

# Direct and correlated responses to selection for larval ethanol tolerance in *Drosophila melanogaster*

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## Abstract

Ethanol is an important larval resource and toxin for natural *Drosophila melanogaster* populations, and ethanol tolerance is genetically variable within and among populations. If ethanol-tolerant genotypes have relatively low fitness in the absence of ethanol, as suggested by the results of an earlier study, genetic variation for ethanol tolerance could be maintained by variation in ethanol levels among breeding sites. I selected for ethanol tolerance in large laboratory populations by maintaining flies on ethanol-supplemented media. After 90 generations, the populations were compared with control populations in egg-to-adult survival and development rate on ethanol-supplemented and unsupplemented food. When compared on ethanol-supplemented food, the ethanol-selected populations had higher survival and faster development than the control populations, but on unsupplemented food, the populations did not differ in either trait. These results give no evidence for a 'trade-off' between the ability to survive and develop rapidly in the presence of ethanol and the ability to do so in its absence. The effect of physiological induction of ethanol tolerance by exposing eggs to ethanol was also investigated; exposing eggs to ethanol strongly increased subsequent larval survival on ethanol-supplemented food, but did not affect survival on regular food, and slowed development on both ethanol-supplemented and regular food, partly by delaying egg hatch.

## Introduction

Genetic variation in traits closely related to fitness, such as fecundity and juvenile survival, is common in natural populations (Mousseau & Roff, 1987; Simms & Rausher, 1989; Via, 1991; Houle, 1992). The persistence of this variation at first seems paradoxical, because one might expect natural selection to eliminate all but a single high-fitness genotype from a population. Nonetheless, there are a number of plausible hypotheses for the maintenance of genetic variation in fitness traits. These include mutation-selection balance (Houle *et al.*, 1996; Charlesworth & Hughes, 1999), antagonistic pleiotropy between different components of fitness (Rose, 1982), frequency-dependent selection (Ayala & Campbell, 1974; Antonovics

& Kareiva, 1988) and environmental heterogeneity (Levene, 1953; Haldane & Jayakar, 1963; Hedrick, 1986; Gillespie & Turelli, 1989).

Indirect evidence that environmental heterogeneity can maintain genetic variation comes from the observation that allowing a population to adapt to one environment often reduces its fitness in another (Ceccarelli, 1989; Hoffmann & Parsons, 1991; Mongold *et al.*, 1996; Bell & Reboud, 1997; Ebert, 1998). For example, in *Drosophila melanogaster*, selection for resistance to a parasitoid wasp resulted in decreased ability of larvae to compete for food (Fellowes *et al.*, 1998, 1999), and culturing populations at 16.5 °C for 5 years resulted in declines in larval viability at 25 °C (Partridge *et al.*, 1994). These sorts of observations suggest that genotypes favoured in some environments are selected against in others, a critical assumption of models for the maintenance of genetic variation by environmental heterogeneity. Nonetheless, another interpretation of the

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observations is possible: fitness of a population in an environment that it does not encounter may erode because of accumulation of deleterious alleles with environment-specific effects. Such alleles might increase in frequency as a result of recurrent mutation (Kawecki *et al.*, 1997), hitch-hiking caused by linkage with advantageous alleles (Peck, 1994) or simply genetic drift (Frankham *et al.*, 1988). Furthermore, even when genotypes with high fitness in one environment initially have low fitness in another (i.e. when a 'trade-off' is initially present), genetic modifiers that mitigate the trade-off may be favoured (McKenzie & Game, 1987; Lenski, 1997), potentially allowing the appearance of a single genotype with maximum fitness in all environments.

The relationship between *D. melanogaster* and environmental ethanol is a promising system to test the role of environmental heterogeneity in maintaining genetic variation for fitness traits. In the wild, *D. melanogaster* larvae can be collected in fermenting fruits with ethanol concentrations up to 7% (McKenzie & McKechnie, 1979; Gibson *et al.*, 1981). Although the species as a whole is comparatively well-adapted to dietary ethanol (David & Van Herrewege, 1983; Merçot *et al.*, 1994), populations respond rapidly to selection for higher ethanol tolerance (David & Bocquet, 1977; Gibson *et al.*, 1979; Oakeshott *et al.*, 1985; Chakir *et al.*, 1996), and mean ethanol tolerance is higher in populations from higher latitudes (David & Bocquet, 1975; Chakir *et al.*, 1996). The factors responsible for maintaining this genetic variation are not known.

A clue that environmental heterogeneity could maintain genetic variation in ethanol tolerance comes from the work of Oakeshott *et al.* (1985). These authors maintained *D. melanogaster* lines from each of eight base populations on each of four ethanol concentrations (0, 3, 6 and 9%). After 30 generations, lines that had been maintained on ethanol-supplemented media had faster development and higher productivity on 9% test medium than did lines that had not previously been exposed to ethanol, indicating that the former lines had adapted to ethanol. Notably, on medium lacking ethanol, the ethanol-selected lines had substantially slower development and lower productivity than the unselected lines, providing evidence that ethanol-tolerant genotypes are at a fitness disadvantage in the absence of ethanol.

Here, I report the results of a new set of selection experiments for larval ethanol tolerance in *D. melanogaster*. This work had three goals. First, I sought to replicate the finding that selecting for ethanol tolerance reduces larval fitness components in the absence of ethanol. To the extent that such declines are reproducible with different base populations and in different laboratories, they are less likely to be caused by chance linkage disequilibrium, genetic drift or other confounding factors. Second, I examined how larval fitness components in the

presence and absence of ethanol changed in populations in which roughly half of the individuals were reared on each medium type each generation. In these 'mixed'-regime populations, alleles that reduce fitness on normal medium without affecting fitness on ethanol-supplemented medium are much less likely to invade, either by genetic drift, hitch-hiking, or recurrent mutation, than in populations maintained only on ethanol-supplemented medium. In addition, if genotypes favoured on ethanol-supplemented food are selected against on normal medium, selection for modifiers that mitigate the trade-off should occur in the mixed populations, possibly allowing these populations to attain the same fitness on normal medium as populations maintained continually on normal medium.

The third goal of this work was to examine how a physiological induction response changed in the populations selected for ethanol tolerance. Exposure of eggs to ethanol has been reported to greatly increase the ability of the resulting larvae to survive on medium containing ethanol (Bijlsma-Meeles, 1979; Kerver & Rotman, 1987). The extent to which ethanol-tolerant genotypes are at a fitness disadvantage in the absence of ethanol depends on the extent to which tolerance is inducible: if the mechanisms responsible for high ethanol tolerance are activated only when an egg or larva comes into contact with ethanol, there is little reason to expect those mechanisms to impose a fitness cost in the absence of ethanol. Therefore, I investigated the effects of exposing eggs to ethanol on subsequent larval fitness in the experimental populations.

## Materials and methods

### Rearing conditions

All flies were reared in 2.5-cm-diameter shell vials at 24–26 °C under continuous light. The medium contained, per litre of water, 77 g cornmeal, 17 g killed brewer's yeast, 8 g agar and 59 mL molasses. To this was added 1.5 g methyl *p*-hydroxybenzoate and 0.3 mL benzyl benzoate, dissolved in 36.5 mL 95% ethanol, as preservatives (this mixture added about 3% ethanol to the food, but as the medium was *ca.* 80 °C when it was added, some of the ethanol probably evaporated immediately). To make ethanol-supplemented medium, the amount of water in the recipe was reduced and 95% ethanol was added to produce the desired concentration after the medium had cooled to 47 °C. Flies were handled under CO<sub>2</sub> anaesthesia.

### Experimental populations

The base population for the selection experiment was derived from 47 isofemale lines that were collected in the vicinity of Raleigh, North Carolina, USA, in 1994. The lines were crossed in a 'round-robin' scheme (line 1

females  $\times$  line 2 males, line 2 females  $\times$  line 3 males, etc.), and equal numbers of progeny from each cross were pooled to form the base population, which was maintained for *ca.* eight generations at a size of several thousand adults. In June 1995, this population was divided into six selection lines, consisting of two replicates of each of three treatments (Fig. 1). The 'R' populations were maintained on normal (regular) medium. The 'E' populations were maintained on ethanol-supplemented medium; the ethanol concentration was 9% for the first 15 generations, 12% from generations 16–30, 10% from generations 31–75 and 12% thereafter. The 'M' populations (for 'mixed') were reared on both medium types; in each generation, half of the adults were placed in vials containing regular food, and half in vials containing ethanol-supplemented food. This treatment was expected to select for ethanol tolerance, but more slowly than in the 'E' populations.

Each population was maintained in 50 vials on 2-week discrete generations. When a new generation of an R or E population was to be set up, all flies emerging from the previous generation's vials were pooled; then 10–15 adults of each sex were introduced into each new vial. When a new generation of an M population was to be set up, flies from the regular food vials and ethanol food vials were pooled separately. The pooled set from regular food usually contained considerably more flies than that from ethanol food; in order to equalize the contribution of the two food types to the next generation (simulating 'soft' selection; Christiansen, 1975), some flies from regular food were discarded until there were approximately

equal numbers of flies in the two groups. The groups were then mixed, and flies from the pooled mixture were placed in 25 new vials of each food type at the same density as above. Flies were allowed to lay eggs for 3 days and then removed.

### Assays of survival and development rate

In order to remove possible maternal effects of the selection environment, flies from each population were reared for two generations on regular food at a density of two pairs per vial before being used to lay eggs for any of the assays described below.

*Initial development rate comparisons.* At generations 49 and 50, the six populations were compared in development rate on both ethanol-supplemented (10%) and regular food. For these comparisons, five pairs of flies were allowed to lay eggs for 5 h in vials containing the appropriate food type. On days 8–14 after the vials were set up, adult progeny were counted and removed at least once per day. On regular food, emergence was complete by day 14; on ethanol food, additional counts were made on days 17 and 19. Average development time of males and females was calculated for each vial. Assays were conducted in two blocks on each food type, with 15 vials per population and block.

*Main experiment.* In the main experiment, conducted between generations 93 and 98, egg-to-adult survival and development time of each of the six populations was measured on each of four ethanol concentrations: regular medium (<3% ethanol), 8, 12 and 16% ethanol. Eggs were collected by allowing flies to oviposit on apple juice-agar medium (Ashburner, 1989; the amount of agar was reduced to 1 g per 100 mL) in the lids of 35 mm plastic Petri dishes. These lids fit neatly into the mouths of standard *Drosophila* half-pint bottles, which were used as the laying chambers. A small smear of yeast paste was placed on the laying medium, and 50–60 adults of each sex were placed in each bottle. Flies were allowed to lay eggs for 3 h. After this time, the yeast was removed by gently rinsing with distilled water.

The primary purpose of the main experiment was to determine whether the E and M populations had higher survival and faster development on ethanol-supplemented food, and lower survival and slower development on regular food, than the R populations. I simultaneously investigated how treating eggs with ethanol affected survival and development rate of the six populations. For this purpose, after the egg-laying period, the discs of apple juice-agar medium were removed from the Petri dishes and cut in half (Fig. 2). One half was placed in a 60-mm Petri dish containing 4 mL of an 18% ethanol solution, and the other half was placed in a similar dish containing a control solution with no ethanol; to avoid

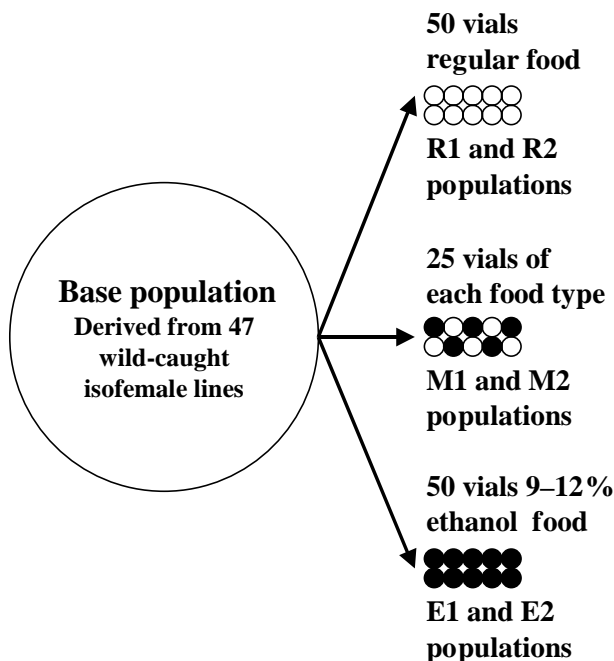


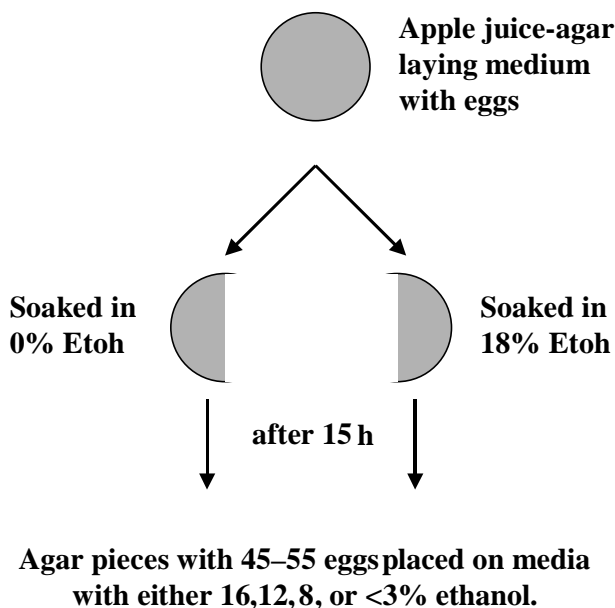
Fig. 1 The three selection regimes; see text for details.

hypotonicity, both solutions contained minerals and tartaric acid in 80% of the concentrations given by Kalmus (1943). Eighteen percent of ethanol was chosen because each half-disc of agar was estimated to contain about 2 mL of liquid; thus the effective ethanol concentration to which the eggs were exposed was approximately 12%, the same concentration as used in the maintenance of the E and M populations. The Petri dishes were sealed with parafilm and incubated for 15–18 h at 22 °C. After this time, the disc halves were rinsed gently with distilled water and blotted dry; small pieces of agar containing 45–55 eggs were then cut from the disc halves and placed in food vials. Emerging adults were counted daily through the 14th day after the vials were set up, by which time emergence had ceased. Analysis variables were the proportion surviving to the adult stage and average development time of males and females.

The main experiment was conducted in eight blocks set up on different weeks, each using genetically independent samples of flies from the populations. Within each block, four vials were set up at each combination of population, egg treatment and ethanol concentration of food medium, for a total of 1509 vials.

#### *Effect of ethanol exposure on development time of eggs.*

The main experiment showed that treating eggs with ethanol increased egg-to-adult development time of all populations, especially that of the R populations. To determine whether this was because of delayed egg hatch



**Fig. 2** Procedure used in the main experiment to quantify the degree of induction of ethanol tolerance as a result of exposing eggs to ethanol; see text for details.

of ethanol-treated eggs, the time required for ethanol-treated and control eggs to hatch was measured, using eggs from the R and E populations. Eggs were collected and treated as above, except that the oviposition period was reduced to 2 h and the eggs were thereafter maintained at 25 °C continuously. After 16 h of incubation in either 0 or 18% ethanol solution, a piece of agar containing 25–150 eggs (usually 50–100) was taken from each disc half. The rate of hatching of eggs on the piece was monitored by counting and removing newly hatched larvae at roughly hourly intervals, until approximately 24 h after the end of the egg-laying period. By this time, over 95% of the eggs that were destined to hatch had hatched, as determined by monitoring a subset of samples for an additional 16 h. The average time until hatching was calculated for each agar piece. The hatching time experiment was conducted in five blocks on different weeks, between generations 105 and 110. Within a block, 4–12 agar pieces were monitored for each combination of population and egg treatment, for a total of 375 pieces.

#### *Survival and development rate under competitive conditions.*

Two additional experiments were conducted to determine if the R and E populations differed in survival and development rate under harsh competitive conditions, in the absence of ethanol. To create harsh conditions, medium containing one-quarter the normal amount of the food ingredients was used, and the populations were reared in competition with a strain bearing the sparkling-poliert eye marker. The competitor strains for the first and second experiments had genetic backgrounds derived predominantly from the R and E populations, respectively. These strains were created by crossing R1 (E1) females to *spa*<sup>pol</sup> males, allowing the F<sub>1</sub> to mate among themselves, collecting F<sub>2</sub> *spa*<sup>pol</sup> males and mating them to R2 (E2) females, allowing the progeny of this cross to mate among themselves, and finally collecting *spa*<sup>pol</sup> virgin females and males to establish the *spa*<sup>pol</sup> stock. Because *spa*<sup>pol</sup> resides on the tiny fourth chromosome, the resulting stocks are expected to have three-quarters of their autosomal genes and five-sixths of their X chromosome genes derived from the R or E populations.

The experiment using the R background competitor was conducted between generations 80 and 84, and used somewhat different methods than any of the other experiments. The dilute food was poured into 35 mm Petri dishes instead of vials, and 75 larvae from each of the competitor strain and an R or E population were placed in each dish. The 35 mm dishes were taped to the bottoms of 100 × 20 mm Petri dishes, which were taped shut. Most larvae pupated on the sides of the small dish or the bottom of the large dish. Adults were counted and removed on days 10, 12, 14, 16 and 19. There were four blocks, with 12–13 dishes per population per block (total 50 dishes per population).

The experiment using the E background competitor was conducted at generations 100 and 101. Vials were used instead of dishes. Eggs were collected on apple-juice laying medium, and agar pieces containing 35 eggs of the competitor and 35 eggs from an R or E population were placed in each vial. (A pilot experiment using 75 eggs each resulted in very low adult emergence; although the volume of dilute food in the vials was similar to that in the dishes of the first experiment, the greater food surface area in the dishes apparently allowed higher survival). Emerging adults were counted daily until day 19. There were three blocks, with 12 vials per population and block.

Analysis variables in the two experiments were the proportion of wild-type (R or E population) flies among the emerging flies, and the average time to develop of the wild-type flies, with sexes not distinguished.

*Statistical analysis.* All experiments were analysed using the 'Mixed' procedure in SAS version 8 (Littell *et al.*, 1996). Except for the initial assays of development rate, in which there were only two blocks, analyses were performed on the block means of each population-treatment combination. In addition to simplifying and balancing the analyses, this resulted in more normally distributed values. Proportions surviving in the main experiment were angular-transformed (before averaging) in order to remove the dependence of the variance on the mean (Larsen & Marx, 1981); means displayed little variation in the other experiments, so transformation was unnecessary. Selection regime (R, E and M), food type (when applicable) and egg treatment (when applicable) were treated as fixed effects, and population within regime and block were treated as random. All possible interactions between these factors were included in the models; interactions involving only fixed main effects were treated as fixed whereas all others were treated as random. In the initial assays of development rate, progeny density was not controlled, and development time increased with increasing numbers of progeny per vial, probably because of crowding. Progeny number was therefore used as a (fixed) covariate; further details are given below.

The 'Mixed' procedure produces variance component estimates and standard errors (SEs) for the random effects, and *F*-tests for the fixed effects. Satterthwaite approximate *F*-tests were obtained using the 'ddfm = Satterth' option. With this option, SAS calculates appropriate denominator mean squares after dropping from the model random effects that explain zero variance; thus, denominator d.f. can vary considerably between different datasets with identical designs, depending on which random effects were dropped. When the effect of selection regime in the main experiment was significant, single d.f. contrasts were performed to determine which selection regimes differed. Variance component estimates and significance tests for the random

effects are not reported in most cases; these effects are usually of little biological interest, and variation caused by them is accounted for in the tests for the fixed effects. In the few cases where random effects were of interest, significance tests were obtained by re-running the analysis with the random effect deleted; under the null hypothesis, twice the difference in log-likelihoods between the models with and without the effect should have a  $\chi^2$  distribution with one d.f. (Littell *et al.*, 1996).

## Results

### Initial development rate comparisons

In the initial development rate comparisons at generation 50, progeny density was not controlled, and there were significant linear and quadratic effects of the number of progeny per vial on development time of both males and females (Table 1). On food supplemented with 10% ethanol, there was significant variation among the three selection regimes in both the intercepts and the linear terms (Table 1A). On regular food, in contrast, there was no significant variation among regimes in either intercepts or linear terms (Table 1B). Adding a fixed interaction between (no. progeny)<sup>2</sup> and selection regime did not significantly improve the fit of the model in any case ( $P > 0.1$ ). On both food types, the random effect of population within regime was not significant in either sex ( $P > 0.25$ ). In addition, on both food types, mean progeny numbers did not differ among regimes ( $P > 0.8$ ), or among populations within regimes ( $P > 0.1$ ).

The estimated relationships between progeny number and development time are shown in Fig. 3. In most cases, development time increased with increasing progeny number, suggesting that development was delayed in the more crowded vials. Nonetheless, on ethanol food, E population flies took less long to develop than flies from the other regimes over the entire range of observed progeny numbers (Fig. 3A & B). R population flies took the longest to develop and development time of M population flies was intermediate. In contrast, on regular food, although development time increased sharply with density, the curves for the three regimes are similar (Fig. 3C & D).

These results indicate that by generation 50, selection had increased development rate of the E and M populations on ethanol-supplemented food, without affecting their development rate on regular food. Because of the strong density effects observed in these assays, the number of eggs per vial was controlled in subsequent assays.

### Main experiment

In this experiment, egg-to-adult survival and development time was measured on media containing 16, 12, 8 and <3% ethanol; before being placed in food vials, eggs

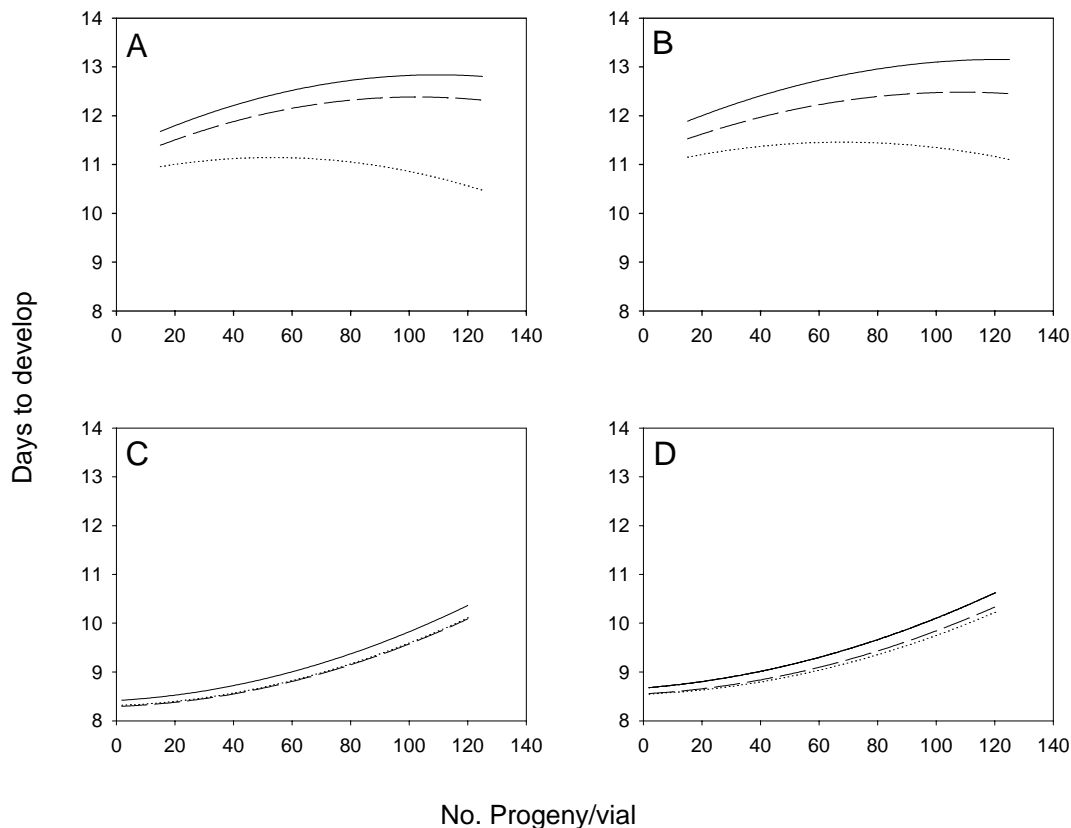
**Table 1** Results of the initial (generations 49–50) development time comparisons. Tests for fixed effects are shown; random effects were block, populations within regimes, block × regime, and block × population (regime). Progeny numbers were expressed as deviations from the mean of all populations.

Effect	Females			Males		
	F	d.f.	P	F	d.f.	P
A. Tested on 10% ethanol food						
Selection regime	16.5	2, 2.27	0.044	14.6	2, 2.35	0.047
No. progeny per vial	151.7	1, 95.6	<0.001	120.0	1, 117	<0.001
(No. progeny) <sup>2</sup>	4.23	1, 171	0.041	3.05	1, 171	0.083
No. progeny × regime	6.77	2, 109	0.002	3.70	2, 129	0.027
B. Tested on regular food						
Selection regime	2.07	2, 2.68	0.286	2.18	2, 2.61	0.278
No. progeny per vial	3.25	1, 159	0.073	4.56	1, 145	0.034
(No. progeny) <sup>2</sup>	10.8	1, 172	0.001	6.30	1, 168	0.013
No. progeny × regime	0.20	2, 167	0.819	0.31	2, 51.3	0.735

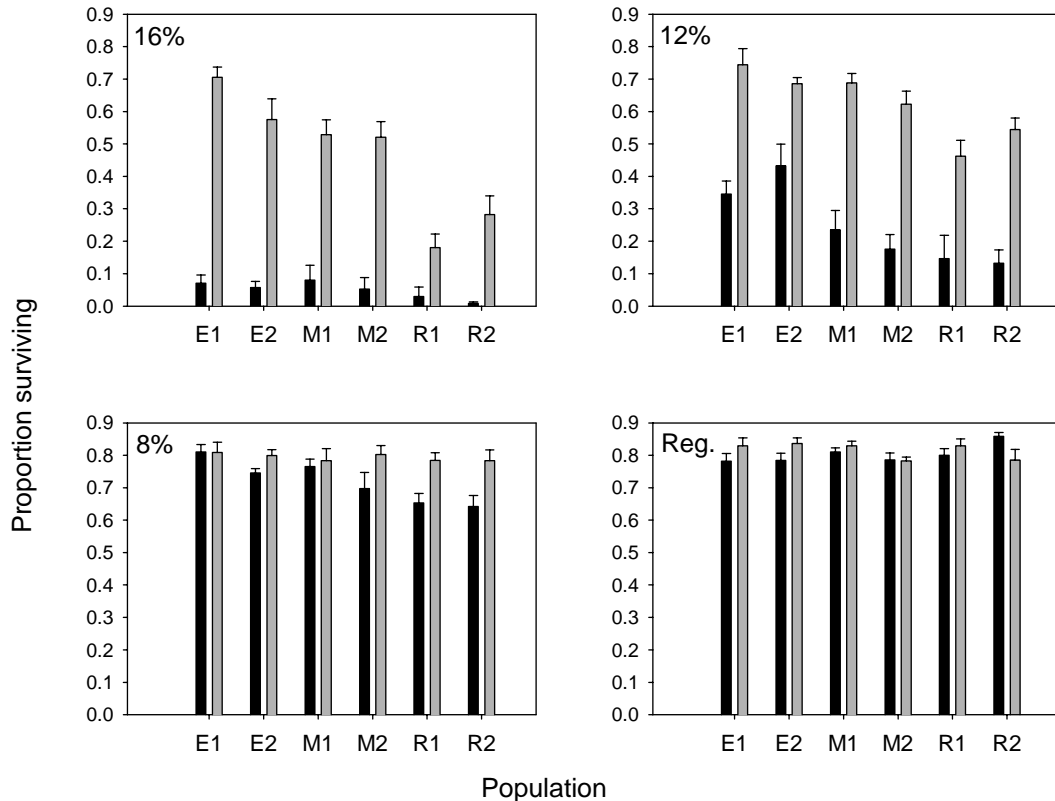
were treated with solutions containing either 0 or 18% ethanol. Results for survival, development time of females, and development time of males are shown in Figs 4–6, respectively.

In the analysis of all four food types together, there were significant main effects of food type, selection

regime and egg treatment on each of the three traits, and the food by regime interaction was significant or nearly so in each case (Table 2). The food by egg treatment interaction was significant for viability but not for development time whereas the regime by egg treatment interactions were significant for development time but



**Fig. 3** Development time in the initial assays at generations 49–50. Lines show predicted values from quadratic regressions of average development time in a vial vs. number of flies emerging from the vial. Solid lines, dashes and dots are for the R, M and E populations, respectively. Curves for each regime are based on the slopes and intercepts for that regime, but share a common quadratic term because the latter did not differ among regimes. Curves extend over the range of progeny numbers observed on each food type. A: females, 10% ethanol food; B: males, 10% ethanol; C: females, regular food; D: males, regular food.



**Fig. 4** Egg-to-adult survival on ethanol-supplemented (16, 12 and 8%) and regular food in the main experiment. Black bars: survival of eggs treated with 0% ethanol; grey bars: survival of eggs treated with 18% ethanol. Bars give SEs among block means.

not viability. The three way interaction was significant only for viability (Table 2).

Turning to analyses of data from each food type separately, treating eggs with ethanol significantly increased survival on all three concentrations of ethanol-supplemented medium (Table 3); on 16 and 12% ethanol food, the effect was substantial (Fig. 4). Selection regime significantly influenced survival on 16 and 12% ethanol, and had a nearly significant effect on 8% ethanol. In single d.f. contrasts, the E populations had significantly higher survival ( $P < 0.05$ ) than the R populations at all three concentrations and had significantly higher survival than the M populations on 12% ethanol. The M populations had significantly higher survival than the R populations on both 16 and 12% ethanol. The interaction between selection regime and egg treatment approached significance on 16 and 8% ethanol (Table 3).

In contrast to its beneficial effect on survival on ethanol-supplemented food, treating eggs with ethanol delayed development on 12 and 8% ethanol food (Figs 5 & 6, Table 3; there were too few survivors on 16% ethanol for analysis of development time). On 12 and 8% ethanol, there were significant effects of selection regime on development time of both males and females. In each case, the E and M populations developed significantly

faster than the R populations, but did not differ significantly from each other. There were no significant interactions between selection regime and egg treatment for development time on 12 and 8% ethanol, although the interaction approached significance in one case (Table 3).

In contrast to the results on ethanol-supplemented food, there was no significant effect of either selection regime or egg treatment on survival on regular food, nor was the interaction between the two significant (Table 3, Fig. 4). The main effects of selection regime on development time on regular food were not significant in either females or males, but there were significant ( $P < 0.01$ ) interactions between selection regime and egg treatment (Table 3). Ethanol treatment of eggs tended to delay development on regular food (as on ethanol food), but did so more for the R populations than for the other populations (Figs 5 & 6); mean delays were 0.05–0.14 day in the E and M populations, compared with 0.36–0.42 day in the R populations. Considering only eggs not treated with ethanol, there were no significant effects of selection regime on development time of either females ( $F_{2,3} = 0.34$ ,  $P = 0.73$ ) or males ( $F_{2,3} = 0.64$ ,  $P = 0.59$ ) on regular food. These results give no evidence that the E and M populations had lower

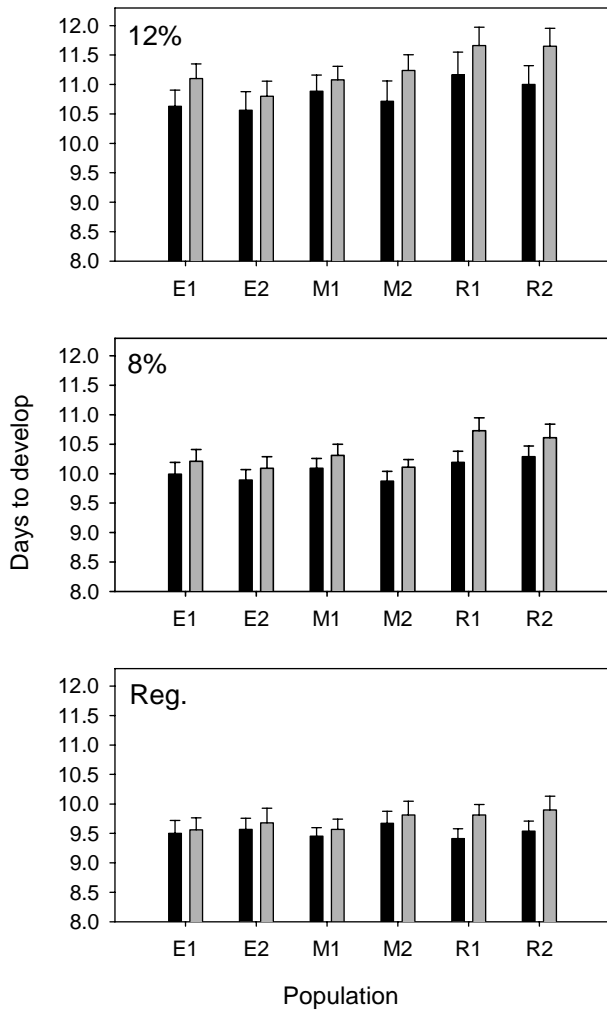


Fig. 5 Egg-to-adult development time of females in the main experiment. See legend to Fig. 4.

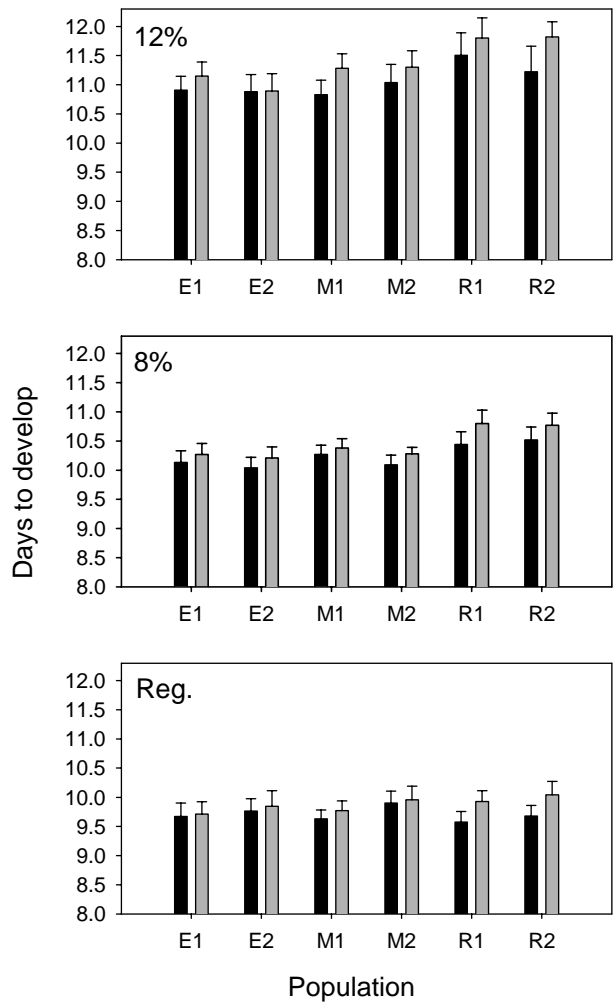


Fig. 6 Egg-to-adult development time of males in the main experiment. See legend to Fig. 4.

**Table 2** Results of *F*-tests for the effects of rearing food (regular medium, 8, 12 or 16% ethanol), selection regime (E, M or R), egg treatment (0 or 18% ethanol) and their interactions on egg-to-adult survival and development time in the main experiment. Random effects were block, populations within regimes, interactions of block with each of the seven fixed effects in the table and interaction of population with each fixed effect except the *F* × *R* × *E* interaction. Development time on 16% ethanol was not analysed as a result of the low numbers of surviving flies.

Effect	Development time								
	Survival			Females			Males		
	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>
Food type ( <i>F</i> )	149.7	3, 23.7	<0.001	20.1	2, 14.6	<0.001	20.7	2, 14	<0.001
Selection regime ( <i>R</i> )	25.4	2, 3	0.013	16.8	2, 11.6	<0.001	22.5	2, 12	<0.001
Egg treatment ( <i>E</i> )	195.6	1, 8.9	<0.001	22.6	1, 6.8	0.002	13.1	1, 7.0	0.009
<i>F</i> × <i>R</i>	13.5	6, 20.3	<0.001	3.31	4, 9.5	0.057	6.08	4, 9.1	0.012
<i>F</i> × <i>E</i>	63.3	3, 23.1	<0.001	2.60	2, 9.4	0.126	1.17	2, 14	0.338
<i>R</i> × <i>E</i>	1.62	2, 19.3	0.224	4.32	2, 10.5	0.043	4.59	2, 19.6	0.023
<i>F</i> × <i>R</i> × <i>E</i>	7.23	6, 19.3	<0.001	0.05	4, 8.2	0.994	0.91	4, 98.8	0.461



**Table 3** Analysis of fixed effects in the main experiment, by food type. d.f.<sub>DEN</sub> = denominator degrees of freedom. Numerator degrees of freedom were 2, 1 and 2, for the three respective effects. Random effects were block, populations within regimes, interactions of block with each of the three fixed effects and interactions of population with the fixed main effects.

Trait	Test ethanol conc. (%)	Selection regime			Egg treatment			Regime × egg treatment		
		F	d.f. <sub>DEN</sub>	P	F	d.f. <sub>DEN</sub>	P	F	d.f. <sub>DEN</sub>	P
Survival										
	16	27.9	3	0.012	357.7	3	<0.001	9.03	3	0.054
	12	22.2	7.38	<0.001	72.2	12.3	<0.001	2.84	4.82	0.153
	8	4.00	8.77	0.058	7.18	8.14	0.028	4.32	5.27	0.078
	Reg. food	0.71	6	0.527	1.04	6	0.347	2.00	6	0.215
Development time										
Females										
	12	12.0	6.29	0.007	22.0	6.25	0.003	0.73	6.25	0.517
	8	11.3	3	0.040	8.80	7	0.021	2.93	38	0.066
	Reg. food	0.29	3.44	0.765	6.55	7	0.038	7.03	17.4	0.006
Males										
	12	13.8	14.2	<0.001	16.8	13.2	0.001	1.93	13.1	0.185
	8	19.7	30.2	<0.001	5.36	7	0.054	1.48	17.8	0.255
	Reg. food	0.19	3.37	0.835	3.88	7	0.090	6.36	16.9	0.009

larval fitness on regular food than the R populations; thus, there is no evidence for a 'trade-off' between fitness in the presence and absence of ethanol.

The extent to which the replicate populations differed in survival or development rate gives information on the extent to which genetic drift may have influenced the results of the selection experiment. In no case was the variance component because of populations within regimes significantly different from zero at the 0.05 level (Table 4), although the variance approached significance in two cases (development time of males and females on regular food). The interaction between replicate population and egg treatment was always nonsignificant (Table 4). These results indicate that there was relatively little divergence between the replicate populations.

#### Effect of ethanol exposure on development time of eggs

The main experiment indicated that treating eggs with ethanol delayed egg-to-adult development and did so more in the R populations than in the other populations; this accounts for the significant regime by egg treatment interactions in the overall analysis (Table 2). I hypothesized that exposure to ethanol delayed egg hatch, and that the E and M populations had partly counteradapted to this adverse effect of ethanol. To test these possibilities, I measured hatching time of both ethanol-treated and control eggs from the R and E populations (Fig. 7). Exposure to ethanol significantly delayed egg hatch ( $F_{1,4.18} = 34.31$ ,  $P = 0.0037$ ), but there was no effect of selection regime on hatching time ( $F_{1,2} = 0.05$ ,  $P = 0.85$ ), nor was the egg treatment × regime interaction significant ( $F_{1,2} = 7.63$ ,  $P = 0.11$ ). The average hatching delay of 84 min accounts for a substantial proportion of the delays in egg-to-adult development on

**Table 4** Variance components because of replicate populations within selection regimes and the population by egg treatment interaction in the main experiment. Variance components and SEs have been multiplied by 1000.

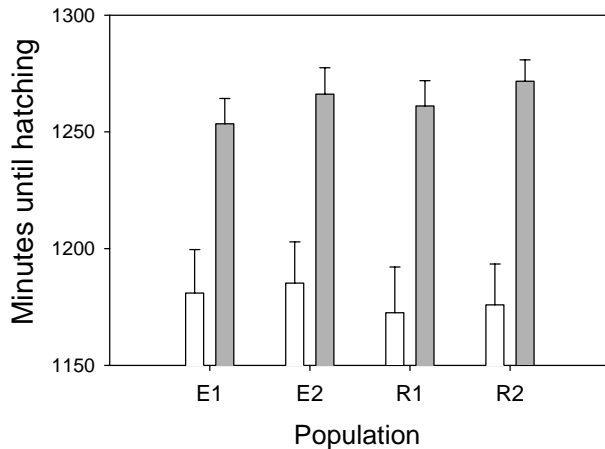
Trait	Test ethanol conc. (%)	Variance component (SE)	
		Population (regime) × egg treatment	Population (regime)
Survival			
	16	0.28 (1.98)	1.78 (2.58)
	12	0	1.43 (2.05)
	8	0	0.54 (0.71)
	Reg. food	0	0.44 (0.64)
Development time			
Females			
	12	0	1.22 (11.86)
	8	3.98 (7.47)	0
	Reg. food	8.29* (10.14)	0
Males			
	12	0	0
	8	0	0
	Reg. food	7.90* (10.58)	0

\*  $0.05 < P < 0.10$ . All others  $P > 0.1$ .

regular food observed when E and M population eggs were treated with ethanol (Figs 5 & 6; average delay of 0.09 day, or 130 min), but accounts for only a small proportion of the delay experienced by R population eggs (average 0.37 day, or 530 min). These results indicate that the egg treatment × regime interactions for egg-to-adult development time on regular food cannot be explained by a similar interaction for hatching time.

#### Competition experiments

When competed on dilute food against a marked competitor strain with genetic background from the R



**Fig. 7** Amount of time required for embryonic development in the E and R populations. See legend to Fig. 4.

populations, the E populations appeared to do marginally better than the R populations, giving rise to higher proportions of wild-type flies, and developing faster by an average of about 1 day (Table 5). The effect of selection regime was not quite significant for either trait, however. When a competitor strain with genetic background from the E populations was used, the R populations gave rise to slightly higher proportions of wild-type flies (Table 5), although again the effect of selection regime was not significant. Development time of the E and R populations was similar in this case.

## Discussion

### Adaptation to ethanol, and absence of evidence for trade-offs

I observed adaptation to ethanol in four large laboratory populations that were maintained wholly (E1 and E2) or partly (M1 and M2) on ethanol-supplemented food for *ca.* 100 generations: when tested on ethanol-supplemented food, these populations had higher egg-to-adult survival and faster development rate than populations (R1 and R2) which had not previously been exposed to

ethanol. In addition, the E populations had higher survival on ethanol food than the M populations, showing that rearing 50% of the flies on regular food each generation significantly slowed the selection response of the latter populations.

In spite of the clear superiority of the E and M populations on ethanol-supplemented food, I found no evidence that these populations were inferior to the R populations in survival or development rate on regular food. In contrast, when Oakeshott *et al.* (1985) selected eight different base populations for tolerance of 9% ethanol food for 30 generations, the selected lines took an average of a day longer to develop on regular food than the unselected lines. Oakeshott *et al.* used a different method for measuring development time, and it is possible that this, as well as the different base populations used, caused the difference between their results and mine. A possibility that needs to be seriously considered, however, is that the declines in development rate on regular food that Oakeshott *et al.* observed were the result of inbreeding depression. Each generation of each selection line was initiated with approximately 40 adults of each sex; even if one generously assumes that effective population size was 75% of actual population size (Frankham, 1995), the inbreeding coefficient after 30 generations would have been 0.22, about the same as for a full-sib mating. Furthermore, inbreeding is likely to have been more severe in the ethanol-selected lines because strong selection reduces effective population size (Robertson, 1961). Although development rate has received relatively little attention in studies of inbreeding depression in *Drosophila*, Roper *et al.* (1993) showed that inbreeding can depress development rate in selected populations. Inbreeding depression does not appear to have had a substantial effect on my results; when I crossed the replicate E and R populations, the hybrids had similar development rate on regular food as the pure populations (J. D. Fry, unpublished data).

An alternative explanation as to why I did not observe trade-offs is that modifiers were selected for in the E and M populations that mitigated initial negative effects of alleles conferring higher ethanol tolerance. Such modi-

**Table 5** Survival and development time on dilute food in competition with strains bearing the *spa*<sup>pol</sup> marker. Results of *F*-tests for the effect of selection regime are given; d.f.<sub>DEN</sub> = denominator degrees of freedom (numerator d.f. = 1). SEs are among block means.

Population	R population competitor strain (SE)		E population competitor strain (SE)	
	Proportion wild-type*	Days to develop	Proportion wild-type*	Days to develop
E1	0.50 (0.020)	13.6 (0.16)	0.49 (0.025)	12.8 (0.39)
E2	0.52 (0.016)	13.3 (0.19)	0.48 (0.054)	12.6 (0.20)
R1	0.46 (0.029)	14.1 (0.32)	0.52 (0.013)	12.5 (0.25)
R2	0.47 (0.024)	14.7 (0.24)	0.51 (0.021)	13.0 (0.31)
<i>F</i>	5.37	6.15	1.48	0.00
d.f. <sub>DEN</sub>	3.64	3.02	8	2
<i>P</i>	0.088	0.089	0.26	0.96

\* (No. of wild-type flies)/(total no. of flies emerging).

fiers would have to have been in high frequency by generation 50, by which time the E and M populations were similar to the R populations in development time on regular food (indeed, the slight tendency at this time was for slower development in the R populations; Fig. 3). There is some reason to doubt the ubiquity of modifiers that can ameliorate negative effects of genes favoured under stressful regimes, however. In spite of the clear example of a modifier of the fitness effects of an organophosphate resistance gene in the Australian sheep blowfly (McKenzie & Game, 1987; Davies *et al.*, 1996), similar resistance modifiers in other insect species do not appear to be common (Roush & McKenzie, 1987). Perhaps more relevant to the current experiment, in at least four experiments with *Drosophila*, apparent negative pleiotropic effects of adaptation to stressful regimes have persisted in experiments lasting about the same number of generations as the current experiment. Selecting for accelerated development for 125 generations resulted in a 10% reduction of larval viability (Chippindale *et al.*, 1997); maintaining populations at 16.5 °C for 5 years resulted in lower survival at 25 °C (Partridge *et al.*, 1994), selecting for large body size for 49 generations reduced larval competitive ability (Partridge & Fowler, 1993); maintaining populations under severe crowding for 50 generations resulted in lower survival to pupation under food-limited conditions (Joshi & Mueller, 1996). In all of these cases, inbreeding depression was either ruled out or does not appear to have been likely as a cause of the declines.

Like those of Oakeshott *et al.* (1985), my ethanol-selected populations did not have lower survival on regular food under uncrowded conditions than the nonselected populations. This is not surprising because survival was uniformly high (*ca.* 80%) in both studies, indicating that uncrowded regular food is a benign environment. Because trade-offs are sometimes easier to detect under harsh conditions than under benign conditions (e.g. Partridge & Fowler, 1993), I also tested the E and R populations for survival and development rate on dilute regular food under competitive conditions. Although the differences were not quite significant, the E populations appeared to do better than the R populations by both measures when the competitor strain was related to the R populations. In contrast, when the competitor strain was related to the E populations there was no difference in development rate, but the R populations may have had slightly higher survival than the E populations, although this difference was also nonsignificant. These results therefore suggest the possibility that each population did worse in competition with the marker stock more closely related to itself than in competition with the less closely related stock. Numerous studies have documented such negative frequency-dependent selection for viability in *Drosophila* (reviewed in Ayala & Campbell, 1974; Antonovics & Kareiva, 1988). The most important conclusion from the competitive

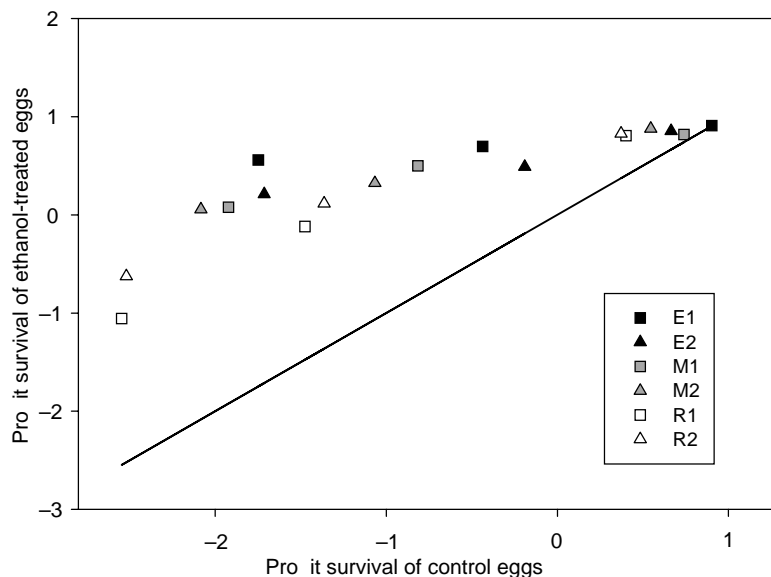
assays, however, is that adaptation to ethanol did not lower the *general* competitive ability of the E populations.

### Induction of ethanol tolerance by exposure of eggs to ethanol

My results confirm that ethanol tolerance in *D. melanogaster* larvae is inducible: larvae that hatch from eggs that develop in contact with ethanol have higher survival on ethanol-supplemented food than those hatching from eggs not exposed to ethanol (Bijlsma-Meeles, 1979; Kerver & Rotman, 1987; Bijlsma & Bijlsma-Meeles, 1991). The inducibility of ethanol tolerance provides a potential mechanism by which ethanol-tolerant genotypes could avoid having low fitness in the absence of ethanol: even if the mechanisms responsible for high ethanol tolerance are costly in the absence of ethanol, the costs will be realized only to the extent that the mechanisms are activated constitutively. If I had observed no difference between the populations in ethanol tolerance of larvae hatching from eggs not exposed to ethanol, there would have been little reason to expect larvae from the E and M populations to have had lower survival or slower development in the absence of ethanol than larvae from the R populations. Such differences were observed, however, both for survival (Fig. 4) and development rate (Figs 5 & 6), indicating that ethanol tolerance in the E and M populations was to some extent constitutive.

My results give some information, albeit highly indirect, on whether activation of mechanisms necessary for high ethanol tolerance would reduce fitness when ethanol is absent. Exposing eggs to ethanol did not lower the subsequent survival of larvae on regular food, giving no evidence for such a cost. However, ethanol treatment of eggs slowed egg-to-adult development on both regular and ethanol-supplemented food, partly by increasing the amount of time it took eggs to hatch. It is possible that the delays in development rate caused by treating eggs with ethanol reflect a cost of induction; alternatively, the delays may simply have resulted from toxic effects of ethanol. Some evidence argues against the former explanation. If the slowing of development rate reflected a cost of induction, then those populations showing greater induction of tolerance would be expected to show greater slowing of development rate when eggs are treated with ethanol. Contrary to this prediction, ethanol slowed the embryonic development period of the E and R populations to a similar extent (Fig. 7) but ethanol tolerance appeared to be more strongly inducible in the E populations than in the R populations. This can be seen from the following comparison (Fig. 4): on 16% ethanol, ethanol treatment of eggs increased survival of the E populations from 0.06 to 0.64, whereas on 12% ethanol, ethanol treatment of eggs increased survival of the R populations from 0.14 to only 0.50. The degree of induction of tolerance in the different populations can also be visualized in a scatter plot of survival of ethanol-

**Fig. 8** Proportion surviving on 16% (six leftmost points), 12% (six middle points) and 8% ethanol (six rightmost points) in the E, M and R populations in the main experiment. The diagonal line denotes equal survival of ethanol-treated and control eggs; the height of a point above the line therefore reflects the degree of induction of ethanol tolerance. The probit transformation was used because it produces approximately linear relationships within each of the populations.



treated eggs on the ordinate vs. survival of control eggs on the abscissa (Fig. 8); for a given survival of control eggs, the distance of a point from the diagonal line reflects the degree to which survival is improved by treating eggs with ethanol. For most of the plot, the points from the R populations lie below those from the other populations (Fig. 8).

One might have predicted that the M populations, in which only half the individuals encounter ethanol each generation, would show a greater degree of inducibility of ethanol tolerance than the E populations, in which every individual encounters ethanol. In the E populations, constitutive tolerance should serve as well as inducible tolerance; in fact, if there is a cost to maintaining mechanisms for responding to ethanol concentration (a.k.a., a cost of phenotypic plasticity; Van Tienderen, 1991), constitutive tolerance might be favoured over inducible tolerance in the E populations. Figure 8 shows, however, that inducibility of tolerance in the M populations was not greater than in the E populations. It is possible that not enough time had elapsed for such a difference to emerge; I plan to retest the populations after another *ca.* 100 generations of selection to check this possibility. It is also possible, of course, that no cost of phenotypic plasticity exists in this system.

On regular food, the development delay of the R populations caused by treating eggs with ethanol was greater than in the E and M populations, and much greater than can be accounted for by the effect of ethanol exposure on egg development. The simplest explanation for this is that the R population larvae were affected by residual ethanol in the agar pieces that were used to transfer eggs to the regular food vials; although the agar was rinsed with distilled water after being soaked in ethanol, some ethanol undoubtedly remained.

In addition to causing high larval mortality, ethanol in the food delays egg-to-adult development considerably. The fact that exposing eggs to ethanol alleviates the first effect but not the second suggests that the lowering of survival and slowing of development may arise from different causes. In support of this view, it appeared that the majority of mortality on ethanol-supplemented food took place within 24 h after the eggs had hatched (J.D. Fry, unpublished observations). The slowing of development, in contrast, seems more likely to have resulted from the effects of feeding on ethanol-supplemented food throughout larval development, rather than being caused only by exposure to ethanol within the first 24 h. Late in larval development, it is likely that most of the ethanol in the medium had been converted to acetic acid by microbial activity (Hageman *et al.*, 1990); therefore it is possible that the slowing of development was partly caused by acetic acid buildup.

The mechanism of the induction of ethanol tolerance is not fully understood. Exposing eggs to ethanol causes an increase in alcohol dehydrogenase activity (Bijlsma-Meeles, 1979; Kerver & Rotman, 1987) but increases the ethanol tolerance of *Adh* null strains as well (Bijlsma & Bijlsma-Meeles, 1991). It is possible that other enzymes involved in ethanol degradation (Geer *et al.*, 1993) are induced by exposing eggs to ethanol. In addition, ethanol induces production of stress (heat-shock) proteins in a variety of organisms (Li & Hahn, 1978; Michel & Starka, 1987; Feder *et al.*, 1995), and this response has been associated with increased ethanol resistance in a bacterium (Michel & Starka, 1987) and yeast (Feder *et al.*, 1995).

The inducibility of ethanol tolerance explains some initially puzzling observations I made. Flies from the R populations produce large numbers of adult progeny when allowed to lay eggs in vials containing 10–12%

ethanol (J.D. Fry, unpublished data). For example, in the initial development rate comparisons on 10% ethanol, in which five females were allowed to lay eggs for 5 h, the mean number of progeny was about 60, and there were no significant differences among the selection regimes in progeny number. At about the same time, however, preliminary comparisons of larval survival were made by transferring larvae to vials with 10% ethanol food. Survival of larvae from the R populations was extremely low, only about 15%, compared with *ca.* 50% survival of larvae from the E populations (J.D. Fry, unpublished data). This difference in results makes sense in light of the induction effect: the eggs laid in ethanol food developed in contact with ethanol whereas the larvae transferred in the survival experiment had not been previously exposed to ethanol. The induction response appears critical for allowing laboratory populations to thrive on medium with 10% or more ethanol. It is notable that even after nearly 100 generations of selection, survival of noninduced eggs from the E populations on 12 and 16% ethanol was lower than that of induced eggs from the R populations (Fig. 4).

## Conclusion

Given that ethanol appears to be an important selective agent in *D. melanogaster* populations (Merçot *et al.*, 1994; Ashburner, 1998), it is not clear why genetic variation for ethanol tolerance is prevalent. The results reported here suggest there is no simple trade-off between the ability to survive and develop rapidly in the presence of ethanol, and the ability to do so in its absence. I have not examined adult fitness traits, so it remains possible that ethanol-tolerant genotypes are at a disadvantage in terms of fecundity, mating success or longevity in the absence of ethanol (cf. Oakeshott *et al.*'s (1985) finding that ethanol-adapted populations had lower productivity on regular food). Another possibility is that selection against ethanol-tolerant genotypes involves some feature of the natural environment that would be difficult to reproduce in the laboratory. For example, Eanes (1999) suggests that the cline in *Adh*-allele frequencies, which accounts for some but not all of the cline in ethanol tolerance (Chakir *et al.*, 1996), may be driven by selection for higher lipid content in high-latitude populations. Much ethanol consumed by both larvae and adults is used in lipid synthesis, and the higher activity *Adh-Fast* allele results in greater lipid synthesis in larvae (Freriksen *et al.*, 1991). The implication of Eanes' hypothesis is that in equatorial populations, flies possessing the fast allele may accumulate more lipid than is optimal for their environment; such selection on lipid levels might be difficult to reproduce in a laboratory setting. Whether correct or not, Eanes' hypothesis illustrates that understanding the maintenance of genetic variation in ethanol tolerance is likely to require identifying the genes involved and characterizing both their physiological and ecological effects.

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## References

- Antonovics, J. & Kareiva, P. 1988. Frequency-dependent selection and competition: empirical approaches. *Phil. Trans. R. Soc. Lond. B* **319**: 601–613.
- Ashburner, M. 1989. *Drosophila: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Ashburner, M. 1998. Speculations on the subject of alcohol dehydrogenase and its properties in *Drosophila* and other flies. *Bioessays* **20**: 949–954.
- Ayala, F.J. & Campbell, C.A. 1974. Frequency-dependent selection. *Annu. Rev. Ecol. Syst.* **5**: 115–138.
- Bell, G. & Reboud, X. 1997. Experimental evolution in *Chlamydomonas* II: genetic variation in strongly contrasted environments. *Heredity* **78**: 498–506.
- Bijlsma-Meeles, E. 1979. Viability in *Drosophila melanogaster* in relation to age and ADH activity of eggs transferred to ethanol food. *Heredity* **42**: 79–89.
- Bijlsma, R. & Bijlsma-Meeles, E. 1991. Induction of tolerance for ethanol in eggs of *Adh* null mutants of *Drosophila melanogaster*. *Dros. Inf. Serv.* **70**: 35–36.
- Ceccarelli, S. 1989. Wide adaptation: how wide? *Euphytica* **40**: 197–205.
- Chakir, M., Capy, P., Genermont, J., Pla, E. & David, J.R. 1996. Adaptation to fermenting resources in *Drosophila melanogaster*: ethanol and acetic acid tolerances share a common genetic basis. *Evolution* **50**: 767–776.
- Charlesworth, B. & Hughes, K.A. 1999. The maintenance of genetic variation in life history traits. In: *Evolutionary Genetics from Molecules to Morphology* (R. S. Singh & C. B. Krimbas, eds), pp. 369–392. Cambridge University Press, Cambridge, UK.
- Chippindale, A.K., Alipaz, J.A., Chen, H.-W. & Rose, M.R. 1997. Experimental evolution of accelerated development in *Drosophila*. 1. Developmental speed and larval survival. *Evolution* **51**: 1536–1551.
- Christiansen, F.B. 1975. Hard and soft selection in a subdivided population. *Am. Nat.* **109**: 11–16.
- David, J.R. & Bocquet, C. 1975. Similarities and differences in latitudinal adaptation of two *Drosophila* sibling species. *Nature* **257**: 588–590.
- David, J.R. & Bocquet, C. 1977. Genetic tolerance to ethanol in *Drosophila melanogaster*: increase by selection and analysis of correlated responses. *Genetica* **47**: 43–48.
- David, J.R. & Van Herrewege, J. 1983. Adaptation to alcoholic fermentation in *Drosophila* species: relationship between alcohol tolerance and larval habitat. *Comp. Biochem. Physiol. A* **74**: 283–288.
- Davies, A.G., Game, A.Y., Chen, Z., Williams, T.J., Goodall, S., Yen, J.L., McKenzie, J.A. & Batterham, P. 1996. Scalloped wings is the *Lucilia cuprina* Notch homologue and a candidate for the modifier of fitness and asymmetry of diazinon resistance. *Genetics* **143**: 1321–1337.

- Eanes, W.F. 1999. Analysis of selection on enzyme polymorphisms. *Annu. Rev. Ecol. Syst.* **30**: 301–326.
- Ebert, D. 1998. Experimental evolution of parasites. *Science* **282**: 1432–1435.
- Feder, M.E., Parsell, D.A. & Lindquist, S.L. 1995. The stress response and stress proteins. In: *Cell Biology of Trauma* (J. L. Lemasters & C. Oliver, eds), pp. 177–191. CRC, Boca Raton, FL.
- Fellowes, M.D.E., Kraaijeveld, A.R. & Godfray, H.C.J. 1998. Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* **265**: 1553–1558.
- Fellowes, M.D.E., Kraaijeveld, A.R. & Godfray, H.C.J. 1999. Association between feeding rate and parasitoid resistance in *Drosophila melanogaster*. *Evolution* **53**: 1302–1305.
- Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. *Genet. Res.* **66**: 95–107.
- Frankham, R., Yoo, B.H. & Sheldon, B.L. 1988. Reproductive fitness and artificial selection in animal breeding: culling on fitness prevents a decline in reproductive fitness in lines of *Drosophila melanogaster* selected for increased inebriation time. *Theor. Appl. Genet.* **76**: 909–914.
- Freriksen, A., Seykens, D., Scharloo, W. & Heinstra, P.W.H. 1991. Alcohol dehydrogenase controls the flux from ethanol into lipids in *Drosophila* larvae. *J. Biol. Chem.* **266**: 21399–21403.
- Geer, B.W., Heinstra, P.W.H. & McKechnie, S.W. 1993. The biological basis of ethanol tolerance in *Drosophila*. *Comp. Biochem. Physiol.* **105B**: 203–229.
- Gibson, J.B., Lewis, N., Adena, M.A. & Wilson, S.R. 1979. Selection for ethanol tolerance in two populations of *Drosophila melanogaster* segregating alcohol dehydrogenase allozymes. *Aust. J. Biol. Sci.* **32**: 387–398.
- Gibson, J.B., May, T.W. & Wilks, A.V. 1981. Genetic variation at the alcohol dehydrogenase locus in *Drosophila melanogaster* in relation to environmental variation: ethanol levels in breeding sites and allozyme frequencies. *Oecologia* **51**: 191–198.
- Gillespie, J.H. & Turelli, M. 1989. Genotype–environment interactions and the maintenance of polygenic variation. *Genetics* **121**: 129–138.
- Hageman, J., Eisses, K.T., Jacobs, P.J.M. & Scharloo, W. 1990. Ethanol in *Drosophila* cultures as a selective factor. *Evolution* **44**: 447–454.
- Haldane, J.B.S. & Jayakar, S.D. 1963. Polymorphism due to selection of varying direction. *J. Genet.* **58**: 237–242.
- Hedrick, P.W. 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annu. Rev. Ecol. Syst.* **17**: 535–566.
- Hoffmann, A.A. & Parsons, P.A. 1991. *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford, U.K.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195–204.
- Houle, D., Morikawa, B. & Lynch, M. 1996. Comparing mutational variabilities. *Genetics* **143**: 1467–1483.
- Joshi, A. & Mueller, L.D. 1996. Density-dependent natural selection in *Drosophila*: trade-offs between larval food acquisition and utilization. *Evol. Ecol.* **10**: 463–474.
- Kalmus, H. 1943. A factorial experiment on the mineral requirements of a *Drosophila* culture. *Am. Nat.* **77**: 376–380.
- Kawecki, T.J., Barton, N.H. & Fry, J.D. 1997. Mutational collapse of fitness in marginal habitats and the evolution of ecological specialisation. *J. Evol. Biol.* **10**: 407–429.
- Kerver, W.J.M. & Rotman, G. 1987. Development of ethanol tolerance in relation to the alcohol dehydrogenase locus in *Drosophila melanogaster*. II. The influence of phenotypic adaptation and maternal effect on alcohol supplemented media. *Heredity* **58**: 239–249.
- Larsen, R.J. & Marx, M.L. 1981. *An Introduction to Mathematical Statistics and its Applications*. Prentice Hall, Englewood Cliffs, NJ.
- Lenski, R. 1997. The cost of antibiotic resistance – from the perspective of a bacterium. *CIBA Found. Symposium* **207**: 131–140.
- Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. *Am. Nat.* **87**: 331–333.
- Li, G.C. & Hahn, G.M. 1978. Ethanol-induced tolerance to heat and to adriamycin. *Nature* **274**: 699–701.
- Littell, R.C., Milliken, G.A., Stroup, W.W. & Wolfinger, R.D. 1996. *SAS System for Mixed Models*. SAS Institute Inc., Cary, NC.
- McKenzie, J.A. & Game, A.Y. 1987. Diazinon resistance in *Lucilia cuprina*; mapping of a fitness modifier. *Heredity* **59**: 371–381.
- McKenzie, J.A. & McKechnie, S.W. 1979. A comparative study of resource utilization in natural populations of *Drosophila melanogaster* and *D. simulans*. *Oecologia* **40**: 299–309.
- Merçot, H., Defaye, D., Capy, P., Pla, E. & David, J.R. 1994. Alcohol tolerance, ADH activity, and ecological niche of *Drosophila* species. *Evolution* **48**: 746–757.
- Michel, G.P.F. & Starka, J. 1987. Preferential synthesis of stress proteins in stationary *Zymomonas mobilis* cells. *FEMS Microbiol. Lett.* **43**: 361–365.
- Mongold, J.A., Bennett, A.F. & Lenski, R.E. 1996. Evolutionary adaptation to temperature. IV. Adaptation of *Escherichia coli* at a niche boundary. *Evolution* **50**: 35–43.
- Mousseau, T.A. & Roff, D.A. 1987. Natural selection and the heritability of fitness components. *Heredity* **59**: 181–197.
- Oakeshott, J.G., Cohan, F.M. & Gibson, J.B. 1985. Ethanol tolerances of *Drosophila melanogaster* populations selected on different concentrations of ethanol supplemented media. *Theor. Appl. Genet.* **69**: 603–608.
- Partridge, L., Barrie, B., Fowler, K. & French, V. 1994. Thermal evolution of pre-adult life history traits in *Drosophila melanogaster*. *J. Evol. Biol.* **7**: 645–663.
- Partridge, L. & Fowler, K. 1993. Responses and correlated responses to artificial selection on thorax length in *Drosophila melanogaster*. *Evolution* **47**: 213–226.
- Peck, J.R. 1994. A ruby in the rubbish: beneficial mutations, deleterious mutations and the evolution of sex. *Genetics* **137**: 597–606.
- Robertson, A. 1961. Inbreeding in artificial selection programmes. *Genet. Res.* **2**: 189–194.
- Roper, C., Pignatelli, P. & Partridge, L. 1993. Evolutionary effects of selection on age at reproduction in larval and adult *Drosophila melanogaster*. *Evolution* **47**: 445–455.
- Rose, M.R. 1982. Antagonistic pleiotropy, dominance, and genetic variation. *Heredity* **48**: 63–78.
- Roush, R.T. & McKenzie, J.A. 1987. Ecological genetics of insecticide and acaricide resistance. *Ann. Rev. Entomol.* **32**: 361–380.
- Simms, E.L. & Rausher, M.D. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. *Evolution* **43**: 573–585.
- Van Tienderen, P.H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* **45**: 1317–1331.
- Via, S. 1991. The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. *Evolution* **45**: 827–852.

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