GENETIC DIVERGENCE UNDER UNIFORM SELECTION.
II. DIFFERENT RESPONSES TO SELECTION FOR
KNOCKDOWN RESISTANCE TO ETHANOL AMONG
DROSOPHILA MELANOGASTER POPULATIONS AND THEIR
REPLICATE LINES

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ABSTRACT

We have tested the hypothesis that genetic differences among conspecific populations may result in diverse responses to selection, using natural populations of Drosophila melanogaster. Selection for ethanol tolerance in a tube measuring knockdown resistance was imposed on five West Coast populations. In 24 generations the selected lines increased their mean knockdown times, on average, by a factor of 2.7. An initially weak latitudinal cline was steepened by selection. The two southernmost populations showed the same increases in the selected character, but differed consistently in their correlated responses in characters related to ethanol tolerance. This result indicates that the populations responded to selection by different genetic changes. Selection decreased female body weight and increased resistance to acetone, suggesting components of the response unrelated to ethanol metabolism. The Adh' allele was favored by selection in all populations at the onset, but increased in frequency only in the selected lines of the southernmost population. There was a correlation between latitude and Adh frequency changes, suggesting that fitnesses of the Adh alleles were dependent on the genetic background. Genetic background also had a large effect on the loss of fitness due to selection. Genetic drift between replicate lines caused more variation in selection response than initial genetic differences between populations. This result demonstrates the importance of genetic drift in divergence among natural populations undergoing uniform selection, since the effective population sizes approached those of small natural populations. Drift caused greater divergence between selected replicates than control replicates. Implications of this result for the genetic model of selection response are discussed.

NOT all differences among species are necessarily adaptations to diverse ways of life; some differences may instead reflect alternative solutions to the same problem (GOULD and LEWONTIN 1979). Even conspecific populations have sometimes responded in different ways to similar selection (reviewed by

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For example, natural selection for malaria resistance in different human populations has resulted in high frequencies of aberrant forms of hemoglobin and hemoglobin expression: populations show high frequencies of sickle-cell hemoglobin in Africa, but have β-thalassemia near the Mediterranean (reviewed by Luzzatto 1979).

Why have similar selection pressures resulted in divergent selection responses among conspecific populations? One possibility is that the selection pressures at different localities were not identical. Artificial selection experiments have demonstrated that small differences in the way selection is practiced can affect the outcome of selection (Schnee and Thompson 1984; Druger 1962). Alternatively, genetic differences between populations before selection may cause the populations to respond differently (Cohan 1984a). This has been investigated recently in replicated artificial selection experiments using *D. melanogaster* (Gibson et al. 1979; Cohan 1984a; Cohan and Graf 1985). Uniform selection for modifiers of the wing venation mutation, *ci*<sup>o</sup>, did not reveal differences among three populations of *D. melanogaster*, either in their rates of response or in the developmental mechanisms underlying their responses (Cohan 1984a). However, replicated selection for ethanol tolerance resulted in different mechanisms of response in two populations (Gibson et al. 1979)—hence, genetic divergence between the populations before selection affected selection responses.

Cohan and Graf (1985) imposed uniform artificial selection for knockdown resistance to ethanol fumes on two replicate lines derived from each of five populations of *D. melanogaster*; two other lines from each population were maintained unselected. Before selection the populations showed a weak latitudinal cline of increasing knockdown resistance in more northern populations; after ten generations of selection the steepness of the cline increased by a factor of 4½. Ten generations of selection exaggerated the initial genetic differences between populations.

In this paper we extend the study of Cohan and Graf (1985) by further examining the nature of genetic background effects on responses to selection. We first show that the latitudinal cline has persisted after 24 generations of selection. In addition to comparing the magnitudes of response of the various populations, we have tested for differences in their mechanisms of response. We have examined correlated responses to selection in order to discern differences among populations in the physiological, biochemical and behavioral changes underlying their increases in knockdown resistance. We contrast the relative similarity among populations for changes in the selected trait to larger differences among populations in their correlated responses. Finally, we investigate the contribution of genetic drift to divergence under uniform selection, by measuring the relative divergence of the selected and control lines from each population. We interpret the greater divergence between replicate selection lines in terms of a model by Cohan (1984b), which predicts the conditions under which the effects of drift will cause greater divergence among uniformly selected populations than among unselected populations.
MATERIALS AND METHODS

Selection for knockdown resistance: The protocols for the establishment of the selected lines and for the first 12 generations of selection were described by COHAN and GRAF (1985). Briefly, 12 isofemale lines were collected from each of five West Coast localities: Lakeside, California (LS); Five Points, California (WE); Hamilton City, California (DR); Aloha, Oregon (AL); and Port Coquitlam, British Columbia (PC), spanning a latitudinal gradient from 32.9° to 49.3° N (Southern California to British Columbia). The 12 isofemale lines from each locality were pooled to form resynthesized populations representing these localities. From each population, four lines each of 256 flies (unsexed) were established; thus, there were ten selected and ten unselected lines. Each line was maintained in eight %-pint bottles on a laboratory medium consisting of semolina, cornmeal, dextrose, sucrose, dead yeast and agar, with propionic acid as a preservative.

Flies to be selected were transferred without anesthesia into a long vertical glass tube through which air with ethanol fumes was pumped (see COHAN and GRAF 1985). As flies succumbed to the fumes they rolled down a series of baffles made from plastic mesh. The baffles ensured that a partially succumbed fly had numerous opportunities to stop itself from rolling to the bottom. Succumbed flies were collected in a dish that was replaced every minute. Flies were counted, yielding a distribution of knockdown times for a trial. Each generation 1.3-1.6 g of flies (1000-1500 individuals) pooled from all bottles of a selection line were put into the tube. From the last quartile to fall, 256 flies (unsexed) were kept as parents for the next generation. Unselected controls were maintained at the same population size. One each day of selection a control line (called C) was run to correct for fluctuations in husbandry and ethanol dosage. This protocol was practiced for 24 consecutive generations, with the exception of one generation of relaxed selection at generation 21.

Measurement of resistance in the knockdown tube: At generation 24, 80 flies (unsexed) from each of the ten selected and the ten unselected lines were divided equally among four bottles containing live yeast, paper towel strips and laboratory medium. Bottles were incubated at 19.6° ± 0.6° for 19 days. The flies were then transferred to fresh medium. Parents were not cleared, but represented only a small portion of these flies because several hundred individuals were collected from each bottle. Two days later flies were pooled across bottles, and 0.32-0.39 g of flies (250-300 individuals) from each line were placed into the knockdown tube under the conditions used for selection. The smaller number of flies permitted sexing during the experiment. Two replicate tests were made for each selected line, and one test for each control line. This experiment was repeated 2 days later with similarly aged flies, but with all the baffles and other components taken out of the knockdown tube. By comparing the knockdown time of a line with and without the baffles, we could examine the contribution of the baffles to the selection response.

Knockdown resistance tests in the desiccator: Flies were cultured as above, except that three culture bottles were used per line. The adult progeny were pooled across bottles, and four groups of 12 males were counted and sorted under CO2 anesthesia into 8-dram vials containing laboratory medium. After 2 days the flies were shaken into empty vials covered by cheesecloth. All 80 vials from the 20 lines were randomized and placed in a desiccator jar with 500 ml of 95% ethanol. The flies were removed after 90 min. This test and all longevity tests described below were carried out at 20.6 ± 0.6°. Within 15 min all vials were scored for the number of flies “knocked down.” Our criterion of “knockdown” was that a fly must be on its side or back at the moment of scoring. The vials were scored “blindly” and in random order, with a code for identification assigned by a third person. This experiment was performed after 20 generations of selection.

Longevity experiments in sealed vials: Flies were cultured, sorted, aged and put into vials without food, as above, except that two culture bottles were used per line and two groups of males were sorted from each bottle. Males from one bottle were
sorted under CO₂, and those from the other bottle were sorted under ether. Culture bottles for these tests, therefore, were associated with a fixed factor (type of anesthesia).

Following the protocol of Starmer, Heed and Rockwood-Sluss (1977), vials were covered with cheesecloth and were then inverted and positioned on top of another vial containing two rayon balls and 10 ml of water or an ethanol test solution. The two vials were hermetically sealed together with Parafilm. In all longevity tests the number of dead flies in each vial was counted at intervals no greater than 12 hr until at least half the flies in a vial had died. The lethal time 50% in hours (LT50) of each vial was linearly interpolated. These tests were performed after 17 generations of selection.

Longevity tests in a desiccator chamber: We experienced some problems with condensation in the sealed vial tests and, hence, used a modified technique in which the test solutions were placed in a desiccator. Flies were cultured and prepared as for the sealed vial tests, except that all flies were sorted under CO₂ and were placed in vials covered by cheesecloth. All 80 vials from the 20 lines were placed in a desiccator jar with one liter of the appropriate solution (water, ethanol, acetaldehyde or acetone). There were condensation problems with acetone; therefore, flies were put into 6-dram vials with a foam plug at the bottom that prevented flies from adhering to condensation after being knocked down. These tests were performed after 17 or 18 generations.

Body size: Approximately 100 adults of each line were fed yeasted medium for 3 days and were then allowed to lay eggs for 4 hr on spoons covered with a medium of grape juice concentrate and 1.5% agar, with a paste of live yeast. From each line, five groups of 50 eggs were placed into separate bottles containing laboratory medium. The adults were pooled over bottles and were arranged randomly on a tray at 40° for 24 hr. From each line, eight replicates of five males and five females were weighed. This test was performed after 24 generations.

Adh frequencies: Estimates of frequencies of fast and slow electromorphs at the Adh locus were based on 25 flies from each selected and control line at generations 2, 6 and 10 (Cohan and Graf 1985), and, again, at generation 16 following the same protocol. At generation 14, each control line was measured for knockdown resistance, and 25 flies from both the most resistant and most sensitive quartiles were assayed for Adh allele frequencies.

Productivity: Four bottles of each line were cultured as for the body size test. One-day-old flies were pooled over bottles and were sorted under CO₂ into groups of three males and three females, with nine replicate groups per line. After one recovery day, flies were transferred by aspiration into vials with fresh medium every day for the next 5 days. Dead flies were replaced by extras maintained identically. Vials were incubated for 14 days at 25 ± 1°. Productivity was scored as the total number of adult progeny to emerge.

RESULTS

The first section of the results considers responses of the selected lines to generation 24. The second section addresses the question of whether populations responded to selection by different mechanisms. The third section describes allele frequency changes at the Adh locus; these provide an estimate of effective population size. In the fourth section we describe line differences in productivity, which we use to examine correlated responses in fitness. The final section presents an analysis of the relative magnitude of replicate line divergence in control and selected lines.

Responses to selection through generation 24

Selection response curves: The knockdown time of the control line C showed a marginally significant increase over the first 20 generations of selec-
Divergence under uniform selection \((b = 0.116 \pm 0.055 \text{ min/generation}, 19 \text{ d.f., } P < 0.06)\). The values predicted from the linear trend for generations 0 and 20 were 10.1 and 12.4 min, respectively. This trend may be due to natural selection or to a gradual, uncontrolled directional change in the conditions of husbandry or testing.

We corrected for this trend and for random fluctuations by calculating the deviation of knockdown time for control line C on each day of testing from its grand mean over generations. This deviation was subtracted from the knockdown times of the selected lines each generation. Linearly predicted values of selected lines were obtained by regression of corrected scores on generation of selection for the first 20 generations (until the generation of relaxed selection).

In 20 generations the selected lines increased their (corrected) knockdown times, on average, by a factor of 2.7, from a mean linearly predicted value of 12.6 min at generation 0 to one of 34.6 min at generation 20. The lines responded continuously over the 20 generations of selection.

**Single-day comparisons of knockdown times:** Each generation the selection response data were collected over a number of days, and day-to-day fluctuations in test conditions can contribute to the error variance. To more accurately compare the selection responses, selected lines were measured on a single day for two consecutive days, and control lines were tested on another day. The mean knockdown times at generations 10, 12 (COHAN and GRAF 1985) and 24 are plotted against the latitudes from which the populations originated (Figure 1). At generation 12 the unselected lines showed a weak but significant latitudinal cline of increasing resistance in more northern populations (cline of \(0.08 \pm 0.02 \text{ min per degree latitude, } 3 \text{ d.f., } P < 0.05\)). At generation 24 the cline among unselected lines was as steep as before \((0.09 \pm 0.06 \text{ min per degree latitude})\), but it was no longer significant. The increase in residual variation may be due to drift or selection under laboratory conditions.

By generation 10 the latitudinal cline among selected lines had increased to \(0.37 \pm 0.04 \text{ min per degree latitude (COHAN and GRAF 1985)}\), and by generation 24 had increased to \(0.65 \pm 0.08 \text{ min per degree latitude (3 d.f., } P < 0.01\)). The mean selection responses of the more northern populations were larger than those of the southern populations, as indicated by the difference in the regression slopes of selected and control lines \((P < 0.01)\). An analysis of variance (ANOVA) of the mean knockdown times of the selected lines indicated that 64.1\% of the variance was due to population differences \((P < 0.05)\), and 13.6\% was due to replicate line divergence \((P < 0.12)\). (Selection and populations were treated as fixed factors, whereas replicate lines constituted a random factor nested within both selection and population.)

We have standardized each population's average selection response by the ratio of its response to the average for all populations:

\[
\frac{(s_i - c_i)}{(\bar{s} - \bar{c})}
\]

in which \(s_i\) and \(c_i\) are the mean phenotype of the two selected and control lines, respectively, from locality \(i\). This measure is useful for comparing the relative magnitude of the selection response with variation among populations.
FIGURE 1.—Latitudinal clines for knockdown resistance among selected and control lines derived from five West Coast populations, based on data from single-day tests. ●, selected (G24); ○, selected (G10); x, control (G24); ▲, control (G12).

The values of this response ratio ranged from 0.85 for the southernmost locality (LS) to 1.17 for the northernmost (PC) (Figure 2).

**Correlated responses—mechanisms of knockdown resistance**

**Knockdown times without baffles:** One mechanism whereby flies may increase knockdown resistance is to reduce their rate of fall over the baffles, so we removed them and tested the flies as in the selection procedure. Because it was impossible to change the baffles every run, we could not randomize with respect to the presence of baffles; therefore, their effect can be assessed only from the mean times of a series of runs with or without baffles.

The knockdown times of each selected and unselected line were lower when tested without baffles in the knockdown tube (Table 1). The mean knockdown times of the control lines decreased, on average, from 16.3 min with baffles to 7.2 min without; the selected lines decreased, on average, from 43.5 to 17.5 min (unweighted means of both sexes). The knockdown times of the unselected lines without baffles were characterized on two occasions, and an ANOVA of mean knockdown times indicated that 59.9% of the variance was
FIGURE 2.—Selection responses for characters related to ethanol tolerance. The ratios of the average changes of selected lines from each population over the grand average of the changes of all selected lines, calculated as \((s_i - c_i)/(\bar{s} - \bar{c})\), in which \(s_i\) is the average phenotype of the two selected lines from population \(i\), and \(c_i\) is the average phenotype of the two control lines of population \(i\). Characters are abbreviated as follows: knockdown time in tube with baffles (KDT); knockdown resistance in desiccator (1); longevity with 2% acetaldehyde (2); longevity with 20% ethanol (3); longevity with 17% ethanol in desiccator (4); longevity with 17% ethanol in sealed vials (5); longevity with 5% ethanol in sealed vials (6).

due to population differences \((P < 0.05)\) and none was due to replicate line divergence.

**Knockdown resistance in the desiccator chamber:** To examine whether the increased knockdown resistance of the selected lines depended on the structure of the tube used for selection, we tested knockdown resistance in a desiccator containing 95% ethanol. The selected lines were significantly more resistant than controls (Table 2), with the selection factor accounting for 64.1% of the variance. After 90 min of exposure to ethanol fumes, 82% of the flies from control lines and 28% of the flies from selected lines had been knocked down. In the same time of exposure in the knockdown tube, all selected and control flies would have been knocked down; thus, these are different measures of
TABLE 1  
Mean knockdown times in the knockdown tube, with and without baffles, after 24 generations

<table>
<thead>
<tr>
<th>Lines</th>
<th>Males with baffles</th>
<th>Females with baffles</th>
<th>Males without baffles (min)</th>
<th>Females without baffles (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS-1</td>
<td>38.9 (12.5)</td>
<td>40.3 (12.2)</td>
<td>14.9 (5.4)</td>
<td>17.5 (6.1)</td>
</tr>
<tr>
<td>LS-2</td>
<td>36.9 (13.3)</td>
<td>40.1 (13.6)</td>
<td>14.0</td>
<td>16.8</td>
</tr>
<tr>
<td>WE-1</td>
<td>37.6 (12.9)</td>
<td>45.7 (12.8)</td>
<td>11.3 (5.2)</td>
<td>13.9 (5.8)</td>
</tr>
<tr>
<td>WE-2</td>
<td>35.9 (11.6)</td>
<td>39.2 (11.5)</td>
<td>13.9</td>
<td>15.1</td>
</tr>
<tr>
<td>DR-1</td>
<td>42.2 (12.9)</td>
<td>47.0 (13.2)</td>
<td>17.7 (6.3)</td>
<td>20.4 (6.2)</td>
</tr>
<tr>
<td>DR-2</td>
<td>40.8 (13.4)</td>
<td>44.3 (14.0)</td>
<td>15.7</td>
<td>16.0</td>
</tr>
<tr>
<td>AL-1</td>
<td>46.0 (16.4)</td>
<td>51.9 (15.5)</td>
<td>19.9 (8.2)</td>
<td>24.5 (9.0)</td>
</tr>
<tr>
<td>AL-2</td>
<td>40.7 (14.3)</td>
<td>44.6 (14.5)</td>
<td>18.8</td>
<td>24.0</td>
</tr>
<tr>
<td>PC-1</td>
<td>48.7 (13.5)</td>
<td>50.9 (15.8)</td>
<td>17.2 (7.0)</td>
<td>18.2 (7.6)</td>
</tr>
<tr>
<td>PC-2</td>
<td>47.4 (15.4)</td>
<td>51.6 (15.6)</td>
<td>18.3</td>
<td>22.6</td>
</tr>
<tr>
<td>Mean</td>
<td>41.5</td>
<td>45.5</td>
<td>16.2</td>
<td>18.9</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LS-3</td>
<td>14.6 (5.7)</td>
<td>14.7 (5.7)</td>
<td>5.5 (2.9)</td>
<td>6.2 (2.9)</td>
</tr>
<tr>
<td>LS-4</td>
<td>16.4 (5.3)</td>
<td>17.9 (6.8)</td>
<td>6.6 (2.4)</td>
<td>7.3 (3.0)</td>
</tr>
<tr>
<td>WE-3</td>
<td>16.6 (7.0)</td>
<td>16.6 (6.3)</td>
<td>7.6 (3.1)</td>
<td>7.9 (3.4)</td>
</tr>
<tr>
<td>WE-4</td>
<td>16.0 (6.2)</td>
<td>15.7 (5.9)</td>
<td>7.5 (3.1)</td>
<td>8.2 (3.3)</td>
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<tr>
<td>DR-3</td>
<td>15.8 (8.0)</td>
<td>15.0 (7.0)</td>
<td>6.7 (2.8)</td>
<td>6.5 (2.9)</td>
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<tr>
<td>DR-4</td>
<td>14.8 (6.1)</td>
<td>15.2 (7.1)</td>
<td>7.6 (3.5)</td>
<td>8.0 (3.6)</td>
</tr>
<tr>
<td>AL-3</td>
<td>14.9 (5.9)</td>
<td>14.5 (6.3)</td>
<td>7.0 (2.9)</td>
<td>7.4 (3.3)</td>
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<tr>
<td>AL-4</td>
<td>16.6 (6.3)</td>
<td>18.7 (8.0)</td>
<td>7.5 (2.9)</td>
<td>9.1 (3.6)</td>
</tr>
<tr>
<td>PC-3</td>
<td>18.2 (7.8)</td>
<td>18.6 (7.9)</td>
<td>7.3 (2.7)</td>
<td>7.7 (3.3)</td>
</tr>
<tr>
<td>PC-4</td>
<td>16.5 (6.2)</td>
<td>17.9 (6.6)</td>
<td>6.0 (2.8)</td>
<td>6.4 (3.3)</td>
</tr>
<tr>
<td>Mean</td>
<td>16.0</td>
<td>16.5</td>
<td>6.9</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Standard deviations among individuals within a run are given in parentheses.  
\(^a\) Means and standard deviations are the average of two runs per line.  
\(^b\) Means are the average of two runs per line; standard deviations are the average of the two replicate selected lines from each population.  
\(^c\) Means and standard deviations represent a single run per line.  

Knockdown resistance to ethanol. There was marginally significant heterogeneity among populations (Table 2), with the LS flies showing lower knockdown resistance in both the selected and control lines.

Figure 2 shows the correlated responses of each population for all ethanol tolerance traits not measured in the knockdown tube. The correlated response shown by the LS population for knockdown resistance in the desiccator is only about one-half as much as that of the other populations, although the selection × population interaction term is not significant.

**Longevity tests with ethanol:** In this section we have characterized the lines for measures of ethanol tolerance and utilization used by other workers (e.g.,
<table>
<thead>
<tr>
<th>Characters unchanged by selection</th>
<th>Selection (1 d.f.)</th>
<th>Populations (4 d.f.)</th>
<th>Replicate lines (10 d.f.)</th>
<th>Selection × populations (4 d.f.)</th>
<th>Error</th>
<th>Longevity</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Water (sv)*</td>
<td>0.0 (59.1)</td>
<td>1.4 (287.6)</td>
<td>17.9** (227.2)</td>
<td>0.0 (142.1)</td>
<td>13.8 (36.7) (39 d.f.)</td>
<td></td>
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</tr>
<tr>
<td>Water (desc)</td>
<td>0.0 (0.0)</td>
<td>0.0 (20.6)</td>
<td>21.1† (46.9)</td>
<td>0.0 (19.8)</td>
<td>78.9 (20.0) (59 d.f.)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5% ethanol (desc)</td>
<td>0.0 (97)</td>
<td>9.6 (986)</td>
<td>15.1† (442)</td>
<td>12.5 (795)</td>
<td>62.8 (224.4) (59 d.f.)</td>
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<tr>
<td>Body weight</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Males</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Males</td>
<td>0.0 (12.25)</td>
<td>0.0 (12.70)</td>
<td>13.5* (24.06)</td>
<td>0.0 (7.47)</td>
<td>86.5 (10.74) (139 d.f.)</td>
<td></td>
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<tr>
<td>Characters changed by selection</td>
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<td></td>
<td></td>
<td>Longevity</td>
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<tr>
<td>Longevity</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>5% ethanol (sv)</td>
<td></td>
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</tr>
<tr>
<td>5% ethanol (sv)</td>
<td>12.1* (38194)</td>
<td>6.6 (11351)</td>
<td>0.0 (3871)</td>
<td>9.5 (9250)</td>
<td>56.7 (4205) (35 d.f.)</td>
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</tr>
<tr>
<td>17% ethanol (sv)</td>
<td>48.5*** (7715)</td>
<td>4.7 (537)</td>
<td>8.6* (247)</td>
<td>0.0 (136)</td>
<td>29.5 (114) (40 d.f.)</td>
<td></td>
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</tr>
<tr>
<td>17% ethanol (desc)</td>
<td>40.9** (3549)</td>
<td>0.0 (180)</td>
<td>20.0* (262)</td>
<td>0.0 (181)</td>
<td>39.1 (71) (60 d.f.)</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>20% ethanol (desc)</td>
<td>77.4*** (7.294)</td>
<td>3.8† (0.210)</td>
<td>3.0 (0.069)</td>
<td>0.0 (0.032)</td>
<td>17.9 (0.042) (60 d.f.)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>41.2*** (3910)</td>
<td>10.7* (507)</td>
<td>0.3 (111)</td>
<td>0.9 (128)</td>
<td>46.8 (108) (60 d.f.)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>10.1* (62.9)</td>
<td>3.5 (18.5)</td>
<td>1.9 (11.3)</td>
<td>3.5 (14.9)</td>
<td>81.0 (10.3) (60 d.f.)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Adh frequencies</td>
<td>4.8 (0.035)</td>
<td>17.8† (0.044)</td>
<td>40.6 (0.016)</td>
<td>36.8† (0.045)</td>
<td></td>
<td>Body weight</td>
<td></td>
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<tr>
<td>Body weight</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Females</td>
<td>10.3** (131.4)</td>
<td>4.3† (31.19)</td>
<td>0.0 (11.13)</td>
<td>0.0 (5.91)</td>
<td>85.4 (12.78) (136 d.f.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Productivity</td>
<td>20.5*** (20201)</td>
<td>3.6† (1965)</td>
<td>0.0 (598)</td>
<td>14.8* (3437)</td>
<td>61.2 (651) (60 d.f.)</td>
<td></td>
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<tr>
<td>Knockdown resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Desc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desc</td>
<td>64.1*** (6.045)</td>
<td>3.9† (0.209)</td>
<td>0.0 (0.064)</td>
<td>1.6 (0.094)</td>
<td>30.4 (0.071) (60 d.f.)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tube with baffles (selected lines only)</td>
<td>64.1* (79.55)</td>
<td>13.6 (12.87)</td>
<td>22.4 (5.82) (10 d.f.)</td>
<td></td>
<td></td>
<td>Tube without baffles (selected lines only)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tube without baffles (selected lines only)</td>
<td>59.9* (39.21)</td>
<td>0.0 (4.50)</td>
<td>40.1 (5.82) (10 d.f.)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations used: sv, sealed vial test; desc, desiccator test.

* Anesthesia contributed 37.8% of variance ($P < 0.001$), and anesthesia by replicate line interaction contributed 29.1% ($P < 0.001$).

* Anesthesia contributed 2.8% of variance ($P < 0.10$), and anesthesia by population interaction contributed 12.4% ($P < 0.10$).

* Anesthesia by replicate line interaction contributed 8.7% of variance ($P < 0.10$).

*1 T50 values were log-transformed so that error variance of selected and control lines would be more similar.

* Mean square of replicate lines was the error term.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; † $P < 0.10$. 
STARMER, HEED and ROCKWOOD-SLUSS 1977; PARSONS, STANLEY and SPENCE 1979). We carried out these tests to clarify the relationship between the different measures of ethanol response and to test for differences in the mechanisms of response of the populations.

ANOVA of longevity (LT50) with only water vapor indicate no effect of selection or population for both the sealed vial and desiccator tests (Table 2); however, the replicate line factor is significant in the sealed vial test and is marginally significant in the desiccator test. These replicate line effects are measures of genetic divergence by drift over the course of the experiment. Since this trait shows no correlated response with selection, it will not contribute to selection effects in the longevity tests with solutions of chemicals.

The mean LT50 scores with 5% ethanol were higher than with water in both the desiccator and sealed vial tests (mean LT50 of control lines tested in sealed vials was 76.9 hr with water and 220.4 hr with 5% ethanol; mean LT50 of control lines in the desiccator was 43.4 hr with water and 155.1 hr with ethanol). These results suggest that the longevity scores with 5% ethanol are a measure of utilization of ethanol as a metabolic resource (STARMER, HEED and ROCKWOOD-SLUSS 1977). The ANOVA of LT50 scores for the sealed vial test indicates that selected lines lived significantly longer than controls (Table 2; mean LT50 of 264.1 hr for selected lines and 220.4 hr for controls), suggesting that they are relatively better at utilizing ethanol as a metabolic resource. In contrast, the selection term is not significant in the desiccator test (mean LT50 of 157.3 hr for selected lines and 155.1 hr for controls). These differences between tests cannot be explained by the amount of error variance, since the error terms account for a similar proportion of the variance. These analyses suggest that the two tests are measuring different traits.

Analyses of the 17% ethanol data indicate significant effects of selection for both tests, with the selection term accounting for almost one-half the variance (Table 2; mean LT50 of 55.2 hr for selected lines in sealed vials and 35.6 hr for controls; mean LT50 of 61.5 hr for selected lines in the desiccator and 48.2 hr for controls). There are also replicate line effects, which are larger in the desiccator test. Longevities were lower in the sealed vials than in the desiccator test, perhaps reflecting increased toxicity of ingested ethanol. These means indicate that the sealed vial test measured ethanol tolerance (since longevity with 17% ethanol was less than with water), whereas this was not the case for the desiccator test (since longevity with 17% ethanol was greater than with water).

There were significant selection and replicate line effects for longevity with 20% ethanol in the desiccator (Table 2; mean LT50 of 32.4 hr for selected lines and 8.0 hr for controls). These longevities are lower than those for the water test, indicating that this trait tests for tolerance.

**Tolerance to acetaldehyde:** Acetaldehyde is metabolically related to ethanol, being an intermediate in its breakdown. It is toxic (i.e., it reduces longevity) at the concentration used (2%).

Selected lines are significantly more tolerant of acetaldehyde than are controls (Table 2; mean LT50 of 35.4 hr for selected lines and 21.4 hr for
controls). Populations are significantly heterogeneous, while there is no replicate line effect. Longevity of selected lines with acetaldehyde is similar to that with 20% ethanol; however, the mean longevity for the control lines is higher with acetaldehyde than with 20% ethanol. Hence, selection accounts for less of the variance than in the case of ethanol tolerance.

**Heterogeneity among populations in correlated responses related to ethanol tolerance:** The effect of genetic background on the selection response is given by the selection × population interaction in the ANOVA. This term is not significant for any of the above traits that are related to ethanol tolerance but are not measured in the knockdown tube. Therefore, background effects on the selection response were small relative to the overall effect of selection. Genetic background effects were also examined for these traits by comparing the correlated responses of each population to the mean responses of all populations according to formula (1) above. The WE population always showed the first or second greatest correlated response, whereas LS was always ranked fourth or fifth (Figure 2). A Friedman analysis of rankings (nonparametric two-way ANOVA; Sokal and Rohlf 1981, p. 446) indicated that the rankings were too consistent to be attributed to chance ($P < 0.01$). Therefore, the LS and WE populations were significantly different in their correlated responses for characters related to ethanol tolerance.

**Tolerance to acetone:** Acetone is not directly related to ethanol metabolism and provides a possible test for nonmetabolic mechanisms of knockdown resistance. It is toxic at the concentration tested (1.5%).

Analysis of LT50s for acetone tolerance indicates a significant effect of selection, although this factor accounts for only 10% of the variance and selected lines differ from controls by less than 2 hr (Table 2; mean LT50 of 28.7 hr for selected lines and 26.9 hr for controls). This result suggests that there is a component of the selection response unrelated to ethanol metabolism.

**Body weight:** Changes in body weight may alter the surface to volume ratio of the flies and affect the rate of entry of ethanol. An ANOVA of the body weights of males (Table 2) indicates no effect of selection, with significant divergence between replicate lines. For females, however, the selected lines were significantly lighter than the controls (Table 2; mean of 0.45 mg for selected lines and 0.49 mg for controls).

**Adh and population size**

**Adh frequencies:** $Adh$ frequencies of populations were estimated from the mean of the replicate lines. In all five populations the frequency of the $Adh'$ allele was higher in the most resistant quartile of the knockdown distribution of control lines than in the most sensitive quartile in generation 14 (mean $Adh'$ frequencies of 0.47 and 0.36 for the most resistant and sensitive quartiles, respectively). A $t$-test of the differences between the average arcsin-transformed frequencies in the two quartiles was significant ($t = 4.59$, 4 d.f., $P < 0.02$). These results suggest that selection favored the $Adh'$ allele at the onset of selection in all populations.
At generation 16 the mean frequency of $Adh^i$ for the selected lines was similar to that of the controls (0.35 and 0.43 for selected and control lines, respectively). An ANOVA on the arcsin-transformed frequencies showed only a small, nonsignificant main effect of selection, contributing 4.8% to the variance (Table 2). Selection clearly did not have a general or consistent effect on $Adh$ frequencies, but may have produced opposite changes at the $Adh$ locus in different populations. The northern populations showed greater decreases in the frequency of $Adh^i$, whereas the southernmost population (LS) showed an increase in $Adh^i$. The regression on latitude of the differences in frequency between selected and control lines is significant ($-0.057 \pm 0.016$, 3 d.f., $P < 0.05$). In addition, the ANOVA indicates that the interaction between population and selection contributed 36.8% of the variance and was marginally significant ($P < 0.10$).

**Estimation of effective population size:** The effective population size ($N_e$) of the control lines was estimated from the divergence in $Adh$ allele frequencies of the two control lines from the initial frequencies in each population over 16 generations. The mean of the two control lines at generation 2 was our estimate for the initial frequency $p$. The variance $V$ among replicate lines was estimated by the divergence between the two controls. We then estimated $N_e$ for each population as follows (Crow and Kimura 1970, p. 328):

$$N_e = \frac{1}{2 \left\{ 1 - \left[ 1 - \frac{V}{p(1-p)} \right]^{1/2} \right\}}$$

in which $t = 16$ generations. The estimates of $N_e$ were 121 for LS, 183 for WE, 144 for DR, 64 for AL and 8840 for PC; this last number was truncated to the census size of 256, yielding an arithmetic mean of 154.

**Correlated responses-productivity**

We examined the effect of selection on a fitness-related trait by scoring the productivities of females from each line over 5 days. This measure encompasses fecundity as well as egg-to-adult survival.

The selected lines had, on average, only 76% of the productivity of the unselected controls (Table 3). The reduction in productivity over the course of selection was unevenly spread across populations: the selected lines of the DR and LS population differed from their controls by 44% and 34%, respectively, whereas the AL and PC selected lines did not differ from their controls. An ANOVA showed that the difference between selected and unselected lines, differences among populations, and selection by population interaction all contributed significantly to the variance, whereas there was no variance component due to replicate lines (Table 2).

**Analysis of replicate line divergence**

In this section we shall discuss to what extent replicate line variance was due to divergence between selected or control replicates. Selected lines founded from the same population may show greater genetic divergence than do control
TABLE 3
Mean productivity (number of progeny over 5 days) based on nine groups of three males and three females of each line

<table>
<thead>
<tr>
<th>Population</th>
<th>Selected lines</th>
<th>Control lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>LS</td>
<td>54.5 (19.8)</td>
<td>68.8</td>
</tr>
<tr>
<td>WE</td>
<td>53.7 (20.6)</td>
<td>57.7</td>
</tr>
<tr>
<td>DR</td>
<td>50.2 (19.1)</td>
<td>62.9</td>
</tr>
<tr>
<td>AL</td>
<td>78.1 (26.8)</td>
<td>100.4</td>
</tr>
<tr>
<td>PC</td>
<td>66.7 (22.8)</td>
<td>76.8</td>
</tr>
</tbody>
</table>

Mean 67.0 88.2

Standard deviations among groups, averaged over replicate lines, are in parentheses.

TABLE 4
ANOVAs for characters that showed replicate line divergence, with selected and control lines analyzed separately

<table>
<thead>
<tr>
<th>Characters unchanged by selection</th>
<th>Variance due to replicate lines</th>
<th>Variance due to error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longevity</td>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Water (sv)</td>
<td>51.52</td>
<td>43.72</td>
</tr>
<tr>
<td>Water (desc)</td>
<td>8.93</td>
<td>4.71</td>
</tr>
<tr>
<td>5% ethanol (desc)</td>
<td>29.45</td>
<td>81.08</td>
</tr>
<tr>
<td>Body weight</td>
<td></td>
<td>0.94</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Characters changed by selection   |                                 | Controls | Selected | Controls | Selected |
| Longevity                         |                                 |          |          |          |          |
| 17% ethanol (sv)                  | -23.84                          | 90.30*   | 161.47   | 66.15    |
| 17% ethanol (desc)                | -5.89                           | 86.52†   | 58.83    | 142.75   |
| 20% ethanol (desc)                | -0.006                          | 0.020*   | 0.033    | 0.051    |
| Knockdown resistance              |                                 | 2.89     | 13.70*   |          |          |

Significance level for the difference between selected and control variance estimates are indicated. Abbreviations used: sv, sealed vial test; desc, desiccator.

* $P < 0.05$; † $P < 0.10$.

lines by two mechanisms. First, the effective population size of selected lines may be lower than that of control lines since selection tends to favor related individuals. Reduction of the effective population size by selection would be expected to increase divergence for unselected as well as selected characters. We compared the phenotypic divergence of selected and control lines for four characters not affected by selection (Table 4). [These characters had at least marginally significant levels of replicate line divergence ($P < 0.12$); for all
other characters, there was no indication of variance among replicate lines \( (P > 0.38) \). Selected lines showed no significantly greater replicate line divergence than did controls (Table 4); therefore, selection had no discernible effect on effective population size.

Selection may increase the divergence between replicate lines by a second mechanism. When the probability of fixation of a favored allele is closer to \( \frac{1}{2} \) under selection (with drift) than under pure drift, replicate selected lines will be more likely than replicate control lines to become fixed for alternative alleles (Cohan 1984b). Whether selection with drift or pure drift results in a greater probability of alternate fixations depends on the initial frequency of the favored allele, the effective population size and the intensity of selection \( (s) \). In general, selection with drift results in a greater number of alternate fixations (or phenotypic variance) than pure drift when the frequencies of the favored alleles are low and \( Ns \) is low.

In contrast to the depression of effective population size, this second mechanism can only increase the divergence among replicate lines at the loci under selection, or at loci closely linked to those under selection. We shall now compare the levels of phenotypic divergence of selected and control replicate lines for characters that were changed by artificial selection. Four such characters showed large replicate line divergence and thus were included in this analysis (Table 4).

Since the means for these characters are higher for the selected lines than for the controls, we must demonstrate that any increase in variance among selected replicates is not an artifact of the higher mean. This can be tested by comparing the error variance of selected and control lines (Table 4). No estimates of the error variances were available for knockdown times, since each control line was run only once. Variances for the selected and control lines do not differ significantly for any of the other three characters.

Any increase in phenotypic variance of the selected replicate lines compared to that of the controls may thus be attributed to genetic variance among lines. The variances are compared for the three longevity characters in Table 4. In each case, all of the replicate line variance was due to the divergence between replicate selected lines. The control line variance estimate was significantly different \( (P < 0.05) \) from the selected line estimate for two characters and was marginally different \( (P < 0.10) \) for the other. For knockdown resistance the estimate of variance of replicate selected lines was significantly higher than that for the control lines, but no independent estimate of error variance was available. Nevertheless, selection did increase the genetic divergence among replicate lines for three characters responding to selection.

Assuming directional selection on each locus, we can show that selection with drift would have caused more phenotypic divergence between replicate lines than drift only if the initial frequencies of the favored alleles were very low. This analysis requires an estimate of the product of the effective population size and the selection coefficient on the genes responding to artificial selection. The selection coefficient can be approximated by \( s = i \cdot (2a/\sigma_p) \), in which \( i \) is the intensity of selection and \( 2a/\sigma_p \) is the standardized effect of the
DIVERGENCE UNDER UNIFORM SELECTION

genes (FALCONER 1981, p. 186). The proportion selected each generation (25%) corresponds to \(i = 1.27\) (FALCONER 1981, p. 175). The standardized effect of the genes is equal to \(2h \sqrt{2/n}\), in which \(h = \) square root of heritability, and \(n = \) number of loci (FALCONER 1981, p. 202). Heritability was estimated at 0.143 by COHAN and GRAF (1985). Thus, the selection coefficient could be as high as 0.961 if only two loci are postulated, or as low as 0.304 if 20 loci are assumed. This value of \(s\) is defined so that the ratio of fitnesses of the nonfavored and favored homozygotes is \(1 - s\), whereas the selection coefficient used in COHAN’s (1984b) model is defined so that the above ratio is

\[
\left(1 - \frac{s}{2}\right) / \left(1 + \frac{s}{2}\right).
\]

The appropriate transformation gives a selection coefficient of 0.358, with 20 loci. Assuming \(N_e = 154\) (see RESULTS), \(N_e s\) is then 55.5. Under these conditions, the divergence under selection with drift would exceed that under pure drift when the initial frequencies of the favored alleles are less than 3.1% (see COHAN 1984b). If fewer loci were postulated, the selection coefficient would have been higher, and the initial frequencies of the favored alleles would have to be lower than 3.1%.

DISCUSSION

We have described a number of correlated responses to selection for knockdown resistance to ethanol. Here, we discuss the nature of the correlated responses and what they tell us about the mechanisms of knockdown resistance. We also consider the association between the selection responses and fitness, as well as the importance of genetic background on the selection response. Finally, we have demonstrated that divergence among replicate lines is greater for the selected lines than for the control lines. We shall discuss implications of this result for the initial frequencies of favored alleles and the genetic model of selection response.

Mechanisms of knockdown resistance: Selection responses for knockdown resistance were associated with increased longevity under exposure to low (5%) and high (17 and 20%) concentrations of ethanol. We interpret the increased longevity under exposure to low concentrations of ethanol as an increased ability to utilize ethanol as a metabolic resource, because flies live longer with ethanol than with water. Associations between ethanol utilization and tolerance have been found by other workers (DAVID et al. 1979; PARSONS 1981; HOLMS, MOXON and PARSONS 1980; VAN HERREWEGE and DAVID 1984; OAKESHOTT, COHAN and GIBSON 1985).

Body weight showed a correlated response to selection only in the females. An explanation for this difference between the sexes is that female body weight was affected by a trait related to reproductive state. One candidate is productivity, which differed overall between the selected and control lines (Table 3). If the lower productivity of females from the selected lines were due to reduced vitellogenesis, then this could lead to a reduced body weight for these females. However, this explanation is unsupported by the interpopulation cor-
relation between female body weight and productivity, which accounts for only 10% of the variance. Body weight appears to be uncorrelated with variation in ethanol tolerance or utilization among isofemale lines (S. M. Stanley, unpublished results), and a response to selection for ethanol utilization may occur independent of body weight (van Herreweg and David 1984).

Flies selected for knockdown resistance to ethanol are more tolerant of acetaldehyde in the desiccator. This result may indicate a simultaneous selection for acetaldehyde tolerance, because ethanol is normally oxidized to acetaldehyde in Drosophila (Deltombe-Lietaert et al. 1979). In addition, mechanisms that increase the metabolism of ethanol may also increase the metabolism of acetaldehyde. Recent studies have suggested that ADH may be largely responsible for the oxidation of acetaldehyde (Heinstr et al. 1983), as well as ethanol. Increased tolerance of both ethanol and acetaldehyde may possibly have occurred by reducing the rate of entry of chemicals into the insect body via the cuticle or spiracles. This explanation is supported by the finding that the selected lines were slightly more tolerant of acetone, which Drosophila cannot metabolize (David et al. 1981), than the controls.

We tested for a behavioral component of knockdown resistance in the “no baffle” test. The importance of the baffles is indicated by the reduced knockdown time of runs undertaken with these components removed. Flies may have increased their knockdown times by clinging to the mesh or remaining motionless after alighting on a baffle. Additionally, morphological traits could retard fall time over the baffle. These traits would be under stabilizing rather than directional selection, since flies which never fell down were not selected.

Changes in fitness during selection: On average, selected lines had lower productivity than the controls, but this is not the case in all populations. This difference among populations is not related to the magnitude of the selection responses because the AL and PC populations attained high levels of resistance with no loss of fitness. Hence, it appears that reduced productivity depended on the genetic background.

One explanation is that the same factors were responsible for the selection responses in all populations, but the resistance factors were linked to deleterious genes in the LS and DR populations. However, linkage between resistance and deleterious genes would have to be reasonably tight. Another possibility is that different resistance factors responded to selection in the various populations, and the selected genes of LS and DR may have been particularly deleterious in their pleiotropic effects. A third alternative is that selection could have favored the same resistance factors in all populations, but in the genetic background of LS and DR these factors might have resulted in particularly deleterious interactions. These mechanisms cannot be distinguished with the present data set, but it is evident that the genetic background can have a profound effect on the extent to which fitness will be altered as a result of directional selection.

Mechanisms of response and genetic background: Twenty-four generations of selection exaggerated initial differences among populations in knockdown resistance. We present two mechanisms to explain both the original cline in
knockdown resistance (COHAN and GRAF 1985) and the greater response rates of the populations with greater initial levels of resistance. First, suppose that in all populations almost all of the genetic variation in knockdown resistance were due to segregation at the same loci, with the northern populations having higher (and more intermediate) frequencies of the resistant alleles. Since the efficiency of selection is proportional to the allele frequency product, the northern populations would show a greater initial selection response.

This model predicts that the populations starting with the greatest knockdown resistance should show the highest levels of realized heritability in the earliest generations of selection, which is shown only if the northernmost population (PC) is excluded (data in COHAN and GRAF 1985). Note that this model does not require that populations responded entirely by changes at the same loci, only that a large fraction of the response is due to shared genetic changes.

An alternative model assumes that loci have unequal effects on knockdown resistance, with the loci of larger effect segregating in more northern populations. If the frequencies of favored alleles were low initially in all populations, an equivalent increase in frequency of these alleles in the different populations could lead to a steeper cline in knockdown resistance.

We shall now discuss the evidence that populations responded to selection, at least partly, by different mechanisms. For the selected knockdown trait, the five populations were similar in their responses to selection, with the average selection responses of each population within 17% of the mean response of all populations (Figure 2). There was much greater diversity among populations for the various correlated responses related to ethanol tolerance. In some of these traits, populations differed from the mean level of response by over 100%. The two southernmost populations, LS and WE, responded almost identically to one another as measured by changes in the selected character, but were consistently different from one another in their correlated responses related to ethanol tolerance (Figure 2). These differences suggest that the populations increased their knockdown resistance by different mechanisms. The LS selected lines are probably superior in some aspect of knockdown resistance that is specific to the conditions of the knockdown tube. We hypothesized that the LS lines might utilize the baffles more than the other populations; however, the selection response of the LS population was affected the least by removal of the baffles.

Another possible background effect is that selection may have favored different alleles at the Adh locus in different populations. The more northern populations showed the greatest decreases in frequency of the Adh' allele, whereas the southernmost population (LS) showed an increase in the frequency of the Adh' allele. This result may not be due to selection at the Adh locus per se, but at the In(2L)t inversion, which may be in linkage disequilibrium with this locus (VOELKER et al. 1978; KNIBB 1983). Whether or not selection acted directly on Adh, these results demonstrate that selection caused diverse genetic changes in different genetic backgrounds.

Effects of genetic background have been reported by others who have investigated the responses of conspecific populations to uniform selection. Selc-
tion for DDT resistance in populations of *Aedes aegypti* (Inwang, Khan and Brown 1967) and selection for decreased sternopleural bristles in populations of *D. melanogaster* (Lopez-Fanjul and Hill 1973) both resulted in different rates of response in different populations, which seemed to be due to changes at the same loci. Selection for ethanol tolerance in two populations of *D. melanogaster* resulted in responses by different mechanisms: replicate selected lines from one population consistently increased their ADH activity, whereas lines from the other population increased their ethanol tolerance independent of ADH (Gibson et al. 1979).

**Replicate line divergence:** Replicate lines diverged more than populations in their responses to selection. Since the effective population sizes in this experiment (average $N_e = 154$) may approach those of populations in nature (Wright 1978, Chap. 2), the divergence caused by drift in this experiment is a measure of how much identical populations in nature may diverge under uniform selection. Our results suggest that genetic drift is likely to have at least as large an effect on divergence as initial genetic differences.

Finally, we have demonstrated that genetic drift caused greater divergence between selected replicates than control replicates. Consider the implications of this result, under the assumption of a nonepistatic model, in which every locus is subject to directional selection. Under this model, selection with drift will result in greater divergence than pure drift when the probability of fixation of favored alleles is closer to $\frac{1}{2}$ under selection than under pure drift, as demonstrated by Cohan (1984b). We have shown that this could only have occurred if the initial frequencies of the favored alleles were less than 3.1%. Such low frequencies, however, are not consistent with other results. For example, selection responses would have started slowly and then increased as the allele frequencies became more intermediate, but this did not occur. Also, it would have been difficult to detect a latitudinal cline if the initial frequencies of resistance alleles were all around 3%.

Consider, instead, the consequences of an epistatic model of selection, with multiple stable endpoints favored by selection, each surrounded by its own basin of attraction. A population that begins close to the boundary of two basins of attraction may wander from basin to basin as a result of genetic drift (Wright 1970). Two replicate selected lines founded from such a population would have a high probability of eventually being drawn to different endpoints. Replicate control lines, on the other hand, would not be subject to these basins of attraction and, thus, would not be drawn to different endpoints after early wanderings by genetic drift.

While this argument does not prove that the populations were subject to multiple basins of attraction, this model is more consistent with the increased effect of drift on the divergence of selected lines found in this experiment. In future work we shall test deductions from the multiple basin model more directly with the selected lines.

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LITERATURE CITED


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