933.
577 (doi:10.1107/S174430911301885X)

578 57. Han J, Ulevitch RJ. 2005 Limiting inflammatory responses during activation of innate
579 immunity. *Nat. Immunol.* **6**, 1198–1205. (doi:10.1038/ni1274)

580 58. Lambeth JD. 2007 Nox enzymes, ROS, and chronic disease: An example of antagonistic
581 pleiotropy. *Free Radic. Biol. Med.* **43**, 332–347.
582 (doi:10.1016/j.freeradbiomed.2007.03.027)

583 59. Regan JC, Khericha M, Dobson AJ, Bolukbasi E, Rattanavirotkul N, Partridge L. 2016
584 Sex difference in pathology of the ageing gut mediates the greater response of female
585 lifespan to dietary restriction. *eLife* **5**, e10956. (doi:10.7554/eLife.10956)

586 60. Ayyaz A, Li H, Jasper H. 2015 Haemocytes control stem cell activity in the Drosophila
587 intestine. *Nat. Cell Biol.* **17**, 736–748. (doi:10.1038/ncb3174)

588 61. Belmonte RL, Corbally M-K, Duneau DF, Regan JC. 2020 Sexual Dimorphisms in
589 Innate Immunity and Responses to Infection in Drosophila melanogaster. *Front.*
590 *Immunol.* **10**, 3075. (doi:10.3389/fimmu.2019.03075)

591 62. Speakman JR. 2008 The physiological costs of reproduction in small mammals. *Philos.*
592 *Trans. R. Soc. B Biol. Sci.* **363**, 375–398. (doi:10.1098/rstb.2007.2145)

593 63. Camus MF, Piper MD, Reuter M. 2019 Sex-specific transcriptomic responses to changes
594 in the nutritional environment. *eLife* **8**, e47262. (doi:10.7554/eLife.47262)

595 64. Bonfini A, Dobson AJ, Duneau D, Revah J, Liu X, Houtz P, Buchon N. 2021 Multiscale
596 analysis reveals that diet-dependent midgut plasticity emerges from alterations in both
597 stem cell niche coupling and enterocyte size. *eLife* **10**, e64125. (doi:10.7554/eLife.64125)

598 65. Vale PF, Fenton A, Brown SP. 2014 Limiting Damage during Infection: Lessons from
599 Infection Tolerance for Novel Therapeutics. *PLOS Biol.* **12**, e1001769.
600 (doi:10.1371/journal.pbio.1001769)

601 66. Vale PF, McNally L, Doeschl-Wilson A, King KC, Popat R, Domingo-Sananes MR,
602 Allen JE, Soares MP, Kümmerli R. 2016 Beyond killing: Can we find new ways to
603 manage infection? *Evol. Med. Public Health* **2016**, 148–157. (doi:10.1093/emph/eow012)

604 67. Ferreira A *et al.* 2011 Sickle Hemoglobin Confers Tolerance to Plasmodium Infection.
605 *Cell* **145**, 398–409. (doi:10.1016/j.cell.2011.03.049)

606 68. Jamieson AM, Pasman L, Yu S, Gamradt P, Homer RJ, Decker T, Medzhitov R. 2013
607 Role of Tissue Protection in Lethal Respiratory Viral-Bacterial Coinfection. *Science* **340**,
608 1230–1234. (doi:10.1126/science.1233632)

609 69. Taracena ML *et al.* 2018 Regulation of midgut cell proliferation impacts Aedes aegypti
610 susceptibility to dengue virus. *PLoS Negl. Trop. Dis.* **12**, e0006498.
611 (doi:10.1371/journal.pntd.0006498)

612 70. Lambrechts L, Saleh M-C. 2019 Manipulating Mosquito Tolerance for Arbovirus
613 Control. *Cell Host Microbe* **26**, 309–313. (doi:10.1016/j.chom.2019.08.005)

614 71. Vale P, Prakash A. 2021 Data and code for Mechanisms of damage prevention,
615 signalling, and repair impact the ability of Drosophila to tolerate enteric bacterial
616 infection. (doi:10.5281/zenodo.5564919)

617 72. Prakash A, Monteith KM, Vale PF. 2021 Mechanisms of damage prevention, signalling,
618 and repair impact the ability of Drosophila to tolerate enteric bacterial infection. *bioRxiv* ,
619 2021.10.03.462916. (doi:https://doi.org/10.5281/zenodo.5564919)

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Supplementary information for

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

Arun Prakash[1,2]*, Katy M. Monteith[1], Pedro F. Vale[1]*

Supplementary figures

Figure SI-1. Schematic representation of *Drosophila* gut-epithelial immune response following enteric infection.

Figure SI-2. Split-vial experimental design.

Figure SI-3. Estimated hazard ratios

Figure SI-4. Bacterial load measured at 24 hours after OD_{600}=25 of oral *Pseudomonas entomophila* infection

Supplementary tables

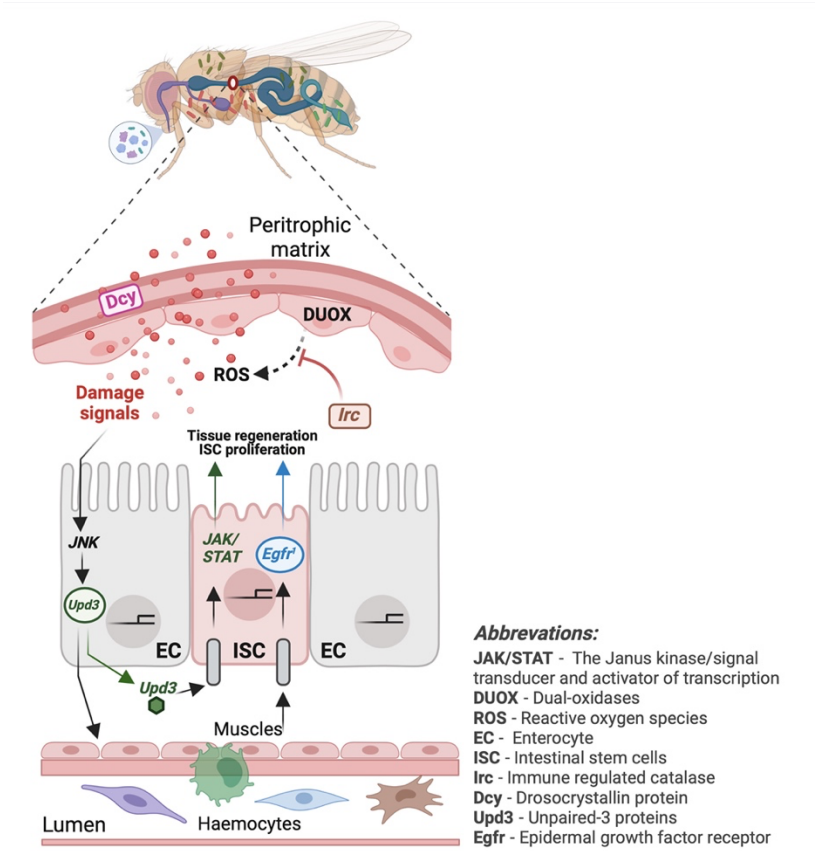
Table SI-1. Summary of mixed effects Cox model,

Table SI-2. Summary of estimated hazard ratio relative to w^{1118} from the cox proportional model.

Table SI-3. Summary of log_{10} transformed bacterial load data after exposure to 3 different concentration (doses) *P. entomophila*

648 **Supplementary figures**

649



650

651 **Figure S1**. Schematic representation of *Drosophila* gut-epithelial immune response following
652 enteric infection. Several stages of damage prevention and repair, epithelial response in the
653 *Drosophila* gut can be found. ***Stage-I***: involves the physical barrier the peritrophic matrix
654 (*PM* - layer of chitin and glycoproteins) that lines the *Drosophila* midgut lumen. A major component
655 of the *PM* is ***dcy*** (*drosocrystallin*) and loss-of-function mutations in *dcy* increase the width of the
656 *PM*, increasing permeability to pathogens. ***II***: production of reactive oxygen species (*ROS*) by the
657 gut epithelia - enteric infections cause massive destruction of the gut epithelium and in response
658 to ingested pathogens, *ROS* production is induced by *nox* (NADPH oxidase) and *duox* (dual
659 oxidase), while ***irc*** (immune-reactive catalase) negatively regulates *ROS* production by
660 suppressing the cytotoxic effects of *ROS* once the infection threat is lessened, avoiding
661 immunopathology. In addition, *ROS* production is known to trigger signalling pathways the *IMD* or
662 *Toll* that leads to the production of antimicrobial peptides (*AMPs*) critical for pathogen clearance
663 in the gut. ***III***: damage renewal process after infection - The final stage in gut defence is to repair
664 the damage caused during enteric infections. Damage-signalling cytokine-like molecules ***upd3***,
665 released from damaged cells trigger the *Jak/Stat*-pathway, stimulating the proliferation of intestinal
666 stem cells (*ISCs*) and their differentiation into enterocytes (*ECs*) via ***egfr***^{1} (epidermal growth factor
667 receptor) signalling which are indispensable for maintaining homeostasis and renewing the
668 damaged cells/tissues. Figure created using Biorender.

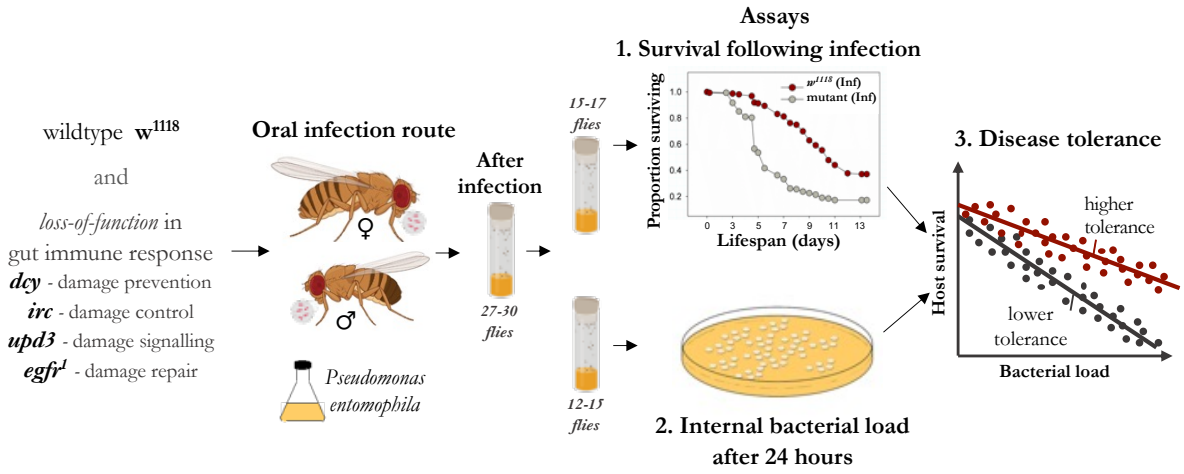


Figure S2. Split-vial experimental design to assay (1) survival (15-17 flies/combination in a vial) and (2) internal bacterial load (12-15 flies/combination in a vial) following oral bacterial infection with Pseudomonas entomophila to test how epithelial immune response including damage prevention and tissue repair mechanisms in Drosophila gut contribute to (3). disease tolerance. For the split-vial design, after oral exposure with each vial containing 25 flies (of each infection treatment, sex, fly line combination) were divided into 2 vials, each for survival (15-17 flies) and bacterial load (12-15 flies). Each point in disease tolerance panel (3) represents replicate-matched data [n=30 vials/infection treatment/sex/fly line – with each vial containing 27-30 flies] from survival (1) and bacterial load (2).

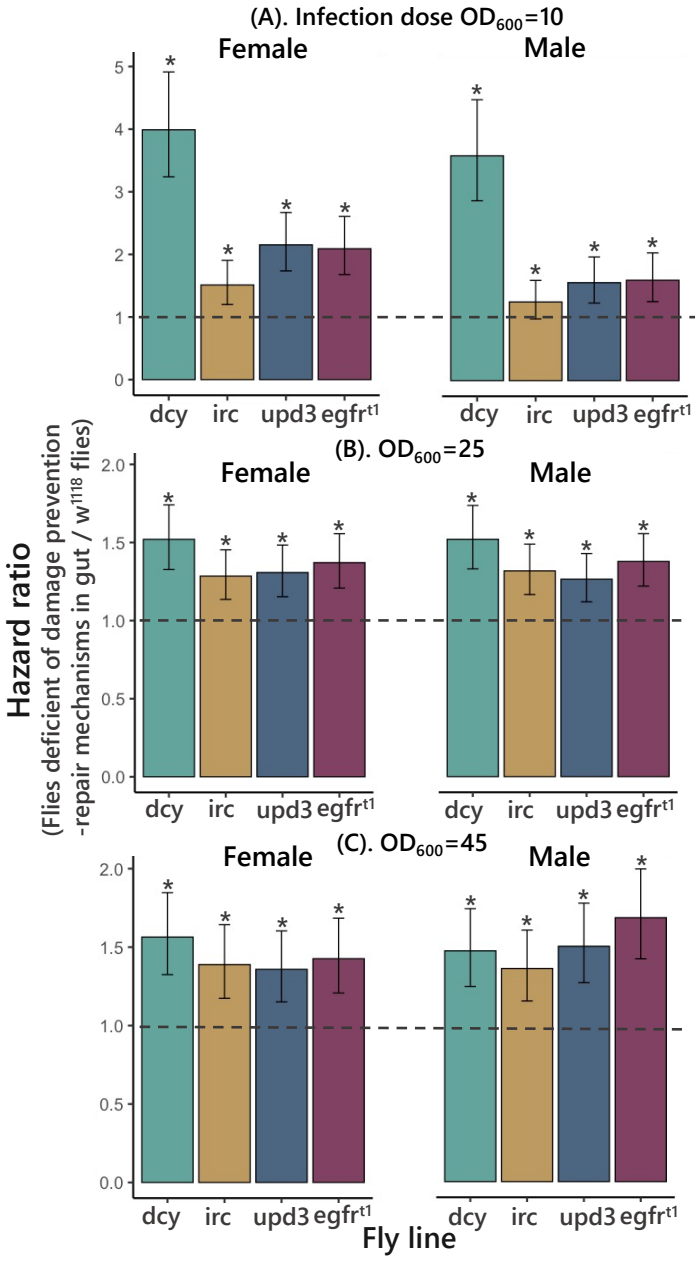


Figure S3.

Estimated hazard ratios for males and female flies (w^{1118} control flies and flies with absence of fully functional gut-epithelial response) calculated from the survival curves. A greater hazard ratio (>1) indicates higher susceptibility to infection. ['*' indicates that the transgenic flies are significantly different from w^{1118} flies for males and females respectively].

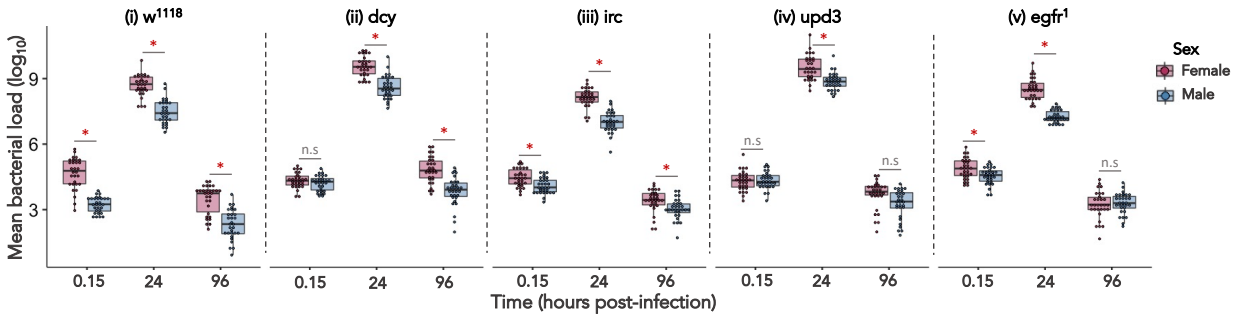


Figure S4. Bacterial load measured at 24 hours after OD_{600}=25 of oral *Pseudomonas entomophila* infection for male & female flies of w^{1118} and flies with deficient damage repair mechanisms in *Drosophila* gut. "*' indicates significant differences in males and females of each fly line (w^{1118} and transgenic flies) analysed using Kruskal-Wallis test.

709 **Supplementary tables**

710

711

712 **Table SI-1.** Summary of mixed effects Cox model, for male and female *w*^{1118} & flies with *loss-*
713 *of-function* in in the gut-epithelial response orally infected with 3 different concentration/doses
714 of *P.entomophila*. We used 3-5 day old adult males and females of each fly lines (w^{1118} & flies
715 with deficient damage repair mechanisms in gut) and specified the model as: Survival ~
716 Treatment x Sex x Fly line (1|vial/block), with 'Treatment', 'Sex' & 'Fly line' as fixed effects,
717 and 'vials' nested in 'block' as a random effects. The table shows model output (ANOVA) for
718 survival post oral infection for *w*^{1118} flies & flies with *loss-of-function* in in the gut-epithelial
719 response.

Infection dose	Source	Loglik	Chisq	df	p
OD_{600}=10	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
	Random effect vial/block	Std dev 8.88E-04			
OD_{600}=25	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
	Random effect vial/block	Std dev 1.92E-03			
OD_{600}=45	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
	Random effect vial/block	Std dev 0.019			

720

721

722 **Table SI-2.** Summary of estimated hazard ratio relative to w^{1118} from the cox proportional
723 model. A hazard ratio (>1) indicates that flies lacking damage prevention and repair
724 mechanisms in gut-epithelia are more susceptible to oral *P. entomophila* infection than w^{1118}
725 flies.
726
727

	Fly line	Sex	Estimate	p	lower 95%	upper 95%
OD10	*dcy*	Female	3.99	<0.01	3.24	4.91
		Male	3.59	<0.01	2.87	4.49
	Egfr[1]	Female	2.09	<0.01	1.68	2.61
		Male	1.60	<0.01	1.26	2.04
	irc	Female	1.51	0.0005	1.20	1.90
		Male	1.26	0.065	0.99	1.60
	upd3	Female	2.15	<0.01	1.74	2.67
		Male	1.56	0.0002	1.24	1.98
OD25						
	dcy	Female	1.52	<0.01	1.32	1.74
		Male	1.52	<0.01	1.32	1.73
	Egfr[1]	Female	1.37	<0.01	1.20	1.55
		Male	1.37	<0.01	1.21	1.55
	irc	Female	1.28	<0.01	1.13	1.45
		Male	1.31	<0.01	1.16	1.48
	upd3	Female	1.31	<0.01	1.15	1.48
		Male	1.26	<0.01	1.11	1.42
OD45	dcy	Female	1.56	<0.01	1.32	1.84
		Male	1.47	<0.01	1.25	1.74
	Egfr1	Female	1.43	<0.01	1.21	1.68
		Male	1.69	<0.01	1.42	2.00
	irc	Female	1.39	<0.01	1.17	1.64
		Male	1.36	0.0003	1.15	1.61
	upd3	Female	1.36	0.0003	1.15	1.60
		Male	1.50	<0.01	1.27	1.78

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731 **Table SI-3.** Summary of log_{10} transformed bacterial load data after exposure to 3 different
732 concentration (doses) *P. entomophila* orally infected flies (w^{1118} & transgenic flies), analysed
733 using non-parametric Kruskal-Wallis test by fitting 'Sex' as categorical fixed-effects for
734 timepoints 15 mins, 24 hours & 96 hours for OD_{600}=25)
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	Sex	Fly line		Mean diff	Z	p
15 min	Female	egfr1	w^{1118}	3.50	0.77	0.43
		irc	w^{1118}	-7.83	-1.73	0.08
		upd-3	w^{1118}	-11.03	-2.44	0.01
		dcy	w^{1118}	-11.16	-2.47	0.01
	Male	egfr1	w^{1118}	29.56	6.55	< 0.001
		dcy	w^{1118}	28.96	6.423	< 0.001
		upd-3	w^{1118}	28.96	6.42	< 0.001
		irc	w^{1118}	28.033	6.21	< 0.001

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	Sex	Fly line		Mean diff	Z	p
24 hours load	Female	dcy	w^{1118}	24.30	5.388	<0.001
		upd-3	w^{1118}	22.23	4.930	<0.001
		egfr1	w^{1118}	-7.233	-1.604	0.108
		irc	w^{1118}	-18.96	-4.206	<0.001
	Male	upd-3	w^{1118}	28.70	6.364	<0.001
		dcy	w^{1118}	25.90	5.743	<0.001
		egfr1	w^{1118}	-7.233	-1.604	0.10
		irc	w^{1118}	-14.16	-3.141	0.001

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	Sex	Fly line		Mean diff	Z	p
96 hours load	Female	dcy	w^{1118}	26.90	5.965	< 0.001
		upd-3	w^{1118}	6.100	1.352	0.17
		irc	w^{1118}	-5.166	-1.145	0.25
		egfr1	w^{1118}	-8.566	-1.899	0.057
	Male	dcy	w^{1118}	26.50	5.876	< 0.001
		egfr1	w^{1118}	22.73	5.041	< 0.001
		upd-3	w^{1118}	20.36	4.516	< 0.001
		irc	w^{1118}	17.56	3.895	< 0.001

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