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The Effects of Nutritional Manipulation and Laboratory Selection on Lifespan in *Drosophila melanogaster*

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There are parallels between the effects of laboratory selection and nutritional manipulation on the expression of lifespan and other fitness-related characters in* Drosophila melanogaster.** ***However, little is known about the effects of laboratory selection and nutritional manipulation when applied simultaneously. Given that* D. melanogaster *is one of the major model organisms for testing theories of aging, simultaneous application of laboratory selection and nutritional manipulation is of considerable interest. To that end, we developed six groups of fivefold replicated populations selected for either early or late fertility. Each of these groups was maintained on either high- or low-nutrition diets. Comparisons among the groups showed that nutrition is neutral in selecting for lifespan. Moreover, the dietary-restriction response can be broken by simultaneous selection and nutritional manipulation. Finally, characters that respond in a parallel manner under selection or nutritional manipulation may not when the two are applied simultaneously.

NUTRITION controls the relationship between survival and reproduction, at least in part, in taxa as phylogenetically diverse as insects and mammals (1). Nutritional control of the relationship between survival and reproduction, or "dietary-restriction," has long been studied and is best documented in laboratory rodents (e.g., 2–9). Rodents whose diets are calorically restricted but otherwise adequate exhibit increased lifespan compared to controls who are fed ad lib. However, there is a penalty paid for increased lifespan in the form of reduced fertility.

Various theories have been advanced to explain this trade-off between reproduction and survival. Some have hypothesized that the dietary-restriction response is an adaptation to dietary uncertainty (10). If food availability is unpredictable, the optimal strategy is to put energy into surviving rather than reproducing, at least until food availability becomes predictable. Others have hypothesized that the degree of dietary restriction exhibited by a particular taxon is a function of the relative amount of energy required for reproduction (11). Species which reproduce relatively early in their lives and often, like laboratory rodents, will exhibit a greater dietary-restriction response than species which reproduce relatively late and infrequently, like many primates, including man. Others ask if the dietary-restriction response is an adaptation to environmental circumstances at all (12). Implicit in this suggestion is an "internal" cause for dietary-restriction, reflecting unnamed constraints caused by a general physiological antagonism between survival and reproduction.

Although it is not yet obvious which, if any, of the hypotheses outlined above best explains the dietary-restriction response, it is clear that the relationship between survival and reproduction can also be changed by natural selection or its laboratory analogue, artificial selection. For example, in various species of *Drosophila*, selection for fertility late in life increases lifespan at the cost of reduced early fecundity (13–15). Other survival characters beside lifespan also exhibit the same selective relationship with reproduction. The ability to resist starvation, which might be thought of as lifespan in the absence of nutrition, trades-off with fecundity early in life under laboratory selection. *Drosophila* artificially selected for increases in the ability to resist starvation exhibit reduced early fecundity (16). Conversely, flies selected for increased early fecundity exhibit decreased starvation resistance, as well as decreased lifespan (17,18).

Interestingly, the relationship between the ability to resist starvation and reproduction is also diet-mediated. *Drosophila* conditioned on relatively restricted diets are much better at resisting starvation, again at the cost of reduced early reproduction. This reverses on rich diets, with early reproduction increasing and starvation resistance decreasing (19).

Apparently the patterns in the survival-reproduction relationship are similar in *Drosophila*, whether dietary restriction or laboratory selection is applied, and whether we consider lifespan or starvation resistance versus early fecundity. Under these circumstances, the interesting question is what happens to the relationship between survival and reproduction when nutritional manipulations *and* laboratory selection for lifespan are simultaneously applied?

There may be practical outcomes resulting from studies that explore this question, as well as important ramifications for understanding how nutrition influences patterns of aging and other survival and reproduction characteristics. Organisms with selectively postponed aging have proven to be important models for testing theories of aging. They have also been highly significant models for understanding the genetics, physiology, and demography of aging (e.g., 20–22). If organisms with selectively postponed aging can be produced more rapidly on particular nutritional regimes, it is to our advantage to know this. But there have been no studies in *Drosophila* of how the simultaneous application of selection and nutritional manipulations influence lifespan.

In particular, we are interested in comparing populations of *Drosophila* which are maintained under different nutritional conditions to determine if nutrition is a neutral factor in selection for increased lifespan or whether it enhances or opposes selection. There is some expectation that nutrition should not be neutral because lifespan is decreased under conditions of high nutrition. Accordingly, one would predict that progress *in selection* for increased lifespan under conditions of high nutrition would be less than in a relatively lower nutritional environment, given that the response to a high nutritional environment is opposed in direction to the response to selection. On the other hand, it is a common obser-

vation that progress in selection is often greater in "bad" environments (23), where a "bad" environment in this case would be one of high nutrition. Under those circumstances, progress in increasing lifespan should be greater with high nutrition than in a "good" or relatively lower nutritional environment. However, it must be immediately added that there is no theoretical justification for the latter expectation, only empirical evidence to that effect (23).

We are also interested in the way survival characters related to lifespan respond to the dietary manipulation and selection on lifespan. One such character is starvation resistance. Does starvation resistance respond similarly to lifespan as might be expected from earlier studies (e.g., 19)? If so, the common physiological basis of both characters so far identified would seem to adequately define their relationship. If not, then starvation resistance would exhibit a degree of independence from lifespan not yet revealed in other studies (cf. 24). In this circumstance, some aspect of the physiology and genetics of starvation resistance does not influence lifespan and the relationship of these two characters is more complex than expected.

Just as there are definable expectations for the response of lifespan to simultaneous dietary manipulation and selection, so are there for early fecundity. Under almost all conditions yet investigated, early fecundity responds in opposition to lifespan (see above). Will this result still occur when lifespan and fecundity are the direct targets of selection in high nutrition treatments? If not, the dietary-restriction response can be broken, with no cost paid in reduced reproduction for increased lifespan.

To explore the responses of survival and reproduction characters to simultaneous dietary manipulation and selection, we created six new groups of populations, each fivefold replicated. These populations were selected for either early or late fertility. Selection for late fertility has been repeatedly shown to increase lifespan substantially (e.g., 14,15). In both cases some groups of populations were also maintained on high or low nutrition diets. In order to facilitate comparisons between different treatments, controls were exposed to their selective environments for exactly the same amount of time as experimental populations.

Materials and Methods

Experimental Populations

All populations of *D. melanogaster* employed in these studies were derived from a group of populations, the Bs, which were selected for fertility at 2 weeks of adult age (15). Six selection treatments were derived, each fivefold replicated. Including the ancestral Bs, 35 populations were tested in these studies. The new treatments were called BH, BL, OL-α, OH, OL-β, and OHL. All like-numbered replicate populations from each treatment were derived from the like-numbered ancestral B population, e.g., $B_i \Rightarrow BH_i$, BL_i, OL-α_i, etc.

The letters in the acronyms indicate the selection treatment, "B" for early fertility or "O" for late fertility, and the nutritional treatment, "L" for low and "H" for high. The ancestral B populations were selected for fertility at 14 days of age, whereas the other "B" treatments were selected for fertility at 18 days of age. The "O" treatments were selected for fertility at increasingly later times in the life cycle during the course of the experiment. The "L" diet consisted of the standard banana-molasses medium, rich in carbohydrates. The "H" diet included the standard banana-molasses medium with the addition of live yeast paste. The live yeast paste was made by mixing 5 g of baker's yeast with 40 mL of deionized water and 2 mL of a 1% acetic acid solution. Although the nutritional or caloric content of the two diets was not measured, earlier work showed large effects of the live yeast treatment on lifespan, starvation resistance, and early fecundity compared to the standard banana-molasses medium (19,25).

At the time of the assays described below, the Bs had undergone selection for more than 400 generations. The BH and BL populations had been selected for about 40 generations, and the late fertility "O" stocks had undergone 27 generations of selection. Even in the case of the late fertility "O" stocks, this is a substantial number of generations of laboratory selection, much greater than the typical laboratory experiment. More often than not, substantial responses to selection occur within 5 to 10 generations in outbred populations of *D. melanogaster* for the characters tested in this experiment (26).

Selection Procedures

All stocks, including the ancestral Bs, were treated identically during the first two weeks of life. Eggs were collected and placed in 8-dram vials provisioned with about 2 cm of standard banana-molasses medium. Because density is known to affect the study characters (27,28), the vials were standardized at relatively uncrowded densities, 60–80 eggs/vial. After 2 weeks in vials, sufficient time for most flies to eclose, the imagoes were transferred to population cages. The B populations, however, were reproduced at 2 weeks, their entire life cycle spent in vials. See Figure 1 for a schematic representation of the selection procedures.

Replicate population sizes were about 8,400 flies in the new treatments and about 2,000 in the ancestral Bs. Studies indicate that there is no measurable inbreeding depression in the Bs, despite the hundreds of generations of selection in the laboratory (29). Accordingly, the new populations were sampled from rel-

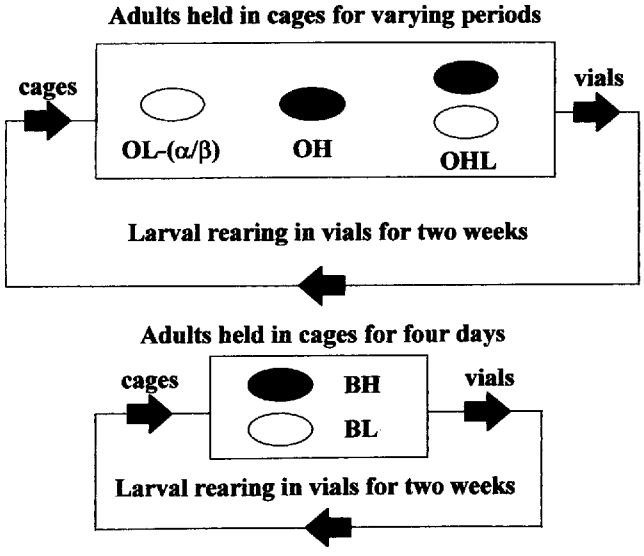

Figure 1. Selection protocol. All populations undergo 2 weeks of larval rearing in vials. Late-fertility–selected populations undergo a cage phase with one of three nutritional regimes: unyeasted plates (OL-α/β), yeasted plates (OH), or alternation between yeasted and unyeasted plates every 3 days (OHL). Early-fertility–selected populations undergo 4 days in cages with one of two nutritional regimes: unyeasted plates (BL) or yeasted plates (BH).

atively large, outbred populations, alleviating the complications caused by inbreeding when interpreting assay results.

These six new treatments were handled in three independent groups. BH and BL were run through their life cycles simultaneously, as were OL-α and OH, and OL-β and OHL. Operationally, this means that when selection is stopped on any replicate population in either of the two paired treatments, it is stopped in all replicates of both treatments. Effectively, each replicate is exposed to its selective environment for the same amount of time as all other replicates in the paired treatments. By so doing, the analyses become interpretable in terms of one or the other nutritional environments causing stronger selection. Differences in the amount of selection experienced by a treatment are indicated by differences in the values of the study characters within paired treatments.

The BH and BL treatments spent 4 days in population cages before egg collection for the next generation, the only difference in handling between the two being nutrition. Fresh food was provided daily. The selection procedures in the other treatments were slightly more complicated because successful selection for late fertility entailed longer periods of time spent in the populations cages across generations. Early on in the experiments, the early-fertility–selected cage populations and the late-fertility–selected ones spent the same amount of time in the cages, i.e., 4 days. By the end of the experiment, however, late-fertility selected populations had experienced 7–9-fold increases in time spent in cages compared to the "B" cage populations.

After 2 weeks in vials, the OL-α and OH stocks were transferred to population cages where only diet differed among the two treatments. Fresh food was offered daily. Three days before the end of the cage cycle, both groups received standard banana-molasses food with a highly concentrated live yeast paste added, four times as concentrated as the standard yeast paste. This food was changed daily before the egg collection. Adding a highly concentrated yeast paste to the diet stimulated egg production, thereby ensuring adequate numbers of adults to start the subsequent generation. In the absence of the highly concentrated yeast paste, insufficient numbers of eggs would be laid to maintain the large population sizes in the late-fertility–selected treatments.

The OL-β and OHL populations were treated similarly to the other late-fertility–selected treatments in most respects. But the nutritional regime of the OHLs involved 3-day periods of low nutrition alternating with 3 days of high nutrition, where this cycle was repeated throughout the entire cage phase. The OL-βs and OHLs received the highly concentrated yeast paste added to the standard medium during the last 3 days of the life cycle to stimulate egg laying. Alternating the diet on this time scale kept the flies in flux physiologically, rather than allowing acclimation to a particular level of nutrition (personal observation). The consequences of a constant flux for the relationship between survival and reproduction are unknown. However, the negative relationship between the two classes of characters may be due to the allocation of energy between the competing functions (30). Under flux conditions, it is not at all clear how the allocation of energy will be affected, or more directly for this study, what the selective consequences for lifespan, starvation resistance, and early fecundity might be.

Assay Procedures

All stocks were assayed for lifespan, starvation resistance, and fecundity at 4 days of adult age. All assays were conducted simultaneously.

Samples of flies from each replicate population were removed from their selective regimes for two generations prior to the assays and handled identically to control for parental effects. Flies from the third generation off selection that emerged on day 11 from the time of egg laying were either allocated to lifespan assays or to nutritional conditioning. Samples nutritionally conditioned were destined for starvation resistance or fecundity assays.

Nutritional conditioning entailed maintaining groups of eight flies (four male and four female) in vials with standard banana-molasses medium or the standard medium enriched with 100 μL of live yeast paste. The concentration of live yeast paste was identical to that used in the selection protocols. After 2 days of conditioning, flies were transferred to fresh vials. Upon completion of the full conditioning period of 4 days, flies were allocated to starvation resistance or fecundity assays. The responses of starvation resistance and early fecundity to the highly concentrated yeast paste diet provide a large contrast to the expression of those characters on standard medium alone. Earlier research showed that most of the response of these characters to yeast is revealed by this range of nutrition (19,25).

Life span.—Lifespan was assayed using the procedures outlined in Rose (15). Forty flies of each sex were assayed for each replicate, for a total of 2,800 flies. Flies were assayed in 8-dram vials with a layer of banana-molasses medium. For each replicate population, 10 vials, each containing four males and four females, were set up. Flies were transferred to fresh vials three times weekly. Each vial was checked daily for mortality. A fly was considered dead on the first check when it did not respond to mechanical stimulation on three consecutive checks.

Starvation resistance.—For each replicate at each of the two nutritional levels, five vials of each sex (four flies/vial) were set up as in Service and colleagues (31). The total number of flies assayed for starvation resistance was 2,800. Flies were placed in the bottom 2 cm of an 8-dram vial and closed off with a sponge stopper. Two cotton balls soaked with 100 μL of water were placed on the opposite side of the sponge and the whole vial was sealed with parafilm. This set-up supplied moisture in the absence of food. Mortality was recorded every 6 hours. A fly was considered dead on the first check if it did not respond to mechanical stimulation on three consecutive checks.

Early fecundity.—Early fecundity was assayed using the procedures of Rose and Charlesworth (32). Forty vials, each containing one male and female, were set up for each replicate population, 20 vials at each nutrition level. A total of 1,400 female flies were assayed. Fecundity vials contained a sucrose and charcoal medium (supplemented with yeast paste in the high nutrition treatment). Flies were discarded after 24 hours and the eggs were counted using a light microscope. The contrast between the medium and egg color facilitates egg counting.

Experimental Design and Statistical Analysis

All hypothesis testing used replicate means as variates. Treatment means and standard errors were calculated from the means of the five replicate populations.

Testing the effects of selection treatment on mean lifespans was accomplished using factorial analysis of variance. Analysis of variance was also used to test the effects of selection treatment

and nutrition level on the fecundity and starvation resistance characters. Orthogonal comparisons were made by decomposing treatment sums of squares. These planned comparisons allowed us to examine two classes of hypotheses: (i) Are there differences in a focal character between paired populations exposed to their selective environments for the same amount of time (i.e., BH vs BL; OL-α vs OH; OL-β vs OHL)? (ii) Are there differences in a focal character as a function of whether populations are selected for early or late fertility (i.e., B, BH, BL vs OL-α, OH, OL-β, OHL)?

Results

Life Span

The results of the lifespan assay are shown in Table 1. Analysis of variance and planned comparisons are shown in Table 2. Analysis of variance showed a statistically significant effect of selection treatment on lifespan ($F = 2.740, p < .05$). A planned comparison of early fertility selected populations, BH and BL, showed no differences in lifespan. Similarly, planned comparisons uncovered no differences in lifespan between the late fertility selected populations, OL-α and OH or OL-β and OHL. However, a comparison of the early-fertility–selected groups to the late-fertility–selected groups showed highly significant differences with the late-fertility–selected populations having a longer lifespan than the early-fertility–selected populations ($F = 15.314, p < .001$).

Starvation Resistance

As has been observed repeatedly, the ability to resist starvation was depressed in flies conditioned on live yeast compared to the standard banana-molasses medium (19,25). In this study, in each selection treatment, flies conditioned on standard medium outperformed their counterparts conditioned on yeasted medium, oftentimes by as much as 50% (see Table 3). Two-factor analysis of variance showed significant differences in the response of starvation resistance to nutrition. Moreover, there were also significant differences among selection treatments for starvation resistance as well (see Table 4). The interaction term between nutrition and selection treatments was not significant.

Given the lack of significance in the interaction term, specific comparisons among selection treatments on the joint response of starvation resistance to the two yeast levels were made (Table 4). Comparisons were made on the sum of the mean values for starvation resistance in both yeast levels for each replicate. As was the outcome of the lifespan comparisons,

Table 1. Summary of the Lifespan Assay

B	BH	BL	OL-α	OH	OL-β	OHL	
26.9	25.9	30.9	30.4	33.8	33.5	38.8	
32.5	29.9	24.9	36.2	36.7	33.2	32.0	
25.6	28.3	29.7	26.6	34.2	32.1	28.4	
26.8	27.3	29.4	33.2	31.2	31.2	27.3	
27.7	32.0	27.7	34.4	31.0	29.5	32.4	
27.9	28.7	28.5	32.2	33.4	31.9	31.8	Mean
1.2	1.1	1.0	1.7	1.1	0.7	2.0	*SE*

Each entry is the mean lifespan (days of adult lifespan) of a replicate. Means of each selection treatment (±*SE*) are also included.

Table 2. Analysis of Variance—Longevity

Source of Variation	*SS*	*df*	*MS*	*F*
Populations	142.726	6	23.788	2.740*
OL-α vs OH	3.721	1	3.721	0.429 (n.s.)
OL-β vs OHL	0.36	1	0.36	0.04 (n.s.)
BH vs BL	0.064	1	0.064	0.007 (n.s.)
Early vs late	132.947	1	132.947	15.31***
Error	243.064	28	8.681	

Single-factor ANOVA with selection treatment as the main effect. The treatment sums of squares were decomposed into orthogonal comparisons. Selection treatments were differentiated for longevity. "Early" refers to the early fecundity selected populations (B, BH, and BL) and "late" refers to the late fecundity selected populations (OL-α, OH, OL-β, OHL). The late treatments had significantly greater lifespans than the early treatments. *$p < .05$; ***$p < .001$; n.s. = nonsignificant.

Table 3. Summary of the Starvation Resistance Assay

B	BH	BL	LOW OL-α	OH	OL-β	OHL	
26.6	22.0	28.7	31.5	31.9	36.6	41.1	
24.0	27.4	25.4	35.7	28.9	26.4	35.3	
28.1	28.3	33.6	36.5	30.9	33.1	25.4	
29.8	28.2	32.3	31.4	32.2	29.3	29.7	
24.1	26.3	26.2	29.8	30.7	34.9	28.5	
26.5	26.4	29.2	33.0	31.0	32.1	32.0	Mean
1.1	1.2	1.6	1.3	0.6	1.9	2.8	*SE*
			HIGH				
19.6	16.6	19.7	20.3	19.2	24.5	21.8	
15.3	17.4	19.1	23.4	21.1	20.3	27.6	
20.8	18.5	22.6	20.3	18.5	21.5	20.7	
20.5	19.2	21.5	19.5	21.7	21.1	18.3	
21.7	21.6	17.9	25.3	24.3	22.9	21.7	
19.6	18.7	20.2	21.8	21.0	22.1	22.0	Mean
1.1	0.9	0.8	1.1	1.0	0.7	1.5	*SE*

Each entry is the mean starvation resistance (hours) of a replicate. Means of each selection treatment (±*SE*) are also included. "LOW" and "HIGH" refer to levels of nutrition.

Table 4. Analysis of Variance—Starvation Resistance

Source of Variation	*SS*	*df*	*MS*	*F*
Population	236.531	6	39.422	4.179**
Nutrition	1507.072	1	1507.072	159.745***
Population × Yeast	32.500	6	5.417	0.574 (n.s.)
OL-α vs OH	20.656	1	20.656	2.189 (n.s.)
OL-β vs OHL	0.025	1	0.025	0.003 (n.s.)
BH vs BL	519.256	1	519.256	55.04***
Early vs late	398.87	1	398.87	42.28**
Error	528.316	56	9.434	

Two-factor ANOVA with selection treatment and nutrition, as measured by the amount of dietary yeast, as main effects. Both treatments had significant effects on starvation resistance. The treatment sums of squares were decomposed into orthogonal comparisons (see text). "Early" refers to the early fecundity selected populations (B, BH, BL) and "late" refers to the late fecundity selected populations (OL-α, OH, OL-β, OHL). The starvation resistance across yeast levels was greater in the BLs than in the BHs. The "late" populations also had significantly greater starvation resistance across yeast levels than the "early" populations. **$p = .002$; ***$p < .001$; n.s. = nonsignificant.

no significant differences were found in starvation resistance for the joint response to yeast between late-fertility–selected populations selected in the presence of yeast and their non–yeast-selected controls. However, the BLs were significantly more starvation resistant than the BHs ($F = 55.04$, $p < .001$). The starvation resistance of the late-fertility–selected populations across yeast levels was significantly greater than that of the early-fertility–selected populations ($F = 42.28$, $p = .002$), ranging from a low of 18.7 hours in the BHs to a high of 22.1 hours in the OL-βs.

Early Fecundity

The results of the early fecundity assay are displayed in Table 5. Analysis of variance showed significant effects of nutritional treatment but not selection treatment (see Table 6). As has been reported before (19,25), fecundity greatly increases with the addition of live yeast paste to the standard medium, by as much as 6–7 times in the current assay.

Discussion

In these studies, late-fertility–selected treatments outlived the early-fertility–selected treatments in comparisons including populations selected under high- or low-nutritional conditions in both groups. Given the robust nature of this result, it is not surprising that other laboratories have successfully selected for increased longevity, despite applying slightly different protocols (e.g., 13,14,33).

What is somewhat surprising is the lack of differentiation for lifespan in comparisons between late-fertility–selected populations in yeasted and nonyeasted environments. Despite the observation that populations conditioned on yeast have decreased longevity (19), evidently *selection* in a yeasted environment did not retard the response to selection for increased longevity. Prediction of the selection response based on a simple nutritional manipulation of the longevity phenotype is thus inappropriate. Effectively, high nutrition, at least as represented by the addition of live yeast paste to the standard medium, is neutral from the viewpoint of selection. These studies, in which populations were exposed to their selective environments for the same duration, show that indistinguishable progress in enhancing lifespan can be made in low- or high-nutritional environments. Put another way, statistically equivalent responses to selection for lifespan occurred in both nutritional environments. This conclusion should not be viewed as a demonstration of substantial equality over environments. Any study of this kind is limited to the statistical power inherent in its design; a considerably larger study might possess enough power to detect differences between nutritional regimes in their effect on selection response. Nonetheless, the data at least indicate an absence of large-magnitude differences between selection responses over different nutritional regimes, whatever the subtler differences that might have been missed.

A corollary of manipulating the lifespan phenotype by nutrition is the accompanying change in early fecundity. As nutrition increases, lifespan decreases and early fecundity increases. However, in these selection studies, increases in lifespan were realized without concomitant measurable decreases in early fecundity. Here there was no cost to enhancing lifespan paid through early fecundity. It might be argued that there was little genetic variation remaining for early fecundity, given that the ancestral B populations had been subjected to selection for early fertility for more than 400 generations at the time of these assays. Nevertheless, the lifespan character and the fecundity character were independent of one another in these analyses and the trade-off pattern so often observed in *Drosophila* (e.g., 14) was broken.

This result must be interpreted cautiously in regard to theories of trade-offs among fitness-related characters. There is no a priori reason why any two fitness-related characters must necessarily trade off (34,35), although trade-offs between some fitness-related characters are expected. In these study populations, it is conceivable, indeed plausible, that trade-offs remain between lifespan and other characters, especially other unmeasured reproductive characters. Moreover, there are theoretical and empirical reasons to expect that any particular trade-off may appear and disappear as a function of the environments in which it is measured, the so-called "Cheshire Cat" phenomenon (26), better known in population genetic terms as genotype-by-environment interactions. In particular, trade-offs may disappear in environments that have not been the historical environment of selection. Modest changes in the environment in which trade-offs are measured may cause large departures from trade-off expression in the historical environment (vid. 25). Finally, trade-offs may evolve, and as suggested above, it might con-

Table 5. Summary of Early Fecundity Assay

			LOW				
B	BH	BL	OL-α	OH	OL-β	OHL	
25.2	21.3	16.6	17.5	13.3	11.3	9.1	
11.6	4.7	15.3	11.0	12.9	9.1	15.9	
9.7	11.4	17.4	9.4	15.1	13.8	13.1	
8.4	8.9	11.1	9.0	13.2	11.5	11.8	
10.3	8.8	15.4	15.0	23.5	12.7	10.2	
13.0	11.0	15.1	12.4	15.6	11.7	12.0	Mean
3.1	2.8	1.1	1.7	2.0	0.8	1.2	*SE*
			HIGH				
69.3	73.3	69.6	80.7	75.3	75.0	84.3	
60.7	65.6	69.2	75.5	75.3	59.9	59.1	
65.2	69.9	60.6	65.7	69.0	64.2	60.5	
73.7	75.9	70.9	60.7	63.6	70.1	59.5	
71.2	67.9	71.1	82.2	65.4	75.6	78.0	
68.0	70.5	68.3	72.9	69.7	68.9	68.3	Mean
2.3	1.8	1.9	4.2	2.4	3.1	5.4	*SE*

Each entry is the mean early fecundity (eggs/hour/female) of a replicate. Means of each selection treatment (±*SE*) are also included. "LOW" and "HIGH" refer to levels of nutrition.

Table 6. Analysis of Variance—Early Fecundity

Source of Variation	*SS*	*df*	*MS*	*F*
Population	70.385	6	11.731	0.323 (n.s.)
Nutrition	55957.639	1	55957.639	1542.340***
Population × Nutrition	113.765	6	18.961	0.523 (n.s.)
Error	2031.736	56	36.281	

Two-factor ANOVA with selection treatment and nutrition, as measured by the amount of dietary yeast, as main effects. Selection treatments were not differentiated for fecundity. However, the response of fecundity to nutrition was highly significant, with fecundity greatly enhanced in the high yeast environment. ***$p < .001$; n.s. = nonsignificant.

ceivably be the case that the ancestral B populations no longer possess significant genetic variation for early fecundity. We conclude that although the trade-off response between early fecundity and longevity appears to have been broken, this does not undermine theories of trade-offs, given the varied expectations for any particular pair of fitness-related characters under these theories.

The patterns in the response of the starvation resistance character were similar in many respects to the response of lifespan. There was significant differentiation for starvation resistance among selection treatments as in the lifespan character. Previous research showed that there is a positive genetic correlation between lifespan and starvation resistance as well as a physiological connection (24,31), so similar responses of these characters are not unexpected. Starvation resistance was also differentiated between the two nutrition levels with much longer survival in flies from the low yeast environment.

We looked for parallel responses of lifespan and starvation resistance in the comparisons among pairs or groups of treatments. Because there was no significant interaction term in the analysis of variance for starvation resistance, we can consider the joint response of starvation resistance to nutrition, i.e., the sum of the responses in both environments, as a single character. Again, the patterns in the comparisons of pairs or groups of populations for starvation resistance were almost identical to those for lifespan. There were large differences between early and late selected populations for both lifespan and starvation resistance, with late-fertility–selected populations having a greater lifespan and starvation resistance than early-fertility–selected populations. For both characters, late-fertility–selected populations that have undergone selection in the yeasted environments were not significantly different than their nonyeasted controls.

Only in the comparison of early-selected populations under different nutritional regimes did the parallelism in the responses of starvation resistance and lifespan break down. Although the early-selected–populations did not differ measurably in lifespan, the low-nutrition populations had significantly greater starvation resistance. Given that both high- and low-nutrition treatments were subjected to the same amount of direct selection on lifespan, and that changes in starvation resistance resulted from indirect selection only, it is not clear why this difference was observed. It cannot reasonably be attributed to the low-nutrition populations having been selected specifically in a "good" (i.e., low nutrition) environment for starvation resistance, because none of the late-fertility–selected populations maintained in low nutrition environments outperformed their yeasted late-fertility–selected counterparts. We leave this problem as a puzzle for future research.

By combining nutritional manipulations with selection in these studies, we uncovered relationships not predicted by studies of either. Nutrition is neutral with regard to selection response, and both early fecundity and starvation resistance can break their usual associations with lifespan measured under environmental manipulation or selection alone. The parallel responses of these characters to environmental manipulation and selection can be lost under simultaneous nutritional manipulation and selection. Thus the relationships between survival and reproductive characters, as revealed by simultaneous application of nutritional manipulations and selection, are more complex than could be predicted by the study of either in isolation.

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