Evolution, 57(3), 2003, pp. 527–535

BREAKDOWN IN CORRELATIONS DURING LABORATORY EVOLUTION. I. COMPARATIVE ANALYSES OF *DROSOPHILA* POPULATIONS

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Abstract.—We provide evidence from comparisons of populations of *Drosophila* that evolutionary correlations between longevity and stress resistance break down over the course of laboratory evolution. Using 15 distinct evolutionary regimes, we created 75 populations that were differentiated for early fecundity, longevity, starvation resistance, desiccation resistance, and developmental time. In earlier experiments, selection for postponed aging produced increases in stress resistance, whereas selection for increased stress resistance produced increases in longevity. Direct estimates of correlations also indicated an antagonistic relationship between early fecundity on one hand and longevity or stress resistance on the other. Laboratory evolution of extreme values of stress resistance, however, led to a breakdown in these evolutionary relationships. There was no evidence that these significant changes in correlation resulted from genotype-by-environment interactions or inbreeding. These findings suggest that correlations between functional characters are not necessarily durable features of a species, and that short-term evolutionary responses cannot be extrapolated reliably to longer-term evolutionary patterns.

Key words.—Antagonistic pleiotropy, correlations, fitness characters, laboratory evolution, longevity, stress resistance selection.

Received January 28, 2002. Accepted November 18, 2002.

To what extent are the evolutionary correlations between characters stable? In the wild, changes in genetic correlations, selection mechanisms, or both may alter associations between characters. When multiple characters are subject to selection in multiple wild populations, for example, heterogeneity among selection pressures can produce a diversity of evolutionary correlations. One character might be selected with consistent directionality in a particular population, whereas another undergoes normalizing selection. Patterns of coordinate evolution can also arise from changes (or differences) in genetic correlations

Changes in breeding systems, too, can produce changes in correlations between functional characters among populations, most obviously when previously outbred populations are abruptly subject to intense inbreeding (Rose 1984a). Inbreeding or hybridization may change a genetic correlation between two characters by a variety of specific mechanisms. One such mechanism arises when inbreeding depression affects one trait but not the other. Because characters that are not connected with fitness are less affected by inbreeding than traits connected with fitness (Wright 1968; Falconer and Mackay 1996), over multiple generations of inbreeding, the means of fitness-related characters are expected to fall, whereas neutral characters often show no such directional change.

Although the inference of evolutionary correlations from data collected in the wild is difficult (cf. Leroi et al. 1994a), laboratory evolution, by contrast, provides a simplified context for studies of evolutionary correlations, as long as artifacts are avoided (Rose et al. 1996). If, during a period of evolutionary change, one character increases as another character increases (or decreases), then the characters are evolutionarily correlated. This relationship must be sustained, however, even under controlled assay conditions, as opposed to being a correlation observed only from in situ data, such as a fossil pattern. Given the difficulties in controlling mating systems in nature, and that general environmental conditions are also less readily managed in nature (although selection differentials may change with the evolution of the populations), evolutionary correlations are unlikely to be observed outside of laboratories.

With control of these potential problems, three remaining factors can still potentially alter genetic correlations between characters during laboratory evolution: (1) changes in linkage disequilibrium, (2) genotype-by-environment interaction, and (3) changes in allele frequency through drift or sustained selection.

The evolutionary correlation we consider here is that between stress resistance and longevity. Longevity is correlated with stress resistance in several species. In yeast, for example, mutational screens for increased stress resistance have been used as a surrogate for selection for longevity itself, with positive results (Kennedy et al. 1995). Similarly, nematode mutants with increased lifespan have increased resistance to heat and other stresses (Jazwinski 1996). In *Drosophila*, a positive correlation between longevity and stress resistance has been observed regardless of whether genetic manipulation (Service et al. 1985; Rose et al. 1992) or nutritional manipulation (Chippindale et al. 1993) is used to vary the traits. Lin et al. (1998) successfully screened for lifespan mutants using enhanced stress resistance. In this paper, however, we provide evidence from comparisons of 75 populations of *Drosophila* that evolutionary correlations between these fitness characters can disappear over the course of long-term laboratory selection.

MATERIALS AND METHODS

Stocks

All populations used in these experiments were ultimately derived from the outbred *D. melanogaster* population studied

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by Ives (e.g. 1970) for several decades and characterized for life-history variation by Rose and colleagues (e.g., Rose and Charlesworth 1981; Rose 1984b; Service and Rose 1985). A sample of this population, called ''IV,'' was collected in 1975 and has been kept as an outbred population in the laboratory, undergoing two-week generations with abundant food for 24 years, more than 500 generations. We describe the selective regime for each of the subsequently derived populations below. Common rearing practices included the following: (1) all eggs were placed at densities of 60–80 eggs/vial for hatching, pupation, and eclosion; (2) all populations were maintained under discrete generations; (3) all selection was stopped when 80% of the original 5000 flies in a population had died and the surviving 20% were given yeast plates and eggs were collected to start the next generation; (4) except during stress selection, flies were maintained with unlimited food.

In all of our procedures and assays, the population was the basic unit of observation and each population's trajectory was a single datum. Consequently, we replicated all of these selection regimes five-fold at the population level and our statistical comparisons test for differences among population means for the different treatments. When we describe the ''D'' and ''C'' treatments below, for example, these refer to five populations each, numbered 1 to 5 (i.e., D_1 to D_5), where each population is derived from the similarly numbered population (i.e., D_1 is derived from O_1, D_2 from O_2, and so on).

In all, 15 different regimes of laboratory selection were employed in this study. Each of these, however, can be classified into one of two distinct categories of selection. In the first category, life-history traits (age-of-reproduction and longevity) were the focus of selection and we observed the correlated changes in stress resistance. In the second category, starvation resistance or desiccation resistance was the focus of selection and we observed correlated changes in longevity. In both categories, we utilized selection protocols both for increasing as well as for decreasing trait values. Because each population was produced concurrently with an appropriate control, the populations are presented below in these groups.

Stocks in Which Life-History Traits Were Manipulated via Selection

Increasing longevity

B and O populations.—In February 1980, 10 derivatives of the IV stock were created. Five derivatives were designated as Bs, numbered B_1–B_5 (Rose 1984b), with eggs collected exactly 14 days following oviposition. At the same time that the B populations were created, an additional five replicate populations, designated as O populations, were created that were selected for late-life fertility. The rearing in this treatment was the same as for the B populations until two weeks following oviposition. At that time, adults were transferred into Plexiglas population cages until the day on which eggs were laid for the next generation. The day of egg collection was progressively postponed. Initially it was 28 days posteclosion, then 35, and after continuous gradual increases the generation time was fixed at 70 days (Rose 1984b). The O populations have been maintained on 70-day generations since early 1982. The effective breeding sizes of these populations have been about 1000 (L.D. Mueller, unpubl. data). During the course of selection, mean longevity among the O populations increased to 60–80 days versus about 25–45 days for the B populations, with no longitudinal trend apparent in the B data (Leroi et al. 1994b).

Decreasing generation time

ACO populations.—In 1991, a selection treatment for accelerated development time was begun, derived from the CO lines described below. The flies in the treatment labeled ACO were maintained as follows (see Chippindale et al. 1997). As the adults emerged from pupae, only the first 20% were transferred to population cages for reproduction. For each replicate, eggs were collected one day after the total number of adults transferred to the cage reached 800–1200 flies. This selection favored early eclosion, early maturation, early mating, and early fecundity. During the course of 130 generations of selection, the rate of development accelerated dramatically; egg-to-egg generation time dropped from 12 days to eight days.

Stocks in Which Stress Resistance Was Manipulated via Selection

Increasing stress resistance

D and C populations.—In 1988, two new treatments were derived from the O populations. One of the treatments was selection for desiccation resistance (D populations) and the other (C populations) was a control treatment (see Rose et al. 1990). The desiccation selection was as follows. Fourteen days following oviposition, the flies from each treatment were transferred to cages and the control (C) flies were given access to agar, providing water but not nutrition, whereas the desiccation-selected flies were given neither food nor water but were instead desiccated by exposure to Drierite (W. A. Hammond Drierite Co., Xenia, OH) in a sealed cage. Selection was halted when all but 20% of the desiccation-selected flies had died. At that time, food was added to both the C and D populations of flies and eggs were collected for the next generation, from both populations simultaneously. During the course of this selection, mean desiccation resistance among the D populations increased to 68 hours versus about 17 hours for the C populations.

SO and CO populations.—In 1989, two new treatments were derived from the O populations. The flies in one of these treatments, labeled SO, were selected for increased starvation resistance and the flies in the other, labeled CO, served as a control treatment (see Rose et al. 1992). The selection regime was as follows. Fourteen days after oviposition, flies from each treatment were placed into population cages and the control (CO) flies were given unlimited food whereas the starvation-selected (SO) flies were given only agar. Selection was halted when all but 20% of the starvation-selected flies had died. At that time food was placed in the cage and eggs were collected for the next generation. Eggs were collected from the control populations simultaneously. During the course of 105 generations of selection, the generation time for the SO and CO flies increased gradually from 17–20 days

TABLE 1. Summary of all populations and selection regimes used in this study. See text for full details of selection procedures.

Stock	Source	Year founded	Generation	Selected for:
IV	Wild-caught flies	1975	520	fertility at 14 days
B	IV	1980	390	fertility at 14 days
O	IV	1980	90	fertility at 70 days
ACO	CO	1992	148	early emergence from pupae and early fecundity
CB	B	1989	91	fertility at 30 days
CO	O	1989	91	fertility at 30 days
SO	O	1989	91	resistance to starvation
D	O	1987	151	resistance to desiccation; moderate resistance to starvation
C	O	1987	151	moderate resistance to starvation
RSO	SO	1996	12	fertility at 30 days
LL	CB	1993	35	fertility at 27 days under low nutrition
HH	CB	1993	35	fertility at 30 days under high nutrition
LSL	CB	1993	35	resistance to starvation under low nutrition
LSH	CB	1993	35	resistance to starvation following low nutrition, with high nutrition prior to egg laying
HSL	CB	1993	35	resistance to starvation following high nutrition with low nutrition prior to egg laying
HSH	CB	1993	35	resistance to starvation following high nutrition with high nutrition prior to egg laying

to 30–35 days as the average time to starvation among the SO flies increased from 65 hours to more than 9 days.

LSH, HSL, HSH, LSL, HH, LL, and CB populations.—In 1993, four additional starvation-resistance selection treatments and two control treatments were begun. These treatments, begun with flies maintained on a two-week generation time and adapted to cage living (labeled CB), were modifications on the SO/CO starvation selection treatment described above; each treatment differed in the nutritional regime under which the flies were maintained before starvation selection and the nutritional regime imposed after starvation but before eggs were laid for the next generation. This selection can be distinguished from the SO treatment as follows. In the SO selection treatment flies were supplied with unyeasted plates of food prior to selection (an ''L'' nutritional regime) and then, following starvation but prior to egg collection, were given plates covered with yeast (an ''H'' nutritional regime). This regime selects for a dietary restriction response. That is, it selects for flies that both (1) in the presence of low yeast respond by lowering their fecundity and increasing their starvation resistance, and (2) in the presence of high yeast respond with enhanced fecundity. We designate this selection regime ''LSH,'' for low nutrition, then starvation, then high nutrition, followed by egg collection. Exactly opposed to this selection regime is an HSL regime, in which high yeast level precedes starvation and is followed by low yeast level just prior to egg laying. In this case, flies that exhibited the reverse response to yeast levels had the selective advantage.

Four additional related treatments were also created: HSH and LSL, in which yeast levels are consistently high or low and there is selection for increased starvation resistance; and L–L and H–H, in which there is no selection for increased starvation resistance but the yeast levels before egg laying are high or low. The first two treatments were created to serve as checks on the effects of starvation selection upon dietary restriction that arise simply from yeast level alone. That is, changes in dietary responses could arise as effects of selection for starvation resistance alone in either high or low yeast treatments. The second two treatments test for effects specific to selection upon fecundity at high and low levels of yeast level. During 35 generations of selection, the starvation-resistance selected populations experienced two- to four-fold increases in starvation resistance, with significant variation in this response depending on nutritional levels. Populations fed high nutrition just prior to starvation selection, for instance, showed approximately double the selection response relative to those given low nutrition prior to selection. Collectively, the LSH, HSL, HSH, LSL, HH, LL, and CB populations were referred to as the ''LuSH'' stocks.

Decreasing stress resistance

RSO populations.—After 94 generations of selection for starvation resistance, an RSO line was derived from the SO line. These populations were placed under a reverse selection regime, released from selection for starvation resistance and instead maintained under identical conditions to the CO populations. This selective regime represents selection only for fertility at 30 days. Twelve generations after these populations were taken off the starvation resistance selection regime, their starvation resistance had decreased by one-third.

The 75 populations—that is, five replicates each of the 15 genetically distinct stocks described here—are summarized in Table 1.

Assay Procedures

The five replicate populations from each selection treatment were assayed for longevity and stress resistance (desiccation resistance and/or starvation resistance). To mitigate nongenetic, maternal effects, all assayed populations were maintained under a common rearing environment for two generations prior to being assayed (Mousseau and Dingle 1991).

Longevity.—Longevity was assayed in groups of eight flies (four males, four females) kept on banana medium in 8-dram vials. Flies were transferred to new vials every 2–3 days (Monday, Wednesday and Friday). Records of flies dying were made each day until all flies in a vial had died a natural death (methodology follows Rose et al. 1992).

Desiccation resistance.—Desiccation resistance was assayed by measuring the amount of time it took a fly to become completely unresponsive to mechanical stimulation under conditions of 0% humidity and no food (to eliminate an important moisture source). The desiccation environment was created by placing flies in groups of four into sealed vials containing Drierite desiccant. (Flies were separated from the desiccant by a cotton ball.) The time, to the nearest hour, of inanition was then used as the time of death (methodology follows Service et al. 1985).

Starvation resistance.—In a manner almost identical to the measurement of desiccation resistance, starvation resistance was assayed by measuring the amount of time it took a fly to become completely unresponsive to mechanical stimulation under conditions of high humidity and no nutrition. The starvation environment was created by placing flies in groups of four into 8-dram vials containing no food and a water-saturated cotton ball. The time (to the nearest six hours) of first inanition was then used as the time of death (methodology follows Service et al. 1985).

Specific experimental designs

We used three experimental approaches—described below—in determining the extent to which genetic correlations between fitness characters were fixed.

1. Genotype-by-environment tests.—Alterations in the relationships among fitness characters may reflect fundamental changes in the nature of the genetic correlations between them. Or they may change as a simple function of the environment in which they are measured. We conducted tests to determine the extent to which changes in the relationships among fitness characters we observed were due to such genotype-by-environment effects.

Using a subset of our populations—the D/C populations, the SO/CO populations, and the B/O populations—we tested whether changes in the relationship between stress resistance and longevity were artifacts of the longevity assay environment. We did this by designing two alternative longevity assay methods.

The first alternative longevity assay method was identical to the longevity assay method described above except that the assay vials throughout the entire assay were kept on their sides. The food in the normal assay vials softens a bit and, because the flies are transferred to new vials only three times a week, can pose a hazard; dead flies sometimes appear to have gotten stuck in the food. By placing the vials on their sides, the flies are able to stand on glass rather than the surface of the food. The second alternative longevity assay method was conducted exactly as the standard longevity assay described above except that flies were transferred to new vials every day.

2. Divergent populations in which the evolutionary histories and/or selection regimes were varied.—All of our experimental treatments used flies derived from the same original ancestral stock. In eight of the 15 treatments (B, O, D, C, SO, CO, ACO, and RSO), however, the criterion for selection was qualitatively different. In some it was applied to a different fitness character (age of reproduction, desiccation resistance, starvation resistance, developmental rate), in others to the same character but in a different direction (e.g., early vs. late reproduction, increased vs. decreased starvation resistance). In other words, we assessed the relationship between fitness characters among a broad demographic spectrum of genetically distinct populations when each had arrived at their particular constellation of life-history traits from disparate starting points.

3. Divergent populations in which only the environment during selection was varied: LuSH stocks.—In contrast to the approach just described, we also implemented 7 selection treatments that did not vary at all in the criterion used for selection or the starting point of the populations but instead differed only in the nutritional environment present during selection. In each of these treatments, all flies were derived from the five replicates of a common ancestral stock (CB) and selected for the same fitness character (increased starvation resistance) in the same manner (only the top 20% were selected each generation). With this protocol we were able to create another broad demographic spectrum of populations with respect to stress resistance and longevity. This time, however, the populations arrived at their different constellations of life-history traits despite originating from identical ancestral stocks and being subjected to the same selective screening; only the nutritional environment differed.

RESULTS

Genotype-by-Environment Effects on Longevity

When longevity was measured in vials kept on their sides, the flies from all selection regimes (SO, CO, D, C, O, and B; males and females combined) lived longer than under the normal assay conditions (only the SO longevity increase was not statistically significant). Among O flies, mean longevity increased by almost 10 days when flies were maintained in vials kept on their sides. Among the shorter-lived B flies, the increase was only five days. Across six different selective regimes, the mean increase in longevity between the normal longevity assay and the side-vial assay was 15% (see Table 2). Conversely, when flies were transferred to new longevity-assay vials every day, their longevity decreased, regardless of the selection regime under which the flies were maintained. Across four selection regimes (SO and CO, O and B) the decrease in longevity was consistent and ranged from 4 to 8% (see Table 2).

Although longevity varied depending upon the assay environment in which it was measured, the amount by which it varied did not differ significantly between any of the selective regimes. This lack of genotype-by-environment effects is presented in Figure 1. In this figure, the difference in longevity between treatment groups and their controls (SO vs. CO, D vs. C, O vs. B) is plotted for each of the longevity assay environments. The difference in longevity between SO and CO flies, for instance, is 9.6 ± 2.6 days in the normal assay environment, with the starvation-selected SO flies liv-

TABLE 2. *Drosophila* longevity under different assay environments. Values are mean and standard deviation for five replicate populations, males and females combined. Normal environment flies were transferred to new vials every M, W, and F. In the Side group, flies were transferred to new vials on the same days but the vials were kept on their sides at all times. In the Every day group, the vials were stood up as in the Normal environment but the flies were transferred to fresh vials every day.

	Longevity (mean ± SD)		
Treatment	Normal	Side	Every day
SO	50.4 (4.2)	53.6 (3.3)	48.4 (6.2)
CO	40.8 (3.6)	46.9 (2.8)	38.8 (4.3)
D	53.0 (1.6)	61.7 (2.0)	
C	49.3 (4.9)	57.1 (5.0)	
O	53.8 (1.6)	63.3 (3.3)	50.5 (2.9)
B	28.4 (2.0)	33.1 (2.5)	26.2 (0.7)

ing longer. Similarly, the SO flies' longevity advantage is 9.6 ± 5.5 days in the ''every day'' environment and 6.7 ± 5.5 days in the side-vial environment. In the parallel comparisons between the O and B treatments and the D and C treatments, the O flies lived longer than the B flies and the D flies lived longer than the C flies in all assay environments. As with the SO versus CO pairing, however, the magnitude by which the O and D longevity exceeded that of the B and C treatments was the same in all assay environments.

Correlated Responses to Selection

Evolutionary correlations between stress resistance and longevity that are positive at low levels of stress resistance break down at the highest levels of stress resistance. The relationship between starvation resistance and longevity among females of 40 populations of flies maintained under eight different selection regimes is revealed in Figure 2. At low starvation resistances (the six treatments with mean starvation resistances less than 85 hours), there is a significant positive linear relationship ($F = 10.1$, $P = 0.004$, $R^2 = 0.26$; using bivariate means for each replicate population). With increased starvation resistance, however, this relationship breaks down; there is no significant linear relationship between these characters when the 10 populations from the two treatments (RSO and SO) that produced the highest starvation resistances are included ($F = 3.5$, $P = 0.07$, $R^2 = 0.08$).

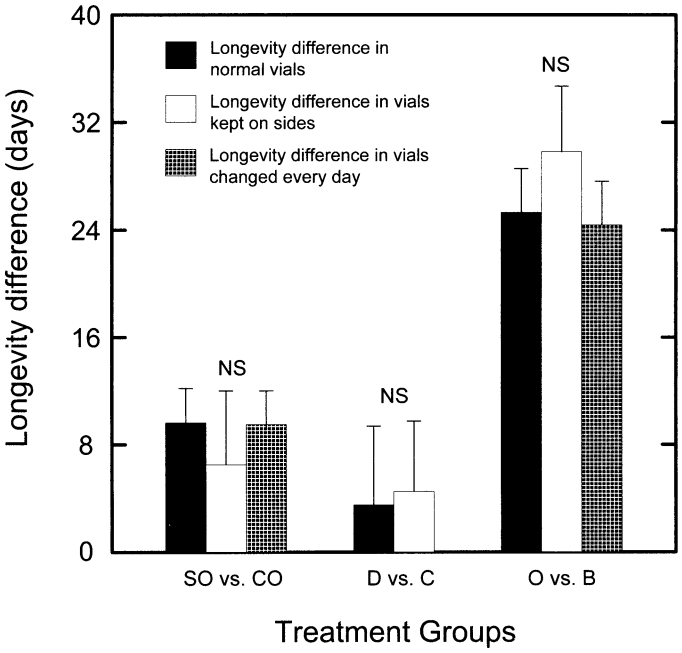

FIG. 1. Test for genotype-by-environment effect on longevity. For each comparison, bar represents difference (mean of five replicate populations) between the first listed treatment (SO, D, O) and their control (CO, C, B, respectively). In each case, the longevity difference between the pairs of treatments is not influenced by the environment in which longevity is measured. NS, not significant.

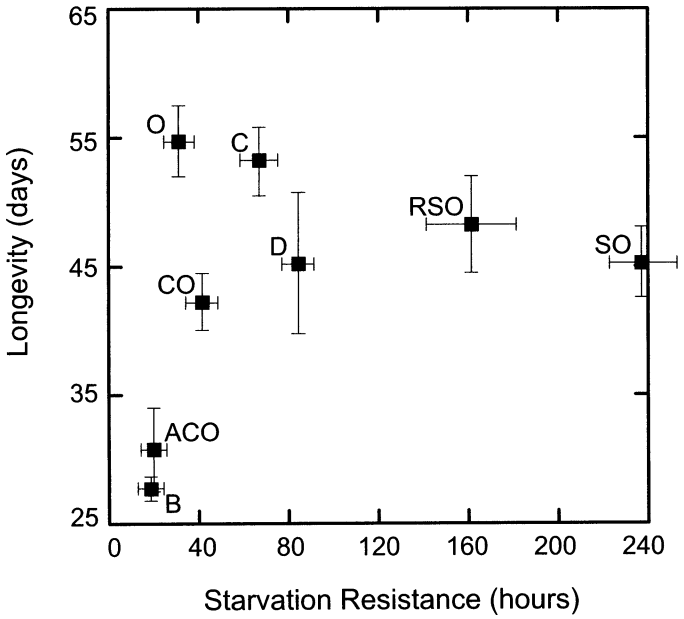

FIG. 2. Female longevity versus starvation resistance. Datapoints represent bivariate means of five replicate populations ± SE.

In Figure 3 we plot the relationship between desiccation resistance and longevity among 35 populations of flies maintained under six different selection regimes. Throughout the relatively low range of desiccation resistances (<20 hours) characterizing all but one of the selection treatments (the D treatment), we found a significant positive linear relationship between desiccation resistance and longevity ($F = 22.0$, $P < 0.0001$, $R^2 = 0.44$; using bivariate means for each replicate population). As with starvation resistance in Figure 2, however, the five populations in the treatment group in which the most extreme stress resistance was achieved—more than three times the mean desiccation resistance of the populations in the treatment with the second highest value—did not show continued corresponding increases in longevity. In fact, longevity decreased. When these populations with extreme desiccation resistance are included, there is no significant linear relationship between the two characters ($F = 1.8$, $P = 0.19$, $R^2 = 0.05$).

The populations comprising the datapoints in Figures 2 and 3 were divergent populations in which the evolutionary histories and/or selection regimes differed from one another, sometimes dramatically. Some were selected for increased or

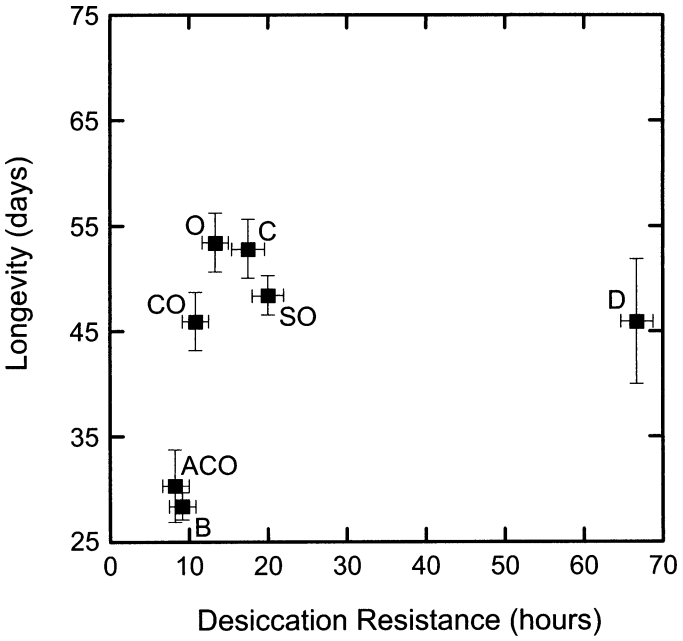

FIG. 3. Female longevity versus desiccation resistance. Datapoints represent bivariate means of five replicate populations ± SE.

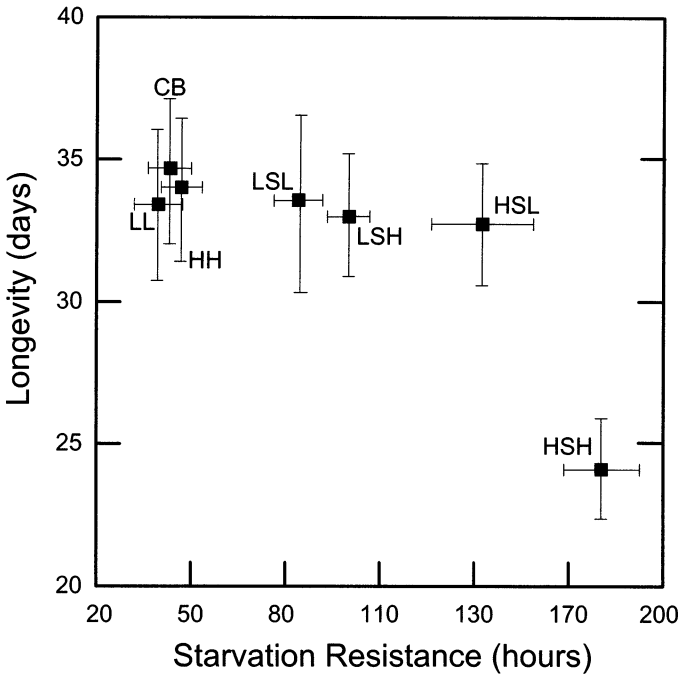

FIG. 4. Female longevity versus starvation resistance among starvation-selected stocks. Datapoints represent bivariate means of five replicate populations ± SE.

decreased starvation resistance (SO and RSO, respectively), some for desiccation resistance and starvation resistance (D), and others for specific fertility patterns (ACO, B, CO, O). They exhibit a variety of stress resistances and corresponding longevities but were not all explicitly selected for stress resistance.

In Figure 4 we examine the relationship between stress resistance and longevity among populations without such a variety of selection pressures. The populations included here varied only in the nutritional regime imposed during selection. These populations all originated directly from a common ancestral stock and were produced via selection on the same character (starvation resistance). Among these populations we observed a nonlinear relationship between starvation resistance and longevity. As with the relationships between starvation resistance and desiccation resistance with longevity shown in Figures 2 and 3, the selection regime that produced the greatest starvation resistance also resulted in the shortest longevity. If we consider only six of the seven selection regimes (ignoring the HSH treatment), the populations' mean starvation resistances ranged more than threefold, from 42 h to 132 h, yet the mean longevities all fell within the narrow range from 32.6 to 34.4 days. In the seventh selection regime, the HSH treatment, starvation resistance was increased even further, reaching 180 hours. This increase, though, was accompanied by a significant and substantial decrease in mean longevity, to only 24.0 days.

DISCUSSION

Summary and Interpretation of the Results: Breakdown of an Evolutionary Relationship

Over the course of long-term selection experiments, incorporating varied selective regimes, we found that the evolutionary correlations between characters among populations changed both quantitatively and qualitatively, and in the absence of identifiable genotype-by-environment interactions.

Before these experiments, we had generally found a simple positive correlation between longevity and starvation resistance. Selection for delayed reproduction (O vs. B) increased both longevity and starvation resistance (Service et al. 1985). Direct selection on starvation resistance increased longevity as it increased starvation resistance (Rose et al. 1992). In each case, these selection experiments suggested a consistent relationship between longevity and starvation resistance.

Further selection on starvation resistance (SO vs. CO), however, produced no corresponding longevity increase, despite continued significant increases in starvation resistance. Moreover, in flies that first were selected for increased starvation resistance for 94 generations and then were released from this selection (the RSO treatment), there was a 33% decrease in starvation resistance in only 12 generations that was actually accompanied by a slight (6%) *increase* in longevity (see Archer et al. 2003). Similarly, among flies selected for desiccation resistance (which also involved weak selection for starvation resistance, because the flies have no access to food while they are being desiccated), as their starvation resistance increased they concurrently exhibited decreased longevity.

Taken together, the results from these selection treatments reveal a complex, nonlinear relationship between starvation resistance and longevity, as shown in Figure 2. In the selection regimes represented in this figure, the evolutionary changes undergone by these two variables allow us to evaluate their relationship over a seven-fold range of starvation resistances and a two-fold range of longevities. Stress resis-

tance and longevity appeared to increase together up to some threshold of starvation resistance, after which increases in starvation resistance were accompanied by decreases in longevity.

A comparable nonlinear relationship between stress resistance and longevity also appears when we consider desiccation resistance. The long-term direct response to selection for desiccation resistance in the D populations was one of continuing increases in desiccation resistance. Similarly, selection regimes that decreased longevity (ACO) or increased longevity (e.g., O) also altered desiccation resistance as if the two traits (longevity and desiccation resistance) were positively, linearly correlated. However, as we found for starvation resistance, considerably greater increases in desiccation resistance were not accompanied by continued increases in longevity. Instead, longevity among the desiccation-selected D lines appears to have reached a plateau and perhaps begun to decrease slightly.

Figures 2 and 3 provide evidence that our stress-selected stocks were in the midst of an evolutionary correlation breakdown. In the case of starvation resistance, selection for this form of stress resistance appeared to continue unfettered. Yet longevity reached a plateau. In the case of desiccation resistance, successful upward selection on desiccation resistance continued whereas longevity, no longer increasing, possibly even started to decline. In neither case was there currently a significant linear relationship.

It is interesting to note that D flies fell nicely on the curve of starvation resistance and longevity (Fig. 2) and SO flies also fell right within the linear portion of the curve of desiccation resistance and longevity (Fig. 3). Selection for increased starvation resistance produced a moderate correlated response in desiccation resistance. Similarly, our selection procedure for desiccation resistance, which actually imposed slight selection pressure for starvation resistance, lead to an elevation in starvation resistance. In each case, however, moderate increases in the stress resistance characters were accompanied by significant increases in longevity.

When laboratory selection first builds flies that are better at resisting stress, it is also apparently building flies that can live longer. Alleles boosting one character seem to boost the other as well. It is only when these selective regimes are pushed to extremes, doubling or tripling mean stress resistance, that accompanying increases in longevity disappear. As selection squeezes out additional increases in stress resistance, longevity no longer increases. It may, in fact, be reduced.

Despite the similarities between Figures 2 and 3, we cannot draw a definite conclusion about the cause of the changing among-population correlation because of the confounding of selection pressures. Few of the populations in the figure were subject to a single, consistent selection regime. Instead, most were subject to a particular selection pressure only after being derived from a population that had experienced laboratory selection for some other trait. For example, in Figure 2, the SO populations were derived from O populations, nine years of strong selection for late fertility followed by selection for starvation resistance. During the subsequent nine years of selection for starvation resistance, selection on late fertility was relaxed. Each of the treatment groups of populations has had a similarly complex evolutionary history. Therefore, the present study is not based on a simple, consistent pattern of selection, but a somewhat natural pattern of complex selection pressures, which limits interpretation.

Stronger support for the inference of correlation breakdown is presented in Figure 4. To produce the 35 populations that comprise this figure, we selected solely on one trait, starvation resistance, and produced stocks exhibiting mean starvation resistances distributed evenly across a spectrum. We then measured the longevities characterizing flies at each starvation resistance.

Populations selected for starvation resistance under varying nutritional regimes exhibited a similarly nonlinear relationship between longevity and starvation resistance. In Figure 4, slight increases in longevity accompany starvation resistance increases to intermediate values. However, among the stocks responding to starvation selection with the greatest increases in starvation resistance, longevity no longer increases but rather is significantly lowered. The HSH selection protocol, for example, led to a four-and-a-half-fold increase in mean starvation resistance—from 40 hours to nearly 180 hours—among the five replicates during 35 generations of selection. During this time, mean longevity *decreased* nearly a third, from 34 to 24 days.

The Changing Evolutionary Correlations Are Not Due to Linkage Disequilibrium, Inbreeding, or Genotype-by-Environment Effects

Of the three ways in which genetic correlations might change in the laboratory, genotype-by-environment interaction (G×E) is perhaps the best studied (Stearns et al. 1991; Morris et al. 1993; Leroi et al. 1994b; Ariyo and Ayo-Vaughn 2000). Via (1984), for example, found that in the vegetable leafminer, *Liriomyza sativae*, the genetic correlations between different characters (pupal weight and development time) across environments did not remain constant. In *Drosophila*, Service and Rose (1985) used correlations between relatives to show that a novel environment changed the genetic correlation between early fecundity and starvation resistance toward higher values, whereas quantitative trait loci studies of recombinant inbred lines of *D. melanogaster* reveal commonplace G×E effects on lifespan (e.g. Vieira et al. 2000).

Table 2 highlights the fact that fitness characters that we measured are indeed strongly environment specific. By measuring longevity in three different ways, we discovered that *Drosophila* longevity varies significantly with environment. There is an approximately 25% difference in mean longevity depending on whether vials are kept vertically and changed every day or kept on their sides and changed only three times each week. This reveals that, among aging flies, the food at the bottom of their vials can pose a significant mortality threat. This suggests that the physical interaction between gravity, vial conditions, and the flies is a significant one. These data also have important implications for the detection of a changing evolutionary correlation between stress resistance and longevity. By measuring longevity under a variety of different conditions, we were testing whether the stress-resistant stocks fail to increase longevity in a linear fashion simply because of G×E interaction. That is, by modifying

the handling of the flies, we attempted to eliminate a possible source of mortality that might have arisen only as a result of changes in the behavior and/or physiology of the flies with extremely high stress resistance.

As we discovered, however, the flies with the most extreme stress resistances—the SO strains for starvation resistance and the D strains for desiccation resistance—did not experience greater increases in longevity in the side-vial assays than did their control groups. Life span increased in every group in the safer environment, but the differences between strains did not significantly change (Fig. 1). There was no apparent G×E interaction.

Linkage disequilibrium, too, can in theory produce genetic correlations between characters that are affected by alleles that have nonrandom phase correlations across loci (Bulmer 1985). When this occurs, genetic correlations will depend on genetic phase disequilibrium. However, this effect is likely to be small for quantitative characters such as stress resistance and longevity, which are affected by many loci; the linkage disequilibria among the many loci determining the characters will tend to have little consistent effect on genetic correlations.

Inbreeding, too, is unlikely to be a significant factor in these results. Hutchinson and Rose (1991) found no evidence for hybrid vigor in crosses of the B and O stocks used here. L. D. Mueller (unpubl. ms.) estimates an effective population size of about 1000 in these stocks. This estimate is based on both multi-generation estimates of population size and the distribution of male mating success. With respect to the inbreeding of neutral alleles, these populations are at most 20–25% inbred. Alleles affecting functional characters, and thus subject to selection, are likely to be subject to a much slower rate of fixation by inbreeding.

Nonlinear Selection versus Allele Frequency Changes?

In addition to changes in linkage disequilibrium and G×E effects, genetic correlations can change as a consequence of selection that changes the frequencies of alleles that vary in their patterns of pleiotropy (Bohren et al. 1966). Laboratory evolution is not a process like artificial selection, which focuses on a single trait or trait index. Multiple characters undergo selection during laboratory evolution. Moreover, the selection differential imposed on these characters will not usually be known. Therefore, although laboratory evolution with bouts of starvation may impose selection for increased starvation resistance, it is conceivable that this same selection procedure incidentally imposes 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.

Alternatively, it is possible that 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 (Gibbs et al. 1997; Djawdan et al. 1998). This extra weight might have deleterious effects on adult survival. In this scenario, selection changes one character, stress resistance, which in turn has a nonlinear relationship with another character, longevity.

Finally, it is possible that there are alleles that happen to enhance both stress resistance and longevity, and they are favored by natural selection in the cages of the SO and D flies. However, once these alleles are fixed by selection, the only remaining alleles that foster increased stress resistance are either neutral or deleterious with respect to longevity.

We do not have the information required to choose among these contrasting scenarios. It is also conceivable that the evolutionary correlation breakdown presented here involved all three of these selective mechanisms. And there are bound to be still other selection scenarios that we have not thought of. But we are willing to conclude that our results indicate that selection, rather than some other process, has acted to break down a well-established correlation. Such a dramatic correlation breakdown in a relatively simple laboratory system is a result of some significance.

Predicting the Course of Evolution

Studies using selection experiments to study correlations usually employ selection for only a relatively small number of generations, often no more than 20 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 life-history traits and provide empirical support for theoretical models predicting changes in genetic correlations between two characters during selection (Slatkin and Frank 1990). But these results do not, in principle, enable predictions about the long-term evolution of these traits, which should inhibit extrapolation from short-term quantitative genetic data to long-term evolutionary patterns and relationships. The present study experimentally illustrates a potential pitfall for such extrapolations. If evolutionary correlations are not durable within the context of selection in a single laboratory, then they are unlikely to be durable over natural environments over the course of millions of years of evolution.

The argument that short-term selection cannot predict the long-term evolution of correlations is not novel (e.g. Sheridan and Barker 1974; Wilkinson et al. 1990; Falconer and Mackay 1996). Turelli (1988) has shown that long-term changes in genetic covariances are not reliably predictable, whereas Leroi et al. (1994c) showed that a genotype-by-environment interaction can evolve and obscure the negative correlation between longevity and fecundity in about 100 generations of laboratory evolution. Likewise, inbreeding can alter correlations, as discussed above. Considered together with our findings that evolutionary correlations can be qualitatively altered in the course of sustained selection, we suggest both that evolutionary correlations not be regarded as durable features of a species and that short-term evolutionary relationships be extrapolated to long-term evolutionary patterns only with great caution.

Acknowledgments

We thank T. Burnham and J. Vallone for helpful comments on the manuscript and discussion. We are also grateful to the editors and the referees for their helpful suggestions. This research was supported in part by grants AG09970 to MRR and AG11644 to J. G. Tower and MRR.

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Corresponding Editor: D. Houle