# Heritability of resistance against ectoparasitism in the *Drosophila–Macrocheles* system

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## Abstract

Ectoparasites are abundant in natural communities, can have pronounced deleterious fitness consequences to their host and are important vectors of transmissible parasitic disease. Yet very few studies have estimated the magnitude of heritable genetic variation underlying resistance against ectoparasitism, which significantly limits our ability to predict the evolution of this ecologically important character. The present paper reports results of artificial selection for increased resistance in *Drosophila nigrospiracula* against ectoparasitic, haematophagous mites, *Macrocheles subbadius*. In this system, which occurs naturally in the Sonoran Desert of North America, ectoparasitism significantly damages the expression of host fitness traits, including longevity, fecundity and male mating success. In the present study, resistance, which was modelled as a threshold trait, responded significantly to selection applied on either sex. Realized heritability, calculated as a mean across four replicates, was estimated to be $0.152 \pm 0.014$ (SE). The heritability estimate from selection on males did not differ from that on females, but both estimates differed significantly from zero. This documented presence of additive genetic variation for resistance, coupled with knowledge of the fitness consequences of ectoparasitism, indicates that the host population possesses significant evolutionary potential. Selection was applied on the pre-attachment phase, thereby targeting behavioural forms of defence. This study therefore establishes parallels between insects and other animals in their ability to protect themselves and evolve behavioural defences against ectoparasites.

## Introduction

The realization that parasites can be harmful to their host in even long-standing, co-evolved host–parasite systems, has contributed, over the past two decades, to the abandonment of the conventional view that 'successful' parasites will evolve to do little or no harm to their host (Price, 1980; May & Anderson, 1983; Toft & Karter, 1990). Through the selection they impose on their hosts, parasites are now recognized as being capable of driving rapid host evolution (e.g. van Ripper *et al*., 1986; Dwyer *et al*., 1990), and altering genetic structure within and between host populations (e.g. Burdon, 1980; Jarosz & Burdon, 1991).

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Understanding the evolutionary potential of parasite-mediated selection requires knowledge of the magnitude of additive genetic variance underlying defensive traits of the host. Much of the evidence for genetic involvement comes from studies of plants documenting differential response of host genotypes to pathogens and insect herbivores (e.g. Berenbaum *et al*., 1986; Burdon, 1987; Alexander, 1989; Burdon & Jarosz, 1991; Karban, 1992; Alexander & Antonovics, 1995). Among insects, some studies have documented genetic variability in resistance by contrasting resistance of different host strains or genetic isolates against a variety of parasitic organisms ranging from viruses, bacteria and protozoa, to helminthes and insect parasitoids (Carton *et al*., 1986;

Shykoff & Schmid-Hempel, 1991; Tanada & Kaya, 1993; Delpuech *et al*., 1994; Henter & Via, 1995; Yan & Norman, 1995). Although these studies indicate that resistance is at least in part under genotypic control, such studies alone are not sufficient evidence for the presence of heritable genetic variation. Recently Kraaijeveld & Godfray (1997) and Fellowes *et al*. (1998) successfully selected *Drosophila melanogaster* for increased resistance against the parasitoids *Asobara tabida* and *Leptopilina boulardi*, respectively, demonstrating heritable genetic variation for endoparasite resistance. Another example is from the work of Collins *et al*. (1986) who report significant responses to artificial selection in mosquito colonies for resistance against *Plasmodium* malarial parasites. In contrast, Klingenberg *et al*. (1997) detected no heritable variation for resistance against gregarine gut parasites in the water strider *Gerris buenoi*.

Thus the evidence suggests that genetic variation in resistance may often be present in natural populations, although in many cases, the extent to which this variation is attributable to heritable differences (i.e. additive effect of genes) remains unknown. Moreover, existing studies of genetic variability within insects typically are restricted to insect-endoparasite systems. Most of what we know about genetic variation and the proximate basis of resistance against ectoparasites comes largely from studies of domesticated vertebrates (Wakelin, 1978, 1984; Marshall, 1981; Wakelin & Blackwell, 1988). Yet ectoparasites, such as mites and ticks (Acarina), bugs (Hemiptera), chewing lice (Mallophaga), and fleas (Siphonaptera), are not trivial ecologically. Indeed, they are abundant in natural communities (Marshall, 1981), and have been shown to reduce host condition (Forbes & Baker, 1991; Polak, 1998; Blanco *et al*., 2001) and fitness components (Waage, 1979; Brown & Brown, 1986; Murray, 1990; Brown *et al*., 1995). Ectoparasites are known vectors to many important parasitic diseases of animals, including humans, and so are of enormous medical and veterinary importance (Marshall, 1981; Lehmann, 1993).

The present study tests for the presence of additive genetic variation underlying resistance against ectoparasites, and reports replicate estimates of realized heritability for this trait. The system involves *D. nigrospiracula* and mites, *Macrocheles subbadius*, a naturally occurring association for which there is a considerable amount already known about the fitness consequences and distribution of parasites in natural populations. The mite, which has been recovered from multiple host species in the North American Sonoran Desert (Polak, 1996), reduces male mating success and generates significant sexual selection in natural host populations (Polak & Markow, 1995). Infested females suffer greater mortality and produce fewer progeny over their lives (Polak, 1996), effects which depend both on the number of parasites per host as well as on the duration of ectoparasitism (Polak, 1998).

In the present study, artificial selection was applied in five replicate host lines recently derived from the field, and the genetic stability of resistance in one line was tracked for 27 generations (*c*. 1.5 years) after selection was relaxed. Selection was applied either in males or females, and responses to these different selection regimes were compared. The selection protocol targeted premite-attachment mechanisms of resistance, so that general inferences regarding the kinds of defensive traits that evolved can be made.

## Methods

### Base populations

Two selection experiments (A and B) were conducted, encompassing five replicate selection lines. Locations and dates of collection of the four base populations from which these lines were derived are presented in Table 1. Base populations were initiated with field-caught flies, and 'mass-cultured' in the laboratory (Polak, 1996) each in six 200 mL bottles per generation, and at 25 °C and a 12D and 12L light cycle.

For experiment A, a first base population was initiated from 140 flies (70 of each sex) collected at one necrotic saguaro cactus along Peralta Road, *c*. 80 km east of Phoenix, Arizona, USA. A collection (75 flies of each sex) for a second base population was made along Cherry Creek Road, *c*. 50 km north-east of the first site. Base populations (200 flies each) for experiment B came from these same sites, but from different cacti (Table 1).

Although flies were derived from two sites, the evidence indicates they represent samples from one panmictic field population. *D. nigrospiracula* is a strong, long-distance disperser (Johnston & Heed, 1976; Markow & Castrezana, 2000), and populations sampled across different cacti within sites, and across geographical regions separated by distances (e.g. 475 km; Pfeiler & Markow, 2001) much greater than between sites sampled

**Table 1** Locations and dates of collection of base populations used in selection experiments derived from nature within the Tonto National Forest, in a vicinity east of Phoenix, Arizona, USA. Selection lines derived from each base population are also given.

| Location | Replicate cactus | Date of collection | Selection line designation |
|---|---|---|---|
| Experiment A | | | |
| Peralta Road* | 1 | August 1995 | S1A |
| Cherry Creek Road† | 2 | November 1995 | S2A, S3A |
| Experiment B | | | |
| Peralta Road | 3 | March 1999 | S1B |
| Cherry Creek Road | 4 | March 1999 | S2B |

*111°22′W, 33°23′N (Maricopa County); †110°56′W, 33°37′N (Gila County).

here, show uniform allele frequencies and heterozygosities (Sluss, 1975; Pfeiler & Markow, 2001).

Base populations were mass-cultured in the laboratory for five generations prior to the commencement of selection to help reduce variation because of maternal effects arising from differences in condition among field-caught females. Flies were not mass-cultured for a great many generations (e.g. Promislow & Tatar, 1998), for one, to avoid accumulating lab-specific mutations (Harshman & Hoffmann, 2000). Long-term culture in the laboratory could alter genetic variability of the population, which is undesirable because the present research attempts to estimate the magnitude of standing genetic variation in nature.

A colony of mites was initiated with several hundred female mites taken from flies captured at the field sites in 1995 and 1999, and cultured (Polak, 1996). All mites extracted from the culture were discarded following use to avoid selection on the mite stock.

## Selection protocol

The selection protocol consisted of exposing 250 flies of each generation to mites for 48 h in four experimental infestation chambers consisting of 300 mL Ball® Mason Jars (Alltrista Corp., Muncie, IN, USA) lined with plaster of Paris and containing medium with mites (Fig. 1). The mouth of the jar was sealed with coarse paper towel. Flies were aspirated into a space excavated within the medium.

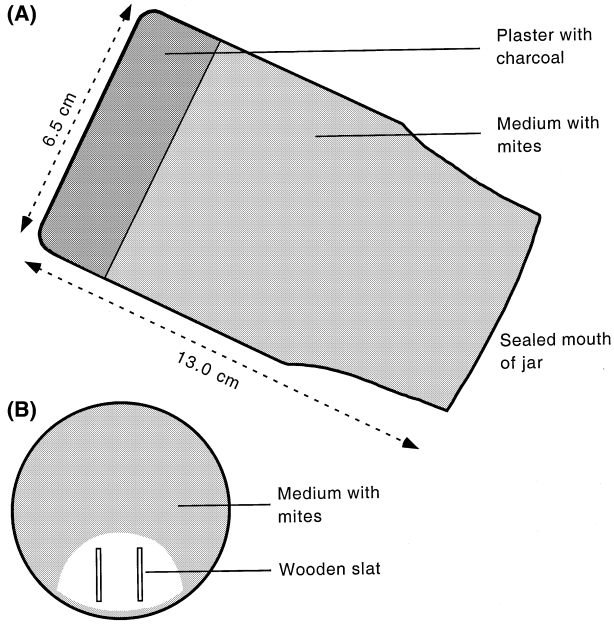


**Fig. 1** Side (A) and cross-sectional (B) schematics of an experimental infestation chamber in which flies were exposed to mites. Cross-sectional view shows wooden slats within the space excavated in the medium, which imitate cactus ribs within pockets of necrotic cactus tissue and serve to increase the area on which mite–fly interactions occur.

Following exposure, all surviving flies were aspirated from chambers, and flies that had never been parasitized were used to seed subsequent generations (parasitized and scarred individuals were discarded). In this way, selection was avoided on putative host resistance mechanisms operating postmite attachment, such as the induction of host haemolymph biochemical effectors that could interact with, and function to dislodge, a feeding mite (e.g. Wang & Nuttall, 1994). A total of 20 (8%) to 50 (20%) (mean = 42.2 ± 2.7 SE) unparasitized flies seeded each new generation. For S1A and S3A, selection was applied on males only, whereas for S2A selection was applied on females only. An equal number of males and females was used to seed each new generation of each line. Selection was continued for 21, 17 and 14 generations in the case of S1A, S2A and S3A, respectively. Selection was applied on males in both S1B and S2B, and was continued for 12 generations in each of these two lines.

A control line was maintained in parallel to each selected line. Each selected line and its paired control were derived from the same base population. At each generation the control line was seeded with the same number of individuals of both sexes as its selected line, but these flies were a randomly chosen subset of the control line that had been held for 48 h in chambers as described above, but without mites. In experiment A, control lines C1A, C2A and C3A, were paired with S1A, S2A and S3A, respectively, whereas in experiment B, control lines C1B and C2B were paired with S1B and S2B, respectively.

Resistance against mites was modelled as a discontinuous or threshold trait, with an expected underlying continuous variable called the liability, influenced by both genetic and environmental factors (Falconer & Mackay, 1996). It was assumed that a single threshold along the liability distribution separated susceptible and resistant forms. Thus, despite the minor fluctuations between generations in the number of unparasitized hosts used as parents (see above), the number of unparasitized individuals recovered from chambers with mites was treated as a random sample of the class desired for selection, and thus, the mean of the recovered group was assumed to be representative of this class.

## Response to selection

To track response to selection within each line, resistance was assayed at various generations of the experiment by contrasting resistance between the selected line and its paired control. In experiment A, S1A and S2A were assayed 12 and five times, respectively, whereas S3A was assayed only after selection in this line was terminated. Realized heritability was therefore estimated for S1A and S2A only. In experiment B, lines S1B and S2B were

assayed five and six times, respectively, and so heritabilities were estimated for both these lines as well.

The resistance assay consisted of aspirating groups of selected and unselected flies together in replicate chambers with mites (Fig. 1). The sexes were exposed separately and no food resource was present within chambers other than that which is in the culture medium (e.g. moisture and nutriment leached from bran flakes), thereby minimizing competitive interactions for mates or concentrations of food resource. Groups ranged from 10 to 70 flies (mean = 21) of each type, and the two groups in any one chamber were always equal in size. The identity of flies was determined by minute wing clips (<2% of total wing area). Prior to the assay, flies were anaesthetized with light $CO_2$, and administered a clip to the tip of either the right or the left wing under a dissecting microscope. The side receiving a clip was alternated between the groups across replicate chambers; every fly received a clip. Flies were given 2 h to recover from the $CO_2$ prior to exposure.

Exposing selected and control flies within a common chamber permitted control of unwanted variation in prevalence, for example, because of mite density, substrate humidity and other chamber characteristics. Such effects were expected to obscure subtle differences in susceptibility between selected and control groups had they been assayed in separate chambers, thereby reducing the sensitivity of the assay. Flies were exposed to mites from 12 to 48 h; they were retained in chambers until it was estimated through observation that 50% of flies had become infested. This rule was adopted instead of fixing the exposure time to help accommodate for variation in mite density and motivation to infest flies. Thus, by virtue of this protocol, prevalence data pertaining to any one type alone cannot meaningfully be used to track change in resistance over the selection experiment. Rather, the metric of change is the *difference* in mean liability values (calculated from prevalence data, see below) between selected and control flies.

Following exposure, all living flies were carefully recovered from chambers using an aspirator, distinguished by type according to their wing clips, and the presence of mites and/or mite-induced scars on each fly were ascertained. The identity of flies was unknown until after they were scored for parasite burden. Prevalence (P) of infestation was calculated as $P = (N_i + N_d)/N_e$, where $N_i$ is the number of flies infested by mites (currently infested plus scarred flies); $N_d$, the number of flies that died because of infestation and $N_e$, the total number of flies exposed to mites.

Whether flies died in experimental chambers because of mites was tested in a separate experiment to validate using $N_d$ as index of parasite-induced mortality. At the terminus of selection in S1A, resistant and control flies were added to chambers with medium but without mites in groups equal in size and distinguished by wing clips. Groups on average consisted of 14.5 flies, and flies of both sexes were represented (each sex was tested separately in two chambers). Following 48 h in chambers, all flies were removed, and of a total of 116, one fly (from the resistant category) was found dead. Thus, mortality in chambers during the resistance assays could be attributed to the mites.

Mean prevalence across chambers for a given assay was transformed into a measure of 'mean liability' for each group (i.e. resistant and control) following Falconer & Mackay (1996, p. 301). Assuming similar variances of liability of each group, the difference in mean liability between selected and control lines was taken as the difference [in standard deviation (SD) units] in their level of resistance (Hill, 1972). This difference is the 'divergence' between groups measured using any assay. The strength of the response to selection in each line was estimated from the regression of divergence on generation number (Muir, 1986); thus, two regression models were fitted to the results in each of the two experiments, A and B.

In a separate analysis, the slopes of the two regression functions for each experiment were compared using a single regression model constructed such that selection line was treated as a binary or qualitative variable. The model was in the form of $Y_i = \beta_o + \beta_1 X_{i1} + \beta_2 X_{i2} + \beta_3 X_{i1} X_{i2} + \varepsilon_i$, where $Y$ is divergence due to selection, $X_1$ is generation number and $X_2$ is the binary variable (coded as 1 or 0). $\beta_2$ Indicates the magnitude of the difference between the intercepts of the two regression lines, and $\beta_3$ indicates the magnitude of the difference between slopes (Neter *et al.*, 1990).

In incorporating data from the control line to estimate the response to selection, it was assumed that the effect of genetic drift on susceptibility divergence was negligible, so that the evolution of resistance could be attributed to selection. In experiment A, this assumption was tested by contrasting degree of susceptibility, after selection was completed, between (1) C1A vs. C1A's initial base population from which it, and S1A, were originally derived, and (2) C2A vs. C3A. The base population was maintained by mass culture throughout the duration of selection. In experiment B, the contrasts were C1B and C2B vs. their respective base populations. Absence of genetic drift will be supported by lack of difference in these contrasts. In the first contrast, the assay consisted of exposing flies in four replicate chambers (20 flies of each type per chamber) whereas the second contrast consisted of three chambers with 20, 36 and 30 flies of each type per chamber. Differences in the frequency at which flies were recovered parasite-free were evaluated with $\chi^2$ tests. These tests were conducted for each comparison on pooled data across chambers; in each case heterogeneity $\chi^2$ was not significant (all *P*-values n.s.), justifying the pooling procedure. The $\chi^2$ statistics on pooled data were calculated using three-dimensional contingency tables (Zar, 1984); line, sex and parasitism (i.e. infested or not) were represented.

Genetic stability of evolved resistance in one experimental line was tested by assaying S1A at six times over a period of 27 generations (*c.* 1.5 years) after selection was terminated in this line. During this period, the selected line and its control were maintained in mass culture under conditions described above. Because sexes were tested separately in only four of these assays, and resistance remained at a relatively constant high value (see Results), data across these four assays were pooled for the purpose of testing for differences in resistance between the sexes. Factorial ANOVA was performed on $\log_{10}$-transformed proportion values (proportion flies infested per chamber) with line (selected and control) and sex entered as factors. Sex effects in all five replicate selection lines were also examined using tests of partial independence in three-dimensional contingency table analysis (Zar, 1984).

## Results

Table 2 presents rates of infestation, expressed as proportion of flies infested, among selected and control flies in each of four replicate lines over the course of selection. Divergence, calculated as the difference between selected and control lines in mean liability, is also presented (Table 2); positive values of divergence indicate a lower prevalence of parasitism in the selected line relative to that in its paired control. In all lines, divergence grew from near zero to progressively higher values over the course of selection. At the terminus of selection, each selected line was significantly more resistant than its respective unselected, control line (Table 3), indicated by chi-square testing (all $P$-values <0.05).

Realized heritability was estimated using each of the four lines for which there were sufficient data tracking changes in resistance throughout the selection experiment (Table 4). Because selection was applied on one sex only, realized heritability of resistance was calculated as twice the slope of each least-squares regression relating cumulated response to generation number. Figure 2 compares regressions calculated using data from lines in which either males (S1A) or females (S2A) were selected; slopes of these regression lines were not significantly different ($\beta_3 \pm \mathrm{SE} = 0.019 \pm 0.018$, $t_{13} = 1.0$, n.s.). In experiment B, in which both selection lines (S1B and S2B) were generated through selection on males, the slopes were also not significantly different from each other ($\beta_3 \pm \mathrm{SE} = 0.019 \pm 0.014$, $t_7 = 1.42$, n.s.). The mean realized heritability (across the four replicates for which heritability estimates were calculated) is given in Table 4. The reported standard error is the empirical standard error (Falconer & Mackay, 1996), estimated directly from the variance of the replicate heritability estimates (Hill, 1971).

Several tests were conducted to determine whether genetic drift could have biased observed divergence in resistance between selected and control lines. In experiment A, line C2A vs. C3A and line C1A vs. its base populations were contrasted using contingency table analysis in which line, sex and parasite status were represented. In neither case was the overall $\chi^2$ significant ($\chi^2_4 = 2.33$ and 3.39, n.s. in both cases), indicating

**Table 2** Results of assays tracking divergence in resistance between selected and control lines in experiments A and B. Generations are number generations of selection preceding a given assay. Values for each line represent prevalences (P) of infestation, averaged across chambers. Values in parentheses are numbers of flies of either group (i.e. selected or control) exposed to mites (total number of flies exposed is twice this value). Divergence is the difference in mean liability between selected and control lines, and is the variable entered into regression analyses for determination of slopes and heritabilities (Table 4).

| | Experiment A | | | | | | Experiment B | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Generation(s) | S1A | C1A | Divergence (SD units) | S2A* | C2A | Divergence (SD units) | S1B | C1B | Divergence (SD units) | S2B | C2B | Divergence (SD units) |
| 1 | 0.55 (241) | 0.57 | 0.05 | – | – | – | 0.62 (47) | 0.62 | 0.0 | 0.36 (60) | 0.35 | –0.03 |
| 2 | – | – | – | 0.47 (15) | 0.53 | 0.15 | – | – | – | 0.33 (43) | 0.35 | 0.06 |
| 3 | 0.37 (59) | 0.45 | 0.21 | 0.13 (24) | 0.30 | 0.39 | – | – | – | – | – | – |
| 4 | 0.63 (96) | 0.65 | 0.05 | – | – | – | 0.58 (62) | 0.71 | 0.35 | 0.49 (75) | 0.60 | 0.28 |
| 5 | 0.53 (100) | 0.73 | 0.54 | – | – | – | – | – | – | – | – | – |
| 6 | 0.60 (60) | 0.87 | 0.83 | – | – | – | – | – | – | – | – | – |
| 8 | 0.14 (70) | 0.35 | 0.70 | – | – | – | – | – | – | – | – | – |
| 9 | – | – | – | 0.07 (30) | 0.30 | 0.95 | – | – | – | 0.39 (42) | 0.55 | 0.41 |
| 10 | 0.42 (90) | 0.74 | 0.85 | – | – | – | 0.61 (62) | 0.87 | 0.85 | 0.47 (105) | 0.68 | 0.54 |
| 11 | 0.15 (20) | 0.50 | 1.04 | – | – | – | 0.63 (100) | 0.92 | 1.07 | | | |
| 12 | – | – | – | – | – | – | 0.33 (72) | 0.68 | 0.91 | 0.34 (74) | 0.65 | 0.80 |
| 14 | 0.72 (67) | 0.97 | 1.22 | 0.34 (24) | 0.66 | 0.82 | – | – | – | – | – | – |
| 17 | 0.64 (81) | 0.91 | 0.98 | 0.54 (101) | 0.84 | 1.09 | – | – | – | – | – | – |
| 18 | 0.51 (38) | 0.93 | 1.45 | – | – | – | – | – | – | – | – | – |
| 21 | 0.58 (80) | 0.97 | 1.68 | – | – | – | – | – | – | – | – | – |

*Identifies the line in which selection was applied on females.

**Table 3** Proportion flies of each type (selected or control) infested (carrying mites and found dead) by mites across replicate chambers; difference between selected and control lines is the net divergence in defence because of selection.

| Experiment / line | Generations of selection | Total number of flies per type (number chambers) | Proportion flies infested (SE*) | |
|---|---|---|---|---|
| | | | Selected | Paired control |
| Experiment A | | | | |
| S1A | 21 | 80 (8) | 0.58 (0.055) | 0.97 (0.035) |
| S2A | 17 | 101 (10) | 0.54 (0.050) | 0.84 (0.037) |
| S3A | 14 | 72 (6) | 0.25 (0.051) | 0.50 (0.059) |
| Experiment B | | | | |
| S1B | 12 | 72 (5) | 0.33 (0.055) | 0.68 (0.054) |
| S2B | 12 | 74 (4) | 0.34 (0.055) | 0.65 (0.055) |

*Standard error calculated using the binomial distribution.

**Table 4** Slopes and intercepts of regression functions relating divergence (in SD units) to generation number in four replicate selection lines. Realized heritability ($h^2$) values are calculated as twice the slope of each regression function.

| Line | Generations of selection | Slope (SE) | *P*-value (*t**) | Intercept (SE) | *P*-value (*t***) | $h^2$ (SE) |
|---|---|---|---|---|---|---|
| Experiment A | | | | | | |
| S1A | 21 | 0.0754 (0.0092) | <0.0001 (8.2) | 0.058 (0.11) | n.s. (0.55) | 0.15 (0.018) |
| S2A | 17 | 0.0568 (0.015) | 0.03 (3.7) | 0.18 (0.16) | n.s. (1.1) | 0.11 (0.030) |
| Experiment B | | | | | | |
| S1B | 12 | 0.0920 (0.012) | 0.005 (7.5) | –0.045 (0.10) | n.s. (–4.3) | 0.18 (0.024) |
| S2B | 12 | 0.0726 (0.0071) | 0.0005 (10.3) | –0.10 (0.051) | n.s. (–2.0) | 0.15 (0.014) |
| Mean | – | – | – | – | – | 0.15 (0.014) |

*$t$ evaluating $H_0$: slope ($\beta_1$) = 0. **$t$ evaluation $H_0$: intercept ($\beta_o$) = 0.

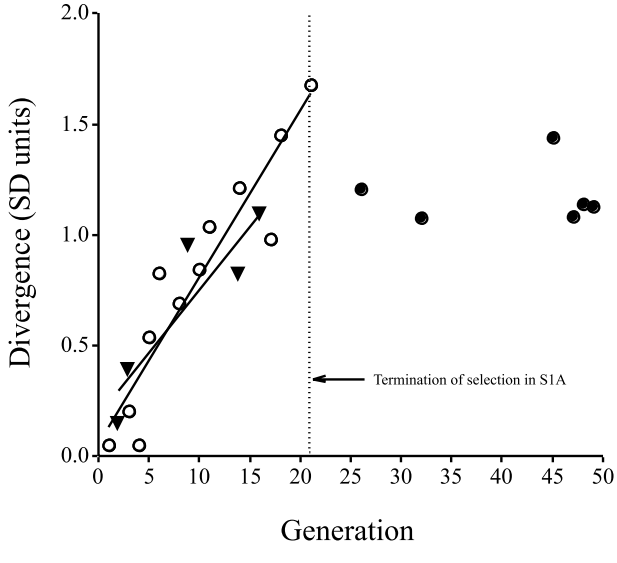


**Fig. 2** Response of resistance against mites to artificial selection. Divergence is the difference in mean liability between selected and control lines, expressed in standard deviation (SD) units (data in Table 2). Empty circles (○) correspond to resistance in S1A during selection, whereas filled circles (●) track resistance in S1A after selection was terminated (termination occurred at dashed line, generation 21). Triangles (▼) correspond to divergence in resistance in S2A from selection on females.

mutual independence among variables. Thus, control lines were stationary in their level of resistance across the experiment, weakening the possibility that genetic drift was an important cause of divergence between these selected and control lines.

In experiment B, there was also no difference in susceptibility between C2B and its base population ($\chi^2_4 = 1.58$, n.s.). In contrast, there was a tendency for C1B flies to be *more* resistant than its base population ($\chi^2_4 = 9.27$, $0.05 < P < 0.10$). Indeed, when the sexes were analysed separately, the reason for this pattern was discovered: C1B females were significantly more resistant than base population females ($\chi^2_1 = 3.96$, $0.025 < P < 0.05$), although in males there was no difference ($\chi^2_1 = 1.31$, n.s.). To determine what effect this divergence, which may have occurred because of genetic drift, could have on the heritability estimate for line S1B, the slope of the selection response was recalculated using assay data for males only. This new value (0.117 ± 0.012 SE) was marginally larger than that of the S1B value reported in Table 4, which was calculated using sexes combined. However, the difference between these two slopes was not significant ($t_8 = 0.78$, n.s.).

Line S1A was assayed six times over a period of 27 generations following selection, and resistant flies were found to be on average 40.0 ± 2.0% SE more resistant than controls. One-way ANOVA on $\log_{10}$-transformed

percentage of selected flies that evaded parasites relative to controls, showed no differences across assays ($F_{4,18} = 0.63$, n.s.), demonstrating genetic stability in evolved resistance over this period (Fig. 2). Moreover, regression performed on divergence data following selection (filled circles, Fig. 2), showed that there was no decrease in divergence across generations (slope $= 5.7 \times 10^{-4} \pm 7.0 \times 10^{-3}$ SE, $t_4 = 0.082$, n.s.) when selection was absent.

Data from four of the six assays in which sexes were distinguished were used to test for sex differences in susceptibility in S1A. Factorial ANOVA on proportion flies infested, with line (selected and control) and sex entered as factors, revealed (as above) a significant effect of line ($F_{1,40} = 38.2$, $P < 0.0001$), but not of sex ($F_{1,40} = 1.5$, n.s.). The line–sex interaction was not significant ($F_{1,40} = 0.13$, n.s.). Sex effects were also examined for each of the five selection lines at the terminus of selection in each line. Using three-dimensional contingency table analysis on fly frequency, a test of partial independence revealed that sex was independent of line and parasitism (S1A: $\chi^2_3 = 0.82$; S2A: $\chi^2_3 = 1.12$; S3A: $\chi^2_3 = 2.30$; S1B: $\chi^2_3 = 6.01$; S2B: $\chi^2_3 = 0.74$; n.s. in all cases). Thus, both sexes responded similarly to selection, despite selection having been applied on one sex only.

Finally, assay data were pooled across all lines to determine whether selected and control flies following selection differed in the frequency at which they carried mite-induced scars. Of the 369 selected and 214 control flies recovered from assay chambers, nine (2.4%) and six (2.8%) flies of each type, respectively, carried scars, a difference that was not statistically significant ($\chi^2_1 = 0.10$, n.s.).

## Discussion

The data indicate that *D. nigrospiracula* contains significant additive genetic variation in resistance against *M. subbadius* mites, at least in the population sampled in the vicinity east of Phoenix, Arizona, which occupies the northernmost fringe of *D. nigrospiracula*'s geographical distribution. Net divergence in resistance following an equivalent number of generations of selection was similar across replicate lines. This similarity occurred despite selection having been applied separately on the sexes. Moreover, the slope of the regression relating response to generation number in the case of males was similar to that in females, which is a further testimony to the consistency of the response, and suggesting a lack of cytoplasmic inheritance underlying resistance. Realized heritability estimates ranged between 12 and 18% across replicate lines of both experiments.

Resistance remained at high and constant values over 27 generations post-selection, indicating genetic stability of evolved resistance. There was, however, ~30% drop in resistance from the end of selection to the first post-selection assay (Fig. 2), suggesting that increased resistance carries with it some fitness cost (Mitchell-Olds & Bradley, 1996). Barring this initial drop, however, the long-term stability of evolved resistance suggests that costs of resistance may be weak or even absent, at least under experimental conditions of the present study. However, these data are from a single resistant line (and its control), so the generality of this pattern of a sudden drop followed by stasis is unknown. Resolution of the question of costs awaits comparison of resistant and control lines with respect to fitness traits in the absence of parasites, and under variable experimental environments.

Few data exist on the heritable genetic basis of resistance against parasites and pathogens in natural populations, and the present study is one of the first to demonstrate a heritable genetic basis for resistance against ectoparasites in an invertebrate host. The evidence for genetic variation for resistance in other *Drosophila* comes from studies of endoparasites, where the defensive mechanisms are physiological and biochemical (reviewed in Fellowes & Godfray, 2000), and undoubtedly very different from the behavioural traits targeted in the present study. Kraaijeveld & Godfray (1997) selected four lines of *D. melanogaster* for increased resistance against the parasitoid *A. tabida*, and estimated narrow-sense heritability ($h^2$) of resistance to be *c.* 25% (Fellowes & Godfray, 2000), a value considerably higher than that for behavioural resistance reported here. The reasons for this difference may relate to the inherent variability, and low repeatability of behavioural traits (Hoffmann, 1999), and/or loss of genetic variation because of strong ectoparasite-mediated directional selection in nature. Henderson (1990) suggests that complex behavioural traits that are composed of integrated component parts should be closely related to fitness, and which could help to explain their lower heritabilities (see Roff & Mousseau, 1987; Hoffmann, 1999).

Which host traits responded to selection in the present study? Although the identity of host resistance mechanisms is unknown precisely, some general inferences can nevertheless be made. In using those flies that neither carried mites nor were scarred by mites as parents, selection was applied only on traits that prevented mite attachment from occurring, in other words, on traits of the preattachment phase. For a mite to penetrate host defences of this phase, it first must make contact with a fly, typically a tarsus, and succeed to climb over the fly's body to its site of attachment. Thus, resistant flies may have become more active generally (exhibit hyperactivity), making them a more difficult target for mites. An interesting contrast would be the work of Sokolowski & Turlings (1987) showing that greater activity levels in larval *D. melanogaster* was associated with a significantly *greater* probability of contact by *A. tabida*, a parasitoid wasp that uses vibrotaxis to locate its host.

Alternatively, resistance could also have been enhanced through a reduction in response time to specific visual and tactile stimuli produced by searching

parasites. Direct observation of fly–mite interactions within experimental infestation chambers show that flies dodge approaching mites and appear to exhibit reflex behaviour in the form of sudden, brisk movements away from a mite, or bursts of flight from the substrate when touched by a mite. When a mite grasps a tarsus, flies can rid themselves of the mite by vigorous grooming and tarsal flicking. A somewhat different mechanism occurs in the damselfly *Ischnura verticalis*, wherein larvae protect themselves against colonization by water mites (*Arrenurus pseudosuperior*) by an explosive extension of the labium (labial strike) directed at the swimming larval ectoparasites (Leung *et al*., 2001).

It remains possible, however, that flies evolved traits that were not behavioural, but rather, those that made them less 'palatable' to mites, for example, through changes in components of their cuticle or haemolymph. The available data, however, argue against this possibility. First, selected and control flies did not differ in the frequency at which they carried scars (if resistant flies were less palatable, the frequency of scarring, indicative of mite feeding and subsequent dislodgement, should have been higher than among controls). Moreover, when flies were experimentally immobilized without anaesthesia (to attenuate any behavioural defences), selected and control flies did not differ in the rate at which they accumulated parasites (Hinn & Polak, in preparation).

Current knowledge of the genetic, demographic and mechanistic basis of ectoparasitism in the present fly mite system establishes parallels between insect and vertebrate hosts in their relationships with ectoparasites (Wakelin, 1978; Murray, 1990; Clayton, 1991; Hart, 1994). In birds, for example, there is also a growing body of research on fitness consequences of ectoparasitism and on the heritability of resistance (e.g. Møller, 1990; Boulinier *et al*., 1997). The available evidence indicates that traits mediating host–ectoparasite associations possess significant evolutionary potential, one critical component of co-evolutionary processes expected to be occurring between host organisms and their natural enemies.

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