Genotype and diet affect resistance, survival, and fecundity but not fecundity tolerance

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Abstract

Insects are exposed to a variety of potential pathogens in their environment, many of which can severely impact fitness and health. Consequently, hosts have evolved resistance and tolerance strategies to suppress or cope with infections. Hosts utilising resistance improve fitness by clearing or reducing pathogen loads and hosts utilising tolerance reduce harmful fitness effects per pathogen load. To understand variation in, and selective pressures on resistance and tolerance we asked to what degree they are shaped by host genetic background, whether plasticity in these responses depends upon dietary environment, and whether there are interactions between these two factors. Females from ten wild-type *Drosophila melanogaster* genotypes were kept on high or low protein (yeast) diets, and infected with one of two opportunistic bacterial pathogens, *Lactococcus lactis* or *Pseudomonas entomophila*. We measured host resistance as the inverse of bacterial load in the early infection phase. The slope linking fly fecundity and individual-level bacteria load provided our fecundity tolerance measure. Genotype and dietary yeast determined host fecundity and strongly affected survival after infection with pathogenic *P. entomophila*. There was considerable genetic variation in host resistance, a commonly found phenomenon resulting from e.g. varying resistance costs or frequency-dependent selection. Despite this variation and the reproductive cost of higher *P. entomophila* loads, the slopes linking bacteria load and fecundity did not vary across genotypes. Absence of genetic variation in tolerance may suggest that at this early infection stage fecundity tolerance is fixed or that any evolved tolerance mechanisms are not expressed under these infection conditions.

Keywords: diet, DGRP, ecological immunology, fecundity tolerance, fitness, *Lactococcus lactis,* pathogen, *Pseudomonas entomophila*, resistance, yeast.

Introduction

The composition of a hosts' microbial community is in part determined by how a host responds towards invading microbes. Such host reactions towards microbes are composed of resistance and tolerance, two disparate strategies whose deployment may ultimately depend on a combination of intrinsic, innate factors and external, environmental factors (Råberg *et al.*, 2009; Graham *et al.*, 2011; Kutzer & Armitage, 2016a). Once an infection becomes established within a host, a host can actively resist the pathogen by clearance or by targeting pathogen replication rate, which can aid host recovery time, but often comes at a cost to host fitness (Kraaijeveld *et al.*, 2002). In contrast, host tolerance limits the deleterious fitness and health effects of a pathogenic infection without targeting pathogen load (Roy & Kirchner, 2000; Råberg *et al.*, 2007). Both strategies can have far reaching impacts on host-pathogen co-evolutionary trajectories (Best *et al.*, 2014).

The co-evolution of host resistance and pathogen virulence has been well characterised (e.g. Masri *et al.,* 2015; Woolhouse *et al.,* 2002). Briefly, when a host resists a pathogenic infection, it reduces pathogen prevalence in a population. After the pathogen counter-adapts to circumvent the host resistance mechanisms, pathogen frequency increases in the host population, resulting in a negative feedback loop and antagonistic co-evolution in both the host and pathogen populations (Roy & Kirchner, 2000). Resistance mechanisms can be highly host-pathogen specific or they can be more general. For example, resistance can result from allelic variation in only a few loci (Luijckx *et al.*, 2013), the same antimicrobial peptide (AMP) can increase in expression to a range of different pathogens (e.g. Lemaitre, Reichhart & Hoffmann 1997), and different AMPs can act synergistically against one pathogen (Marxer *et al.*, 2016).

Models predict that the evolution of host tolerance can act in two ways in a population, depending upon whether hosts show fecundity- or mortality-tolerance (Best *et al.*, 2010, 2014). Mortality-tolerance is the ability to reduce the negative effect of infection on host survival, and is important for pathogen prevalence. If an infected host lives longer, then the pathogen also has a greater chance of being transmitted among hosts, which could lead to disease reservoir expansion, and greater mortality in the host population (Roy & Kirchner, 2000; Miller *et al.*, 2006; Best *et al.*, 2008; Vale *et al.*, 2011). Fecundity tolerance, which we test here and is the ability to reduce the negative effect of infection on host fecundity, should be neutral to pathogen prevalence because the pathogen's infectious period is neither prolonged nor shortened (Best *et al.*, 2010). However, if fecundity tolerance comes at a cost to host lifespan, the pathogen's infectious period will be reduced along with host lifespan, which can lead to a negative feedback and potentially, genetic variation in fecundity tolerance (Best *et al.*, 2008).

Numerous mechanisms can lead to disease tolerance in animals, and these seem to be dependent on pathogen and host type (Ayres & Schneider, 2012). Both hosts and pathogens have optimal fitness strategies (Råberg, 2014), but these need not be fixed in their respective populations (Best *et al.*, 2008). Host tolerance can be, but is not necessarily, genetically determined (e.g. Råberg *et al.* 2007; Blanchet, Rey & Loot 2010; Sternberg *et al.* 2013; Howick & Lazzaro 2014; Parker, Garcia & Gerardo 2014), and it can also be a plastic response, where its expression is determined by the host environment, for example concentration of dietary glucose (Howick & Lazzaro, 2014) or yeast (Kutzer & Armitage 2016b). Thereby, variation in defense strategies within and between populations can be attributed to genetic (G) and environmental (E) factors or a combination of both (i.e. G x E interactions), but studies exploring how different populations express resistance and tolerance

92 in response to changing environmental factors are under-represented (but see Howick &
93 Lazzaro 2014). Genetic variation in host immune function in particular can be maintained and
94 selected for by fluctuations in the host environment (Mitchell *et al.*, 2005; Lazzaro & Little,
95 2009; Hawley & Altizer, 2011; Sadd, 2011).
96
97 Resource availability and acquisition are important for mounting and maintaining an effective
98 immune response. Hosts can mask the deleterious effects of infection by increasing their
99 resource intake (Ayres & Schneider, 2009; Bashir-Tanoli & Tinsley, 2014), therefore
100 manipulating dietary components like protein or carbohydrates may uncover trade-offs or
101 costs that are not present under *ad libitum* conditions (Moret & Schmid-Hempel 2000;
102 Sternberg *et al.* 2012; Howick & Lazzaro 2014; Kutzer & Armitage 2016b). Such
103 physiological trade-offs (i.e. immune function versus fitness) are central to life history theory,
104 and can be either genetically fixed or variable, which will ultimately determine if the trade-off
105 is selected for in a population (Flatt *et al.*, 2011). In *Drosophila melanogaster*, experimental
106 dietary manipulation has mixed effects on the immune response, giving weight to the idea that
107 these relationships are largely context dependent (Vale *et al.*, 2011). For example, dietary
108 yeast restriction uncovered pathogen dependent, intra-genotypic variation in host tolerance
109 but not resistance in a single population of flies infected with *Escherichia coli* (Kutzer &
110 Armitage, 2016b), but in a separate study, resistance to *E. coli* was improved in flies with *ad*
111 *libitum* access to food compared with their counterparts on standard medium (McKean &
112 Nunney, 2005). Infections can impose considerable costs on hosts by competing for host
113 resources, decreasing host reproductive output, and causing host death (Stearns, 1992; Hurd,
114 2009), so hosts may use different immune strategies depending on an infection's pathology.
115 That is, fecundity compensation or reduction may be caused by infection or it may be a host
116 strategy (Hurd, 2001), which should be intimately connected to host defense strategies like

117 resistance and tolerance. For example a pathogenic infection may result in a host allocating
118 resources away from resistance to reproduction, appearing tolerant in the short-term (Vale &
119 Little, 2012; Leventhal *et al.*, 2014).
120
121 Resistance and tolerance can be plastic responses, changing over the course of an infection
122 (Howick & Lazzaro, 2014; Lough *et al.*, 2015; Kutzer & Armitage, 2016b; Louie *et al.*,
123 2016), but we were curious to know to what extent these responses show environmental
124 plasticity and genetic variability. Therefore, our novel approach was to test whether dietary
125 restriction through yeast (protein) limitation affects resistance and tolerance, and examine the
126 environmental interaction with genotype by testing ten wild-type *D. melanogaster* genotypes.
127 We infected flies with one of two opportunistic bacterial pathogens,
128 *Pseudomonas entomophila* and *Lactococcus lactis*, with different infection progressions and
129 contrasting short-term pathogenicity, and examined acute-phase resistance and tolerance to
130 infection to explore the extent to which these strategies are affected by genotype and the
131 environment. Here we defined acute phase infections as early stage infections occurring
132 between 0 and 72 hours post infection when pathogen levels are at their peak (e.g. Howick &
133 Lazzaro 2014). We measured range tolerance (Little *et al.*, 2010), where the slope of the
134 regression that results from the pathogen load and fecundity for every individual in the group,
135 describes tolerance for each treatment group (Råberg *et al.*, 2007; Graham *et al.*, 2011;
136 Lefèvre *et al.*, 2011). This provides more information than a single mean value for bacterial
137 load because host tolerance is measured over a range of pathogen loads. A group with a
138 steeper negative slope is less tolerant than a group with a flatter slope, because the former
139 loses their fitness more rapidly as pathogen load increases. Our use of range tolerance
140 contrasts with other studies on *D. melanogaster* (except Kutzer & Armitage 2016b), which
141 used means per group or different individuals for estimates of fecundity and bacteria load

(Corby-Harris *et al.*, 2007; Ayres & Schneider, 2008; Howick & Lazzaro, 2014). Because the expression of defense strategies could be determined by a combination of infection pathology, resource availability, and genetic factors, we predicted that 1. there is genetic variation for resistance and tolerance, 2. dietary restriction would uncover costs manifested as reduced fecundity tolerance in response to infection with a pathogenic bacterium, *P. entomophila*, and 3. dietary restriction may uncover trade-offs between resistance and tolerance. We find that while fecundity and survival are determined by host genotype and dietary yeast, resistance is largely genetically determined, and fecundity tolerance is unaffected by either genotype or environment.

Methods

Drosophila melanogaster *culture conditions*

We used ten wild-type populations. The locally-collected population used in this study (1_4WS; Kutzer & Armitage 2016b) was maintained in a population cage with overlapping generations. Nine populations with variable fecundity (Ral208, Ral350, Ral367, Ral373, Ral375, Ral379, Ral406, Ral509, Ral765) from the *Drosophila* Genetic Reference Panel (DGRP) originating from North Carolina, USA (Mackay *et al.*, 2012) were maintained in vials and placed onto new food every two weeks. For the purposes of this study we consider each of these populations as being a distinct genotype (e.g. Mackay *et al.*, 2012), but we note that the populations will inevitably differ from one another not only in their genetics, but also in factors such as the microbiota that they contain. Therefore, we use 'genotype' in a broader sense. All stocks were kept at 25 °C, 70 % relative humidity on a 12-12 hour light-dark cycle, and were reared on a standard sugar, yeast, agar medium (SYA medium: 1.5 % agar, 5 % sugar, 10 % brewer's yeast [inactive *Saccharomyces cerevisiae* that is approximately 45 %

166 protein], 3 % nipagin, 0.3 % propionic acid) (Bass *et al.*, 2007). The procedures described
167 below were repeated independently to give a total of seven experimental replicates.
168
169 *Experimental animals and dietary treatments*
170 The individuals used in the experiment, as well as their parents, were reared at constant larval
171 density following protocols described in Kutzer & Armitage (2016b) with the following
172 modifications. Between 300 – 500 flies from each DGRP genotype were placed in embryo
173 collection cages to generate the F1 generation for each of the seven replicates. We collected
174 approximately 400 to 500 larvae of each of the ten genotypes for both the F1 and F2
175 generations. After the F2 generation eclosed, virgin females were allocated in groups of 20 to
176 one of the two dietary treatments, SYA or reduced yeast (RY) medium. RY medium
177 contained 25 % of the yeast contained in the SYA medium (Kutzer & Armitage, 2016b).
178 Males were kept in groups of 20 on SYA medium until mating.
179
180 *Mating assay and diet treatments*
181 Five to six days after adult eclosion we performed group mating assays at room temperature.
182 Beginning at 9:00 am, 10 male flies were placed into vials with 10 virgin females and allowed
183 30 minutes to mate. Limiting the time to 30 minutes decreased the chance of remating, which
184 could affect the immune response (Short *et al.*, 2012). Female and male flies were separated
185 by brief CO_2 anaesthetization. Males were discarded and females were individualised on a RY
186 diet or on an added yeast (AY) diet. The AY diet was SYA medium supplemented with active
187 baker's yeast granules, giving *ad libitum* access to yeast.
188
189 *Bacterial preparation and infections*

We chose two infective bacteria species with distinct infection dynamics. *L. lactis* does not cause significant host death between 0 and 24 hours post-injection (hpi) when injected with the dose we use below, but replicates rapidly in the host from 0 to 24 hpi (Kutzer & Armitage, 2016b). *P. entomophila* is comparatively more pathogenic hence the lower injection dose used below, and can cause host death beginning approximately 20 - 22 hpi (personal observation). Our *L. lactis* strain (gift from Brian Lazzaro) was isolated from a wild caught *D. melanogaster* in State College, Pennsylvania (Lazzaro, 2002). The *P. entomophila* strain was isolated from a wild caught fruit fly in Guadeloupe (gift from Bruno Lemaitre) (Vodovar *et al.*, 2005; Vallet-Gely *et al.*, 2008). Both are opportunistic pathogens of *D. melanogaster*. Aliquots of *L. lactis* and *P. entomophila* were stored in 34.4 % glycerol at -80 °C. *L. lactis* was plated on lysogeny broth (LB) agar and *P. entomophila* was plated on LB agar containing 1 % milk to select for protease positive clones (Neyen *et al.*, 2012), after which, bacterial preparation and infections were carried out following Kutzer & Armitage (2016b) using a randomized block design of 60 total treatment groups (10 genotypes x 2 diets x 3 infection treatments). In each experimental replicate we processed 3 flies per treatment group, giving 21 flies per genotype x diet x infection treatment, i.e. 1260 flies in total. A volume of 18.4 nL of bacterial or a control solution was injected into the lateral side of the thorax using a fine glass capillary attached to a Nanoject II^{TM} (Drummond). For *P. entomophila* we injected 18.4 nL of a 5 x 10^6 cells mL^{-1} bacterial solution where the bacteria was suspended in *Drosophila* Ringer's solution (Werner *et al.*, 2000), which was equivalent to approximately 92 bacteria per individual. In preliminary experiments with the 1_4WS genotype we found that this dose resulted in about 10 % mortality 24 hpi. Flies infected with *L. lactis* were injected with 18.4 nL of a 1 x 10^8 cells mL^{-1} bacterial solution, which was equivalent to 1840 bacteria per fly. Control flies were injected with 18.4 nL of *Drosophila* Ringer's solution. Females were returned to 25 °C, 70 % relative humidity after infection. We diluted the leftover injection

bacteria aliquots to 1 x 10^3 cells/ mL^{-1} and plated 50 µl of each on LB plates, which should have yielded 50 CFUs. Bacterial counts from each aliquot ranged from 30 to 76 CFUs for *L. lactis* and 23 and 67 for *P. entomophila*. We found no evidence of contamination for any replicate.

Fitness measure

We measured pre-infection fitness as the total number of adult offspring produced by females in the ~26 hours between mating and injections. Infected fitness was the total number of adult offspring produced by each individual female in the 24 hpi. After we had removed females from their vials for the bacterial load assay (below), the vials were kept at 25 °C until the offspring had completed development and eclosed. Flies on the AY medium were given 12 days to complete development and those on the RY medium were given 17 days. The vials were then turned upside down, frozen, and the offspring were counted after the experiment ended.

Standardising fecundity for fecundity tolerance

There were considerable genotypic differences in the number of adult offspring produced by uninfected flies (general vigour), which will partly determine fly fecundity when infected. Following the example of Graham *et al.* (2011) we therefore assessed fecundity as the cost of infection. We standardised the values by calculating the percent change in adult offspring number relative to uninfected Ringer's controls as our response variable. The calculations were performed using the mean fecundity of the Ringer's group for each genotype/diet combination. The percent change for each individual was therefore calculated as ((individual infected fecundity ω_i – mean Ringer's group fecundity ω_0) / ω_0) x 100. For statistical reasons,

we standardized the change in fitness in this way to make the fecundity values more comparable across the two dietary treatments (Kutzer & Armitage 2016).

Bacterial load assay

We assayed bacterial load at 24 hpi. The inverse of load determines the resistance of individual flies (methods as described in Kutzer & Armitage 2016b). In brief, after surface sterilisation, we serially diluted homogenates of whole flies infected with *L. lactis* in LB medium at 1:1, 1:100 and 1:1000 and homogenates of flies infected with *P. entomophila* were diluted 1:1 and 1:50 for each replicate. We plated 50 µl of each dilution onto LB agar and incubated the plates at 30 °C for 20 hours and then counted bacterial colony forming units (CFUs). We did not homogenize control flies injected with *Drosophila* Ringer's solution because we found from previous work that these were usually negative for bacterial growth (e.g. Kutzer & Armitage, 2016b, 7 % of all treatment groups had colony morphology that was inconsistent with the injected bacteria). If a plate contained too many CFUs to count at the highest dilution (~2%), we assigned the value as the greatest number of CFUs counted in the genotype/treatment group (e.g. Vincent & Sharp, 2014).

Statistical analyses

Statistical analyses were performed in R version 3.3.1 (R Core Team, 2016). The statistical models are detailed in Appendix S1, and model parameter estimates and standard errors are in Tables S1-S4. Because of the substantial mortality in flies infected with *P. entomophila* on RY medium we removed this group from all analyses except for tests on survival.

Genome wide association study for resistance to P. entomophila *infection*

We took advantage of the availability of whole genome sequences for the 9 DGRP genotypes, and performed an exploratory genome wide association study to test for associations between SNPs/INDELs and resistance to *P. entomophila*, using median bacterial load per genotype for the AY environment. We used *P. entomophila* load because we reasoned that it showed the strongest phenotypic differences across genotypes (see Results).

Results

Effect of diet and genotype on survival

Survival 24 hpi with Ringer's or *L. lactis* was high (mean % survival ± SE: Ringer's: 98.8 ± 0.47 %; *L. lactis*: 98.6 ± 0.61 %) and unaffected by either diet or fly genotype (Figs 1A and B; Table 1). However, after infection with *P. entomophila*, the genotypes differed in the degree to which diet reduced their survival (Fig. 1C, interaction between genotype and diet in Table 1). A reduced yeast diet strongly reduced survival, and there were significant differences in how well the genotypes could survive infection over this short time (Fig. 1C, Table 1).

Effect of diet and genotype on bacterial load and bacterial load correlations

Fly genotypes varied in their *L. lactis* bacterial loads (Fig. 2A, Table 2). However, we found no evidence for a dietary effect or genotype-by-diet interaction on *L. lactis* load. There was a marked difference in *P. entomophila* load across genotypes (Fig. 2B, Table 2). We were not able to test whether there was a dietary effect on bacterial load because of the high mortality we observed in the RY treatment (Fig. 1C). There was no relationship between *L. lactis* and *P. entomophila* load across the ten genotypes using mean (Spearman's rank correlation, rho = -0.55, p = 0.10) or median load (rho = 0.17, p = 0.65).

Effect of diet, infection status and genotype on fecundity

Post-injection fecundity, i.e. adult offspring, was unaffected by infection treatment (Ringer's, *L. lactis*, *P. entomophila*) in flies on AY media (Table 3, Model 3a). However, fecundity varied significantly across genotypes (Figs 3A, B and C; Table 3) and there was a strong positive correlation between pre- and post-infection fecundity (Table 3). We found interactions between diet x genotype and genotype x infection status when comparing post-infection fecundity among dietary treatments in flies injected with Ringer's solution or *L. lactis* (Figs 3A, B, D and E, Table 3, Model 3b). Diet, genotype and pre-infection fecundity were also significant predictors of post-infection fecundity in this model. A number of females did not produce adult offspring, which may have been due to protein restriction and/or the possibility that the flies did not mate during the group mating assay. However, female fecundity after group matings in this experiment was comparable to fecundity after observations of single pair matings: in this experiment 17 % of 1_4WS females on RY medium produced zero offspring, which is similar to a previous experiment in which we observed single pair matings (22 % with zero offspring, genotype 1_4WS on RY medium, (Kutzer & Armitage, 2016b)

Effect of diet and genotype on fecundity tolerance

We found no effect of bacterial load on fecundity tolerance to *L. lactis*. Tolerance towards *L. lactis* did not vary by genotype or diet, or a combination of both, which is illustrated by the lack of significant interactions between these factors and bacterial load (Fig. 4A, Table 4). However, genotype and diet affected percent change in adult offspring (Table 4).

Fecundity tolerance to *P. entomophila* tended to decrease as bacteria load increased, which was independent of genotype (Fig. 4B). We observed no effect of genotype on fecundity tolerance to a *P. entomophila* infection (Table 4).

Genome wide association study for resistance to P. entomophila *infection*

We found no significant associations between median *P. entomophila* load per genotype and any of the SNPs or INDELs present in those genotypes (Fig. S1), which is most likely due to a lack of power from only nine genotypes. Therefore, we do not discuss this analysis further.

Discussion

We tested the degree to which host genotype, dietary environment and G x E interactions influence survival, fecundity, resistance, and fecundity tolerance to two acute phase bacterial infections in ten wild-type *D. melanogaster* populations. Host genotype strongly predicted variation in fecundity and resistance, but not tolerance, after infection with both *L. lactis* and *P. entomophila.* As expected, a lower dietary yeast environment reduced fecundity, however, it did not affect host resistance or tolerance to *L. lactis*. In contrast, lower dietary yeast markedly reduced survival after a *P. entomophila* infection, which in combination with genotype and a G x E effect on survival suggests that dietary environment and genetic background play central roles in host defense during the early stages of a pathogenic infection.

Host genotype and diet determine survival after P. entomophila *infection*

The importance of nutrition on traits including immune function, reproduction, and lifespan cannot be disputed (Moret & Schmid-Hempel, 2000; Siva-Jothy & Thompson, 2002; McKean *et al.*, 2008; Ayres & Schneider, 2009; Sadd, 2011; Sternberg *et al.*, 2012; Stahlschmidt *et al.*,

2013; Howick & Lazzaro, 2014; Kutzer & Armitage, 2016b). In our study, adult dietary yeast restriction had a stronger negative effect on the survival of some genotypes infected with *P. entomophila*, indicating a G x E interaction for this phenotype, which may suggest genotypic variation for adult nutritional acquisition or energy storage (e.g. Bashir-Tanoli & Tinsley, 2014; Unckless *et al.*, 2015a, b). Furthermore, lower yeast availability consistently reduced the survival of *P. entomophila*-infected flies across the ten genotypes tested, suggesting that resource acquisition and/or availability is an important determinant of infection outcome in this host-pathogen interaction and highlights the importance of considering the environmental context in immune studies. It is interesting that survival after systemic infection of *P. entomophila* in eight of the DGRP genotypes found in this study is positively correlated (Spearman's rank correlation: rho = 0.84, p = 0.009) with survival three days after an oral *P. entomophila* infection using the same genotypes in another study (Sleiman *et al.*, 2015, Ral367 was not used by these authors), despite the fact that the studies used different infection routes and were done in different laboratories. Survival in the *L. lactis* and Ringer's treatments was high and unaffected by any of our experimental factors.

Genotypic variation in resistance to acute infections

Evolutionary models predict that individuals within a host population will vary in their ability to ward off infection (Miller *et al.*, 2007; Duffy & Forde, 2009; Boots *et al.*, 2012). In addition, environmental heterogeneity can alter the expression of host susceptibility to pathogens and resistance. However, adult dietary manipulation did not result in diet-induced variability in resistance in the *L. lactis* infected groups, or G x E effects, and we were unable to test this hypothesis explicitly in the *P. entomophila* infected groups because of high mortality on the reduced yeast diet. The *L. lactis* observations were consistent with the results of a previous study, where reduced access to protein did not affect bacterial load within a

single wild-type genotype (Kutzer & Armitage, 2016b). We found genotypic variation for resistance (the inverse of bacteria load) in response to acute stage infection with both bacteria species. Resistance is predicted to vary among genotypes or populations (Miller *et al.*, 2007; Duffy & Forde, 2009; Boots *et al.*, 2012; Vale & Little, 2012), so it is not unexpected that these 10 genotypes exhibit variation in their capacity for resistance. The genotypic variation in resistance to a *P. entomophila* infection was considerable, highlighting the importance of testing infections across different host genetic backgrounds and the difficulty in making generalisations from single genotypes, as suggested by Sleiman *et al*. (2015). The five-fold change in median *P. entomophila* load from the most to the least resistant genotype could result from both variation in host immune responses and, potentially, the ability of the bacteria to grow inside the host, for example if resources available for bacterial growth vary across hosts. Genotypic variation in resistance is pervasive, it has been found across *D. melanogaster* genotypes (Lazzaro *et al.*, 2006; Magwire *et al.*, 2012; Hotson & Schneider, 2015; Unckless *et al.*, 2015b), including those orally infected with *P. entomophila* (Sleiman *et al.*, 2015), or injected with *L. lactis* (Lazzaro *et al.*, 2006). Lazzaro, Sackton & Clarke (2006) found that many genotypes infected with *L. lactis* displayed a narrow phenotypic distribution, which they suggest is driven by high bacteria loads. In the present study, we observed high loads and a flat distribution at the maximum load, and there seemed to be an infection ceiling of approximately 1 x 10^8 bacteria per fly, which may indicate that *L. lactis* reaches a growth plateau within the fly independent of genetic variation. Furthermore, the individual bacterial loads of *L. lactis* infected flies were surprisingly variable, especially given that the DGRP genotypes were initially inbred for 20 generations. Individual variation for resistance therefore seems surprising, but it is noteworthy that considerable phenotypic variation within DGRP genotypes has been observed for traits such as antiviral resistance and susceptibility,

sleep, and food acquisition (Magwire *et al.*, 2012; Harbison *et al.*, 2013; Garlapow *et al.*, 2015).

Genotype and diet, but not infection, influence post-infection fecundity

Hosts must balance the costs of mounting an immune response, infection clearance, and repairing infection induced- or self- damage, with life history traits such as reproduction. Insects are sensitive to changes in their dietary environment and rapidly adjust egg production accordingly (e.g. Kutzer & Armitage 2016a), therefore we predicted that adult offspring numbers would decrease in response to protein restriction and that there would be variation across genotypes, which was the case. We observed a marginal effect of infection and genotype on host fecundity but this may have been driven by the addition of diet in the model, as we found no interaction effect when we compared fecundity within the *ad libitum* yeast groups. This may have been due to the ability of the flies to compensate for the effects of infection with a pathogenic bacterium like *P. entomophila*, which reduces survival (e.g. McKean *et al.* 2008). Interestingly, we also found that the degree of reduction in adult offspring after yeast restriction varied across genotypes.

No evidence for variation in host tolerance

The expression of host tolerance within populations should be dependent upon disease pathology and host immunopathology. Pathogen infection dynamics vary according to infective dose, the route of infection, and infection outcome (Schmid-Hempel, 2011; Schneider, 2011). Considering this, we used two bacteria with different infection dynamics and differing degrees of virulence to explore the relationship among host fitness, diet, and bacteria load to estimate fecundity tolerance.

We predicted genetic variation for tolerance phenotypes in response to infection and environment with at least one bacteria species, given that a number of studies have found such effects (e.g. Råberg *et al.*, 2007; Blanchet *et al.*, 2010; Graham *et al.*, 2011; Adelman *et al.*, 2013; Sternberg *et al.*, 2013; Parker *et al.*, 2014). For example, dietary manipulation of glucose lead to a genotype-by-environment effect for *D. melanogaster* fecundity tolerance that was most pronounced early during a *Providencia rettgeri* infection (Howick & Lazzaro, 2014). However, we found no inter-genotypic differences in fecundity tolerance in response to infection with either bacteria species, and fecundity tolerance to *L. lactis* was not affected by changes in host diet. The latter result confirms a previous study, where it was shown that under similar experimental conditions the 1_4WS genotype does not show environmentally induced variation for tolerance in response to infection with *L. lactis* (Kutzer & Armitage, 2016b). We also note that the considerable variation in our response variables, fecundity and bacteria load, makes it potentially difficult to detect relationships between these two variables.

Despite striking differences in bacterial load across genotypes, all genotypes showed similar reductions in fitness with increasing *P. entomophila* load, indicating a general reproductive cost to an increasing bacteria load. It is possible that assaying fecundity at an earlier infection time point would have uncovered genotypic variation in host tolerance (e.g. Howick & Lazzaro, 2014; Kutzer & Armitage, 2016b), or that fecundity tolerance is somehow fixed or has reached saturation (e.g. Miller *et al.*, 2006). It is also possible that the co-expression of host immune strategies shows temporal variation in this system, with flies surviving infection by *P. entomophila* expressing resistance early on and then expressing tolerance later in the infection compared to non-survivors (e.g. Lough *et al.*, 2015), which may explain the considerable variation in resistance we see across these ten genotypes.

Eco-immunological studies have found support for genetic variation in host tolerance but such variation is not the rule. For instance mortality tolerance declined in families of monarch butterflies as *Ophryocystis elektroscirrha* inoculation dose increased, but was unaffected by genotype (Lefèvre *et al.*, 2011), and aphid genotypes displayed variation in fecundity tolerance but not in mortality tolerance (Parker *et al.*, 2014). A common theme that emerges from these studies is the importance in the choice of the fitness or health measure and its relationship to the pathogen in question, i.e. the relationship between fitness and disease pathology. We suggest that fitness measures should be carefully considered in light of disease pathology and infection dynamics.

Conclusion

Here we tested the effects of dietary environment and genotype on resistance and tolerance to two acute phase bacterial infections. Genotype and dietary environment were strong predictors of mortality from an infection with *P. entomophila* as well as predictors of fecundity. We show that there is considerable genetic variability in resistance to infection, while tolerance does not vary among genotypes or according to environment in the ten tested genotypes. Context dependence is a recurring theme in the resistance and tolerance literature. The expression of these immune strategies depends on genetics, environment and the unique infection trajectories of the pathogens. Plotting detailed infection trajectories that cover an entire course of infection whether at the individual or genotype level, as well as successively quantifying within host damage, will help to tease apart the mechanisms governing the expression of resistance and tolerance.

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461

462 **Data Accessibility**

463 Data from the manuscript will be made accessible upon acceptance in the Dryad database.

464 **References**

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Online Supporting Information

Appendix S1. Statistical analyses.

Figure S1. P-value plots of GWAS.

Table S1-S4. Parameter estimates for statistical models.

Tables

Table 1. The effect of diet and genotype on fly survival 24 hours after injection with either Ringer's, *L. lactis* or *P. entomophila.*

	Tested effect	*df*	*Dev*	*Resid.df*	*Resid.dev*	*P*
Model 1a: Ringer's	Diet	1	0.2	138	34.85	0.65
	Genotype	9	6.9	129	27.96	0.65
	Genotype x Diet	9	6.85	120	21.12	0.65
Model 1b: *L. lactis*	Diet	1	0.69	138	39.29	0.41
	Genotype	9	9.02	129	30.27	0.44
	Genotype x Diet	9	7.81	120	22.45	0.55
Model 1c: *P. entomophila*	Diet	1	202.99	138	214.49	**<0.0001**
	Genotype	9	69.05	129	145.45	**<0.0001**
	Genotype x Diet	9	22.94	120	122.51	**0.006**

Table 2. The effect of diet and genotype on bacteria load 24 hours post infection. The dashes (-) indicate that diet and diet x genotype could not be tested.

	Model 2a: *L. lactis*				Model 2b: *P. entomophila*			
Tested effect	*numDF*	*denDF*	*F*	*P*	*numDF*	*denDF*	*F*	*P*
Diet	1	369	0.58	0.446	-	-	-	-
Genotype	9	369	4.57	**<0.0001**	9	150	19.25	**<0.0001**
Diet x Genotype	9	369	0.50	0.874	-	-	-	-

Table 3. The effect of diet, genotype and infection status on post-infection fecundity, measured as adult offspring number. Pre-infection fecundity was included as a covariate. High mortality in the *P. entomophila* group on reduced yeast (RY) precluded testing one fully factorial fecundity model. Therefore Model 3a tests fecundity of the three infection groups, i.e., injection of Ringer's, *L. lactis* or *P. entomophila*, where flies had only *ad libitum* (AY) access to yeast. The dashes (-) indicate that diet and interactions with diet could not be tested. Model 3b tests the fecundity of only Ringer's and *L. lactis* injected flies, on either AY or RY.

	Model 3a: AY fecundity			Model 3b: AY vs RY fecundity		
Tested effect	*df*	*F*	*P*	*df*	*F*	*P*
Diet	-	-	-	1	63	**<0.0001**
Genotype	9	9.22	**<0.0001**	9	12.006	**<0.0001**
Infection status	2	0.13	0.88	1	1.569	0.211
Diet x Genotype	-	-	-	9	9.46	**<0.0001**
Diet x Infection status	-	-	-	1	1.441	0.23
Genotype x Infection status	18	0.69	0.82	9	1.931	**0.045**
Pre-infection fecundity	1	233.23	**<0.0001**	1	46.51	**<0.0001**

Table 4. The effects of bacteria load (CFU), diet and genotype on post-infection fecundity (measured as % adult offspring produced relative to the uninfected Ringer's control) after infection with *L. lactis* or *P. entomophila.* Significant interactions between CFU and either or both of the other factors would indicate variation in fecundity tolerance. The dashes (-) indicate that diet and interactions with diet could not be tested.

	Model 4a: *L. lactis*				Model 4b: *P. entomophila*			
Tested effect	*numDF*	*denDF*	*F*	*P*	*numDF*	*denDF*	*F*	*P*
CFU	1	349	2.77	0.097	1	140	11.1	**0.001**
Diet	1	349	19.62	**<0.0001**	-	-	-	-
Genotype	9	349	4.83	**<0.0001**	9	140	1.63	0.114
CFU x Diet	1	349	0.01	0.907	-	-	-	-
CFU x Genotype	9	349	0.96	0.475	9	140	0.57	0.821
Diet x Genotype	9	349	1.39	0.191	-	-	-	-
CFU x Diet x Genotype	9	349	1.24	0.271	-	-	-	-

Figure legends

Figure 1. *D. melanogaster* survival 24 hours post injection. Females were kept on a diet of *ad libitum* yeast (AY) or reduced yeast (RY) and injected with (A) Ringer's solution as an injection control, or one of two bacteria, (B) *L. lactis*, or (C) *P. entomophila*. Each line represents the reaction norm of one of ten genotypes. Some reactions norms overlap. Each dot represents the proportion of 21 flies that survived.

Figure 2. Bacteria load for each genotype 24 hours post infection. Females were injected with either (A) *L. lactis* or (B) *P. entomophila*. Bacteria load was quantified as the number of colony forming units (CFUs) counted on agar plates containing individual whole fly homogenates. There was no effect of diet on *L. lactis* loads, so AY and RY individuals are combined in (A). Genotypes are arranged in ascending order and diamonds represent medians calculated from between 37 and 42 female flies in (A) and between 15 and 21 female flies in (B). To visualise the data on a log scale, we added 1 to all CFU counts. The dotted lines indicate the approximate infection doses.

Figure 3. Fecundity measured as the number of adult offspring produced in the 24 hours post injection. Female flies were subjected to one of the following injection treatments and diet combinations: (A) Ringer's injected AY medium, (B) *L. lactis* injected AY medium, (C) *P. entomophila* injected AY medium, (D) Ringer's injected RY medium, (E) *L. lactis* injected RY medium. Diamonds represent medians calculated from between 15 and 21 female flies per treatment group. Genotypes are arranged in descending order of offspring numbers. Data points with darker shades of grey indicate where the values of more than one individual overlap.

Figure 4. Fecundity tolerance after infection with *L. lactis* and *P. entomophila*. (A) Fecundity tolerance after *L. lactis* infection is unaffected by diet or genotype; (B) Fitness decreases in response to increasing *P. entomophila* load regardless of genotype. The natural log of bacterial load (CFU) is plotted against the percent change in adult offspring number. Each data point represents the bacteria load and fitness of one female fly. Reaction norms are plotted for AY (solid lines) and RY (dashed lines; *L. lactis* only) for each genotype 24 hours post injection. Data points with darker shades of grey indicate where the values of more than one individual overlap. Mortality was too high in *P. entomophila* infected individuals on the RY medium to include them in the analysis.