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ORIGINAL ARTICLE

Environment-dependent trade-offs between ectoparasite resistance and larval competitive ability in the *Drosophila–Macrocheles* system

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Costs of resistance are expected to contribute to the maintenance of genetic variation for resistance in natural host populations. In the present study, we experimentally test for genetic trade-offs between parasite resistance and larval competitive ability expressed under varying levels of crowding and temperature. Artificial selection for increased behavioral resistance was applied against an ectoparasitic mite (*Macrocheles subbadius*) in replicate lines of the fruit fly *Drosophila nigrospiracula*. We then measured correlated responses to selection in larval competitive ability by contrasting replicate selected and control (unselected) lines in the absence of parasitism. Experiments were conducted under variable environmental conditions: two temperatures and three levels of larval density. Our results reveal a negative genetic correlation between resistance and larval-adult survival under conditions of moderate and severe intra-specific competition. At both low and high temperature, percent emergence was significantly higher among control lines than selected lines. This divergence in larval competitive ability was magnified under high levels of competition, but only at low temperature. Hence, the interaction between selection treatment and larval density was modified by temperature. As predicted, larvae experiencing medium and high levels of competition exhibited an overall reduction in female body size compared to larvae at low levels of competition. Female flies emerging from selected lines were significantly smaller than those females from control lines, but this effect was only significant under conditions of moderate to severe competition. These results provide evidence of environment-dependent trade-offs between ectoparasite resistance and larval competitive ability, a potential mechanism maintaining genetic polymorphism for resistance.
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Introduction

Parasites are ubiquitous in nature and can cause significant damage to host fitness (Ewald, 1980, 1983; Price, 1980). Natural selection is expected to drive resistance-conferring genes to fixation, reducing additive genetic variation for resistance (Fisher, 1930; Falconer and Mackay, 1996). Yet, genetic polymorphisms for resistance exist in most natural host populations (Parker, 1991; Henter and Via, 1995; Kraaijeveld *et al*., 1998). The cost of resistance hypothesis provides a potential mechanism for the maintenance of this variation (Sheldon and Verhulst, 1996; Gemmill and Read, 1998; Rigby *et al*., 2002; Brown, 2003; Sandland and Minchella, 2003). This hypothesis is based on a fundamental assumption underlying life-history theory, that the evolution of fitness traits is constrained by universal trade-offs among them (Reznick *et al*., 2000; Roff, 2002; Sgrò and Hoffmann, 2004). Similarly, if resistance-conferring genes damage the expression of other fitness traits, the evolution of resistance in a population may be constrained, thus contributing to the maintenance of genetic polymorphism for parasite resistance (Simms and Rausher, 1987; Simms, 1992; Mitchell-Olds and Bradley, 1996; Gemmill and Read, 1998; Brown, 2003).

Costs of resistance may be conceptualized as falling into two general categories: the costs associated with actual defense, which require energy and resources to deploy an immune response, and genetic costs associated with the evolution and maintenance of resistance (see review by Rolff and Siva-Jothy, 2003). Physiological costs of immune system deployment have been demonstrated in a growing number of studies involving vertebrate (Lochmiller and Deerenberg, 2000; Bonneaud *et al*., 2003; Hanssen *et al*., 2004) and invertebrate hosts (Ferdig *et al*., 1993; Fellowes *et al*., 1999b; Moret and Schmid-Hempel, 2000; Hoang, 2001; Ahmed *et al*., 2002; Kraaijeveld *et al*., 2002; Armitage *et al*., 2003; Jacot *et al*., 2004; Fedorka and Mousseau, 2007). Many of these studies have assayed for costs following the activation of the immune system with either a metabolically active or inert parasite. A powerful method of detecting *evolutionary costs* of resistance, particularly those resulting from antagonistic pleiotropy, involves measuring correlated responses to selection for resistance (Rose, 1984; Partridge and Fowler, 1992; Reznick *et al*., 2000). Negative genetic correlations between selection for parasite resistance and other host fitness traits have

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previously been demonstrated in plants (Bergelson and Purrington, 1996; Mitchell-Olds and Bradley, 1996; Hare *et al*., 2003; Tian *et al*., 2003) and animals (Boots and Begon, 1993; Yan *et al*., 1997; Webster and Woolhouse, 1999; Hurd *et al*., 2005). For example, *Drosophila melanogaster* selected for parasitoid resistance pay a fitness cost in terms of reduced larval survivorship, but only under conditions of moderate to severe competition (Kraaijeveld and Godfray, 1997; Fellowes *et al*., 1998).

Most life-history trade-offs are measured under only one set of environmental conditions (see review by Sgrò and Hoffmann, 2004). Yet, when genotype-environment interactions are taken into consideration, context-dependent genetic correlations are often detected among life-history traits (Service and Rose, 1985; de Jong, 1990; Stearns *et al*., 1991). Varying environmental conditions, such as temperature and resource availability, have been shown to generate a change in the direction and/or magnitude of correlations between life-history traits not involving resistance (Service and Rose, 1985; Gebhardt and Stearns, 1988; Reznick *et al*., 2000; Rigby *et al*., 2002; Messina and Fry, 2003; Sgrò and Hoffmann, 2004). Environmental stressors in also modify the expression and magnitude of costs of resistance among plant-pathogen associations (see Bazzaz *et al*., 1987; Bergelson and Purrington, 1996; Sandland and Minchella, 2003). Similar environment-dependent expression of costs have been detected in animal-parasite systems as well (Kraaijeveld and Godfray, 1997; Fellowes *et al*., 1998; Lochmiller and Deerenberg, 2000; Moret and Schmid-Hempel, 2000; Hoang, 2001). For example, Rigby and Jokela (2000) showed that the threat of predation modified the costs of immune defense. Hence, investigating trade-offs under variable, ecologically relevant, environmental conditions is crucial for predicting the importance of costs of resistance in natural host populations.

In addition to environmental variation, different stages of host ontogeny can influence the expression of costs (Kraaijeveld *et al*., 2002; Sandland and Minchella, 2003). Previously, we demonstrated that resistance-selected lines experience a significant reduction in female fecundity, and that this effect is temperature-dependent (Luong and Polak, 2007). In the present study, we test for genetic trade-offs between ectoparasite resistance and larval competitive ability in lines of *Drosophila nigrospiracula* selected for resistance against an ectoparasitic mite. In this system, resistance is mediated by behavioral forms of defense, in which flies avoid approaching mites with sudden reflex movements, tarsal flicking and bursts of flight (Polak, 2003). Parasitized females suffer reduced longevity and fecundity (Polak, 1996), and infested males exhibit decreased body condition and copulatory success (Polak *et al*., in press; Polak and Markow, 1995). Given the considerable fitness consequences of parasitism, and hence directional selection for increasing resistance, fly populations, all else being equal, are expected to evolve highest values of resistance against ectoparasitism. However, significant genetic heterogeneity for resistance persists in natural populations (Polak, 2003; Luong and Polak, 2007).

In the present study, we test for trade-offs between experimentally evolved resistance and other host fitness traits in the absence of parasitism. Using three replicate resistant lines coupled with their respective control, unselected lines, we measured larval competitive ability against a genetic marker strain originally extracted from nature. We predicted that resistance-selected lines would experience compromised larval competitive ability relative to control lines, as well as to the base population from which all the lines were originally derived. Few studies assay costs using replicated selection lines (but see Boots and Begon, 1993; Kraaijeveld and Godfray, 1997; Yan *et al*., 1997; Fellowes *et al*., 1998), and even fewer still provide a baseline comparison between the control lines and the base population.

We also tested the hypothesis that the expression of costs is context-dependent by performing the experiments under two sources of environmental variation. Larval competitive ability was assayed at 25° and 29°C, with the latter temperature serving as a form of thermal stress for developing larvae (Gibbs *et al*., 2003). Within each temperature regime, competition experiments were performed at three larval densities. Mangan (1982) demonstrated a negative effect of increasing density on *D. nigrospiracula* mortality, size and larva–pupa development time. We made the specific prediction that the expression of costs will be magnified under conditions of heightened temperature stress and crowding.

Materials and methods

Study system

The facultative, ectoparasitic mite *Macrocheles subbadius* Berlese (Acari: Macrochelidae) occurs naturally with its host *D. nigrospiracula* Patterson and Wheeler (Diptera: Drosophilidae) in the necrotic cacti (*Carnegiea gigantea*) of the Sonoran Desert. Mites attach to the abdomens of adult flies, on which they rely for dispersal and nutrient consumption (Polak, 1996). The prevalence and intensity of parasitism vary in both space and time, and depend on the extent of the cactus necrosis. Parasitized females experience attenuated longevity and fecundity, and infested males suffer reduced mating success. Further, the extent of the host pathology depends on the intensity of infestation and duration of infestation (Polak and Markow, 1995; Polak, 1996, 1998).

Behavioral forms of defense mediate parasite resistance in this system; flies actively avoid mite attack by engaging in sudden reflex movements and bursts of flight from the substrate when a mite makes contact. Moreover, heritable genetic variation for this behavioral form of resistance has been documented in natural populations. Heritability for resistance was previously estimated to be 0.12–0.15 (Polak, 2003; Luong and Polak, 2007), demonstrating that additive genetic variation for ectoparasite resistance exists in natural populations.

Base population and mite culture

Adult flies ($n > 250$ per sex) were collected in the field at necrotic saguaro cacti (*C. gigantea*) and used to established laboratory cultures. Flies were first cleared of mites and then mass-cultured at standard laboratory light and temperature conditions (12 h light, 25°C/12 h dark, 23°C). The flies from which mites were removed were combined with unparasitized hosts to found the base population, and cultured (Polak, 1996). The base population was mass-cultured for four generations before commencing artificial selection, and thereafter

mass-cultured in 8–10 bottles for incorporation into the cost assays; the minimum number of flies that seeded any new generation of the base population was 400.

Mites were harvested from infested flies collected in the field, and reared on artificial media (Polak, 1996). In order to avoid selection on the parasite, the mites were discarded following use.

Artificial selection and resistance assays

The artificial selection protocol followed that of Polak (2003). Briefly, three resistance-selected lines and three unselected control lines were derived from the base population (described above). For each of the selected lines, 250 adult males were exposed to mites in four infestation chambers. The chambers consisted of mite media and a space excavated within the medium to mimic the internal pockets of a necrotic cactus, where flies and mites naturally interact. After 48 h of exposure, live flies were recovered from the chambers with an aspirator and, under a stereomicroscope, checked for the presence parasitism. For each selected line, males carrying neither mites nor mite-induced scars were used to sire the next generation, along with exactly 75 virgin females. These females were never exposed to mites, and were taken from the same generation and line as the males subject to selection. Control lines were maintained in parallel and with an equal number of males and females as its corresponding selected line. The minimum number of flies used to seed a subsequent generation of any line was 100. Selection was imposed for 14 generations, after which time the lines were mass-cultured without selection for at least one generation before the cost assays.

Just before commencing the cost assays, we performed resistance assays on both males and female flies to verify significant divergences in resistance between selected and control lines. For each line, 20 flies from each treatment category were simultaneously exposed to mites in an infestation chamber; two chambers were utilized per sex. We distinguished treatment categories (that is, selected vs control) by applying a small clip to the tip of either the left or right wing, alternating sides between treatment categories and across assay chambers. Wing clips were made 24 h before commencing the resistance assay. Once the flies were loaded into the chambers, they were left for 24 h, after which time live flies were removed and checked for the presence of mites and scars, as above. The wing clip status was noted but not decoded until after all the flies were recovered and assessed for infestation; thus, prevalence was scored blind with respect to the categories to which flies belonged. The death of flies was attributed to parasitism; negligible mortality occurs in chambers without mites (Polak, 2003).

The probability of infestation was analyzed with logistic regression, in which the categorical response variable was whether or not a fly became infested; the former event was specifically modeled. Predictor variables were line (1, 2, 3), selection treatment (selected, control), sex and infestation chamber (4 per line). The term 'estimate' refers to the estimated coefficient, or slope, for a particular predictor in the model. The prevalence of infestation for each treatment category was also calculated as the proportion of selected or control flies parasitized per chamber, respectively. These data were analyzed in a mixed-model analysis of variance (ANOVA): selection treatment and sex were fixed factors, whereas line and chamber (nested within line) were treated as random factors. Data were arcsine(sqrt)-transformed, transformed to achieve normality and homogeneity of variance; back-transformed means $[(\sin x)^2]$ are reported for ease of interpretation; standard errors were calculated from untransformed data.

Larval competitive ability

Before commencing the cost assays, each line was mass-cultured in the absence of selection, in four bottles containing 25 flies per sex. Flies from resistance-selected and unselected-control lines, as well as from the base population were simultaneously assayed for larval competitive ability. By including the latter category, we were able to test for any difference between the base and control lines. This baseline check provides a means of evaluating whether differential inbreeding depression may be playing a role in the expression of any fitness costs, or whether larval competitive ability evolved in the control lines during the course of the experiment, for example, by random genetic drift; either of these factors can confound interpretation of any observed differences between selected and control lines (Luong and Polak, 2007).

We tested for a correlated response in larval competitive ability resulting from direct selection for increased ectoparasite resistance by contrasting the larval-adult survivorship of the experimental (selected, control, base) groups at three different levels of competition. Eighty males and 80 females (8–9 days post-eclosion) were placed into half-pint milk bottles containing standard medium (see above). Adult flies were removed the following day, so the maximum difference in larval age never exceeded 24 h. Bottles were maintained at standard rearing conditions (see above), and from which early third-instar larvae (*D. nigrospiracula* have four larval instars) were harvested. The experiment was conducted at two temperature regimes and three larval density levels, representing low, medium and high levels of competition. Experimental lines were competed against genetically marked 'tester' flies (Santos *et al*., 1992), a *vermillion-eye* adult phenotype (caused by an autosomal recessive allele) isolated from natural populations. For the low-density treatment, 20 larvae from the experimental line were placed in food vials with 20 larvae from the tester stock. The latter serves as a source of background competition against the experimental lines. Medium and high-density vials were seeded with 40:40 and 80:80 experimental and tester larvae, respectively. Comparable density levels have been shown to generate negative effects on survival, body size and developmental time in *D. nigrospiracula* (Fellows and Heed, 1972; Mangan, 1982). Each treatment × temperature × density combination was replicated across two vials. All vials had the same amount of food medium, consisting of 0.9 g mashed potatoes, 0.25 g *Drosophila* instant, 5 ml of water, 10–15 mg active dry yeast and 1–2 drops of autoclaved cactus juice. Experimental vials were randomly assigned to either a low or high-temperature regime, 25°C light/23°C dark and 29°C light/27°C dark, respectively. When

fourth stage larval instars began migrating up the vials in search of pupation sites, the cotton stoppers were temporarily replaced with perforated aluminum foil to preclude larvae from burrowing into the cotton and dying; once pupation was complete, we resumed using cotton stoppers. This experiment was replicated over time in three blocks. We recorded the number of experimental and tester adult flies that emerged daily until all flies emerged, and measured female thorax length as an estimate of body size (block 2 only).

Survival data (proportion of selected, control or base flies emerging from the total number of experimental larvae seeded) were analyzed with a mixed-model ANOVA; block, selection treatment, temperature and density were entered as fixed factors, and line and vial (nested within line) as random factors. A low-density treatment was not performed in the first block, so it was treated as missing data in the analysis. The data were arcsine(sqrt)-transformed to satisfy the assumptions of ANOVA; means were back-transformed to the original scale for interpretability. The three-way interaction between selection, temperature and density (see Results) was further examined for each temperature and density level; whereby the data were sorted by temperature *and* density, and the simple effects of selection at each temperature by density treatment combination were analyzed with separate ANOVAs (Winer, 1971; Sokal and Rohlf, 1995).

Data on female size were analyzed in a mixed-model ANOVA similar to survivorship, but did not require transformation. In a separate analysis, differences (selected vs control) in body size at each of the three levels of competition were examined. The data were first sorted by larval density, then the simple effect of selection on thorax length was computed with an *lsmean* statement by specifying a *slice = density* option; generating separate ANOVAs at each density level. All statistical analyses were performed in SAS (2002).

Results

Response to selection

Logistic regression analysis showed a significantly lower probability of infestation among the selected lines after 14 generations of selection (estimate: −0.89, 0.25 s.e., $\chi^2 = 12.8$, $P = 0.0003$). The interaction terms for selection treatment × sex ($\chi^2 = 0.17$, $P = 0.68$), and selection treatment × line ($\chi^2 = 1.09$, $P = 0.58$) were non-significant. The results of ANOVA were similar: the mean prevalence of infestation was significantly lower among selected lines compared to control lines (Figure 1). This difference in ectoparasite susceptibility was significant ($F_{1,13} = 32.7$, $P < 0.0001$), but the line ($F_{2,13} = 1.08$, $P = 0.37$) and treatment by line interaction were not ($F_{2,13} = 0.61$, $P = 0.56$). The prevalence of infestation was significantly lower among selected lines (mean ± s.e. = 26.3 ± 3.18%) compared to the control lines (52.0 ± 3.18%). Differences in prevalence were comparable across all three replicate lines (control–selected: line 1 = 23.3%, line 2 = 33.2%, line 3 = 38.3%).

Competitive ability

We tested for correlated response to selection in larval competitive ability by contrasting selected, control and base flies under three different levels of competition against a tester strain (Figure 2). The effects of selection, temperature and line were significant (Table 1). In general, larval-adult survivorship was higher at 25°C (mean ± s.e. = 76.3 ± 1.36%) compared to 29°C (51.1 ± 1.36%), and a greater proportion of flies emerged from the control lines (72.5 ± 2.56%) than from selected lines (55.4 ± 2.56%, *post hoc* Tukey's test, $P < 0.0001$); the former was comparable to the base population (Tukey's test, $P = 0.66$).

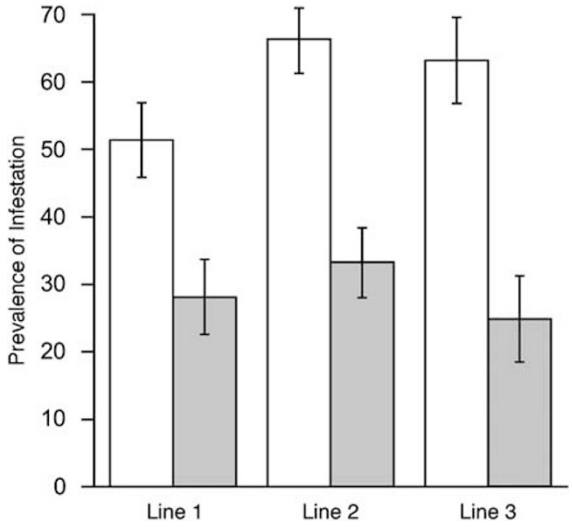

Figure 1 Results from the resistance assays after 14 generations of selection. For each of the three replicate lines, the mean prevalence of infestation was higher among unselected-control lines (white bars) compared to resistance-selected lines (gray bars). Error bars represent ± standard error.

Although the overall effect of the density treatment was not significant, a significant three-way interaction was detected between selection, density and temperature (Table 1). An analysis of the simple effect of selection by temperature and density (Table 2) showed that under low-larval density, there was no significant difference in survival between the selected and control lines, at either temperature. Differences in competitive ability were detected at levels of moderate and severe competition. At 25°C and medium levels of larval density, selected lines exhibited a significant reduction in survivorship compared to that of control lines; and at high-larval density, the difference in emergence rates between selected and control lines were magnified (Figure 2a). At 29°C, control lines performed better than selected lines; this degree of divergence was significant at moderate and high levels of competition (Figure 2b). At low-larval density (29°C), there was an overall drop in percent emergence, relative to the low-temperature regime. This is most likely a consequence of temperature-mediated mortality; that is, when larval densities are too low (for example, <20) and there is insufficient feeding activity to turn up the media, the mortality rate becomes exasperated. Overall, these results suggest that the interaction between selection and larval density is modified by temperature. As expected, the percent emergence was not significantly different between any of the three control lines and the base stock (*Post hoc* Dunnett's test, α-level = 0.05).

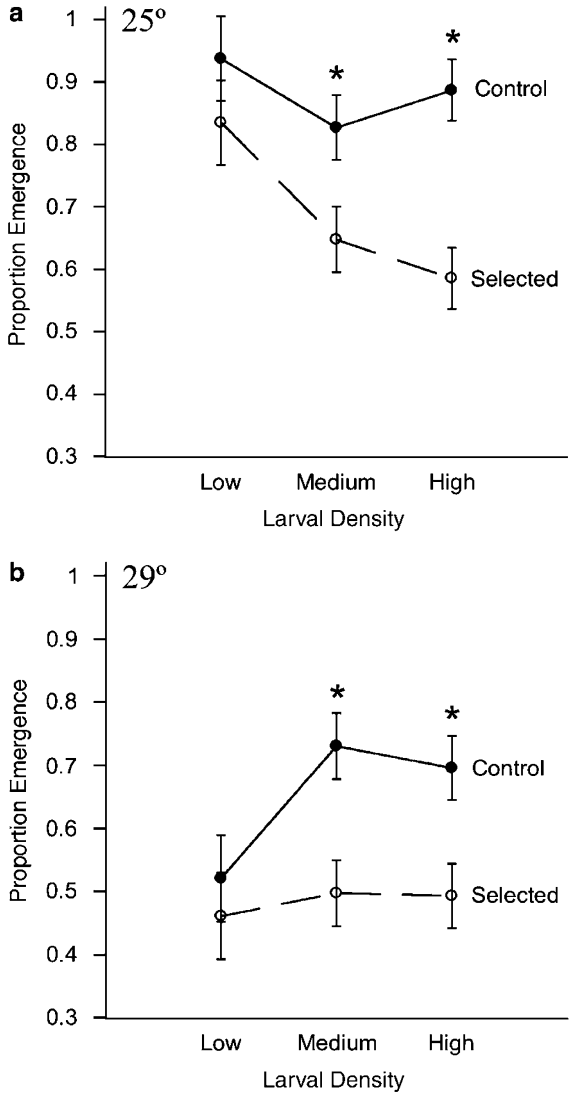

Figure 2 Larval-adult survivorship results from the larval competition experiments conducted at (**a**) 25°C and (**b**) 29°C. At both temperatures, low-larval densities were insufficient to generate a difference in larval competitive ability. At moderate and severe levels of competition, there was a significant divergence in survival between the control and selected lines. Error bars represent ± standard error. Asterisks indicate significant differences between control and selected lines.

Table 1 Results of ANOVA on larva–adult survival

Source	*d.f.*	*MS*	*F*	P
Block	2	1.940	29.1	<0.0001
Line	2	0.501	2.10[a]	0.269
Vial (line)	3	0.239	3.58	0.014
Selection[b]	2	0.843	12.6	<0.0001
Temperature	1	5.117	76.7	<0.0001
Density	2	0.061	0.91	0.404
Selection × density × temperature	6	0.278	4.17	0.0005
Error	259	0.067		

Abbreviations: ANOVA, analysis of variance; d.f., degrees of freedom; MS, mean square.
All two-way interaction terms were non-significant.
[a]Computed using the vial(line) error: d.f. = 3, $MS = 0.239$.
[b]Selection treatments: selected, control and base.

Table 2 A summary of the ANOVA conducted on each of the six temperature by density treatment combinations, testing the simple effects of the selection treatment on larval-adult survivorship

Larval density	*25°C*			*29°C*		
	Statistic[a]	P-*value*	*Tukey*[b]	*Statistic*	P-*value*	*Tukey*[b]
Low	$F_{2,25} = 1.51$	0.241	0.212	$F_{2,25} = 1.01$	0.377	0.811
Medium	$F_{2,40} = 4.87$	0.013	0.021	$F_{2,40} = 6.43$	0.004	0.045
High	$F_{2,40} = 8.27$	0.001	0.002	$F_{2,40} = 5.18$	0.010	0.017

Abbreviation: ANOVA, analysis of variance.
[a]Selection treatments of ANOVA: selected, control and base; *P*-value corresponds to *F*-test.
[b]*P*-values of *post hoc* Tukey comparison between selected and control lines.

Table 3 The simple effect of selection[a] on female body size, computed at each density level

Larval density	*d.f.*	*MS*	*F*	P
Low	1	3.22×10^{-4}	0.64	0.427
Medium	1	2.89×10^{-3}	5.75	0.020
High	1	3.35×10^{-3}	6.67	0.012
Error	58	4.94×10^{-4}		

Abbreviations: d.f., degrees of freedom; MS, mean square.
[a]Selection treatments: selected and control only.

An analysis on female body size revealed significant effects of temperature ($F_{1,87} = 144.3$, $P < 0.0001$) and selection × line ($F_{4,53} = 3.22$, $P = 0.02$), but none of the other interaction terms were significant. The thorax length (mm) for females that enclosed at 25°C (mean ± s.e. = 1.414 ± 0.003) was generally greater than that of females emerging at 29°C (1.361 ± 0.003). The size of flies emerging under conditions of low competition (1.412 ± 0.004) was greater than that of flies emerging at moderate (1.384 ± 0.004) or high (1.367 ± 0.004) levels of competition ($F_{2,87} = 36.3$, $P < 0.0001$). Moreover, selection for resistance had a negative effect on average female body size ($F_{2,87} = 6.59$, $P = 0.002$): resistance-selected flies (1.376 ± 0.004) were on average smaller than control flies (1.394 ± 0.004; Tukey's test, $P = 0.004$). Females from each of the control lines were comparable in size to the females assayed from the base population (Dunnett's test, α-level = 0.05). A separate analysis of the *simple effect* (Table 3) of selection by density revealed that selected lines maintained under conditions of low-larval crowding did not differ in body size from the control lines; but at moderate levels of competition selected flies were significantly smaller compared to control flies. This difference was more apparent under conditions of severe competition (Figure 3). The combined effect of selection and competition on body size is consistent with the results on survivorship.

Discussion

The results show a negative genetic correlation between ectoparasite resistance and larval competitive ability. At both temperatures, low levels of competition were insufficient to generate significant differences in survival between control and selected lines. At moderate levels of

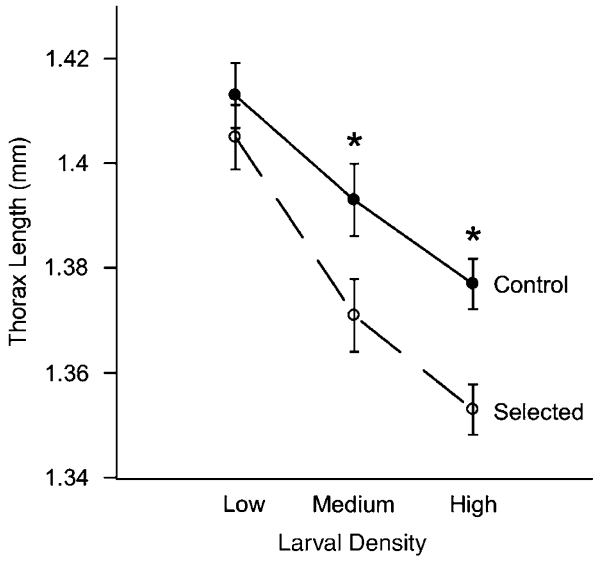

Figure 3 The effect of selection and intra-specific competition on female body size (pooled across temperatures). Flies emerging from control lines (closed circles) were significantly larger than flies emerging from selected lines (open circles). Differences in thorax length were only detected under condition of moderate and severe competition, which is consistent with the survivorship results. Error bars represent ± standard error. Asterisks indicate significant differences between control and selected lines.

competition, selected lines suffered a significant reduction in larval-adult survivorship. The difference in survival between control and selected lines was magnified nearly twofold under conditions of severe competition, but only at low temperature.

Differences in larval competitive ability were also manifested through female body size, such that flies emerging from the selected lines were significantly smaller than flies from the control lines. These findings are consistent with the survivorship results above, in that significant differences in body size were detected only under conditions of moderate and severe competition. Decreased body size can have potentially deleterious effects on host lifespan, dispersal capabilities, male mating success, female fecundity and desiccation tolerance (Roff, 1977; Mangan, 1982; Heed and Mangan, 1986; Polak, 1998; Gibbs *et al*., 2003). Indeed, larval competition for food has been shown to have significant fitness consequences in natural *Drosophila* populations (Atkinson, 1979; Mangan, 1982; Santos *et al*., 1999). The correlated response to selection for resistance on survivorship and body size is evidence of a genetic trade-off between ectoparasite resistance and larval competitive ability.

Our results also support the hypothesis that the expression and magnitude of trade-offs are context-dependent, and that costs are most likely to be detected under stressful conditions. The interaction between selection and larval density was modified by temperature, as reflected by the significant three-way interaction between these three factors. In the field, temperatures outside and inside cactus rot pockets fluctuate seasonally and daily (Gibbs *et al*., 2003), so adult and larval stages experience pronounced variation in temperature at different times of the year and day. Thus, the complex interaction we detected here may be operating in the fly's natural environment. Additionally, larval densities can vary and potentially reach high levels in natural rot pockets (Fellows and Heed, 1972; Mangan, 1982). Although exact estimates of larval densities are not available for natural populations of *D. nigrospiracula*, Breitmeyer and Markow (1998) reported a mean population size of 5123 ± 1713 s.e. adult flies at a single necrosis ($n = 18$ rots, sampled during the Fall, Winter and Spring months); such values are likely to translate into high-larval densities. Furthermore, population sizes vary depending on the size and duration of necroses that differ temporally and spatially (Breitmeyer and Markow, 1998). Hence, *D. nigrospiracula* larvae may experience variable levels of competition over time and space. By measuring costs in two different types of environmental variation (temperature and larval density), we were able to assess the complex interactions between multiple environmental conditions. Our findings highlight the importance of measuring trade-offs in variable environments, as this variation clearly may affect the magnitude of the correlation between traits (and see Sgrò and Hoffmann, 2004).

Raymond *et al*. (2005) showed that diamondback moths (*Plutella xylostella*) resistant to the *Bacillus thuringiensis* toxin Cry1Ac had reduced survival at high-larval densities. Antagonistic trade-offs between immune defense and larval competitive ability were also demonstrated in replicate lines of *D. melanogaster* selected for resistance against parasitoids (Kraaijeveld and Godfray, 1997; Fellowes *et al*., 1998). In these systems, the primary defense against parasitoid attack is an internal, cellular encapsulation response. In contrast, our study focuses on an external form of defense that is behaviorally mediated. By choosing only flies that were free of mites and feeding scars to seed the next generation, we selected for traits that enhanced mite avoidance during the pre-attachment period. Another distinction between our study and the previously cited studies is that we tested for trade-offs at two different temperatures, an additional source of variation that evidently modulates the expression of costs in the *Drosophila–Macrocheles* system (also see Luong and Polak, 2007).

There are several possible explanations underlying the trade-off between selection for increased resistance and larval competitive ability. Selected lines may be less tolerant to the build-up of waste products due to crowding, that is, lines selected for increased ectoparasite resistance in this study might suffer also from a lower tolerance to toxins. However, the decline in body size observed among selected lines in our study suggests that resistant flies are less able to consume and/or assimilate food as efficiently as control flies. Kraaijeveld *et al*. (2001) suggested that the differences in larval competitive ability between resistance-selected and unselected *D. melanogaster* may be a result of an overall shift in general energy investment from trophic function to immune function. Since larval access to food is often dependent on scramble or exploitive competition, feeding rate may be an important determinant of larval competitive ability (Burnet *et al*., 1977; Joshi and Mueller, 1988). Indeed, Fellowes *et al*. (1999a) found that diminished larval competitive ability among resistant *D. melanogaster* was associated with lower feeding rates. More work is needed to determine whether similar mechanism(s) are

mediating the trade-off between resistance and larval competitive ability in the *Drosophila–Macrocheles* system.

The negative genetic correlation detected between resistance and larval competitive ability is due to either antagonistic pleiotropy or linkage disequilibrium (Crow and Kimura, 1970; Parker, 1991). The former occurs when resistance-conferring gene(s) interfere with the expression of other fitness traits (Williams, 1957; Antonovics and Thrall, 1994; Mitchell-Olds and Bradley, 1996; Partridge, 2001). Alternatively, selection on resistance-conferring genes may result in the 'hitch-hiking' of deleterious genes(s) in linkage disequilibrium (Williams, 1957; Antonovics and Thrall, 1994; Partridge, 2001). We do not think genetic hitch-hiking is an important factor here because our selection lines were derived from a large outbreeding population, and the correlated decline in larval-adult survivorship was consistent across all three independent replicate lines. Further, the decrease in competitive ability is unlikely a result of differential inbreeding effects between selected and control lines. Efforts were taken to minimize differences in inbreeding depression by maintaining a paired control line in parallel with each of the selected lines, using exactly the same numbers of reproductive males and females each generation for a given pair of lines. Additional evidence that inbreeding depression played a negligible role in our study is the consistent *lack* of difference in survivorship between the base and control lines (also see Luong and Polak, 2007). This result also indicates that the observed divergence between the selected and control lines is not confounded by inadvertent selection on larval competitive ability among the control lines. Therefore, the negative genetic correlation detected in our study is most likely a consequence of antagonistic pleiotropy of genes influencing ectoparasite resistance and larval competitive ability.

We experimentally demonstrated an evolutionary cost of resistance in the form of reduced survival under conditions of moderate and severe intra-specific competition. These costs combined with the fecundity costs detected in a previous study (Luong and Polak, 2007) provide strong evidence for genetic trade-offs between resistance and other host fitness traits, in the absence of parasitism. Evolutionary trajectories predicted from genetic correlations are likely to depend on the conditions experienced by flies from one generation to the next. In addition to fluctuations in temperature and larval density, the selection pressure of parasitism can also vary temporally and spatially in the field depending on the age of the cactus necrosis (Polak and Markow, 1995). Hence, the rate and direction of the evolution of resistance will depend on the intensity of the selection pressure imposed by parasites and the magnitude of the fitness costs associated with resistance, which we have shown can interact in a complex fashion with ecologically relevant environmental variation.

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References

Ahmed AM, Baggott SL, Maingon R, Hurd H (2002). The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *Oikos* **97**: 371–377.

Antonovics J, Thrall PH (1994). Cost of resistance and the maintenance of menetic polymorphism in host-pathogen systems. *Proc R Soc Lond B* **257**: 105–110.

Armitage SAO, Thompson JJW, Rolff J, Siva-Jothy MT (2003). Examining costs of induced and constitutive immune investment in *Tenebrio molitor*. *J Evol Biol* **16**: 1038–1044.

Atkinson WD (1979). A field invvestigation of larval competition in domestic *Drosophila*. *J Anim Ecol* **48**: 91–102.

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF (1987). Allocating resources to reproduction and defense. *Bioscience* **37**: 58–67.

Bergelson J, Purrington CB (1996). Surveying patterns in the cost of resistance in plants. *Am Nat* **148**: 536–558.

Bonneaud C, Mazuc J, Gonzalez G, Haussy C, Chastel O, Faivre B *et al*. (2003). Assessing the cost of mounting an immune response. *Am Nat* **161**: 367–379.

Boots M, Begon M (1993). Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Funct Ecol* **7**: 528–534.

Breitmeyer CM, Markow TA (1998). Resource availability and population size in cactophilic *Drosophila*. *Funct Ecol* **12**: 14–21.

Brown JKM (2003). A cost of disease resistance: paradigm or peculiarity? *Trends Genet* **19**: 667–671.

Burnet B, Sewell D, Bos M (1977). Genetic analysis of larval feeding behaviour in *Drosophila melanogaster*. II. Growth regulations and competition between lines. *Genet Res* **30**: 149–161.

Crow JF, Kimura M (1970). *An Introduction to Population Genetic Theory*. Harper & Row: New York.

de Jong G (1990). Quantitative genetics of reaction norms. *J Evol Biol* **3**: 447–468.

Ewald PW (1980). Evolutionary biology and treatment of signs and symptoms of infectious disease. *J Theor Biol* **86**: 169–176.

Ewald PW (1983). Host-parasite relations, vectors, and the evolution of disease severity. *Annu Rev Ecol Syst* **14**: 465–485.

Falconer DS, Mackay TF (1996). *Introduction to Quantitative Genetics*. Longman: Essex.

Fedorka KM, Mousseau TA (2007). Immune system activation affects male sexual signal and reproductive potential in crickets. *Behav Ecol* **18**: 231–235.

Fellows DP, Heed WB (1972). Factors affecting host plant selection in desert-adapted cactiphilic *Drosophila*. *Ecology* **53**: 850–858.

Fellowes MDE, Kraaijeveld AR, Godfray HCJ (1998). Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Proc R Soc Lond B* **265**: 1553–1558.

Fellowes MDE, Kraaijeveld AR, Godfray HCJ (1999a). Association between feeding rate and parasitoid resistance in *Drosophila melanogaster*. *Evolution* **53**: 1302–1305.

Fellowes MDE, Kraaijeveld AR, Godfray HCJ (1999b). The relative fitness of *Drosophila melanogaster* (Diptera, Drosophilidae) that have successfully defended themselves against the parasitoid *Asobara tabida* (Hymenoptera, Braconidae). *J Evol Biol* **12**: 123–128.

Ferdig MT, Beerntsen BT, Spray FJ, Li JY, Christensen BM (1993). Reproductive costs associated with resistance in a mosquito-filarial worm system. *Am J Trop Med Hyg* **49**: 756–762.

Fisher RA (1930). *The Genetical Theory of Natural Selection*. Clarendon Press: Oxford.

Gebhardt MD, Stearns SC (1988). Reaction norms for developmental time and weight at eclosion in *Drosophila mercatorum*. *J Evol Biol* **1**: 335–354.
Gemmill AW, Read AF (1998). Counting the cost of disease resistance. *Trends Ecol Evol* **13**: 8–9.
Gibbs AG, Perkins MC, Markow TA (2003). No place to hide: microclimates of sonoran desert *Drosophila*. *J Therm Biol* **28**: 353–362.
Hanssen SA, Hasselquist D, Folstad I, Erikstad KE (2004). Costs of immunity: immune responsiveness reduces survival in a vertebrate. *Proc R Soc Lond B* **271**: 925–930.
Hare JD, Elle E, van Dam NM (2003). Costs of glandular trichomes in *Datura wrightii*: a three-year study. *Evolution* **57**: 793–805.
Heed WB, Mangan RL (1986). Comuunity ecology of Sonoran Desert *Drosophila*. In: Ashburner M, Carson H, Thompson JN (eds). *The Genetics and Biology of Drosophila*. Academic Press: New York, pp 311–345.
Henter HJ, Via S (1995). The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* **49**: 427–438.
Hoang A (2001). Immune response to parasitism reduces resistance of *Drosophila melanogaster* to desiccation and starvation. *Evolution* **55**: 2353–2358.
Hurd H, Taylor PJ, Adams D, Underhill A, Eggleston P (2005). Evaluating the costs of mosquito resistance to malaria parasites. *Evolution* **59**: 2560–2572.
Jacot A, Scheuber H, Brinkhof MWG (2004). Costs of an induced immune response on sexual display and longevity in field crickets. *Evolution* **58**: 2280–2286.
Joshi A, Mueller LD (1988). Evolution of higher feeding rate in *Drosophila* due to density-dependent natural selection. *Evolution* **42**: 1090–1093.
Kraaijeveld AR, Godfray HCJ (1997). Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**: 278–280.
Kraaijeveld AR, Ferrari J, Godfray HCJ (2002). Costs of resistance in insect-parasite and insect-parasitoid interactions. *Parasitology* **125**: S71–S82.
Kraaijeveld AR, Limentani EC, Godfray HCJ (2001). Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Proc R Soc Lond B* **268**: 259–261.
Kraaijeveld AR, Van Alphen JJ, Godfray HC (1998). The coevolution of host resistance and parasitoid virulence. *Parasitology* **116** (Suppl): S29–S45.
Lochmiller BL, Deerenberg C (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**: 87–98.
Luong LT, Polak M (2007). Costs of resistance in the *Drosophila–Macrocheles* system: a negative genetic correlation between ectoparasite resistance and reproduction. *Evolution* **61**: 1391–1402.
Mangan RL (1982). Adaptation to competition in cactus breeding *Drosophila*. In: Barker JSF, Starmer WT (eds). *Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System*. Academic Press: Sydney, pp 257–272.
Messina FJ, Fry JD (2003). Environment-dependent reversal of a life history trade-off in the seed beetle Callosobruchus maculatus. *J Evol Biol* **16**: 501–509.
Mitchell-Olds T, Bradley D (1996). Genetics of *Brassica rapa*. 3. Costs of disease resistance to three fungal pathogens. *Evolution* **50**: 1859–1865.
Moret Y, Schmid-Hempel P (2000). Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**: 1166–1168.
Parker MA (1991). Nonadaptive evolution of disease resistance in an annual legume. *Evolution* **45**: 1209–1217.
Partridge L (2001). Evolutionary theories of ageing applied to long-lived organisms. *Exp Gerontol* **36**: 641–650.
Partridge L, Fowler K (1992). Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* **46**: 76–91.
Polak M (1996). Ectoparasitic effects on host survival and reproduction: the *Drosophila–Macrocheles* association. *Ecology* **77**: 1379–1389.
Polak M (1998). Effects of ectoparasitism on host condition in the *Drosophila–Macrocheles* system. *Ecology* **79**: 1807–1817.
Polak M (2003). Heritability of resistance against ectoparasitism in the *Drosophila–Macrocheles* system. *J Evol Biol* **16**: 74–82.
Polak M, Luong LT, Starmer WT (in press). Parasites physically block host copulation: a potent mechanism of parasite-mediated sexual selection. *Behav Ecol*.
Polak M, Markow TA (1995). Effect of ectoparasitic mites on sexual selection in a Sonoran desert fruit fly. *Evolution* **49**: 660–669.
Price PW (1980). *Evolutionary Biology of Parasites*. Princeton University Press: Princeton, NJ.
Raymond B, Sayyed AH, Wright DJ (2005). Genes and environment interact to determine the fitness costs of resistance to *Bacillus thuringiensis*. *Proc R Soc Lond B* **272**: 1519–1524.
Reznick D, Nunney L, Tessier A (2000). Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol Evol* **15**: 421–425.
Rigby MC, Jokela J (2000). Predator avoidance and immune defense: costs and trade-offs in snails. *Proc R Soc Lond B* **267**: 171–176.
Rigby MC, Hechinger RF, Stevens L (2002). Why should parasite resistance be costly? *Trends Parasitol* **18**: 116–120.
Roff DA (1977). Dispersal in dipterans: it's costs and consequences. *J Anim Ecol* **46**: 443–456.
Roff DA (2002). Inbreeding depression: tests of the overdominance and partial dominance hypotheses. *Evolution* **56**: 768–775.
Rolff J, Siva-Jothy MT (2003). Invertebrate ecological immunology. *Science* **301**: 472–475.
Rose MR (1984). Artificial selection on a fitness component in *Drosophila melanogaster*. *Evolution* **38**: 516–526.
Sandland GJ, Minchella DJ (2003). Costs of immune defense: an enigma wrapped in an environmental cloak? *Trends Parasitol* **19**: 571–574.
Santos M, Eisses KT, Fontdevila A (1999). Competition and genotype-by-environment interaction in natural breeding substrates of *Drosophila*. *Evolution* **53**: 175–186.
Santos M, Fowler K, Partridge L (1992). On the use of tester stocks to predict the competitive ability of genotypes. *Heredity* **69**: 489–495.
SAS Institute (2002). *SAS User's Guide*. SAS Institute: Cary, NC.
Service PM, Rose MR (1985). Genetic covariation among life-history components: the effect of novel environment. *Evolution* **39**: 943–945.
Sgrò CM, Hoffmann AA (2004). Genetic correlations, tradeoffs and environmental variation. *Heredity* **93**: 241–248.
Sheldon BC, Verhulst S (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol* **11**: 317–321.
Simms EL (1992). Costs of plant resistance to herbivory. In: Fritz RS, Simms EL (eds). *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics*. University of Chicago Press: Chicago, pp 392–425.
Simms EL, Rausher MD (1987). Costs and benefits of plant resistance to herbivory. *Am Nat* **130**: 570–581.
Sokal RR, Rohlf FJ (1995). *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman: New York.
Stearns S, Dejong G, Newman B (1991). The effects of phenotypic plasticity on genetic correlations. *Trends Ecol Evol* **6**: 122–126.
Tian D, Traw MB, Chen JQ, Kreitman M, Bergelson J (2003). Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. *Nature* **423**: 74–77.

Webster JP, Woolhouse MEJ (1999). Cost of resistance: relationship between reduced fertility and increased resistance in a snail-schistosome host-parasite system. *Proc R Soc Lond B* **266**: 391–396.

Williams GC (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**: 398–411.

Winer BJ (1971). *Statistical Principles in Experimental Design*. McGraw Hill: New York.

Yan G, Severson DW, Christensen BM (1997). Costs and benefits of mosquito refractoriness to malaria parasites: implications for genetic variability of mosquitoes and genetic control of malaria. *Evolution* **51**: 441–450.