RESEARCH NOTE

A possible tradeoff between developmental rate and pathogen resistance in *Drosophila melanogaster*

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Introduction

The possible involvement of immune function in tradeoffs with life-history related traits is increasingly being recognized as an important aspect of life-history evolution. However, even in model organisms for life-history evolution studies such as *Drosophila*, there is little empirical information on the genetic correlations between immune function and life-history traits. One hypothesis about immune function related tradeoffs is that they are mediated via the conflicting demands of resource allocation to immune defense and other life-history related traits, and there is now some empirical evidence for this from studies on *Drosophila* (McKean *et al*. 2008). In *Drosophila*, selection for faster development is known to lead to the correlated evolution of smaller body size, indicating reduced resources available to the flies. Thus, faster development can be expected to tradeoff with immune function. We tested this hypothesis by assaying the pathogen resistance of *D. melanogaster* populations subjected to selection for faster development, as well as their ancestral controls. The faster developing populations show significantly lower pathogen resistance than controls, indicating a negative genetic correlation between developmental rate and adult pathogen resistance, and showing that resistance to microbial pathogens can indeed decrease as a correlated response to selection on life-history traits in *Drosophila*.

In recent years, possible costs of investment in immune responses have been receiving attention in the context of life-history evolution, with immunocompetence now believed to tradeoff with major life-history-related traits (Sheldon and Verhulst 1996; McKean and Nunney 2001; Zuk and Stoehr 2002; McKean *et al*. 2008). Since pathogens and parasites are ubiquitous in nature, ability to resist them is likely to be an important component of fitness, along with other life-history-related traits. In *Drosophila*, many species of which breed on or around decaying fruits and domestic garbage dumps, exposure to microbial pathogens and hence, selection for pathogen resistance is expected to be high. Laboratory selection experiments over the past few decades have greatly enhanced our understanding of patterns of tradeoffs among life-history-related traits and life-history evolution in *Drosophila* (reviewed in Prasad and Joshi 2003). There is also evidence that reproductive activity reduces immunocompetence in *D. melanogaster* (McKean and Nunney 2001).

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The view that pathogen resistance may tradeoff with other traits important to fitness is strengthened by the observation that although directional selection plays an important role in immune system evolution (Schlenke and Begun 2003), organisms generally do not exhibit maximal immune responses (reviewed in Zuk and Stoehr 2002). The evolutionary cost of maintaining and mounting immune responses is thought to be due to conflicting demands on resource allocation to immune function versus other life-history traits, leading to suggestions that immune function may tradeoff with traits like competitive ability, developmental time and fecundity (Roff 1992; McKean *et al*. 2008). Empirical evidence for such tradeoffs between immune function and life-history traits is, as yet, meagre. *D. melanogaster* larvae selected for increased resistance against parasitoid wasps, have been shown to be less competitive under crowding (Kraaijeveld and Godfray 1997). Immunocompetence has also been shown to tradeoff with reproductive investment in yellow-dung flies, *Scathopaga sterocoraria* (Hosken 2001), and selection for higher resistance in the Indian meal moth, *Plodia interpunctella*, resulted in correlated evolution of longer development time (Boots and Begon 1993). There is, at this time, only one study reporting genetic correlations between pathogen resistance and major life-history traits in *Drosophila* (McKean *et al*. 2008), and that study utilized a neo-classical quantitative genetics approach. We infer genetic correlations through correlated responses to selection in the context of an experimental evolution study.

Selection for faster development in *Drosophila* typically results in the evolution of smaller adult body size (Chippindale *et al*. 1997; Prasad *et al*. 2000). Reduced energy reserves due to faster pre-adult development are likely to provide the basis for a tradeoff between developmental rate and adult traits requiring allocation of reserves accumulated during the larval stage (Prasad and Joshi 2003). It has recently been shown that recording the time to death of *Drosophila* reared in the presence and absence of *E. coli* constitutes a reliable mass-scale assay of pathogen resistance: rearing in the presence of live *E. coli*, but not streptomycin attenuated or heat-killed *E. coli* reduces the time to death of adult flies under starving conditions (Sharmila Bharathi *et al*. 2004, 2007). In this study, we examine the genetic correlation between developmental rate and pathogen resistance in *D. melanogaster*, by assaying pathogen resistance in four populations subjected to selection for faster development and early reproduction, and four populations that are ancestral controls to the selected populations.

Materials and methods

We assayed time to death in the presence and absence of growing *E. coli* culture on virgin male and female flies from eight populations of *D. melanogaster*. Four of them had been subjected to selection for faster egg-to-adult development and early reproduction ($FEJ_{1–4}$) for 244 generations at the time of this study. The remaining four populations ($JB_{1–4}$), described in detail by Sheeba *et al*. (1998), were ancestral controls, maintained on a three week discrete generation cycle without any conscious selection for reduced pre-adult development time or early reproduction. In the FEJ populations, only the first 25% of the eclosing flies from each vial were transferred to the breeding-population cages, and eggs laid by these flies were collected after about 60 h to initiate the next generation (for details, see Prasad *et al*. 2000). Each FEJ population was derived from one JB population; thus JB_i and FEJ_i are more closely related than JB_i and JB_j or FEJ_i and FEJ_j; i, j = 1–4. Consequently, control and selected populations with identical subscripts were treated as blocks in the statistical analysis.

To avoid any non-genetic parental effects, flies from the selected and control populations were reared under common maintenance conditions, in which all flies were allowed to eclose, for one complete generation prior to assay. Eggs collected from these flies were distributed into vials (9 × 2.4 cm) at a density of 60–80 eggs per vial, and freshly eclosed (within 6 h post eclosion) virgin adults from these vials were used to set up the pathogen resistance assay, following the methods of Sharmila Bharathi *et al*. (2004). For each combination of block × selection regime × sex, eight vials were set up with 3 mL of Luria Bertani (LB) agar medium containing ampicillin on which *E. coli* strain DH5a carrying an amp^R gene was streaked. These vials were then incubated at 37°C for 24 h to allow bacterial growth. At this point, a lawn of bacteria was visible on the surface of the medium in the vials. Eight control vials containing LB agar with ampicillin, but not inoculated with *E. coli*, were also set up in a similar manner for each block × selection regime × sex combination. In each treatment or control vial, either five males or five females were placed, and the vials were then monitored every 2 h, and the death of any fly during the previous 2 h period recorded. This process was continued until all the flies had died. Thus, time to death was recorded for a total of 1280 flies (four blocks × two selection regimes × two sexes × two assay conditions × eight vials, with five flies each). To compare pathogen resistance across selection regimes and sexes, we transformed the primary data on time to death of individuals in the vials with *E. coli* by dividing it by the mean time to death averaged across the control vials for that particular block × selection regime × sex combination, as in Sharmila Bharathi *et al*. (2004). The transformed data on individual flies were subjected to analysis of variance (ANOVA), treating vials as a random factor nested within the three-way interaction between the fixed factors selection regime and sex, and the random factor, block.

Results and discussion

The faster developing flies (FEJ) had a lower mean time to death (14 h) compared to the control flies (JB) (48 h) in the presence of *E. coli*, as well as in the absence of the

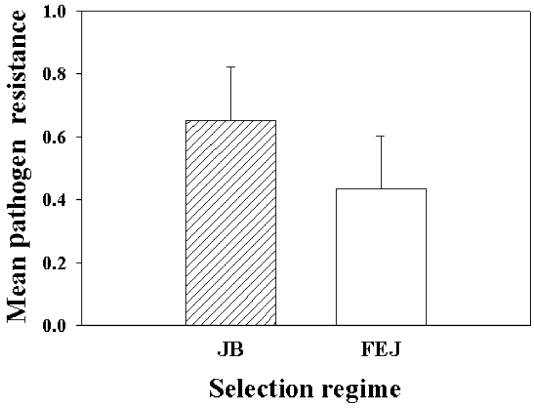

Figure 1. Mean (± 95% c.i.) pathogen resistance measured as time to death in presence of *E. coli* expressed as a fraction of the time to death in absence of *E. coli*. Confidence intervals were calculated using the appropriate MS term from the ANOVA and hence can be used for visual hypothesis testing.

pathogen (FEJ mean = 33 h; JB mean = 74 h). Scaling by time to death in the absence of the pathogen, revealed that in the presence of *E. coli* the JB flies lived 65% and FEJ flies only 43% as long as their respective counterparts in the control vials (figure 1). The ANOVA on this measure of pathogen resistance revealed a significant main effect of selection regime, but no significant effect of sex or the selection × sex interaction (table 1). Pathogen resistance of JB males and females was 66% and 65% respectively, whereas that of the FEJ males and females were 45% and 41%, respectively.

It is clear from the results that the detrimental effect of *E. coli* on adult flies was significantly more severe in FEJs compared to JBs, reflecting a lower pathogen resistance in the faster developing populations. This result indicates a negative genetic correlation between developmental rate and pathogen resistance, complementing observations that selection for increased pathogen resistance leads to longer development time (Boots and Begon 1993). The physiological underpinnings of this tradeoff between developmental rate and pathogen resistance; however, remain obscure.

Table 1. Results of ANOVA on pathogen resistance.

Effect	df	MS	*F*	*P*
Selection regime	1	7.681621	16.86	0.026
Block	3	0.3467	5.95	0.001
Sex	1	0.105935	0.66	0.478
Vial	112	0.058283	1.21	0.088
Selection regime × Block	3	0.455663	7.82	0.001
Selection × Sex	1	0.032552	1.70	0.283
Block × Sex	3	0.161723	2.77	0.045
Selection Regime × Block × Sex	3	0.01912	0.33	0.805
Error	512	0.048152		

Given the substantially smaller size and lipid content of the FEJs (Prasad 2004), the tradeoff is probably mediated through reduced resource availability for investment in immune function. It is also possible; however, that the FEJs, due to their much shorter duration of development (78% that of the JBs at the time of the assay), may have poor immunocompetence due to developmental anomalies. An alternative possibility that cannot be ruled out at this juncture is that the FEJs actually mount an equal or greater immune response than JBs to *E. coli*, and then succumb faster to the starving conditions of the assay vials as they have less lipid reserves to start with. The observation in *D. melanogaster* of a tradeoff between fecundity and immune competence in poor nutrition, but not high nutrition, environments (McKean *et al.* 2008) suggests that this explanation is plausible. This possible explanation could be tested by directly assaying components of the immune response in adult JBs and FEJs. However, the present results clearly show that FEJs ultimately have reduced fitness in the presence of *E. coli*, compared to the JBs, at least under the starving conditions of this assay. Functionally, therefore, there is a possible tradeoff between developmental rate and pathogen resistance in these populations, although whether it is specifically underpinned by a tradeoff between immune competence and developmental rate is not as yet clear.

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