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Direct and correlated responses to selection for desiccation resistance: a comparison of *Drosophila melanogaster* and *D. simulans*

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Abstract

Replicate lines of *Drosophila melanogaster* and *D. simulans* originating from the same location in Australia were selected at two selection intensities (50%, 85% mortality) for increased resistance to desiccation, and scored for correlated responses to see if similar physiological changes were associated with the selection responses. Realized heritabilities were much higher in *D. melanogaster*. Selected lines of both species were more resistant than control lines to starvation and a toxic ethanol concentration. Both species also showed similar correlated responses for traits underlying the selection response: selected lines lost water at a slower rate and had reduced activity levels in a dry environment, but they did not differ in wet or dry body weight or in water content. For *D. melanogaster*, realized heritabilities for lines selected at 85% mortality were higher than for lines selected at 50% mortality, but there was no effect of selection intensity for *D. simulans*. Comparative studies of this nature may be useful in predicting the extent to which species can adapt to stress in the wild.

Introduction

Genetic differences for quantitative traits are usually greater between populations of *Drosophila melanogaster* than between populations of its sibling species *D.*

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simulans (Singh, 1989). This pattern is evident for several comparative studies of morphological traits (e.g., Hyytia et al., 1985; Watada et al., 1986). It is also evident for stress resistance traits: *D. melanogaster* populations are differentiated for resistance to stresses such as ethanol and desiccation (David and Bocquet, 1985; Parsons, 1980), but *D. simulans* populations differ much less for such traits (e.g., David and Bocquet, 1975; Anderson and Oakeshott, 1986).

Some evidence suggests that genetic variation for quantitative traits is also higher within *D. melanogaster* populations than within *D. simulans* populations. Tantawy et al. (1964) used parent-offspring comparisons to look at genetic variation for wing length in *D. melanogaster* and *D. simulans* from Egypt and found somewhat higher heritabilities for *D. melanogaster* at three temperatures. In addition, they selected for increased and decreased wing length under different conditions and found a slow response to selection in two of the four *D. simulans* lines, whereas all the *D. melanogaster* lines responded rapidly. Cohan and Hoffmann (1989) compared the response of replicate *D. melanogaster* and *D. simulans* lines from the same location to selection for ethanol knockdown resistance. The selection response in the *D. melanogaster* lines was twice that in the *D. simulans* lines. Moreover, mechanisms underlying responses in these species may not have been the same because physiological traits showed different patterns of responses.

The main aim of this paper is to see whether resistance to another stress is genetically more variable within *D. melanogaster* populations, by comparing the response of *D. melanogaster* and *D. simulans* to selection for increased resistance to desiccation. This trait is ecologically important and varies geographically with climate (Hoffmann and Parsons, 1991). In a previous study on desiccation resistance in *D. melanogaster*, we found a rapid response to selection and estimated the realized heritability to be 0.65 ± 0.04 (Hoffmann and Parsons, 1989a, b). In contrast, Ringo and Wood (1984) estimated a realized heritability for desiccation resistance of less than 0.2 for *D. simulans*. Here we confirm this apparent species difference in heritability by selecting replicate lines originating from the same location at two selection intensities.

This study also has two subsidiary aims. First, we wanted to see whether selection responses are based on the same physiological mechanisms. Previous studies have shown that increased resistance to knockdown by ethanol fumes can result in different physiological changes that are associated with the selection response in different sibling species of *Drosophila* (Cohan and Hoffmann, 1989). Thus closely related species do not necessarily evolve the same phenotype when exposed to the same pressure. This has also been shown for different populations/strains of the same species (e.g., Lenski, 1988; Cohan and Hoffmann, 1989). We have therefore compared correlated selection responses for several traits in both species. Second, we wanted to test the generality of some of our results obtained with *D. melanogaster* (Hoffmann and Parsons, 1989a, b) on genetic correlations between responses to different stresses. We previously found that lines with increased desiccation resistance also have increased resistance to a range of stresses including starvation, toxic ethanol concentrations, heat and radiation. Here we show that selection for increased desiccation resistance also increases resistance to some of these stresses in *D. simulans*.

Materials and methods

Stocks

Selection was undertaken on mass-bred *D. melanogaster* and *D. simulans* stocks initiated with the progeny of 30 inseminated females. Flies of both species were collected at the same time from an orchard near Melbourne, which has a temperate climate but with dry, hot periods during summer months. Three replicate lines per species were set up soon after flies were introduced into the laboratory, although selection was not started until after each of the lines had been maintained in the laboratory for six generations. Flies were cultured on a sugar-yeast medium at 19–21° C under continuous light.

Selection

Lines were selected for 10 generations at two intensities by breeding from the last 50% of the flies to survive (50% lines) or the last 15% to survive (85% lines). Three replicate selection lines and three replicate control lines were maintained at each selection intensity for each species. Culture techniques and selection follows Hoffmann and Parsons (1989a) who describe the selection of the 85% *D. melanogaster* lines from the same population. Briefly, selection was carried out on inseminated females which were desiccated over silica gel at 25° C. Females had been separated from males after a brief exposure to CO_2 anaesthesia. By setting up large numbers of flies for selection, the census size of each line was maintained at 100 females even when 85% mortality was used. Unselected lines were kept at the same census size as selected lines. For *D. melanogaster*, a control line (line C) was also maintained (see Hoffmann and Parsons, 1989a) and tested for resistance in each desiccator to correct for day-to-day variation in conditions for desiccation. No such control was used for the *D. simulans* lines. Flies were removed from desiccators when approximately 50% or 85% had died. There was some additional mortality as flies recovered, and the exact selection intensity was determined from the number of females contributing to the next generation.

Comparing selected and control lines

The desiccation resistance of the selected and unselected lines was compared after nine generations of selection as in Hoffmann and Parsons (1989a). Two 600 ml bottles were set up per line at a density of 20 males and 20 females per bottle. Adults were cleared after a day to ensure that larvae developed in uncrowded conditions. Progeny emerging from the bottles were kept separate to test for an effect of variation in culture conditions. Progeny were aged for 2–4 days (sexes mixed) and three vials each with 20 females were set up per bottle. The vials were placed at the periphery of glass desiccators to facilitate counting of dead progeny.

Each replicate vial was placed in a different desiccator. The time taken for 50% of the flies to die (LT50) was linearly interpolated for each vial. The design for this experiment is given by

$$Y_{ijklm} = a + b_i + c_{j(i)} + d_{k(ij)} + e_l + f_{m(ijkl)} \quad (1)$$

where a is the grand mean, b_i the selection term, $c_{j(i)}$ the replicate line term nested in selection, $d_{k(ij)}$ the nested culture bottle term, e_l the block (desiccator) effect and $f_{m(ijkl)}$ the error term.

Resistance to ethanol and starvation

Experiments on correlated responses including resistance to other stresses were not carried out with the 50% *S. simulans* lines which only showed a weak response to selection (see below). Correlated responses for the 85% *D. melanogaster* lines are described elsewhere (Hoffmann and Parsons, 1989a, b) and this paper focusses on a comparison of the 85% *D. simulans* and 50% *D. melanogaster* lines which showed a similar level of response to selection (see below). Lines were tested for correlated responses within four generations of selection being completed.

Starvation resistance was measured following Hoffmann and Parsons (1989a). Flies were cultured and collected as above. Vials with females were placed in a hydration chamber containing distilled water. The number of dead flies was scored at 10–14 h intervals until more than half the females had died.

To score ethanol resistance, an ethanol solution was placed into a vial containing cotton wool. This was covered with gauze before a second vial with flies was inverted over it and the two vials were sealed together. For *D. melanogaster*, an ethanol solution of 15% (v/v) was used, whereas for *D. simulans*, a 5% solution was used. Both treatments are toxic because they reduce longevity below that experienced with water (Parsons et al., 1979). Because *D. simulans* is much more sensitive to ethanol than *D. melanogaster*, we expected the longevity of females of the two species to be reduced by a similar proportion.

The experimental design follows (1), except for the *D. simulans* starvation experiment where there is no block effect because all vials were placed in the same hydration chamber. The block effect in the ethanol resistance experiments reflects the fact that replicates were set up sequentially rather than in a randomized order.

Activity and weight loss

Activity was measured following Hoffmann and Parsons (1989a). Briefly, paper strips with lines 10 mm apart were taped to the back of vials. Twenty females were placed into a vial and vials were arranged along the periphery of a desiccator. Activity was scored 3, 4 and 5 hr after vials were set up by counting the number of females that stayed still. By watching 5 females at each time, activity was scored as the number of females out of 15 that stayed still in each vial.

To look at weight loss after 3 h of desiccation, groups of 20 females were weighed to the nearest 0.1 mg before and after desiccation. Weight loss was expressed as the percentage of the initial wet weight lost by the females.

Weight changes in individual flies

To see if differences in desiccation resistance between the lines were associated with weight loss at death or with the water content of the flies, females were desiccated individually. Flies were weighed on a microbalance and placed in small tubes as described in Hoffmann and Parsons (1989a). Females were desiccated in these tubes, and reweighed within 40 minutes after they died. Flies were then weighed a third time after drying at 37° C for 5 days. Differences between the initial wet weight and weight after desiccation provides an estimate of the weight loss flies could tolerate before death. This weight loss includes a component due to water loss as well as a component due to glycogen and lipid metabolized during desiccation (J. Graves, pers. comm.). Differences between weight at death and weight after drying provides an indication of the remaining water content of the flies.

Results

Selection response

Genetic gains for each line were estimated from the selection response over 10 generations. For *D. melanogaster*, the method of Muir (1986) was used to correct for day-to-day fluctuations in desiccation resistance as measured by the LT50s of the control line. The selected phenotype was modelled as a polynomial function of the cumulative selection intensity (i) and the control phenotype (c) as given by

$$Y = a + b_1 i + b_2 i^2 + b_3 c + E,$$

where a is the intercept, b_js the partial regression coefficients and E the error term. The selection intensity measures how much the mean of the selected individuals differs from the mean of the population, expressed in units of phenotypic standard deviations. It depends only on the proportion of individuals selected. Because the quadratic term was not significant for any of the lines it was excluded in the final equations. Genetic gain per unit of selection is therefore given by b_1.

For *D. simulans*, this approach could not be used because the resistance of a control line was not tested each generation. The $b_3 c$ term was therefore excluded from the equation. Quadratic terms were also left out because these were not significant.

Genetic gains can be used to compare the species responses and are given in Table 1, along with the cumulative selection intensities. Genetic gains should not be equated with heritabilities because resistance was measured on groups of females. Heritability estimates can only be obtained once the phenotypic standard deviation

Table 1. Responses of *D. melanogaster* and *D. simulans* to selection.

	Line 1	Line 2	Line 3
D. melanogaster (85%) [1]			
cumulative selection intensity [++]	13.59	13.20	13.56
genetic gain	0.87	0.82	0.74
heritability	0.69	0.65	0.59
D. melanogaster (50%)			
cumulative selection intensity	7.15	7.61	7.31
genetic gain	0.70	0.55	0.69
heritability	0.56	0.44	0.55
D. simulans (85%)			
cumulative selection intensity	13.85	13.79	13.57
genetic gain	0.37	0.30	0.26
heritability	0.32	0.26	0.22
D. simulans (50%)			
cumulative selection intensity	7.11	6.88	7.39
genetic gain	0.30	0.19	0.25
heritability	0.26	0.16	0.21

[1] From Hoffmann & Parsons (1989a).

[++] Expressed in units of phenotypic standard deviations.

for the resistance of individual females to desiccation is known. For *D. melanogaster*, the phenotypic standard deviation was estimated as 2.50 (Hoffmann and Parsons, 1989a). An estimate was also obtained for *D. simulans* by setting up 10 replicate vials with 20 females, and scoring mortality at 30 minute intervals. The mean phenotypic standard deviation for mortality within a vial was 2.33. Heritabilities were obtained by dividing genetic gains by this estimate and multiplying by two because selection was only carried out on one sex.

The resulting estimates (Table 1) suggest lower genetic gains and heritabilities for *D. simulans* than for *D. melanogaster*. The *t*-tests indicate significant differences between the heritabilities of both the 50% lines ($t = 6.38$, $df = 4$, $P = 0.003$) and the 85% lines ($t = 9.17$, $df = 4$, $P = 0.001$) of these species.

In *D. simulans*, selection intensity did not influence genetic gain, but in *D. melanogaster* genetic gains of the 85% *D. melanogaster* lines may be higher than those of the 50% lines (two-tailed *t*-test, $P = 0.06$). A possible explanation for this apparent difference is that the response to selection becomes more rapid as higher levels of desiccation resistance are attained. However, quadratic terms were not significant in regression analyses of the 85% lines, suggesting that the selection response was linear. Moreover, the 85% lines had genetic gains of 0.88, 0.82 and 0.90 after 5 generations of selection, when the cumulative selection intensities were similar to those of the 50% lines after 10 generations. These gains differ significantly by a *t*-test ($P = 0.02$) from those of the 50% lines.

Table 2. Desiccation resistance of lines after nine generations of selection. Mean LT_{50}s (h) and standard deviations are based on six replicates.

	D. mel (50%)	*D. sim* (50%)	*D. sim* (85%)
Means (SD)			
Selected lines			
1	23.7(1.7)	13.8(0.3)	15.8(0.8)
2	23.8(1.4)	13.4(0.7)	15.2(0.6)
3	23.8(1.2)	13.5(0.5)	16.1(1.0)
Control lines			
1	19.5(0.9)	11.8(0.7)	12.6(0.9)
2	18.1(0.9)	12.9(0.5)	12.7(0.9)
3	17.5(0.5)	12.1(0.9)	12.5(0.9)
Mean squares for ANOVAs			
Selection (1 d.f.)	259.5***	142.7*	85.2***
Replicate line (4 d.f.)	3.2	11.5	0.7
Bottle (6 d.f.)	2.1	6.5	1.4
Desiccator (2 d.f.)	2.4	3.5	0.2
Error (22 d.f.)	1.1	3.5	0.6

* $P < 0.05$, *** $P < 0.001$.

Response after nine generations

Selected and control lines have diverged significantly (Table 2) as expected from the above analysis. Resistance in the 50% *D. melanogaster* selected lines had increased by a mean of 5.4 h, compared to 1.3 h in the 50% *D. simulans* selected lines. When an ANOVA is carried out on the means of these lines given in Table 2, there is a significant species by selection interaction ($F_{(1,8)} = 35.43$, $P < 0.001$), confirming that the response to selection was greater in *D. melanogaster* than in *D. simulans*. For the 85% lines, the mean difference between the selected and unselected lines was 3.1 h in *D. simulans*. This compares to a mean difference of 10.0 h in *D. melanogaster* (Hoffmann and Parsons, 1989a). Species differences in responses were therefore evident at both selection intensities.

There was no evidence for significant divergence among the replicate lines in any of the ANOVAs. Variation among culture bottles also had little effect on resistance. The variance component due to the selection term accounted for 94% of the total variance in the *D. melanogaster* comparison, and this component accounted for 58% and 84% respectively of the total variance in the 50% and 85% *D. simulans* line comparisons.

Resistance to ethanol and starvation

As expected, the ethanol concentrations we used were toxic and adult longevity was reduced compared to longevity in the hydration chambers (Tables 3, 4).

Table 3. Resistance of lines to ethanol. Mean LT_{50}s (h) and standard deviations are based on six replicates.

	D. melanogaster (50%)	*D. simulans* (85%)
Means (SD)		
Selected lines		
1	60.3(8.2)	35.2(5.4)
2	54.8(7.5)	28.2(8.6)
3	57.6(7.7)	28.9(3.4)
Control lines		
1	39.0(6.0)	19.7(6.5)
2	47.6(4.2)	20.8(3.8)
3	40.0(6.5)	20.0(4.0)
Mean squares for ANOVAs		
Selection (1 d.f.)	2129.9***	1005.0**
Replicate line (4 d.f.)	88.4	45.2
Bottle (6 d.f.)	41.9	14.8
Block (2 d.f.)	18.8	90.7
Error (22 d.f.)	50.5	30.5

** $P < 0.01$, *** $P < 0.001$.

Selection significantly increased ethanol resistance in both species. The increase in resistance was considerable, leading to a mean difference between the selected and control lines of 15.4 h (36% of control line resistance) for *D. melanogaster* and 10.5 h (52%) for *D. simulans*.

Selected lines of both species were also significantly more resistant to starvation than control lines (Table 4). Selected *D. melanogaster* lines had their resistance increased by a mean of 18.3 h (26% of controls) and selected *D. simulans* had resistance increased by a mean of 9.7 h (21%).

Activity and weight loss

Selection had a slight but significant ($P < 0.05$) influence on the activity of *D. melanogaster* and *D. simulans* (Table 5). Activities of flies from the selected lines were lower in both species. This is consistent with previous findings on females from the 85% *D. melanogaster* lines (Hoffmann and Parsons, 1989a) and suggests that selection for desiccation resistance decreases the activity of flies under low humidity.

Females from the selected lines of both species lost water at a slower rate than those from the controls (Table 6). The mean difference between the lines was 2.1% for *D. melanogaster* and 1.2% for *D. simulans*. Wet weight of the flies was not altered by selection in either species (Table 6), in agreement with results for the 85% *D. melanogaster* lines (Hoffmann and Parsons, 1989a).

Table 4. Resistance of lines to starvation. Mean LT_{50}s (h) and standard deviations are based on six replicates.

	D. melanogaster (50%)	*D. simulans* (85%)
Means (SD)		
Selected lines		
1	86.5(8.2)	57.5(2.4)
2	95.7(6.9)	58.4(3.3)
3	87.3(10.5)	52.0(5.7)
Control lines		
1	82.1(4.6)	45.3(7.2)
2	69.2(11.3)	50.2(4.0)
3	63.3(9.5)	43.3(5.2)
Mean squares for ANOVAs		
Selection (1 d.f.)	3016.9*	844.9*
Replicate line (4 d.f.)	352.3	74.0*
Bottle (6 d.f.)	143.1	15.5
Chamber (2 d.f.)	10.6	–
Error (22 d.f.)	65.7	26.1

* $P < 0.05$.

Table 5. Activity of females from the lines. Means are based on six replicates and represent the number of flies (out of 15) that moved in a 30-second interval obtained by following five females on three separate occasions (3, 4, and 5 h after the start of desiccation).

	D. melanogaster (50%)	*D. simulans* (85%)
Means (SD)		
Selected lines		
1	10.2(1.3)	9.3(1.5)
2	11.2(1.6)	11.0(2.9)
3	11.0(1.3)	10.3(2.2)
Control lines		
1	12.3(1.2)	11.8(1.5)
2	12.3(1.4)	12.3(0.8)
3	14.2(0.7)	11.2(2.6)
Mean squares for ANOVAs		
Selection (1 d.f.)	39.2*	21.8 +
Replicate line (4 d.f.)	4.5	3.1
Bottle (6 d.f.)	1.5	5.2
Chamber (2 d.f.)	2.0	6.2
Error (22 d.f.)	1.7	3.6

+ $P < 0.06$, * $P < 0.05$.

Table 6. Wet weight and percentage of wet weight lost by females after desiccation. Means and standard deviations are based on six replicates. Wet weight prior to desiccation ($g \times 10^{-2}$) and % of wet weight lost during 3 h of desiccation were measured on groups of 20 females. For weight loss, ANOVAs were carried out on arcsin transformed proportions of wet weight lost ($\times 100$).

	D. melanogaster (50%)		*D. simulans* (85%)	
	weight	% lost	weight	% lost
Means (SD)				
Selected lines				
1	3.10(.08)	5.1(1.6)	2.83(.19)	7.8(1.0)
2	3.21(.11)	6.1(1.2)	2.77(.16)	7.4(1.7)
3	3.09(.16)	7.3(2.0)	2.62(.07)	7.8(1.1)
Control lines				
1	3.10(.15)	8.2(1.6)	2.74(.12)	9.0(1.3)
2	3.12(.12)	8.5(2.5)	2.66(.11)	8.2(1.3)
3	3.19(.05)	7.9(1.6)	2.77(.08)	9.3(1.0)
Mean squares for ANOVAs				
Selection (1 d.f.)	0.0	38.8*	4.4	12.3*
Rep. line (4 d.f.)	197.9	4.1	448.6	1.2
Bottle (6 d.f.)	103.8	4.1	279.7	1.1
error (24 d.f.)	148.1	3.1	138.7	1.8

* $P < 0.05$.

Weight changes of individual flies

D. melanogaster females lost around 40% of their weight before they died, and more than 70% of their weight after drying (Table 7). There were no significant differences between the selected and control lines for these traits or for dry weight, suggesting that differences in total water content and the amount of water/metabolites lost at death did not contribute to the selection response.

D. simulans females tended to die after losing less of their weight than *D. melanogaster* females even though the water content of the two species was similar (Table 7). There was a significant difference between the selected and control lines for weight loss at the time of death, but not for water content or dry weight. Differences between the lines were contrary to expectations because females from selected lines had lost less of their weight at time of death than those from control lines. Culture conditions influenced dry weight.

Discussion

The results clearly indicate a difference between *D. melanogaster* and *D. simulans* in their response to selection for desiccation resistance. This difference was apparent at both selection intensities, and in a direct comparison of the selected and control

Table 7. Weight loss of individual females from the 50% *D. melanogaster* and 85% *D. simulans* lines. Means and standard errors are based on the three replicate selected and control lines. ANOVAs on weight loss were carried out on arcsin transformed proportions (× 100), and the ANOVA on dry weights was carried out on untransformed data (× 10).

	% of initial weight lost		Dry weight ($g \times 10^{-2}$)
	At time of death	After drying	
D. melanogaster			
Means (SE)			
Selected lines	42.9(0.97)	74.1(0.76)	4.1(0.14)
Control lines	41.3(1.92)	74.1(0.39)	4.1(0.08)
ANOVA (mean squares)			
Selection (1 d.f.)	37.9	0.3	0.2
Replicate line (4 d.f.)	106.8	33.5	48.7
Bottle (6 d.f.)	64.7	9.7	11.6
Error (43 d.f.)	65.5	18.8	33.8
D. simulans			
Means (SE)			
Selected lines	31.2(0.55)	72.5(0.26)	3.8(0.14)
Control lines	34.7(0.59)	73.8(0.23)	3.6(0.05)
ANOVA (mean squares)			
Selection (1 d.f.)	205.1*	37.8	38.82
Replicate line (4 d.f.)	16.2	5.5	30.5
Bottle (6 d.f.)	13.7	3.2	121.6***
Error (43 d.f.)	31.2	15.0	21.5

* $P < 0.05$, *** $P < 0.001$.

lines as well as in a comparison of the selection responses. Two factors suggest that the reduced response in *D. simulans* reflects lower genetic variance rather than a higher level of environmental variance. First, both species were tested and cultured in the same manner and were therefore exposed to a similar degree of environmental variation. Second, estimates of the phenotypic variance for desiccation resistance were similar in the two species.

The realized heritabilities and phenotypic variances can be used to obtain estimates of the genetic and environmental variances. If additive factors account for most of the genetic variance in desiccation resistance, then the equation $V_P = V_A + V_E$ can be used to estimate the different components. This seems a reasonable assumption on the basis of results from crosses between the 85% *D. melanogaster* lines (Hoffmann and Parsons, 1989b) and other data on stress resistance traits (Hoffmann and Parsons, 1991). In the 85% *D. melanogaster* lines, the values 2.50 for V_P and 0.65 for the heritability give estimates of 0.87 for V_E and 1.63 for V_A. In contrast, in the 85% *D. simulans* lines, the value 2.33 for V_P and 0.27 for the heritability gives an estimate of 1.70 for V_E and 0.63 for V_A. Similar calculations can be made for the 50% lines using the above V_P values.

For *D. simulans*, V_A is estimated as 0.49 and V_E as 1.84, whereas for *D. melanogaster* V_A is estimated as 1.20 and V_E as 1.30. Genetic variance estimates in both sets of lines therefore suggest that V_A is twice as large in *D. melanogaster* as in *D. simulans*.

The correlated responses suggest that selection responses were based on similar physiological changes in the two species. Selected lines of both species lost water more slowly which is consistent with previous findings for *D. simulans* (Ringo and Wood, 1984) and for the 85% *D. melanogaster* lines (Hoffmann and Parsons, 1989a). Activity of the selected lines was reduced in both species. This suggests that increased resistance may be associated with a decrease in the active metabolic rate of the flies. Although metabolic rate was not measured directly here, it has previously been associated with increased desiccation resistance in the 85% *D. melanogaster* lines (Hoffmann and Parsons, 1989a, b). A reduction in metabolic rate may lead to a decreased rate of water loss through the spiracles because of a reduced demand for gaseous exchange. The selection responses of the two species were also similar in not involving changes in wet weight, dry weight or water content. It should be emphasized that other factors not measured in this study could also contribute to genetic variation for desiccation resistance, in particular the amount of glycogen flies have in reserve which has been associated with desiccation resistance in *D. melanogaster* (Graves et al., 1992).

A possible response difference between the species involves the weight lost by flies at the time of their death. In *D. simulans*, females from the selected lines lost less of their weight before dying than control females, contrary to expectations. The reduced rate of weight loss in the selected lines presumably compensates for death at a heavier weight. Weight loss is normally equated with water loss, and insects are often assumed to lose water at a constant percentage of that present until death. When insects are exposed to dry air, the water content at time t (m_t) is given by (Wharton, 1985)

$$m_t = m_0 \, e^{-kt},$$

where k is the percentage lost in unit time and t is the time elapsed between m_t and m_0. By using the means for the selected lines in Tables 6 and 8, this equation can be solved for t, expressed in terms of a three-hour interval because weight loss was scored over this interval. This leads to a predicted mean time of death of 15.9 h for both the selected and control lines, which is similar to the mean LT50 of the selected *D. simulans* lines in Table 2, but higher than that of the control lines. Control flies would therefore have to lose weight non-linearly, and at a faster rate as death approaches.

The correlated responses for ethanol and starvation resistance suggest that genes in *D. melanogaster* and *D. simulans* which increase desiccation resistance also increase resistance to other stresses. An alternative explanation is that starvation resistance was inadvertently selected because flies were desiccated in vials without food. However this seems unlikely because *D. melanogaster* have not exhausted their food reserves at the time of death from desiccation (Da Lage et al., 1990) as reflected by the large differences in LT50s under the two stresses. Evidence against

inadvertent selection was obtained by Hoffmann and Parsons (1989b, 1993) for the 85% *D. melanogaster* lines.

Generalized resistance to different environmental stresses may be associated with a decrease in metabolic rate (Hoffmann and Parsons, 1991). A reduced metabolic rate could increase starvation resistance by decreasing the rate at which energy reserves are utilized, while ethanol resistance might be increased by a reduced rate of entry of this chemical. Nevertheless, part of the genetic variance controlling resistance to desiccation will probably be independent of that influencing starvation and other stresses. In particular, desiccation resistance has been associated with glycogen levels whereas starvation resistance has been related to lipid content (Graves et al., 1992).

The response differences between *D. melanogaster* and *D. simulans* may be related to differences between these species at the molecular level. Genetic variation in *Drosophila melanogaster* and *D. simulans* populations has been compared at various levels with the conclusion that there are differences between the species depending on the type of variation examined. Enzyme variation is similar in populations of these sibling species (Choudary and Singh, 1987). However, DNA sequence variation in some regions such as *rosy*, *Adh* and *per* is much greater in *D. simulans* populations than in *D. melanogaster* populations from the same area (Aquadro et al., 1988; Begun and Aquadro, 1991). In contrast, there is much less insertion-deletion variation in *D. simulans*, which suggests a lower abundance of transposable elements than in *D. melanogaster* (Aquadro et al., 1988). It is possible that quantitative genetic variation is mostly associated with insertion-deletion variation rather than DNA sequence variation. Supporting evidence has recently been provided in *D. melanogaster* by Mackay and Langley (1990) who associated variation in bristle number with insertion variation.

Future studies could address the importance of insertion/deletion variation by analyzing selected lines at the molecular level using candidate genes likely to be involved in stress resistance. Possible genes include those controlling heat shock proteins, those coding for enzymes controlling the rate of metabolism, or those influencing the storage of high energy reserves such as the *adipose* gene (Clark and Doane, 1983). Other quantitative traits also need to be examined, and comparative studies are needed on species pairs other than *D. melanogaster*/*D. simulans*.

Finally, in extrapolating to natural populations, it is relevant that desiccation resistance is an ecologically important phenotype since it can be associated with habitats in a wide range of animal and plant taxa, both at the intra- and interspecific levels (Hoffmann and Parsons, 1989a, 1991). Since resistance to desiccation is correlated with a wide range of generalized stresses, comparative studies of closely related species based upon directional selection should assist in evaluating the extent to which species can adapt to the escalating stresses apparently arising in the world. The higher stress sensitivity of *D. simulans* coupled with a lesser response to selection for desiccation resistance compared with *D. melanogaster* suggests a lower potential for adaptation to environmental change in *D. simulans*. This may explain, for instance, why *D. simulans* does not occur in some areas occupied by *D. melanogaster*, such as around Darwin in northern Australia

which suffers from very dry winters. Similar comparisons of other species of the *melanogaster* subgroup would be interesting, as some are restricted to the tropics and more susceptible to stress than *D. simulans* (Parsons, 1987).

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