

LETTER

Gut physiology mediates a trade-off between adaptation to malnutrition and susceptibility to food-borne pathogens

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Abstract

The animal gut plays a central role in tackling two common ecological challenges, nutrient shortage and food-borne parasites, the former by efficient digestion and nutrient absorption, the latter by acting as an immune organ and a barrier. It remains unknown whether these functions can be independently optimised by evolution, or whether they interfere with each other. We report that *Drosophila melanogaster* populations adapted during 160 generations of experimental evolution to chronic larval malnutrition became more susceptible to intestinal infection with the opportunistic bacterial pathogen *Pseudomonas entomophila*. However, they do not show suppressed immune response or higher bacterial loads. Rather, their increased susceptibility to *P. entomophila* is largely mediated by an elevated predisposition to loss of intestinal barrier integrity upon infection. These results may reflect a trade-off between the efficiency of nutrient extraction from poor food and the protective function of the gut, in particular its tolerance to pathogen-induced damage.

Keywords

Adaptation, *Drosophila*, enteric infections, experimental evolution, host–parasite interactions, innate immunity, nutritional stress, *Pseudomonas entomophila*, stress tolerance, trade-offs.

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INTRODUCTION

By precluding a ‘master of all trades’, ecological trade-offs play a key role in the evolution and maintenance of biodiversity. Yet, although evidence for trade-offs is ubiquitous, we still know little about their detailed mechanisms. Most trade-offs are assumed to result from the allocation of limited resources to competing demands of different functions of the organism, such as growth, reproduction, somatic maintenance, storage or immune defence (Van Noordwijk & de Jong 1986; Stearns 1989; Roff & Fairbairn 2007; Edward & Chapman 2011). Allocation trade-offs between two functions can be alleviated by acquiring more resources or increasing allocation to both functions at the expense of a third function (Roff & Fairbairn 2007). In contrast, this is not an option for allocation-independent ‘design’ trade-offs, which result from the involvement of the same organs, cells or molecules in multiple functions that impose conflicting selection pressures on their properties (Agrawal *et al.* 2010; Edward & Chapman 2011). Thus, design trade-offs may be biologically more fundamental than allocation trade-offs, and in the long term more important in constraining evolution; yet, we know surprisingly little about their ecological significance and mechanisms.

Here, we reveal details of a design trade-off between adaptation to nutritionally poor diet and susceptibility to food-borne pathogens. Most animal species are exposed to a plethora of intestinal pathogens and parasites, as well as being confronted with periods of nutrient shortage long enough to

impose physiological stress (malnutrition). The physiological effects of these ecological stress factors often exacerbate each other (Schaible & Kaufmann 2007; Ponton *et al.* 2011), having more-than-additive effects on individuals, populations and communities (Sheldon & Verhulst 1996; Rolff & Siva-Jothy 2003; Schulenburg *et al.* 2009; Hawley & Altizer 2011; Lulubin *et al.* 2014), with relevance to human public health (Schaible & Kaufmann 2007). Because both malnutrition and intestinal parasites reduce Darwinian fitness, they should generate natural selection favouring adaptations that help the animal cope better with them. Such adaptations are expected in particular to involve the digestive system, which, in addition to its role in nutrient acquisition, acts as an immune organ and a physical barrier to food-borne pathogens. While these two functions of the digestive system have been studied for decades, we do not know to what degree evolution can optimise them independently. Some aspects of digestive system physiology contribute to both functions; for example the low pH of the anterior gut region (stomach in tetrapods) contributes to both digestion of food and killing of microbes (Apidianakis & Rahme 2011). Some other aspects are mainly implicated in one function and could potentially mediate a trade-off. For example intestinal mucus (or its insect analog, the peritrophic matrix) protects the gut wall from pathogens and toxins (Gill *et al.* 2011; Lemaître & Miguel-Aliaga 2013), but it may be costly to secrete and might slow the diffusion of digestive enzymes and digested nutrients between the lumen and the gut epithelium. Similarly, there could be a trade-off

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between secretion of digestive enzymes and immune effectors, either at the histological level (where they are produced by different cells, as is the case in mammals; Gill *et al.* 2011) or at the cellular level (where, as in insects, they are produced by the same cells and thus might depend the same transcriptional and translational machinery; (Lemaitre & Miguel-Aliaga 2013). Such trade-offs might thus involve mechanisms that inhibit the proliferation of or kill pathogens (i.e. immune resistance), and mechanisms that limit or alleviate the consequences of gut damage inflicted by the pathogen and the host's immune response (referred to as tolerance or resilience; Raberg *et al.* 2009; Little *et al.* 2010).

Trade-offs between the efficiency of the gut in extracting nutrients from low-quality food and its protective function against pathogens would constrain evolution of the ability to tackle these common ecological stressors. Such trade-offs could contribute to the maintenance of genetic susceptibility to opportunistic intestinal pathogens that are not specifically selected to overcome the defences of the particular host species (Brown *et al.* 2012; Antonovics *et al.* 2013). Immune resistance reduces pathogen transmission whereas tolerance may enhance it, and so knowing if these trade-offs mainly affect resistance vs. tolerance would help to predict its consequences for epidemiology and pathogen evolution (Roy & Kirchner 2000; Raberg *et al.* 2009; Vale *et al.* 2014). Finally, understanding the mechanisms of these trade-offs would also contribute to understanding of the interactions between malnutrition and intestinal infections.

Yet while the potential trade-off mechanisms mentioned above may sound plausible, experimental evidence pertaining to their existence and importance is indirect and scarce. A substantial number of studies do report genetically based survival and fecundity costs of increased resistance or tolerance to diverse parasites, either detected as correlated responses to experimental selection or as genetic correlations (reviewed in Lazzaro & Little 2009; Martins *et al.* 2013). However, except for numerous studies addressing costs of resistance to the biological control agent *Bacillus thuringiensis* evolved by insect pests (Gassmann *et al.* 2009), few studies tested for the genetic fitness costs of resistance or tolerance to gut parasites (Zhong *et al.* 2005; Vijendravarma *et al.* 2008; Kamath *et al.* 2014). In some studies these costs were found to be more pronounced under nutritional limitation, but this was the case for gut parasites (Vijendravarma *et al.* 2008; Shikano & Cory 2014) as well as for systemic bacterial infections (e.g. McKean *et al.* 2008; Howick & Lazzaro 2014) or parasitoid infections (Fellowes *et al.* 1998). These cases may thus reflect allocation trade-offs, which are predicted to become more acute under nutrient limitation (Stearns 1989), rather than a design trade-off with the ability of the gut to extract nutrients from low-quality food. In one case where the latter was addressed, a *B. thuringiensis*-tolerant strain of a noctuid caterpillar was found to have slightly higher efficiency of nutrient absorption than a susceptible control strain; its slower growth was mediated by a slower ingestion rate (Shikano & Cory 2014). Thus, it remains unclear whether adaptations of the gut to maximise nutrient extraction interfere with its ability to protect from parasites or vice versa. In particular, whether and to what degree evolutionary adaptation to malnutrition has negative

consequences for resistance and tolerance to food-borne infections has not been tested.

Here, we demonstrate that evolution under nutrient limitation adversely affects the ability of the gut to act as a barrier to an opportunistic food-borne pathogen. We take advantage of six *D. melanogaster* populations maintained for over 160 generations on an extremely nutrient-poor larval medium, on which non-adapted larvae suffer high mortality, take twice as long to develop, and emerge as adults of half the size of those raised on standard food (Kolss *et al.* 2009). During this experimental evolution, these selected populations became genetically adapted to this regime of chronic larval malnutrition. In particular, their larvae grow substantially faster than those from control populations on the poor (but not standard) food, suggesting that they manage to extract more nutrients from the poor food (Kolss *et al.* 2009; Vijendravarma & Kawecki 2013). These malnutrition-adapted populations thus offer a unique opportunity to study the consequences of adaptation to malnutrition for resistance and tolerance to food-borne pathogens. We use a natural opportunistic pathogen of *Drosophila*, the bacterium *Pseudomonas entomophila*, which is virulent to larvae and adults upon ingestion (Vodovar *et al.* 2005).

We first show that, compared to the control populations, the malnutrition-adapted populations suffer increased mortality both as larvae and adults upon intestinal infection with *P. entomophila*. We then quantify several parameters relevant to the immune response (expression of a key antimicrobial peptide, inhibition of translation and ROS production) and the bacterial load in the course of infection. These experiments indicate that the immune resistance of the malnutrition-adapted flies is not impaired and their pathogen load is slightly lower. Rather, their increased infection-induced mortality is largely mediated by a greater vulnerability to pathogen-induced loss of gut wall integrity. Thus, adaptation to chronic malnutrition in these populations resulted in reduced tolerance to *P. entomophila* infections, mediated by reduced resilience of the gut to pathogen-induced damage.

MATERIAL AND METHODS

Experimental evolution of the fly populations

The six selected and six control populations and the regimes under which they evolved are described in detail elsewhere (Kolss *et al.* 2009). Briefly, all 12 originated from a single, laboratory-adapted base population. The selected populations have been maintained on a poor larval food regime for over 160 generations; the control populations have been concurrently maintained on standard food (15 g agar, 30 g sucrose, 60 g glucose, 12.5 g dry yeast, 50 g cornmeal, 0.5 g MgSO₄, 0.5 g CaCl₂, 30 mL ethanol, 6 mL propionic acid and 1 g nipagin per litre of water; Kolss *et al.* 2009). The poor larval food contains 25% of the amounts of sugars, yeast and cornmeal of the standard food. Upon emergence, adult flies from both regimes are always maintained on standard food with supplemented yeast. The populations are maintained and all experiments were performed at 25 °C, 60% humidity and a 12 : 12 h

light:dark cycle. Prior to the assays described below, all populations were reared for 2–3 generations on standard food to eliminate maternal effects (Vijendravarma *et al.* 2010) and thus to ensure that the observed differences have a genetic basis. The assays were performed on 3rd instar larvae collected 96 h from egg laying and on adult females aged 3–5 days from eclosion. Unless mentioned otherwise, the assayed individuals were raised on standard food.

From the viewpoint of the hypotheses tested here, the replicate selected and control populations represent the main level of biological replication. Therefore, most of the experiments reported here (11 out of 13) were performed on all six selected and six control populations, with replicate populations treated in the analyses as a random factor nested within the evolutionary regimes. However, two assays (organ-specific *Diptericin* expression and translation activity measurement) were only performed on one selected and one control population (S1 and C1). These assays aimed to confirm the absence of differences between selected and control populations inferred from other experiments; because we indeed found no differences between the two populations and these assays were labour-intensive, we did not extend them to all populations.

Bacterial strains and infection procedures

Two strains of *Pseudomonas entomophila* were used in this study; a virulent wild-type strain isolated in Guadeloupe and the avirulent GacA mutant derived from the virulent strain (Vodovar *et al.* 2005). The experimental infection was carried out by feeding third instar larvae and adult female flies with food laced with the bacteria following standard protocols (Liehl *et al.* 2006). However, we used lower infective doses because the standard doses used in that paper cause nearly 100% mortality. Larvae were infected by feeding for 30 min with a 1/10 of bacterial concentration used by Liehl *et al.* (2006). For adults we used 1/4 of the standard concentration; the length of infection period differed according to the assay: 18 h for mortality, between 45 min and 10 h for other assays according to the expected dynamics of the response, as specified below. For the 'uninfected' treatment the larvae and flies were treated in the same way, but their food was only supplemented with the saline buffer (for details see Supporting Information Methods).

Survival upon infection

To test whether evolutionary adaptation to chronic malnutrition had been associated with changes in susceptibility to *P. entomophila*, we compared infection-induced mortality of larvae and adult flies from the selected and control populations (3 replicate vials of 15 larvae or 30 adults per population). To verify that the observed mortality reflects pathogen virulence rather than adverse nutritional effects of bacteria or remains of their medium, we also assayed the mortality of larvae and adults infected with the avirulent GacA mutant. Arcsine-square root-transformed proportions of surviving individuals in each vial were compared with an ANOVA (populations nested within selection regimes).

Immune response

The immune response to intestinal infection by *P. entomophila* includes the activation of the *Imd* pathway, inducing the production of antimicrobial peptides, notably *Diptericin* (Vodovar *et al.* 2005; Lemaitre & Hoffmann 2007). To test if differences in the expression of the *Imd* pathway are responsible for differences in mortality upon infection, we used quantitative RT-PCR to measure the relative expression of *Diptericin*, both at its baseline and induced level (i.e. in uninfected and infected individuals). This was done on whole larvae (6 h post infection) and adults (10 h from the onset of infection) from all selected and control populations (see Supporting Information Methods). Because we found no differences (see Results), we additionally dissected adults from one selected and one control population and quantified *Diptericin* expression separately in the gut and the fat body, which contribute, respectively, to the local and systemic immune response (Buchon *et al.* 2009b). This was done to verify that a lower expression in the gut is not masked by a higher expression in the fat body or vice versa.

The pathology induced by *P. entomophila* includes inhibition of translation in the intestine, blocking the synthesis of antimicrobial peptides and the renewal of the gut epithelium (Chakrabarti *et al.* 2012). If this occurred to a different degree in selected vs. control populations, the production of antimicrobial peptides could differ even if their expression at the mRNA level were the same. To address this possibility, we quantified the global rate of translation in the gut of infected and uninfected flies. The method involves feeding flies with fluorescently labelled methionine analogue and subsequently dissecting the gut and quantifying the amount of fluorescence relative to the cellular background, which corresponds to the amount of the labelled methionine incorporated in newly synthesised proteins (see Supporting Information Methods).

Intestinal infection with gram-negative pathogens also induces the synthesis of ROS (Bae *et al.* 2010; Lemaitre & Girardin 2013). Even though ROS appear ineffective against *P. entomophila* (Chakrabarti *et al.* 2014), we compared ROS production in infected and uninfected flies from the selected and control populations, using a peroxidase assay, which quantified the amount of H₂O₂ in dissected guts (one sample of 10 guts per population and treatment, see Supporting Information Methods). Because the ROS is produced in a fast burst (Chakrabarti *et al.* 2014), its level was assessed 45 min from the onset of infection.

The normalised relative *Diptericin* expression and translation activity are ratios and were therefore log-transformed before analysis. The transformed values, as well as untransformed ROS measurements, were analysed with ANOVA.

Food intake and bacterial load

Differences in mortality upon infection could result from differences in the amount of bacteria ingested. We showed earlier that larvae from our selection regimes did not differ in their food intake rate (Vijendravarma *et al.* 2012), but this had not been verified for adults. We therefore estimated the food intake of infected and uninfected adults from all populations

using an established protocol which involves allowing flies to feed on food supplemented with a dye for 15 min and quantifying the amount of dye ingested (Kaun *et al.* 2007). To make the intake measure most relevant from the viewpoint of the infectious dose received by the flies, in the 'infected' treatment we quantified the intake of the infectious medium after the flies had already been exposed to the infectious medium for 10 h (see Supporting Information Methods). The amount of dye ingested was quantified as optical density (OD_{633}) and analysed using a mixed-model ANOVA (3 replicate samples of five adults per population and treatment).

To assay the dynamics of infection over time, we estimated the bacterial load of infected flies 2, 4 and 6 h from the onset of the infection protocol. This was done by the standard colony-forming units method (Liehl *et al.* 2006), with one sample of five infected flies per population and time point. The flies were dipped in 70% ethanol thrice (5 s) to sterilise the body exterior and then homogenised in 100 μ L of phosphate buffer. The homogenate was serially diluted and 10 μ L droplets were plated on Luria-Bertani agar plates. The number of viable cells within an individual was estimated by counting the number of colonies formed after incubation. The dilutions to be counted were chosen so that the colony counts ranged between 10 and 46; the same dilution was used for all samples from a given time point. The counts were analysed using a generalised mixed model with Poisson error distribution, log link and an overdispersion parameter; selection regime and time were the fixed factors and population was a random factor nested in the selection regime (PROC GLIMMIX of SAS v. 9.3, SAS Institute Inc., Cary, NC, USA). For plotting, the counts were converted to the estimated number per fly.

Gut wall integrity assays

Repair of the gut wall is an important mechanism of resilience to infection; this mechanism often fails upon infection with high doses of *P. entomophila* leading to loss of gut wall integrity (Buchon *et al.* 2009a; Chakrabarti *et al.* 2012). To compare the proneness of selected and control populations to infection-induced loss of gut wall integrity, we used the 'smurf' assay (Rera *et al.* 2012), which involves feeding larvae or adult flies with food coloured with a non-toxic water-soluble food dye. In individuals with intact intestinal walls, the dye remains contained in the digestive tract, whereas in individuals with intestinal barrier dysfunction, the dye leaks into the haemocoel, colouring the body blue (the 'smurf' phenotype). Larvae and female flies infected with *P. entomophila* or sham-treated with saline for 30 min and 10 h, respectively, were transferred onto standard food containing the blue dye (2.5% w/v) for 8 h (two samples of 15 larvae or 17–20 adults per population and treatment). The proportion of individuals showing the 'smurf' phenotype was subsequently recorded. The arcsine-square root-transformed proportion of 'smurf' individuals were compared between the regimes using a nested ANOVA.

To test the link between the infection-mediated loss of intestinal integrity and mortality, in an independent experiment we separated infected adult flies from each population according to whether or not they showed the 'smurf' phenotype 18 h after the onset of infection (40 flies of each pheno-

type per population). These flies were transferred to fresh food vials (without dye) and their survival was monitored over the next 22 h. The proportion of flies alive at each time point was arcsine-square root-transformed and analysed with ANOVA, with the initial phenotype ('smurf' vs. 'non-smurf') and regime as fixed factors, and population as a random factor nested within regime.

RESULTS

Mortality upon infection

Both larvae and adults from the selected populations suffered higher mortality after infection than those from the control populations (Fig. 1a and b, circle symbols). Almost no mortality was observed in larvae and adult flies infected with the avirulent *GacA* mutant of *P. entomophila* (Fig. 1a and b, square symbols), thus confirming that the high mortality upon infection with the virulent *P. entomophila* strain is indeed mediated by the pathogen's virulence.

Furthermore, a very similar pattern of mortality upon infection, with flies of the selected populations dying faster than controls, was observed when all flies were raised on the poor food (Fig. 1c), indicating that the increased susceptibility of malnutrition-adapted flies is not diet-specific. Thus, evolutionary adaptation to chronic malnutrition in our selected populations was associated with increased genetic susceptibility of both larvae and adults to intestinal infection with *P. entomophila*.

Immune response

As expected, infection with *P. entomophila* induced more than a 100-fold increase in *Diptericin* expression relative to saline-fed controls (Fig. 2a and b; larvae: $F_{1,10} = 249.8$, $P < 0.0001$; adults: $F_{1,9} = 238.8$, $P < 0.0001$). However, *Diptericin* expression did not differ between the selection regimes in either larvae (Fig. 2a; regime: $F_{1,10,9} = 0.6$, $P = 0.44$; regime \times infection treatment: $F_{1,11,3} = 0.16$, $P = 0.69$) or adult flies (Fig. 2b; regime: $F_{1,10} = 0.006$, $P = 0.94$; regime \times infection treatment: $F_{1,9} = 0.28$, $P = 0.61$).

Two additional assays were performed on flies from one selected and one control population. First, although expression of *Diptericin* increased upon infection both in the gut (Fig. 2c; $F_{1,9} = 22.7$, $P = 0.001$) and the fat body (Fig. 2d; $F_{1,9} = 193.6$, $P < 0.0001$), it did not differ between the two populations (Fig. 2c and d; regime – gut: $F_{1,9} = 0.21$, $p = 0.7$; fat body: $F_{1,9} = 0.68$, $P = 0.43$; regime \times infection treatment – gut: $F_{1,9} = 0.0004$, $P = 0.98$; fat body: $F_{1,9} = 0.09$, $P = 0.77$). Second, although we detected a mild reduction in translation in infected flies (Fig. 2e; $F_{1,64} = 7.9$, $P = 0.007$), translation activity was not different between the populations (regime: $F_{1,64} = 0.02$, $P = 0.89$; regime \times infection: $F_{1,64} = 0.14$, $P = 0.71$). Altogether, these results suggest that AMP-based immune response is not impaired in the selected populations.

The baseline ROS level in the guts of uninfected flies did not differ between regimes (Fig. 2f; $F_{1,10} = 1.04$, $P = 0.3$). As expected, the ROS concentration increased upon *P. entomophila* infection in both regimes (Fig. 2f; $F_{1,10} = 52.2$,

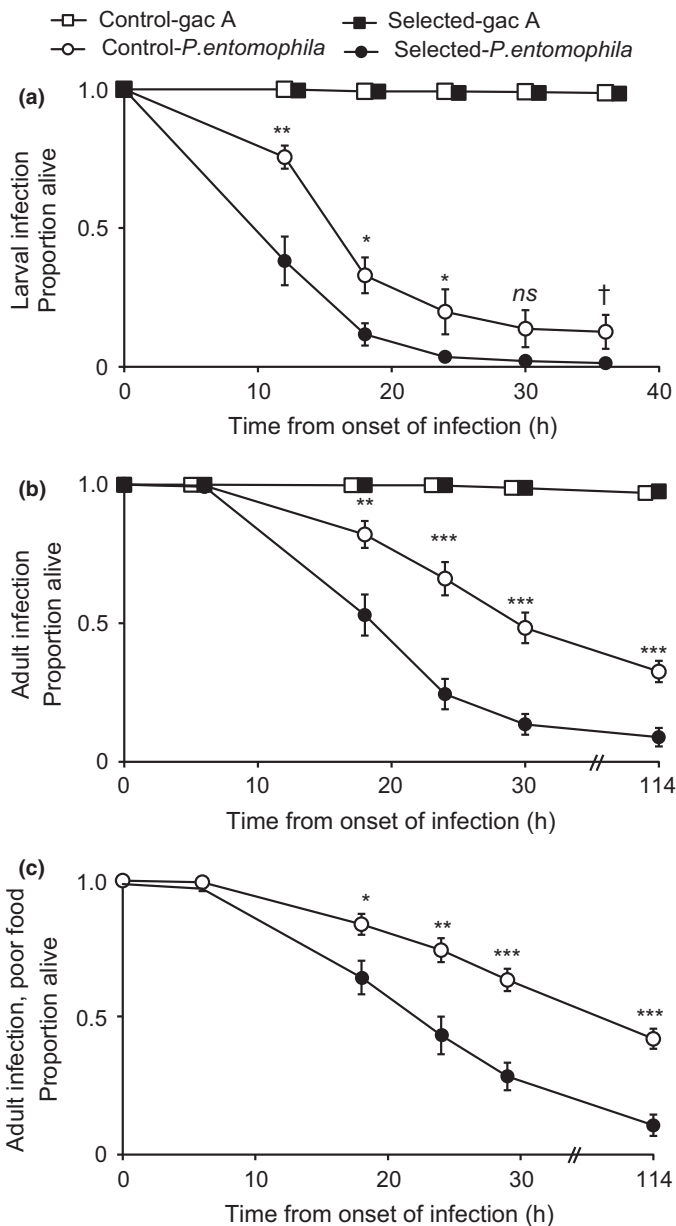


Figure 1 Increased susceptibility to intestinal infection with *P. entomophila* in *Drosophila* populations adapted to malnutrition. (a–c) Larval and adult survival curves of control (open symbols) and selected (closed symbols) populations upon infection with *P. entomophila* (circle symbols) and avirulent GacA mutant (square symbols); each data point indicates the mean \pm SE of six independent replicate populations evolved under each regime. (a) Larvae raised on standard food infection. (b) Adults raised as larvae on standard food. (c) Adults raised as larvae on poor food (infection with GacA mutant was not included in this assay). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, † $P < 0.1$; ns: $P > 0.1$.

$P < 0.0001$), but only by about half as much in the selected as in the control populations (regime \times infection treatment: $F_{1,10} = 11.4$, $P < 0.007$). As a consequence, the induced ROS levels of selected populations were 21% lower than those of control populations (Fig. 2f; $F_{1,10} = 17.9$, $P = 0.002$). This does not, however, necessarily imply reduced defence against *P. entomophila* (Chakrabarti *et al.* 2014); we return to this point in the Discussion.

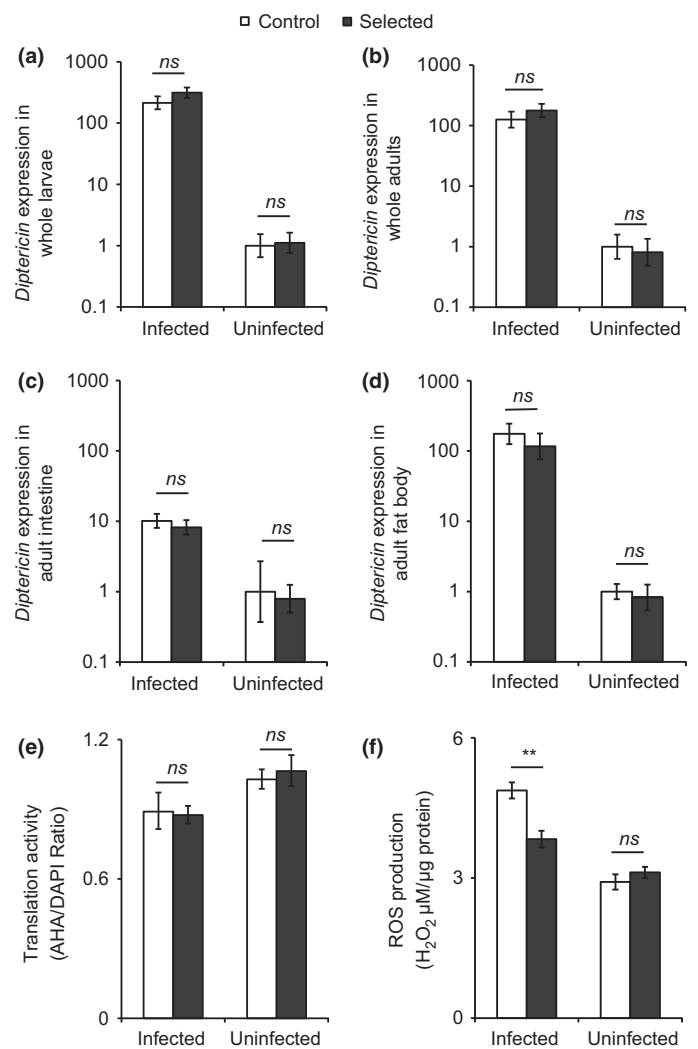


Figure 2 Immune response to intestinal infection by *P. entomophila* in selected and control populations. (a and b) Relative expression level of *Dipterucin* in infected and uninfected whole larvae (a) and whole adults (b) of all selected and control populations. (c) *Dipterucin* expression in adult intestine and (d) in adult fat body in one selected and one control population. (e) Translation of nascent proteins in intestines of one control and one selected population. (f) The mean level of ROS production in all selected and control populations. The bars represent mean \pm SE; where only one population per regime was used (panels c–e) the SE reflects variation among replicate vials, in other cases SE is based on variation among replicate populations within regimes. ** $P < 0.01$, ns: $P > 0.1$.

Food intake and pathogen load

Uninfected adult flies from the selected populations had a lower food intake than those from control populations (Fig. 3a; $F_{1,10} = 8.19$, $P = 0.016$). We observed a similar difference between the selection regimes in intake of infectious medium by flies already exposed to this medium for 10 h (Fig. 1d; $F_{1,10} = 10.95$, $P = 0.008$).

As has been previously reported (Liehl *et al.* 2006), the bacterial load decreased over time, but across the time points it was slightly lower in the selected than in control populations (regime: $\chi^2_1 = 8.2$, $P = 0.004$; regime \times time: $\chi^2_2 = 3.1$, $P = 0.2$), although when analysed separately for each time

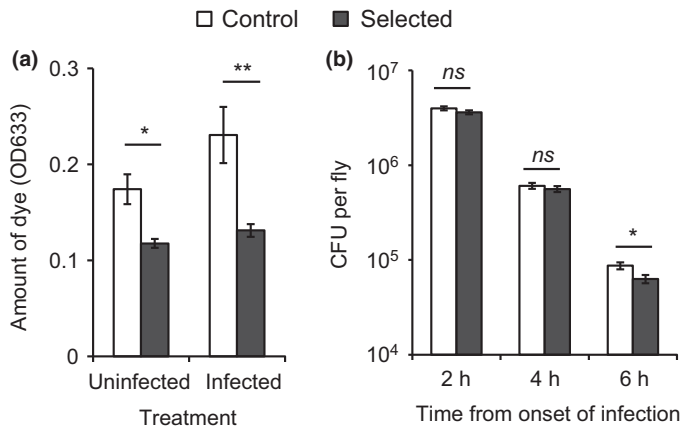


Figure 3 Food intake and bacterial load. (a) The food intake of uninfected and infected flies from all selected and control populations, quantified as the amount of dyed food ingested during 15 min; for the ‘infected’ treatment the measure is the intake of the infectious medium. (b) Within host bacterial load in the time course of infection. Bars represent means \pm SE; ** $P < 0.01$, * $P < 0.05$.

point the difference was only significant at 6 h (Fig. 3b). Thus, the higher mortality of the selected flies cannot be attributed to higher bacterial loads or their slower clearance from the gut.

Infection-mediated loss of intestinal integrity in malnutrition-adapted populations

Despite having lower bacterial loads, 8 h after infection about 31% of larvae from the selected populations showed loss of intestinal barrier integrity, compared to about 18% for control populations (Fig. 4a and b; $F_{1,10} = 11.2$, $P = 0.0074$). Similarly, 18 h from the onset of infection, adults from the selected populations were twice as likely to show the ‘smurf’ phenotype as the control populations (Fig. 4c and d; $F_{1,10} = 42.7$, $P < 0.0001$). No uninfected larvae and very few uninfected adults showed the ‘smurf’ phenotype (Fig. 3b and d).

For both selected and control populations, over 25% of infected flies showing the ‘smurf’ phenotype died within 4 h compared to < 5% infected ‘non-smurf’ individuals (Fig. 4e; $F_{1,10} = 309.6$, $P < 0.0001$); after 22 h these proportions were about 71% vs. 22% (Fig. 4e; $F_{1,10} = 138.6$, $P < 0.0001$). Although both ‘smurf’ and ‘non-smurf’ flies from the selected populations appeared to die faster than flies from control populations showing similar phenotype, the proportion surviving after 22 h was not significantly different between the selection regimes (regime $F_{1,10} = 1.7$, $P = 0.22$; ‘smurf’ phenotype \times regime $F_{1,10} = 0.1$, $P = 0.78$). Thus, the loss of intestinal integrity is a good predictor of imminent death in infected flies and explains most of the difference in infection-induced mortality between the selected and control populations.

DISCUSSION

The ability to deal with two common ecological challenges – nutrient shortage and food-borne pathogens – to a large

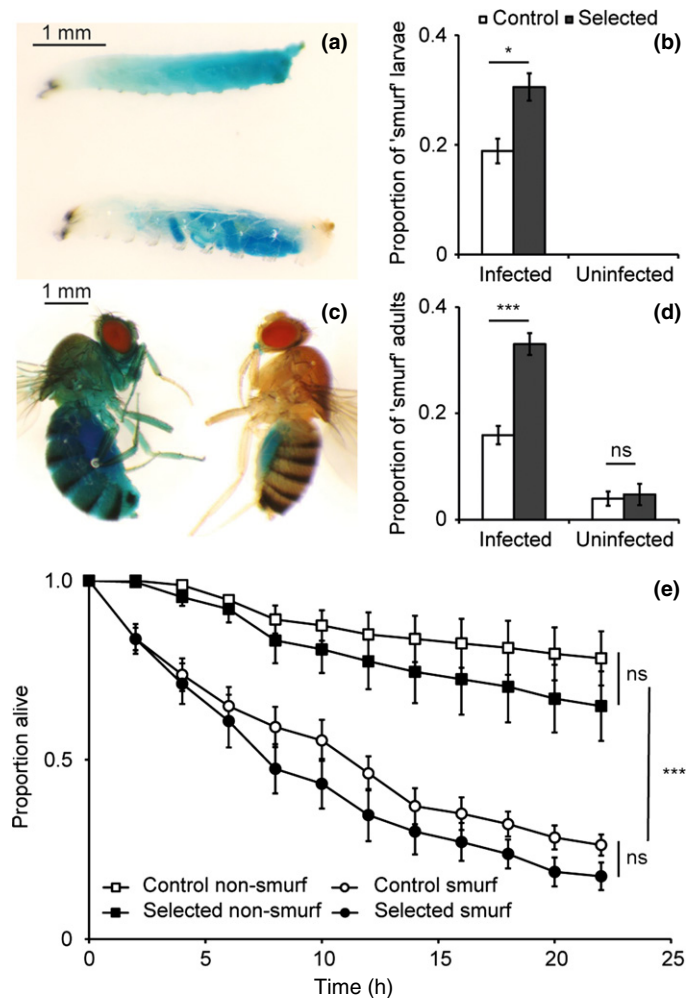


Figure 4 Infection mediated intestinal dysfunction in populations adapted to malnutrition. (a and c) The ‘smurf’ assay reveals loss of gut wall integrity as the presence of ingested dye throughout the body in the larvae (a, top larva) and adults (c, left fly); in individuals with intact intestinal wall the dye remains confined to the gut (a, bottom larva; c, right fly). (b and d) The proportion of smurf individuals found in control (white bars) and selected (dark bars) populations in larvae (b) and adults (d). (e) Survival of infected flies from control (open symbols) and selected (closed symbols) populations showing ‘smurf’ (squares) and ‘non-smurf’ (circles) phenotypes. Each data point indicates the mean of the six replicate populations \pm SE based on variation among populations within regimes; *** $P < 0.001$, ns: $P > 0.1$.

degree relies on adaptations of the gut. Our study suggests that such adaptations are at least partly mutually incompatible – *Drosophila* populations that evolved an improved ability to grow and survive on a poor-quality larval food suffer increased mortality from intestinal infections with *P. entomophila*. This is the case for both larvae and adult flies, and is observed in flies raised under both standard and malnutrition conditions. Our results thus support the notion of an evolutionary trade-off between adaptation of the digestive system to poor-quality food and its protective function against food-borne pathogens.

Our previous work has shown that several aspects of the adaptation of our selected populations to the poor larval

food regime involved genotype \times nutritional environment interactions – improved survival and growth are only observed on the poor food (Kolss *et al.* 2009). This is not the case for susceptibility to *P. entomophila*: the selected flies suffer a higher mortality upon infection than controls even if raised on the poor food, despite being substantially better adapted to this food. This indicates that genotype \times nutritional environment interactions for pathogen defence played at most a minor role, despite having been reported in some other host–pathogen systems (Lazzaro & Little 2009). The reduced tolerance of the selected populations to *P. entomophila* appears to be mediated by mechanisms relatively insensitive to the level of nutrition.

Mechanisms of defence against pathogens can be roughly divided into those that directly inhibit the proliferation and kill the pathogen (i.e. immune resistance), and those that alleviate the consequences of damage inflicted by the pathogen and the host's immune response (referred to as tolerance or resilience; Raberg *et al.* 2009; Little *et al.* 2010). Both play important complementary roles in *Drosophila* infected with intestinal pathogens (Lemaitre & Hoffmann 2007; Ferrandon 2013), and thus reduced efficacy of either or both might explain the greater susceptibility of our malnutrition-adapted populations to *P. entomophila* infection. The main mechanisms of immune resistance against intestinal infections with gram-negative bacteria are the expression of antimicrobial peptides (AMP) mediated by the Imd pathway (Liehl *et al.* 2006) and the production of ROS (Lemaitre & Girardin 2013). We found no evidence for reduced AMP production: neither the baseline level of expression of *Diptericin* (a standard readout for the Imd pathway) nor the degree of its upregulation upon infection differed between the selected and control populations. Furthermore, while the overall rate of protein synthesis in the intestine was, as expected (Chakrabarti *et al.* 2012; Lemaitre & Girardin 2013), reduced in infected flies, it was nearly identical between the selected and the control population assayed, suggesting that similar levels of *Diptericin* mRNA correspond to similar production of the peptide. Infected flies from the selected populations did produce less ROS in their guts than those from the control populations. However, even though a reduction in ROS production has been shown to increase susceptibility to several other pathogens (Ha *et al.* 2005, 2009; Chakrabarti *et al.* 2014), a *Drosophila* mutant for p38 mitogen-activated protein kinase (MAPK p38c), which produces less ROS, is actually more resistant to *P. entomophila* (Chakrabarti *et al.* 2014). Thus, the lower levels of ROS in the selected populations may not necessarily mean reduced immune defence against *P. entomophila*. Consistent with this, the flies from the selected populations did not have higher bacterial loads in the gut than flies from the control populations. All these lines of evidence imply that the greater mortality of selected populations upon infection with *P. entomophila* was not primarily caused by reduced ability to fight the pathogen.

Rather, their higher mortality upon infection seems to be mostly due to lower tolerance, i.e. to greater susceptibility to damage induced by the pathogen and/or the hosts own immune defence. In particular, we show that both larvae and

adult flies from the selected populations are much more prone to loss of intestinal integrity. Furthermore, this loss of intestinal integrity is a very good predictor of mortality within the subsequent 24 h; its incidence as revealed by the 'smurf' assay explains almost all of the difference in infection-induced mortality between the selected and control populations. This is consistent with other studies that implicate damage to intestinal epithelium and blockage of its repair as a major facet of *P. entomophila*-induced mortality in *Drosophila* (Chakrabarti *et al.* 2012).

We do not know the mechanism of the greater susceptibility to loss of gut integrity nor why it should be favoured by selection under malnutrition. It might be a consequence of reduced allocation of the limited resources to defence-related mechanisms, e.g. epithelium repair (Lemaitre & Girardin 2013). However, if this had been the case, we should have seen a difference between selected and control flies in translation activity after infection, and the difference in mortality between the selected and control populations should have been more pronounced on the poor food; neither was the case. Rather, these results indicate a design trade-off, whereby some aspects of the gut structure or physiology favoured by selection under malnutrition make it more vulnerable to pathogen damage. The fact that the larvae of the selected populations grow faster than control larvae on poor food but not on standard food, (Kolss *et al.* 2009; Vijendravarma & Kawecki 2013) suggests that they are able to acquire nutrients from the poor food at a higher rate. While it remains to be shown directly, it is likely that the gut characteristics which increase the vulnerability of the malnutrition-adapted populations to *P. entomophila* have evolved because they enhance nutrient acquisition from low-quality food. Intriguingly, an analogous design trade-off between nutrient acquisition and resistance to damage by antibiotics or detergents occurs in *E. coli* and is mediated by membrane permeability and expression of porin proteins (Phan & Ferenci 2013).

While susceptibility to specialised obligate parasites can be explained by their continuous adaptation to their host species (in relation to prevailing epidemiological conditions), opportunistic facultative pathogens are often thought not to be specifically selected to overcome host defences (Brown *et al.* 2012; Antonovics *et al.* 2013). This raises the question why genetic susceptibility to such opportunistic infections persists during evolution despite their negative consequences for Darwinian fitness. Our results suggest that genetic susceptibility to opportunistic enteric pathogens could be in part a cost of adaptation to deal with periods of malnutrition, in particular during the juvenile growth phase.

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AUTHORSHIP

R.K.V., B.L. and T.J.K. designed the experiments, analysed the results and wrote the manuscript; R.K.V., S.N., S.C., A.B. and S.K. performed the experiments.

REFERENCES

- Agrawal, A.A., Conner, J.K. & Rasmann, S. (2010). Tradeoffs and negative correlations in evolutionary ecology. In: *Evolution since Darwin: The First 150 Years*. (eds Bell, M.A., Futuyma, D.J., Eanes, W.F., Levinton, J.S.). Sinauer Sunderland, Massachusetts, pp. 243–268.
- Antonovics, J., Boots, M., Ebert, D., Koskella, B., Poss, M. & Sudd, B.M. (2013). The origin of specificity by means of natural selection: evolved and nonhost resistance in host-pathogen interactions. *Evolution*, 67, 1–9.
- Apidianakis, Y. & Rahme, L.G. (2011). *Drosophila melanogaster* as a model for human intestinal infection and pathology. *Dis. Model Mech.*, 4, 21–30.
- Bae, Y.S., Choi, M.K. & Lee, W.-J. (2010). Dual oxidase in mucosal immunity and host-microbe homeostasis. *Trends Immunol.*, 31, 278–287.
- Brown, S.P., Cornforth, D.M. & Mideo, N. (2012). Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. *Trends Microbiol.*, 20, 336–342.
- Buchon, N., Broderick, N.A., Chakrabarti, S. & Lemaître, B. (2009a). Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev.*, 23, 2333–2344.
- Buchon, N., Broderick, N.A., Poidevin, M., Pradervand, S. & Lemaître, B. (2009b). *Drosophila* intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe*, 5, 200–211.
- Chakrabarti, S., Liehl, P., Buchon, N. & Lemaître, B. (2012). Infection-induced host translational blockage inhibits immune responses and epithelial renewal in the *Drosophila* gut. *Cell Host Microbe*, 12, 60–70.
- Chakrabarti, S., Poidevin, M. & Lemaître, B. (2014). The *Drosophila* MAPK p38c regulates oxidative stress and lipid homeostasis in the intestine. *PLoS Genet.*, 10, e1004659.
- Edward, D.A. & Chapman, T. (2011). Mechanisms underlying reproductive trade-offs: costs of reproduction. In: *Mechanisms of Life History Evolution* (eds Flatt, T., Heyland, A.). Oxford University Press, Oxford, pp. 137–152.
- Fellowes, M.D.E., Kraaijeveld, A.R. & Godfray, H.C.J. (1998). Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Proc. R. Soc. B*, 265, 1553–1558.
- Ferrandon, D. (2013). The complementary facets of epithelial host defenses in the genetic model organism *Drosophila melanogaster*: from resistance to resilience. *Curr. Opin. Immunol.*, 25, 59–70.
- Gassmann, A.J., Carrière, Y. & Tabashnik, B.E. (2009). Fitness costs of insect resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol.*, 54, 147–163.
- Gill, N., Wlodarska, M. & Finlay, B.B. (2011). Roadblocks in the gut: barriers to enteric infection. *Cell. Microbiol.*, 13, 660–669.
- Ha, E.M., Oh, C.T., Bae, Y.S. & Lee, W.J. (2005). A direct role for dual oxidase in *Drosophila* gut immunity. *Science*, 310, 847–850.
- Ha, E.-M., Lee, K.-A., Seo, Y.Y., Kim, S.-H., Lim, J.-H., Oh, B.-H. *et al.* (2009). Coordination of multiple dual oxidase-regulatory pathways in responses to commensal and infectious microbes in *Drosophila* gut. *Nat. Immunol.*, 10, 949–957.
- Hawley, D.M. & Altizer, S.M. (2011). Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. *Funct. Ecol.*, 25, 48–60.
- Howick, V.M. & Lazzaro, B.P. (2014). Genotype and diet shape resistance and tolerance across distinct phases of bacterial infection. *BMC Evol. Biol.*, 14, 56.
- Kamath, P.L., Turner, W.C., Kusters, M. & Getz, W.M. (2014). Parasite-mediated selection drives an immunogenetic trade-off in plains zebras (*Equus quagga*). *Proc. R. Soc. B*, 281, 20140077.
- Kaun, K.R., Riedl, C.A.L., Chakaborthy-Chatterjee, M., Belay, A.T., Douglas, S.J., Gibbs, A.G. *et al.* (2007). Natural variation in food acquisition mediated via a *Drosophila* cGMP-dependent protein kinase. *J. Exp. Biol.*, 210, 3547–3558.
- Kolss, M., Vijendravarma, R.K., Schwaller, G. & Kawecki, T.J. (2009). Life-history consequences of adaptation to larva nutritional stress in *Drosophila*. *Evolution*, 63, 2389–2401.
- Lalubin, F., Deledevant, A., Glaizot, O. & Christe, P. (2014). Natural malaria infection reduces starvation resistance of nutritionally stressed mosquitoes. *J. Anim. Ecol.*, 83, 850–857.
- Lazzaro, B.P. & Little, T.J. (2009). Immunity in a variable world. *Philos. Trans. R. Soc. B-Biol. Sci.*, 364, 15–26.
- Lemaître, B. & Girardin, S.E. (2013). Translation inhibition and metabolic stress pathways in the host response to bacterial pathogens. *Nat. Rev. Microbiol.*, 11, 365–369.
- Lemaître, B. & Hoffmann, J. (2007). The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.*, 25, 697–743.
- Lemaître, B. & Miguel-Aliaga, I. (2013). The digestive tract of *Drosophila melanogaster*. *Annu. Rev. Genet.*, 47, 377–404.
- Liehl, P., Blight, M., Vodovar, N., Boccard, F. & Lemaître, B. (2006). Prevalence of local immune response against oral infection in a *Drosophila/Pseudomonas* infection model. *PLoS Pathog.*, 2, 551–561.
- Little, T.J., Shuker, D.M., Colegrave, N., Day, T. & Graham, A.L. (2010). The coevolution of virulence: tolerance in perspective. *PLoS Pathog.*, 6, e1001006.
- Martins, N.E., Faria, V.G., Teixeira, L., Magalhaes, S. & Sucena, E. (2013). Host adaptation is contingent upon the infection route taken by pathogens. *PLoS Pathog.*, 9, e1003601.
- McKean, K.A., Yourth, C.P., Lazzaro, B.P. & Clark, A.G. (2008). The evolutionary costs of immunological maintenance and deployment. *BMC Evol. Biol.*, 8, 76.
- Phan, K. & Ferenci, T. (2013). A design-constraint trade-off underpins the diversity in ecologically important traits in species *Escherichia coli*. *ISME J.*, 7, 2034–2043.
- Ponton, F., Wilson, K., Cotter, S.C., Raubenheimer, D. & Simpson, S.J. (2011). Nutritional immunology: a multi-dimensional approach. *PLoS Pathog.*, 7, e1002223.
- Raberg, L., Graham, A.L. & Read, A.F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Phil. Trans. R. Soc. B*, 364, 37–49.
- Rera, M., Clark, R.I. & Walker, D.W. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. *Proc. Natl Acad. Sci. USA*, 109, 21528–21533.
- Roff, D.A. & Fairbairn, D.J. (2007). The evolution of trade-offs: where are we? *J. Evol. Biol.*, 20, 433–447.
- Rolff, J. & Siva-Jothy, M.T. (2003). Invertebrate ecological immunology. *Science*, 301, 472–475.
- Roy, B.A. & Kirchner, J.W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, 54, 51–63.
- Schaible, U.E. & Kaufmann, S.H.E. (2007). Malnutrition and infection: complex mechanisms and global impacts. *PLoS Med.*, 4, 806–812.
- Schulenburg, H., Kurtz, J., Moret, Y. & Siva-Jothy, M.T. (2009). Ecological immunology. *Philos. Trans. R. Soc. B-Biol. Sci.*, 364, 3–14.
- Sheldon, B.C. & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.*, 11, 317–321.
- Shikano, I. & Cory, J.S. (2014). Dietary mechanism behind the costs associated with resistance to *Bacillus thuringiensis* in the cabbage looper, *Trichoplusia ni*. *PLoS ONE*, 9, e105864.
- Stearns, S.C. (1989). Trade-offs in life-history evolution. *Func. Ecol.*, 3, 259–268.
- Vale, P.F., Fenton, A. & Brown, S.P. (2014). Limiting damage during infection: lessons from infection tolerance for novel therapeutics. *PLoS Biol.*, 12, e1001769.

- Van Noordwijk, A.J. & de Jong, G. (1986). Acquisition and allocation of resources - their influence on variation in life-history tactics. *Am. Nat.*, 128, 137–142.
- Vijendravarma, R.K. & Kawecki, T.J. (2013). Epistasis and maternal effects in experimental adaptation to chronic nutritional stress in *Drosophila*. *J. Evol. Biol.*, 26, 2566–2580.
- Vijendravarma, R.K., Kraaijeveld, A.R. & Godfray, H.C.J. (2008). Experimental evolution shows *Drosophila melanogaster* resistance to a microsporidian pathogen has a fitness cost. *Evolution*, 63, 104–114.
- Vijendravarma, R.K., Narasimha, S. & Kawecki, T.J. (2010). Effects of parental larval diet on egg size and offspring traits in *Drosophila*. *Biol. Lett.*, 6, 238–241.
- Vijendravarma, R.K., Narasimha, S. & Kawecki, T.J. (2012). Adaptation to abundant low quality food improves the ability to compete for limited rich food in *Drosophila melanogaster*. *PLoS ONE*, 7, e30650.
- Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P. *et al.* (2005). *Drosophila* host defense after oral infection by an

entomopathogenic *Pseudomonas* species. *Proc. Natl Acad. Sci. USA*, 102, 11414–11419.

- Zhong, D.B., Pai, A. & Yan, G.Y. (2005). Costly resistance to parasitism: evidence from simultaneous quantitative trait loci mapping for resistance and fitness in *Tribolium castaneum*. *Genetics*, 169, 2127–2135.

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