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BREAKDOWN IN CORRELATIONS DURING LABORATORY EVOLUTION. II. SELECTION ON STRESS RESISTANCE IN *DROSOPHILA* POPULATIONS

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Abstract.—We trace the evolutionary correlation between stress resistance and longevity in populations of *Drosophila melanogaster* selected for stress resistance over many generations. Females selected for desiccation resistance and both females and males selected for increasing starvation resistance initially show concurrent increases in longevity, but then begin to decrease in longevity, even as stress resistance continues to increase. We demonstrate that the correlation between two fitness traits can change and that this change is due to sustained selection rather than a genotype-by-environment interaction or inbreeding depression. The breakdown in evolutionary correlation we report underscores the difficulty of extrapolating the results from short-term selection experiments to predictions of long-term evolution.

Key words.—Antagonistic pleiotropy, evolutionary trajectories, genetic correlations, laboratory evolution, long-term selection, longevity, stress resistance.

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Laboratory selection for enhanced stress resistance initially increased longevity among populations of *Drosophila melanogaster* (Rose et al. 1992). Similar results have also been found in other organisms (Rose and Archer 1996). It has been suggested that stress resistance and lifespan are generally positively correlated (e.g. Service et al. 1985; Jazwinski 1996). Recent assays of the selected *D. melanogaster* populations, however, suggest that this relationship is not permanent. Rather, continued increases in stress resistance have been accompanied by *decreases* in longevity. Phelan et al. (2003) provide evidence for the breakdown of an evolutionary correlation between stress resistance and longevity by comparing 75 *Drosophila* populations subjected to a variety of selection regimes for stress resistance or life-history characters. They showed that two initially positive evolutionary correlations broke down during sustained selection. In addition, they argued that this breakdown did not result from (1) inbreeding (cf. Rose 1984a), (2) changes in linkage disequilibrium (cf. Bulmer 1985), or (3) genotype-by-environment interaction (cf. Leroi et al. 1994a).

In documenting the breakdown of an evolutionary correlation, Phelan et al. (2003) were able to avoid many of the problems often associated with the inference of evolutionary mechanisms and patterns (cf. Harvey and Pagel 1991; Leroi et al. 1994b; Rose et al. 1996). They reduced the confounding effects of genetic drift and linkage disequilibrium by maintaining large effective population sizes. They assayed characters under a variety of environments and found no significant genotype-by-environment interactions. Nonetheless, despite the fact that the phylogeny of the experimental populations was known, their study was comparative; the populations under study had complex differences in their evolutionary histories and were exposed to selection on characters other than stress resistance.

The relationship between *Drosophila* stress resistance and

longevity can also be elucidated by tracing well-defined evolutionary trajectories involving these characters. In this study, we trace precisely such trajectories in replicate populations of *D. melanogaster* selected for stress resistance, directly measuring the evolution of the correlation between two traits. In characterizing these population trajectories, we illustrate not just that there are costs that arise at high levels of stress resistance, but that the evolutionary relationship between two functional traits may be complex, and long-term selection with controls can reveal this complexity.

MATERIALS AND METHODS

Experimental Strategy

To evaluate the stability of the evolutionary relationship between the fitness characters of longevity and stress resistance, we created new populations of *D. melanogaster* selected for desiccation resistance (designated as populations NDO_{1–5}) together with control populations (designated NDCO_{1–5}; see Figure 1). We assayed these populations for desiccation resistance and longevity at their inception and regularly thereafter throughout the 37 generations of the selection experiment. This enabled us to characterize the initial, short-term, evolutionary trajectory relating desiccation resistance and longevity. To characterize the long-term evolutionary relationship between these two traits, we assayed five replicate populations that had already been maintained for 184 generations under selection for desiccation resistance (populations D_{1–5}) and their controls (C_{1–5}; Rose et al. 1992).

To characterize the long-term evolutionary trajectory relating longevity and a different index of stress resistance, starvation resistance, we assayed these two traits in five populations selected for starvation resistance (SO_{1–5}) and their controls (CO_{1–5}) and combined these data with similar assay data collected at earlier generations from these same populations, as reported by Rose et al. (1992).

Finally, if at extreme values stress resistance becomes negatively correlated with longevity, selection that moves populations from these extreme values back to more moderate

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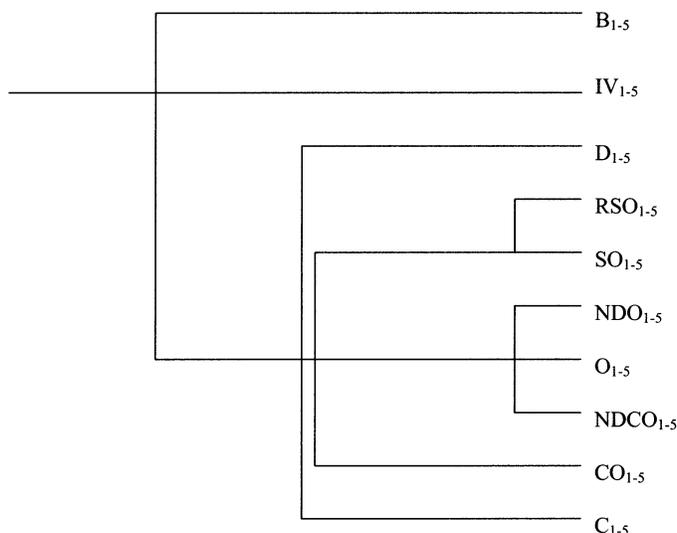


FIG. 1. A phylogeny showing the general relationship between the derivations of the IV, O, B, D, C, SO, CO, NDO, NDCO, and RSO populations. Not drawn to any specific scale.

values ought to be accompanied by increased longevity. To test this prediction, we created five reverse-selected populations from SO_{1-5} and designated them RSO_{1-5} . Although we expected that the RSO populations would decrease in starvation resistance due to the reverse selection results from earlier experiments (Service et al. 1988), we made the additional prediction that longevity would increase in these RSO populations.

Selection Procedures

All populations used in this study are descended from a population of *D. melanogaster* studied by Ives (1970). In 1980, 10 populations were derived from a sample of this population, five called the "O" populations and five called the "B" populations (Rose 1984b). The O populations were selected for late-life reproduction by gradually extending the age of reproduction to 10 weeks (two weeks in 8-dram rearing vials followed by eight weeks in Plexiglas cages), whereas the B populations were maintained with a generation time of two weeks (in vials only).

The following 35 populations were used in this study. All were derived from the O populations except the RSO populations, which were derived from the SO populations (Figure 1).

D and C populations.—In 1988, the D and C populations were derived from the O populations; the selection protocol is described in Rose et al. (1990; see also Rose et al. 1992). Briefly, populations D_{1-5} were selected for desiccation resistance and populations C_{1-5} served as controls. For each of the replicate populations, 14 days after eggs were collected, approximately 4200 flies (60 vials, each containing 60–80 flies) were transferred from the rearing vials into Plexiglas cages containing 175 g of desiccant to extract virtually all water from the air (T. Bradley, pers. comm.). Selection was stopped after approximately 80% mortality. The remaining flies were given plates containing yeast for three days at which point eggs were collected for the next generation. The

control populations were handled the same, except their Plexiglas cages contained agar as a water source. Neither the treatment nor control group cages contained food during the selection period.

NDO and NDCO populations.—The NDO and NDCO populations were derived in 1995 using the same protocol as had been used in creating the D and C populations, respectively. The NDO and NDCO populations were established to obtain evidence about initial evolutionary changes following imposition of the selection regimes.

SO and CO populations.—The SO and CO populations were derived in 1989 using the starvation-resistance selection protocol described by Rose et al. (1992). Briefly, the selection protocol for the SO and CO populations was similar to the D and C populations, except that following transfer to the Plexiglas cages the SO population (selected populations) received agar as a water source but no food medium. The CO populations (control populations) received food medium.

RSO populations.—These reverse-selected populations were derived from the SO populations at generation 94 of starvation selection. Selection for starvation resistance was ceased in these populations by implementing the same selection protocol as the CO populations.

Assay Procedures

All populations were regularly assayed for stress resistance and longevity. The NDO and NDCO populations were assayed at selection generations 0, 5, 8, 11, 14, 17, 20, 26, and 37 for desiccation resistance and longevity. The D and C populations were assayed at generations 161, 166, and 184. For the SO and CO populations, previously published data were used, including longevity data for generations 0, 4, 8, 12, 16, and 19, and starvation resistance data for generations 0, 3, 7, 11, 15, and 19 (Rose et al. 1992). Additional new data were collected at generations 76, 102, 105, and 113 for both longevity and starvation resistance in populations SO_{1-5} and CO_{1-5} . The RSO populations were assayed for starvation resistance and longevity at generations 9, 12, and 20.

Rearing experimental flies for each assay.—Prior to collection of any assay data, experimental flies were taken off selection for two generations to establish a common rearing environment and to remove any parental effects (Mousseau and Dingle 1991). Within each generation, the flies were reared in 8-dram vials (approximately 60 eggs per vial) for 14 days. The flies were then transferred into cages and given ample food for no more than two weeks. Twenty vials were used per population, maintaining approximately 1200 flies per generation. After two generations, the flies used for the assay were collected at exactly 60 eggs per vial and reared in 8-dram vials at 25°C. Flies used in the longevity assays were collected from these vials within 24 hours of eclosion. The experimental flies used in the desiccation assay were not transferred after eclosion, and were three to five days old.

Desiccation assay.—This assay measured the length of time in which a fly lives under completely dry conditions. This procedure is described in more detail in Service et al. (1985). Briefly, four flies of the same sex were placed in an empty 8-dram vial. A sponge plug was used to separate the flies from the desiccant and 5 ml of Drierite (W. A. Hammond

Drierite Co., Xenia, OH) desiccant was placed on top of the sponge. Finally, the top of the vial was sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI). The desiccant removed all humidity in the vial. The flies were checked every hour for deaths. A total of 40 males and 40 females from each population were assayed.

Starvation assay.—This assay measured the length of time in which a fly lives without food, but in the presence of water. This procedure is described in more detail in Service et al. (1985). This assay is similar to the desiccation assay, with a single difference: in place of the desiccant, a piece of cotton containing 3 ml of water was used. In this assay, vials were examined for deaths every six hours instead of every hour. A total of 40 males and 40 females were assayed from each population.

Longevity assay.—This assay measured the life span of each fly. The procedure followed that of Rose et al. (1992). Four males and four females were placed in the same 8-dram vial containing banana agar medium. The flies were transferred every two to three days into fresh medium. Dead flies were not replaced. The vials were checked for death everyday. A total of 40 males and 40 females were assayed from each population.

Statistical Analyses

For starvation-resistance and desiccation-resistance values reported, all replicate means are based on approximately 40 individual samples per population per generation. All treatment means and standard errors are based on the unweighted means of the five replicate populations for each treatment (with the exception of generation 20 for the RSO populations, for which only four replicates were available).

We considered three different subsets of the data in our analyses of the relationship between longevity and stress resistance, including (1) NDO and D females, (2) NDO and D males, and (3) SO males and females combined. Data subsets 1 and 2 reveal an evolutionary trajectory relating desiccation resistance and longevity. Data subset 3 reveals an evolutionary trajectory relating starvation resistance and longevity. Although the NDO and D populations are distinct, the stocks were derived from the same populations, and the selection regime for each stock was identical. The only difference between these stocks is that the D populations were derived from the O populations 29 O-generations earlier than the NDO populations. Longevity in the O populations, relative to the B populations, did not change during the period separating the derivation of the NDO and D populations.

To determine whether the evolutionary correlation between stress resistance and longevity is no longer present as stress resistance increased along these trajectories, we compared linear regression relating the stress resistance and longevity of the initial trajectory to a linear regression relating the stress resistance and longevity of the complete trajectory. Within each data subset, the initial trajectory is defined as generation 0 to that generation in which the flies selected for stress resistance lived longest relative to their controls. If the linear relationship between stress resistance and longevity is significant and positive initially compared to the linear relationship between stress resistance and longevity over the en-

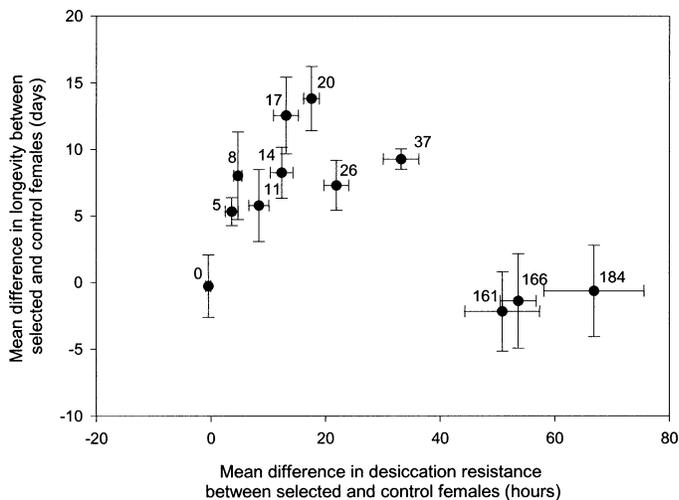


FIG. 2. The evolutionary trajectory showing the relationship between the mean differences in longevity and desiccation resistance between selected and control females. Numbers refer to each generation assayed. NDO and NDCO females were assayed at generations 0, 5, 8, 11, 14, 17, 20, 26, and 37. D and C females were assayed at generations 161, 166, and 184. Standard error bars were calculated using the five replicate means of the difference between selected and control females.

tire trajectory, we define this as a breakdown in the evolutionary correlation between stress resistance and longevity. To further characterize the remaining part of each trajectory, we used a linear regression to examine the relationship between stress resistance and longevity between the generation in which flies selected for stress resistance lived longest compared to their controls and the last generation in the trajectory. In all of these analyses we used the mean difference between the experimental populations and their corresponding control populations rather than the actual values of the experimental populations. This minimized the influence of any environmental variation between the assays conducted at different generations. To evaluate whether reducing stress resistance increased longevity in the RSO populations, we compared the mean difference between each RSO population and its corresponding SO population for longevity and starvation resistance at generations 9, 12, and 20 with the mean difference between the RSO and SO populations for longevity and starvation resistance at generation 0. These comparison use a one-tailed t-test. All analyses were completed using JMP (ver. 4.0, JMP Software, Cary, NC).

RESULTS

Desiccation-Resistance Selection in Females

The relationship between desiccation resistance and longevity in females selected for desiccation resistance is shown in Figure 2. These females exhibited the greatest longevity in generation 20; thus, to test whether there was a positive relationship between desiccation resistance and longevity at low desiccation resistance, we evaluated the trajectory from generations 0 to 20. The linear regression for this initial relationship was significant and positive ($F = 23.56$, $P = 0.005$, $R^2 = 0.82$). A linear regression of the complete trajectory

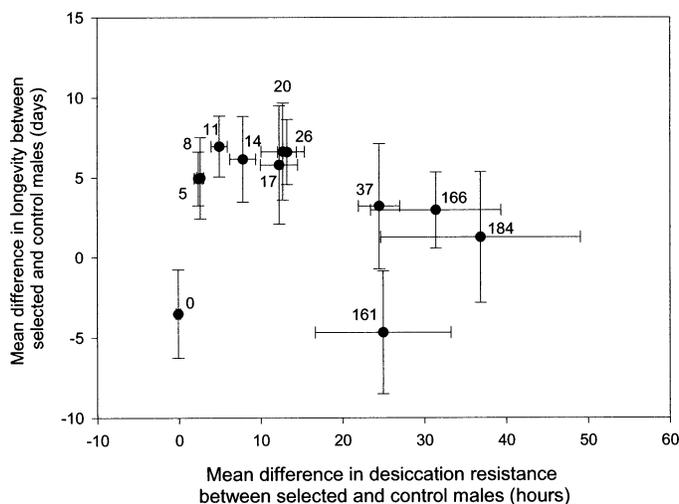


FIG. 3. The evolutionary trajectory showing the relationship between the mean differences in longevity and desiccation resistance between selected and control males. Numbers refer to each generation assayed. NDO and NDCO males were assayed at generations 0, 5, 8, 11, 14, 17, 20, 26, and 37. D and C males were assayed at generations 161, 166, and 184. Standard error bars were calculated using the five replicate means of the difference between selected and control males.

shows no statistically significant relationship between these two traits ($F = 3.99$, $P = 0.073$, $R^2 = 0.28$). In fact, the slope of this overall line is negative, although only marginally significant, suggesting a reversal of the relationship between desiccation resistance and longevity. Further, the relationship between these two traits between generations 20 and 184 was significantly negative ($F = 16.07$, $P = 0.016$, $R^2 = 0.80$). Therefore, as desiccation resistance increased in females selected for desiccation resistance, longevity initially increased but then decreased as desiccation resistance increased.

Desiccation-Resistance Selection in Males

In Figure 3, we present the relationship between desiccation resistance and longevity in males selected for desiccation resistance. On average, the males selected for desiccation resistance lived longest at generation 11 compared to their controls. Therefore, a linear regression was used to compare the initial relationship between desiccation resistance and longevity at generations 0, 5, 8, and 11. This relationship is marginally significantly positive ($P = 0.077$), although the R^2 value is 0.85 ($F = 11.47$). A linear regression of the entire trajectory reveals no relationship ($F = 0.83$, $P = 0.38$, $R^2 = 0.077$). As with the females, the trajectory after the highest longevity value (generations 11, 14, 17, 20, 26, 37, 161, 166, and 184), reveals a significant negative linear regression between these two traits ($F = 5.98$, $P = 0.044$), although this relationship is not as strong as for females selected for desiccation resistance ($R^2 = 0.46$). In summary, males selected for desiccation resistance initially had slightly significant positive relationship between desiccation resistance and longevity but this relationship disappeared as desiccation resistance increased and eventually became significantly negative.

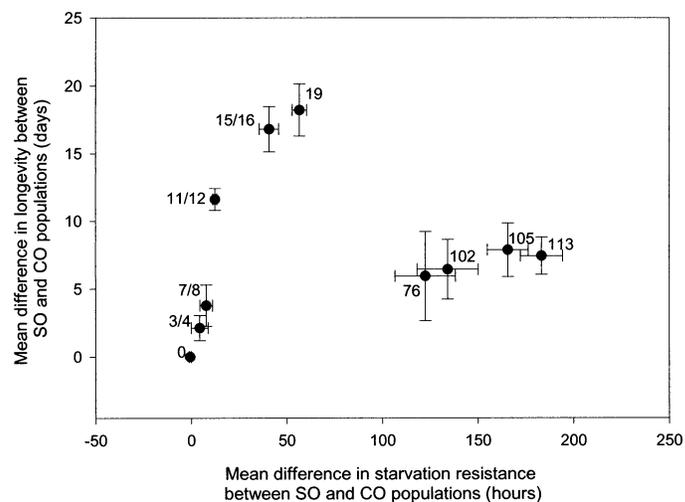


FIG. 4. The evolutionary trajectory showing the relationship between the mean differences in longevity and starvation resistance between SO and CO populations. Numbers refer to each generation assayed. Longevity was assayed at generations 0, 4, 8, 12, 16, 19, 76, 102, 105, and 113. Starvation was assayed at generations 0, 3, 7, 11, 15, 19, 76, 102, 105, and 113. Standard error bars were calculated using the five replicate means of the difference between selected and control populations.

Starvation Resistance

The relationship between starvation resistance and longevity in populations selected for starvation resistance is depicted in Figure 4. These flies lived the longest at generation 19 so the initial trajectory was evaluated between generations 0 and 19. The initial relationship between starvation resistance and longevity in these flies was significantly positive ($F = 24.29$, $P = 0.008$, $R^2 = 0.86$). In contrast, the relationship between these two traits over the entire trajectory was nonexistent ($F = 0.058$, $P = 0.82$, $R^2 = 0.007$). Analysis of the last half of the trajectory shows no relationship between starvation resistance and longevity, suggesting a plateau ($F = 5.04$, $P = 0.11$, $R^2 = 0.63$). Overall, the relationship between starvation resistance and longevity in males and females selected for starvation resistance initially was positive but as starvation resistance further increased, longevity neither increased nor decreased but leveled off.

RSO males and females.—In Table 1 we present stress resistance and longevity data from the reverse-selected RSO populations. Combining the male and female data, the mean difference in starvation resistance between the RSO and SO populations increased significantly for each successive generation after taking the RSO populations off the starvation-resistance selection. By generation 20, across the five replicate populations the mean starvation resistance had decreased by 53% in the RSO populations relative to the SO populations (Table 1a). For both males and females the mean difference in starvation resistance between the RSO and SO populations increased significantly between generation 0 and generations 9, 12, and 20. The mean difference in starvation resistance between the RSO and SO females, however, is approximately 39–45% higher than the mean difference in males at each generation analyzed.

Combining the male and female data, the mean difference

TABLE 1. The mean difference between RSO and SO populations (RSO-SO mean) for starvation resistance and longevity at generations 9, 12, and 20 were compared to the mean difference between RSO and SO populations at generation 0 using a one-tailed *t*-test (*P*-values are shown). The mean difference between RSO and SO populations at generation 0 is zero since RSO and SO populations are the same. The sexes are reported both combined and separately. For the comparisons between generations 0 and 9, and 0 and 12, *df* = 4; for the comparison between generations 0 and 20, *df* = 3.

(a) Starvation resistance						
Generation	Combined		Female		Male	
	RSO-SO mean	<i>P</i> -value	RSO-SO mean	<i>P</i> -value	RSO-SO mean	<i>P</i> -value
9	62.66	0.001	72.91	0.001	52.41	0.001
12	65.47	0.001	77.55	0.001	53.39	0.002
20	114.55	<0.001	134.18	<0.001	94.91	<0.001
(b) Longevity						
Generation	Combined		Female		Male	
	RSO-SO mean	<i>P</i> -value	RSO-SO mean	<i>P</i> -value	RSO-SO mean	<i>P</i> -value
9	3.31	0.062	5.89	0.028	0.72	0.353
12	0.09	0.486	2.57	0.172	-2.38	0.258
20	-0.30	0.454	2.89	0.235	-3.49	0.0934

in longevity between the RSO and SO populations increased from 0 days at generation 0 to 3.31 days at generation 9, although this increase was only marginally significant (Table 1b). This initial increase was followed by a decrease in longevity after 12 and 20 generations of reverse selection, at which times longevity did not differ significantly with the mean difference in longevity at generation 0 (Table 1b).

Analysis of only the female RSO data reveals a significant increase in the mean difference in longevity from generation 0 to generation 9, although the increase from generation 0 to generations 12 and 20 are not significant (Table 1b). For males, on the other hand, the mean difference in longevity between the reverse selected populations and their controls did not differ at generations 9, 12, and 20 when compared with generation 0 (Table 1b).

DISCUSSION

In populations of *D. melanogaster* selected for stress resistance, we found that longevity increases initially and then decreases as stress resistance increases. Theoretically, the change in this relationship might be attributed to a genotype-by-environment (G×E) interaction, inbreeding depression, linkage disequilibrium, or sustained selection. Our data indicate, however, that the first three possibilities are unlikely and that the last most likely accounts for our observations.

Genotype-by-environment interaction.—Although G×E interactions are sometimes anticipated (e.g. Hardin et al. 1967; Bennett and Lenski 1993), they may also occur unexpectedly and obscure the interpretation of experimental results (e.g. Leroi et al. 1994a). For this reason, it is necessary to estimate genetic correlations in more than one environment before concluding that they have changed entirely (Stearns 1989; Rose et al. 1996).

In the present populations selected for extreme stress resistance, the observed decrease in longevity might have been caused by a G×E interaction. Phelan et al. (2003) tested this hypothesis by assaying populations selected for stress resistance and their controls for longevity in two environments: “normal” and “side.” They found that D and C flies, and CO males lived significantly longer in the side environment

than in the normal environment, whereas the CO females and SO males and females showed no difference in longevity between the two environments. Nonetheless, there was no detectable G×E interaction in any of these populations (Phelan et al. 2003).

Inbreeding depression.—The disappearance of the positive correlation between longevity and stress resistance was not a consequence of inbreeding depression in any of the populations in this study. We estimated the inbreeding coefficient for the D populations relative to the C populations after 184 generations of selection and for the SO populations relative to the CO populations after 113 generations of selection. Determination of the inbreeding coefficient first relies on an estimate of the effective population size (N_e). Although N_e of the D and SO populations was not available, it was recently estimated in five populations of *D. melanogaster* from our laboratory that have been selected for early fecundity (B populations). N_e was estimated in the B populations by first estimating four parameters (L.D. Mueller, pers. comm.): (1) sex ratio over 52 generations, spanning a two year period; (2) variance in male reproductive success (Joshi et al. 1999); (3) variance in female reproductive success; and (4) the change in the total population size over 52 generations. Using the methods of Mueller et al. (1985) and their estimates of these four parameters, we computed one-generation estimates of N_e for each generation and a final estimate of N_e using the harmonic mean. The ratio of the effective population size to the census size (N_c) in the B populations was estimated to be between 0.56–0.69. (N_c = 1600 individuals and N_e = 900–1100 individuals).

The D and SO populations used in this study are likely to have similar, if not larger, N_e values. For these populations, the variance in male and female reproductive success is probably similar to that in the B populations since these flies inhabited the same general laboratory environment and were derived from the same natural population. Furthermore, the census size just before reproduction in the D and SO populations was similar to the B populations, at 1440–1920 individuals. Additionally, after selection was terminated in the D and SO populations, an overwhelmingly disproportionate

number of females remained. Many of these females were inseminated with sperm from deceased males, so the effective population size could approach twice the census size. Because the fluctuation in census size each generation was small, we can infer that the ratio of N_e to N_c in the D and SO populations is similar to the B populations, if not higher. If we use the conservative estimate of $N_e/N_c = 0.56$, and a census size of 1440 individuals, then the inbreeding coefficient after 184 generations is 0.11 ($F_t = 1 - (1 - 1/(2N_e))^t$ where F_t = the inbreeding coefficient after t generations) for the D populations (Falconer and MacKay 1996). After 113 generations, it is 0.07 for the SO populations.

Because the evaluation of the selected populations is always relative to their control groups, we need to calculate the inbreeding coefficient for the C and CO populations as well. For the C populations, the actual population sizes are approximately 2160–2880 individuals per generation. Using the same rationale as above, we can estimate that $N_e/N_c = 0.56$ so the conservative estimate of N_e is 1210 individuals for the C populations, giving an inbreeding coefficient after 184 generation of 0.07. The percent of increased inbreeding occurring in the D populations relative to the C populations, therefore, is 4% (0.11–0.07). However, it should be noted that this is a maximal estimate of the differential effect of inbreeding depression, because the estimated effective population size of the selected D population is highly conservative. It could in fact be much closer to that calculated for the C populations.

For the CO populations, N_c is approximately 2400–3200 individuals per generation. Using the same N_e/N_c ratio, a conservative estimate of their effective population size is 1344 individuals and the inbreeding coefficient is 0.04. This means there was approximately 3% more inbreeding in the SO populations relative to the CO populations (0.07–0.04). Again, this estimate is biased in favor of the CO populations.

Following Falconer and Mackay (1996, p. 248, table 14.1), we estimate that the mean value of a fitness character should decrease by 1.48% if the inbreeding differential is 4%, and by 1.11% if the inbreeding coefficient is 3%. Considering the points at which we found the greatest difference in longevity between selected and control populations (13.8 days at generation 20 for the longevity difference between the D and C populations, 18.22 days at generation 19 for the difference between the SO and CO populations), the subsequent reduction in the mean longevities of the selected population is too great to be explained by inbreeding depression. The maximum reduction in longevity based on the difference in inbreeding between selected lines and controls would be about 0.2 days for both desiccation and starvation selection. This is nearly two orders of magnitude smaller than the change observed.

Linkage disequilibrium.—Although the loci underlying desiccation resistance could be physically linked with many of the loci underlying longevity, we find this to be an unlikely explanation for our results. It has been shown by two-dimensional gel electrophoresis that approximately 200 loci are involved in longevity (Fleming et al. 1993). More recently, high-density microarrays give estimates of 1000–2000 for the aging gene number (Pletcher et al. 2002), although there are reasons for thinking that these are overestimates (Rose

and Long 2002). Moreover, it has been shown that many traits, including increased bulk water content, decreased cuticle permeability, and possibly even increased glycogen content, are involved in desiccation resistance (Graves et al. 1992; Gibbs et al. 1997), suggesting that numerous loci are also involved. The probability of some physical linkage in such a large collection of loci is high. However, in general they must be loosely linked and therefore unlikely to maintain gametic disequilibria. Further, even if this were the case in the past, the large effective sizes at which these populations were kept would likely break up this linkage disequilibrium in a few generations (Falconer and Mackay 1996), thus reducing further the likelihood that gametic phase disequilibrium could be responsible for these results.

Sustained selection.—The remaining explanation for this sudden decrease in longevity during ongoing selection for stress resistance is that selection changed the genetic relationship between these traits. A variety of selection scenarios might have been involved, as described by Phelan et al. (2003). First, consider scenarios with multiple selection mechanisms. Although laboratory evolution with bouts of starvation or desiccation may impose selection for increased stress resistance, it is conceivable that this same selection procedure might incidentally impose selection on longevity, without any genetic correlation connecting the characters. Under these conditions, it is also conceivable that natural selection for increased longevity might soon cease, perhaps because of stabilizing selection, whereas selection for increased starvation resistance continues to higher and higher levels of starvation resistance.

Second, scenarios based on the organismal effects of increased stress resistance might have been involved. Laboratory selection for increased stress resistance might have increased longevity as a secondary effect, at low to moderate levels of stress resistance. However, very high levels of stored fat, glycogen, and water are critical in the continued enhancement of stress resistance (Djawdan et al. 1998; Gibbs et al. 1997). This extra weight might have deleterious effects on adult survival, reducing longevity. As selection continues for greater stress resistance, there is a continued positive effect on stress resistance but the effect on longevity may become negative due to pleiotropic deleterious effects associated with hypertrophy of stress resistance. In effect, the pleiotropic effect of increased stress resistance changes from being beneficial for longevity, at moderate levels of stress resistance, to deleterious, at very high levels. In this model, alleles have similar effects across loci. It is the joint physiological effects on stress resistance and longevity that change. In effect, high levels of stress resistance have an unavoidable cost.

Third, it is possible that there are alleles that happen to enhance both stress resistance and longevity, and these alleles are favored by natural selection in the cages of the SO and NDO flies. However, once these alleles are fixed by selection, the remaining alleles that foster increased stress resistance may be neutral or deleterious with respect to longevity. Selection therefore favors alleles with positive effects on stress resistance that also have positive effects on longevity, but through an independent physiological pathway. As selection continues, alleles at loci that have a positive effect on stress

resistance but a negative effect on longevity through an independent physiological pathway are favored, because they increase stress resistance in these flies. In this model, there is heterogeneity among the loci affecting stress resistance with respect to their pleiotropic effects on longevity.

We do not have the information required to choose among these contrasting scenarios. It is also conceivable that the correlation breakdown presented here and in the accompanying paper involved all three of these selective mechanisms, as well as others, as yet undescribed. It is also important to remember that the trajectories presented included data combined from separate experiments conducted years apart. The possible introduction of confounding experimental factors was partially mitigated, however, through the consistent use of comparisons only between treatment groups and their appropriate concurrently maintained controls.

The Relevance of This Study

Several other studies have inferred nonlinear relationships between fitness characters, particularly stabilizing selection. These include correlative studies on body size and fitness (MacArthur 1949; Falconer 1953; Falconer 1965) that suggest that an intermediate body size maximizes fitness (Falconer and Mackay 1996), phenotypic manipulation studies on clutch size (Gustafsson and Sutherland 1988; Sinervo and Licht 1991), and studies on selection for increased and decreased bristle numbers (Kearsey and Barnes 1970).

In this study, we demonstrate that selected populations of *D. melanogaster* that have intermediate levels of stress resistance live longer than populations with low or high levels of stress resistance; a pattern like that of stabilizing selection, except that longevity is essentially a neutral character in these stress-selected populations. This result extends the findings of the above studies in two ways. First, by using a selection experiment, we show that this breakdown in trait association is genetically based, whereas phenotypic correlation studies measure the confounded effects of genotype and environment, and manipulation studies measure only environmental effects. Second, we followed the trajectory relating these two traits through evolutionary time, whereas the above studies report only a few points on the evolutionary trajectory.

Long-Term Predictions

Many selection experiments proceed for a relatively small (<22) number of generations (e.g. Dempster et al. 1952; Friars et al. 1962; Sen and Robertson 1964; Burris and Bell 1965; Sheridan and Barker 1974; Scheiner and Istock 1991). These types of experiments are useful in that they begin to unravel the mechanisms that may enhance or constrain functional traits. Their results, however, may not reveal—and may even obscure—the long-term evolutionary trajectories of these traits. Our study is the first in which an initial evolutionary correlation between two traits is monitored over many generations as it breaks down. This result calls into question predictions of very long-term evolutionary patterns based on either variance components obtained from correlations of relatives or a few generations of selection.

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