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SELECTION ON STRESS RESISTANCE INCREASES LONGEVITY IN *DROSOPHILA MELANOGASTER*

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Abstract — Tests for the causal involvement of specific physiological mechanisms in the control of aging require evidence that these mechanisms can be used to increase longevity or reproductive lifespan. Selection for later reproduction in *Drosophila* has been shown to lead to increased longevity, as well as increased resistance to starvation and desiccation stresses. Selection for increased resistance to starvation and desiccation in *Drosophila melanogaster* is here shown to lead to increased longevity, indicating that alleles that increase stress resistance also may increase longevity. The responses of desiccation and starvation resistance to selection are partly independent of each other, indicating a multiplicity of physiological mechanisms involved in selectively postponed aging, and thus aging in general.

Key Words: stress, selection, *Drosophila*, longevity

INTRODUCTION

ONE OF the central problems of experimental gerontology is the delineation of the physiological mechanisms that causally determine rates and patterns of aging. There are three obvious methods of approaching this problem: (1) study of the temporal correlates of aging in defined cohorts, (2) comparison of organisms undergoing accelerated aging with normal organisms, and (3) comparison of organisms undergoing postponed or slowed aging with normal organisms. Some decades of research using the first two approaches have not led to much in the way of strong inferences concerning the physiological mechanisms controlling aging (Comfort, 1979; Finch, 1990). There are self-evident reasons for this failure. Temporal correlates of aging need not be causally significant, and the problem of delineating such causality requires other experimental methodologies. Organisms with shortened lifespan could be dying because of novel pathologies unrelated to normal aging. For these reasons, it has been argued that the study of organisms undergoing postponed aging is likely to be the most successful approach (Hutchinson and Rose, 1987; Johnson, 1987; Rose, 1991, chap. 8).

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The obvious experimental design for the study of organisms whose aging is genetically postponed is to compare the physiological properties of such organisms with those from control populations (e.g., Rose *et al.*, 1984; Service *et al.*, 1985; Service, 1987; Luckinbill *et al.*, 1988). An analogous strategy can be used with organisms that have undergone physiological postponed aging as a result of an environmental treatment, such as dietary restriction (reviewed by Masoro, 1988; Weindruch and Walford, 1988). But there are several problems with either approach. With environmental manipulations that postpone aging, it remains uncertain whether a specific induced physiological difference *itself* causally postpones aging, or is merely a secondary effect of either the treatment or a mechanism that is causally involved in postponing aging. With genetically differentiated stocks, it is analogously unclear whether the physiological difference is a result of accidentally fixed alleles, or alleles changed in frequency because of linkage disequilibrium with mutant or selectively favored alleles. Either of these genetic difficulties could produce a spurious correlation between postponed aging and physiological differentiation.

To some extent, these problems with genetically postponed aging can be alleviated using replication, large population sizes, or further genetic analysis. When there are only a few stocks with postponed and normal aging, the problem of accidental genetic differentiation during mutagenesis or selection is profound. Differentiation of experimental populations due to genetic sampling effects is one of the best studied phenomena of population genetics (e.g., Wright, 1977). Given enough generations, each population becomes a unique population-genetic entity. For this reason, a relative comparison of two such populations has zero degrees of freedom for statistical inference; it is only when such comparisons are replicated over distinct pairs of populations that there is any statistical power for inference. Thus, a number of studies of genetically postponed aging (e.g., Pretzlaff and Arking, 1989) with one postponed aging population and one control population amount to anecdotal information at the genetic level. In such studies, the populations examined may indeed be differentiated as inferred but repetitions of the experiment involving different populations selected in the same manner may not give consistent results. This arises because of between-population, within-treatment variance, a variance component that such experiments systematically neglect. However, the solution to this problem is straightforward: multiple replication of selected and control populations from the outset of the experimental program (see Luckinbill *et al.*, 1984; Rose, 1984; Service *et al.*, 1985; Hutchinson and Rose, 1991).

Linkage disequilibrium is a somewhat related problem, and replication within and between populations can alleviate it to some extent. The reason for this is simply that the more generations allowed for recombination to breakdown a linkage association, the greater the approach to linkage equilibrium. Similarly, the more generations of selection in a selection experiment, the greater the opportunity for recombination to break down an association. In a genetic experiment where the position of the allele is known, the greater the number of crosses that are performed, the greater the opportunity for separating the relevant allele from others.

In this article, we present a method for forcing a breakdown in artifactual associations between physiological mechanisms and postponed aging. The method is selection upon the correlated physiological character(s) in replicated lines that are paired with control lines. Selection would be applied in the same direction that the character is differentiated in outbred stocks that have genetically postponed aging, with the goal of further postponing aging. The advantages of this procedure are that additional lines can be created, more generations may be generated in which recombination can break down linkage disequilib-

rium, and the genetic correlation between a given character and aging can be immediately tested. In the case of wholly accidental associations between the test character and postponed aging, this type of test should be definitive. With respect to linkage disequilibrium, this type of test can only quantitatively increase confidence in the mechanistic association between the character and postponed aging. However, any such increase is nonetheless worthwhile. The two characters that are tested in the present report are starvation resistance and desiccation resistance, both of which have been found to be associated with selectively postponed aging in *Drosophila melanogaster* (Service *et al.*, 1985, 1988).

MATERIALS AND METHODS

Stocks

All selection experiments used populations derived originally from a South Amherst, Massachusetts, *Drosophila melanogaster* population studied by P.T. Ives (1970). Specifically, the experimental stocks started from a set of 10 populations derived from this Ives stock in February 1980, with five kept under an early reproduction (at 2 weeks of total age) regime (B) and five kept with later culture reproduction, at 10 weeks of total age, since 1981 (O) (Rose, 1984; Hutchinson and Rose, 1991). The "Os" have greater longevities than the "Bs." (In 1990, mean adult male longevity over five B (about generation 270) populations $\pm$ standard error was 39.3 $\pm$ 1.0 days; for O (about generation 60) populations ($\pm$SE), it was 72.4 $\pm$ 1.9 days, $p < 0.01$ [t test].) These B and O stocks also have large differences in age-specific fecundity (Rose, 1984), morphology (Rose *et al.*, 1984), stress resistance (Service *et al.*, 1985; Service, 1987; Service *et al.*, 1988), metabolic rate (Service, 1987), locomotion (Service, 1987; Graves and Rose, 1990), and caloric reserves (Service, 1987; Graves *et al.*, in press.).

Selection procedures

In 1988, two populations were derived from each of the O populations, O_1–O_5. One population of each pair was selected for desiccation resistance by placing the young adults (both sexes) of each generation in sealed cages containing desiccant and no food for enough time to kill most (70–90%) of the adults. Surviving adults were then allowed to feed and reproduce. These selected populations were designated D_x, the subscript giving the O population of origin. The other populations were used as controls and handled like the D_x populations, except that nonnutritive agar rather than desiccant was supplied. This procedure killed very few of these control (C_x) flies. The first six generations of the response to such selection are discussed elsewhere (Rose *et al.*, 1990).

The second experiment, begun in 1989, was analogous to the first in design, except that (1) both sets of B and O populations were used to found pairs of selected and control lines, giving 20 new populations, and (2) selected flies (both sexes) were subjected to starvation rather than desiccation. Control flies had access to food medium throughout life. The control populations are designated CB_x and CO_x according to the population of origin, while the selected are SB_x and SO_x, analogously.

In addition, four desiccation-selected lines were derived from the Ives stocks in 1990 in order to test for effects of stock background on the response to selection for desiccation resistance.

The complete set of stocks of interest are outlined in Table 1.

TABLE 1. A LIST OF ALL THE DIFFERENT TYPES OF LABORATORY *D. MELANOGASTER* POPULATIONS RELEVANT TO THE PRESENT STUDY

Name	*Source*	*Year founded*	*Number*	*Selected for*
IV	Wild	1975	1	2-week net fertility
B	IV	1980	5	2-week net fertility
O	IV	1980	5	10-week net fertility
C	O	1988	5	3-week net fertility
D	O	1988	5	Resistance to desiccation
ID	IV	1990	4	Resistance to desiccation
CB	B	1989	5	4-week net fertility
SB	B	1989	5	Resistance to starvation
CO	O	1989	5	4-week net fertility
SO	O	1989	5	Resistance to starvation

Assay methods

Starvation resistance was assayed in vials of 4–5 flies provided with a humid atmosphere only, following Service *et al.* (1985). Desiccation resistance was assayed using vials containing desiccant (Service *et al.*, 1985). Longevity assays were made using vials of culture medium, three males and three females per vial, with transfers every two to three days. Dead flies were not replaced.

Statistical methods

Data were analyzed using comparisons of population means and their linear regressions on generation number, as in previous studies (e.g., Service *et al.*, 1988). The population mean is the appropriate test statistic in selection studies since quantitative genetics indicates it is the parameter that performs predictably (Falconer, 1981).

RESULTS

The response to selection for starvation resistance is indicated in Figs. 1 and 2. Starvation resistance increased substantially in the SB and SO populations over the course of selection, but not in the controls. Figure 2 shows the indirect response of longevity (with adequate nutrition) to selection for starvation resistance. Both SB and SO populations increased substantially in mean adult longevity, with total increases of about 12 and 17 days, respectively. CO populations did not change significantly in mean longevity, while CB populations did. However, even if the statistical test for significant response of longevity to starvation selection is made relative to these controls, it is still significant in both SO and SB treatments. Figure 3 gives survivorship curves for generation 19 of this experiment, males and females plotted separately.

The cause of the CB longevity increase may be the change in demographic regime imposed during selection. In response to selection, the SB and SO populations evolved greatly increased starvation resistance (Fig. 1), forcing an increase in the generation time

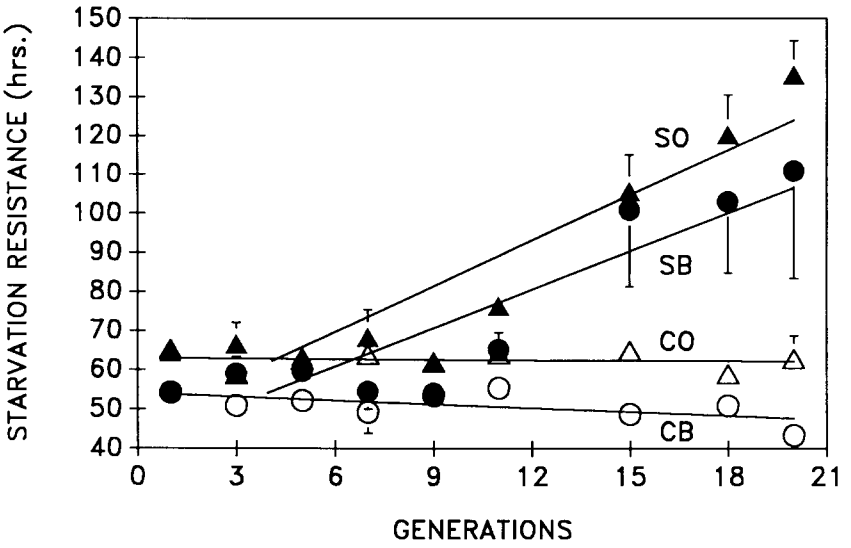

FIG. 1. The direct response to selection for increased starvation resistance. The solid symbols indicate the average of the lines undergoing selection. The hollow symbols indicate the average of lines not undergoing additional selection. The triangles indicate the lines derived from longer-lived populations created by selection in the laboratory. The circles indicate the lines derived from short-lived controls. The error bars indicate 95% confidence intervals for each treatment in each generation; where none is indicated, the confidence interval is narrower than the width of the symbol. Only the SB and SO treatments have average slopes that are significantly different from zero. For SB, the average slope over treatments ($\pm$ SE) is 3.40 $\pm$ 0.75. For SO, the average slope is 3.84 $\pm$ 0.20. These slopes are not significantly different from each other.

(from 14 days to about 28 days) that must have led to selection for a shift to later reproduction and thus postponed aging (e.g., Rose, 1984) in the CB and SB populations. The O populations had already been selected for later reproduction, so this is not expected to arise with CO and SO treatments.

There was also a significant response of desiccation resistance to selection for starvation resistance. In generation 18 of selection for starvation resistance, the desiccation resistances (mean $\pm$ SE) were as follows: CB—9.1 $\pm$ 0.1 h; SB—12.6 $\pm$ 0.2 h; CO—11.3 $\pm$ 0.4 h; SO—15.1 $\pm$ 0.2 h (CB vs. SB, $p < 0.01$, paired t test; CO vs. SO, $p < 0.01$, paired t test). In both cases, the starvation-selected lines have significantly increased desiccation resistance.

Table 2 shows the response to selection for desiccation resistance at generations 19, 30 or 31, and 45. Desiccation resistance increased substantially. Again, male and female longevities increased over the course of selection. These results, together with those of Fig. 2, indicate that longevity can be increased by selection for characters that are positively correlated with longevity in demographic selection experiments, like stress resistance. However, starvation resistance did not significantly increase.

By contrast, in the four desiccation-selected lines derived from the Ives stocks there was a statistically detectable increase in starvation resistance (average net response $\pm$ SE: $+16.5 \pm 2.5$ h, $p < 0.01$).

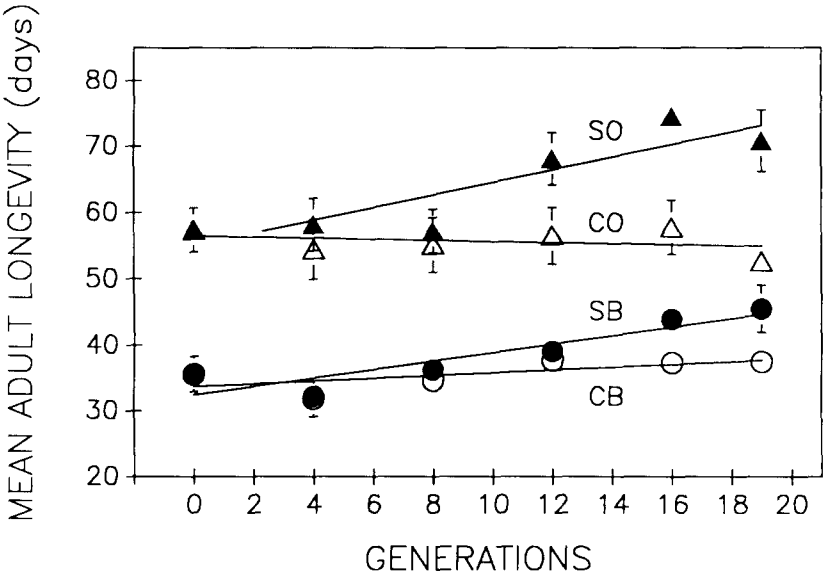

FIG. 2. The indirect response of longevity to selection for increased starvation resistance. These data average the males and females together. Analysis of the sexes separately gives similar results, with one exception noted below. The symbols indicate the same treatments as in Fig. 1. The CB, SB, and SO treatments have average slopes that are significantly different from zero: CB—0.21 ± 0.07; SB—0.66 ± 0.07; SO—0.94 ± 0.03. The SB and SO slopes are significantly greater than the CB and CO slopes, respectively. The SO slopes are significantly greater than the SB slopes for the average of the two sexes and for males, but not for females.

DISCUSSION

The present results show fairly clearly that indirect selection upon longevity-related characters can further increase longevity, and thus postpone aging. A number of additional questions remain. First, the response of longevity was significantly slower in SB males compared with SO males, though not in females. This suggests the possible importance of genetic background. However, it would be unwise to conclude much from this result, because selection differentials were not monitored. Without them, genetic differences cannot be inferred from the pattern of selection response with certainty. On the other hand, the fact that the *direct* response to selection was similar in SB and SO populations while the indirect response was not, suggests that at least the direct response to selection was similar in the two types of line. That leaves only inadvertent indirect selection on the line types as a source of artifactual differences in the indirect response of longevity, other than a difference in initial genetic structure. Note that derivation from the longer-lived O populations did not impose any limitation on the further increase of longevity under selection, relative to the shorter-lived B populations.

Second, are desiccation resistance and starvation resistance genetically correlated to such an extent that the present selection experiments are acting upon the same loci? Table 2 indicates that selection for increased desiccation resistance does not significantly increase starvation resistance, as was found in an earlier study (Rose *et al.*, 1990). How-

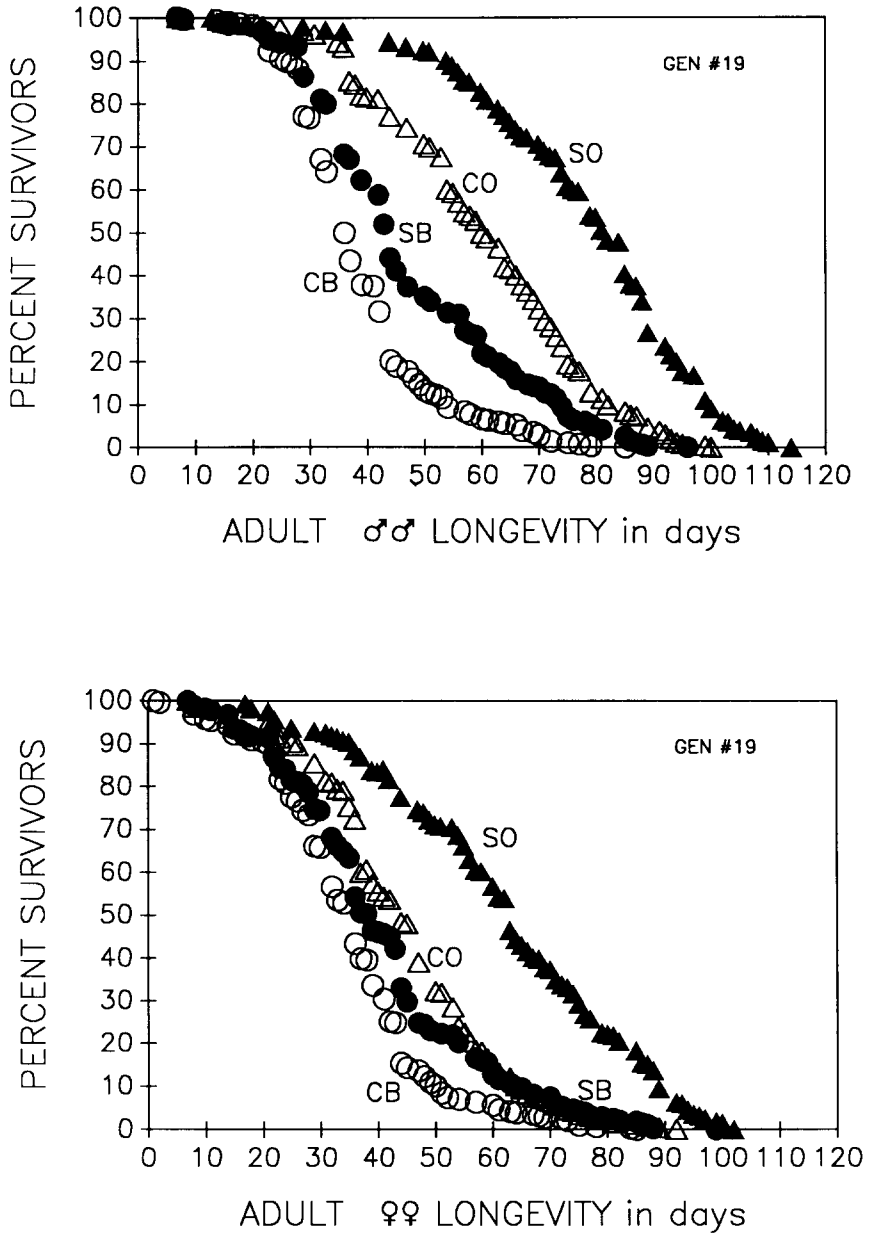

FIG. 3. Survivorship curves for generation 19 of the starvation selection experiment. Top: males; bottom: females. The solid symbols indicate the average of the lines undergoing selection. The hollow symbols indicate the average of the lines not undergoing additional selection. The triangles indicate the lines derived from longer-lived populations created by selection in the laboratory. The circles indicate the lines derived from short-lived controls.

TABLE 2. SELECTION RESPONSE WITH DESICCATION SELECTION. AVERAGE RESPONSE OF D_X STOCKS AND C_X STOCKS

	Generation					
	19		*30/31*		*45*	
Character	*D*	*C*	*D*	*C*	*D*	*C*
Desiccation resistance (h)	30.3**	19.3	29.5*	16.5	34.4*	14.7
Ethanol resistance (h)	53.0**	37.9	67.2*	35.2	77.9*	31.3
Starvation resistance (h)	64.7	60.4	64.9	54.6	69.5	77.9
Male longevity (days)	—	—	54.3**	39.4	61.2*	49.6
Female longevity (days)	—	—	64.7**	56.5	66.6**	60.4

All hypothesis tests are two tailed; all use pairwise comparison comparisons of D and C populations according to their O population of origin. All consistently significant single-generation effects are also significant when analyzed over all generations, but not otherwise (* $p < 0.05$; ** $p < 0.01$). Longevity was not measured in generation 19. Some of the stress resistance characters were measured in generation 30, and the rest in generation 31.

ever, selection for starvation resistance gave a significant increase in desiccation resistance, totalling about 3–4 h. Earlier work on the physiology of starvation resistance indicated that lipid levels were a major factor in the determination of starvation resistance (Service, 1987). In addition, our unpublished studies of glycogen levels in these flies indicate that a major determinant of desiccation resistance is glycogen level (Graves *et al.*, in press). However, glycogen is also an energy source, like lipid. O flies have increased levels of lipid relative to B flies (Service, 1987). If we assume that starvation resistance selection acts to increase both lipid and glycogen levels, with the former quantitatively more important, while desiccation selection primarily acts upon glycogen, then the present results could arise from a proportionally slight effect of increased glycogen levels upon starvation resistance in the D flies, which are derived from high-lipid O flies. The greater effect of starvation selection upon desiccation resistance found in the SO and SB populations could then be interpreted in terms of large increases in both lipid and glycogen level with sustained starvation selection. A critical test of this hypothesis is that the effect of desiccation selection upon starvation resistance should be more detectable when lipid levels are low, as in the B and Ives stocks. This is exactly the result observed when desiccation-selection was imposed on derivatives of the Ives stock.

Third, it should be noted that ethanol resistance, another character associated with increased longevity in previous studies (Service *et al.*, 1985), increases in parallel with desiccation resistance (Table 2), as found in earlier work (Rose *et al.*, 1990). There may be considerable genetic redundancy between ethanol resistance and desiccation resistance.

The present selection results can also be used to calculate roughly the contribution of starvation and desiccation resistance to postponed aging in the original O stocks, providing complete additivity of effects is assumed. (Evidence for average additivity of starvation resistance alleles has been provided elsewhere [Hutchinson and Rose, 1991].) The average total increase in starvation resistance in the SB and SO lines is about 53 h, which was associated with an average total increase in longevity of about 14.5 days. The average total increase in desiccation resistance in the D lines is about 20 h, while the average total

increase in longevity is about 9 days in the D lines. If we assume that all of the increase in longevity in the D lines is due to increases in desiccation resistance, then the effect upon longevity is +0.45 days for each hour of increased desiccation resistance. In the original O stocks in 1984, desiccation resistance was increased by about 3 h (Rose, 1984), suggesting that about 1.35 days of the longevity difference of 12 days between O and B stocks was due to increased desiccation resistance, or about 11% of the total longevity increase. The average increase in desiccation resistance in the SB and SO lines was about 3.7 h, which (again assuming additivity) would give rise to an increase of about 1.6 days in longevity. Thus, the residual impact attributable to the physiological effect of increased starvation resistance alone would be about 12.9 days of increased longevity. The corrected proportionality between starvation resistance alone and longevity is about 0.24 days increased longevity for each hour of increased starvation resistance. The original difference between B and O in starvation resistance was about 11 h, which would then produce about 2.7 days of increased longevity, approximately 22% of the total longevity difference. To perhaps an order of magnitude, then, the loci determining starvation resistance and desiccation resistance are responsible for a total of about 33% of the increased longevity of the O populations. This indicates that changes in just two physiological mechanisms can substantially postpone aging. It also indicates that additional mechanisms are involved, mechanisms that might serve as further targets of selection to postpone aging.

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