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DOES PHENOTYPIC PLASTICITY FOR ADULT SIZE VERSUS FOOD LEVEL IN *DROSOPHILA MELANOGASTER* EVOLVE IN RESPONSE TO ADAPTATION TO DIFFERENT REARING DENSITIES?

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Recent studies with *Drosophila* have suggested that there is extensive genetic variability for phenotypic plasticity of body size versus food level. If true, we expect that the outcome of evolution at very different food levels should yield genotypes whose adult size show different patterns of phenotypic plasticity. We have tested this prediction with six independent populations of *Drosophila melanogaster* kept at extreme densities for 125 generations. We found that the phenotypic plasticity of body size versus food level is not affected by selection or the presence of competitors of a different genotype. However, we document increasing among population variation in phenotypic plasticity due to random genetic drift. Several reasons are explored to explain these results including the possibility that the use of highly inbred lines to make inferences about the evolution of genetically variable populations may be misleading.

KEY WORDS: ***Drosophila*, density-dependent selection, drift, experimental evolution, phenotypic plasticity.**

The phenotypes of many organisms vary in a predictable way in response to environmental variables. Sometimes this phenotypic plasticity may be adaptive. For instance, many organisms with determinant growth, such as toads (Wilbur, 1977) and fruit flies (Mueller et al. 1991a) may metamorphose into a small adult when larval resources are limited. This ability permits the individual to survive and reproduce, albeit at a reduced size, rather than die. Population crowding naturally limits food availability and thus organisms whose size shows phenotypic plasticity with food levels may survive bouts of very low food levels when the population is crowded. If a population evolves in a constantly crowded, low food environment it is not unreasonable to expect the evolution of genotypes that exhibit the ability to become larger on limited food even if it results in a reduced ability to produce large individuals when food is abundant. In other words, evolution may favor a different phenotypically plastic response for adult size versus food level.

The genetic basis of phenotypic plasticity of body size versus food level in *Drosophila melanogaster* has been studied recently by Bergland et al. (2008). Bergland et al. measured female body size at four different larval food levels in a large number of recombinant inbred lines. This study revealed genetic variation and genotype by environment interactions for the plasticity of body size. Based on these observations, Bergland et al. concluded "the evolution of ovariole number and thorax length is highly integrated and environmentally sensitive; the evolution of one character will affect the evolution of the other and will do so in an environment-specific fashion." These conclusions suggest that

populations of *Drosophila* that evolve at very different densities and therefore larval food levels, may also evolve very different plasticity for adult size versus food level.

There are a number of research paradigms that have been used to study adaptation but two important methods are (1) experimental evolution and (2) quantitative genetic analysis of phenotypes important to adaptation. The techniques of experimental evolution permit scientists to create and control environments of interest and then observe the genetic and phenotypic changes that take place (Garland and Rose 2009). However, since these techniques require dozens of generations of observation and large numbers of individuals, there are a limited number of species that can be studied by these techniques. Alternatively, the quantitative genetic parameters of many organisms can be measured in one or a few generations yielding estimates of additive genetic variance, the covariance between different traits, or the genotype by environment correlations of traits. A number of short-term evolutionary predictions are then possible with this sort of information (Lande and Arnold 1983; Wade and Kalisz 1989; Kingsolver et al. 2001; Hereford et al. 2004; Hansen and Houle 2008). Bergland et al. (2008) have used the quantitative genetic paradigm to study the plasticity of size versus food level. We propose next, that the evolution of phenotypic plasticity for size versus food level can be studied using experimental evolution techniques.

If replicate populations of *Drosophila* were kept at very different larval food levels then we could directly observe evolution in these different environments. The results of Bergland et al. (2008) suggest that the genotypes that do best (i.e., produce the biggest females) in high food environments may be different than those genotypes that would do best in low food environments. Estimates of the phenotypic responses to varying food levels in populations that have evolved at extreme food levels should reveal any evolved differences in the plasticity of size versus food level.

As it turns out, an experiment like this was done by one of us about 30 years ago (Mueller and Ayala 1981a). In these experiments, food levels differed due to the different larval densities maintained in the low density or *r* populations and the high density or *K* populations. We have previously documented numerous genetically based changes due to adaptation to these different densities including, population growth rates (Mueller and Ayala 1981a; Mueller et al. 1991b); pupation heights (Mueller and Sweet 1986; Guo et al. 1991), larval feeding rates (Joshi and Mueller 1988), and larval competitive ability for food (Mueller 1988). During the study of the larval competitive ability for food, we collected data on the thorax length of female flies raised at different yeast levels but never fully analyzed these data (Mueller et al 1991a). In this study, we subject those data to a full analysis to test the notion that the phenotypically plastic relationship between adult size and larval food level will respond to evolution in these two different density environments.

Methods

The experimental methods have been previously described in Mueller et al. (1991a). Some key details are reviewed below.

EXPERIMENTAL POPULATIONS

The experimental system consisted of six independent populations initially derived from the same source population: three were kept at low population density (50 adults) and are called *r* selected, and three have been kept at high densities (-1,000 adults) by a serial transfer system (Mueller and Ayala 1981b) and are called *K* selected. Each *r* and *K* population was randomly assigned an index from one to three. At any one time, experiments were conducted on matched populations, for example, r_1 with K_1, r_2 with K_2, and so forth. The three *r* populations were created simultaneously as were the three *K*-populations. Thus, the pairing of r_1 with K_1 is completely random. Any differences between the three *r* or three *K* populations at their time of creation would only be differences that could arise through random sampling. Likewise, any initial difference between an *r*/*K* pair would reflect this random variation and the direction of the difference should vary between matched pairs. Ultimately, our testing procedure will look at the phenotypic differences between matched pairs of *r* and *K* populations. In addition to these six populations, two other populations, called rF_1 and KF_1, which were F_1 hybrids of all three *r* and all three *K* populations, respectively, were studied. These populations were created by making all possible crosses; for example, for the rF_1 population there were six crosses (male × female): $r_1 \times r_2$, $r_1 \times r_3$, $r_2 \times r_3$, and the reciprocal crosses. At the time these experiments were initiated, the *r* and *K* populations were in their respective environments for 125 generations. All experiments were conducted at 23°C on a schedule of 12 h of light and 12 h of darkness.

ADULT SIZE MEASUREMENTS

Experiments were initiated by removing adults from the running *r* and *K* populations of a matched pair. About 100 adults were placed in half-pint cultures with standard cornmeal-flour-sugar-agar *Drosophila* medium and allowed to lay eggs for 24 h. Two weeks later adults were collected from these cultures to provide first-instar larvae for experimental measurements. Eggs were laid on nonnutritive agar (to prevent larvae from growing) and 100 larvae were placed in 8-dram vials (22 × 95 mm) at each of 10 different food levels.

Each vial was filled with 10 mL of non-nutritive Kalmus medium: 11.3 g agar, 1.54 g KH_2PO_4, 2.06 g $(NH_4)_2SO_4$, 0.51 g $MgSO_4$ $7H_2O$, and 5.1 mL propionic acid per 1,000 mL water. Live yeast and water (2.67 mL/g yeast) were added to each vial about 2 h before the larvae were added. Food levels varied from 25 mg to 158 mg per 100 larvae. In the first series of experiments, the 100 larvae added to each vial were all from the same

population (monocultures). To estimate relative competitive ability, two additional types of experiments were conducted. The second type of experiment created a mixture of 50 larvae from either the r or K population, with 50 larvae from a population homozygous for the white (w) allele (even-mixture experiments). The third type of experiment created mixtures of 67 r or K larvae and 33 w larvae (uneven-mixture experiments). There are a number of reasons for preferring to begin these experiments with larvae rather than eggs which are reviewed in Mueller et al. (1991a) and Bakker (1961). In short, it is well known that females may fertilize some eggs hours before they are laid and other eggs right at the time they are laid. The early fertilized eggs then hatch earlier and that early access to limited larval food provides a substantial, unintended advantage in growth and competition (Bakker 1961). Counting out larvae eliminates this potential source of bias.

Adults emerged 11–14 days after larvae were placed in the vials. At daily intervals, the thorax length of all females was measured to the nearest 0.05 mm. Vials were checked for newly emerged adults for up to 12 days after the first adults appeared. In this study, we analyze the results from 8,971 females.

STATISTICAL METHODS

Modeling thorax size

In our original paper (Mueller et al. 1991a), we used a four-parameter model to describe the relationship between female size and yeast level. In this study, we found that at least two parameters of that model had very high variance estimates suggesting that their values were not well estimated and perhaps not necessary. Accordingly, we settled on a simple two-parameter model to describe the relationship between female thorax length [$s(k)$] and yeast level, k,

$$s(k) = \frac{a}{1 + \exp(-bk)}. \tag{1}$$

This model predicts an asymptotic approach to the maximum thorax size, a, with increasing yeast levels. The rate of the approach to this maximum is proportional to b. Thus, b can be interpreted as indicating how efficiently females convert food into biomass at different yeast levels.

Testing the effects of selection

The experimental data consists of the size of a female, y_{ijmk}, where i indicates the selection regime ($1 = K$, $2 = r$), j indicates competition level (1 = control, 2 = even, 3 = uneven), m indicates block ($m = 1, \ldots, 29$), and k is the yeast level. The blocks refer to the different calendar dates a pair of r and K populations was tested. In each block, one r population and its matched K population were tested. Each population was tested in at least six different blocks. Thus, the rF_1 population was tested with the KF_1 population, the r_1 population was tested with the K_1 population and so on. The blocks are treated as random effects whereas selection and competition are fixed treatment effects. These assumptions give rise to the following statistical model for female size:

$$y_{ijmk} = s\left(\theta_{ijm}, k\right) + \varepsilon_{ijmk}, \tag{2}$$

where θ_{ijm} is vector of model parameters (a_{ijm}, b_{ij}) and ε_{ijmk} is the error term. The size function can now be written as

$$s\left(\theta_{ijm}, k\right) = \frac{a_{ijm}}{1 + \exp\left(-b_{ij}k\right)}, \tag{3a}$$

$$a_{ijm} = \alpha_1 + \gamma_1\delta_i + \varphi_1\delta_j + \pi_1\delta_{j-1} + d_m, \tag{3b}$$

$$b_{ij} = \alpha_2 + \gamma_2\delta_i + \varphi_2\delta_j + \pi_2\delta_{j-1}, \tag{3c}$$

where $\delta_p = 1$ if $p = 2$ and 0 otherwise. The parameter γ measures the effects of selection on a and b whereas the parameter φ measures the effects of even competition and π measures the effects of uneven competition. In the formulation of the model above only the parameter a is affected by random block variation. The random components ε_{ijkm} and d_m are assumed to be normally distributed with zero means and variances σ_1^2 and σ_2^2, respectively. We tested a model with block variation in a and b but this model show a high correlation between the variation in a and b and failed to converge. We also compared a model with block variation in only a to a model with block variation only in b and found the former to have a higher likelihood and lower values of the Akaike and Bayesian information criterion. Accordingly, we have used the model with block variation in only parameter a (eq. 3b) in our analysis. Our statistical results were derived from the nonlinear mixed effects package in R, version 2.11.1 (R Development Core Team 2010). This program provides maximum likelihood estimates of the model parameters in equation (3) (Pinheiro and Bates 2000; chapter 7)

DRIFT EFFECTS

The three r and three K populations were maintained independently of each other and thus were subject to independent realizations of genetic drift. The r populations were maintained with exactly 50 adults each generation. The K population size was not regularly counted but in these environments the carrying capacity of the populations is about 1,000 adults (Mueller and Ayala 1981b). Consequently, we would expect among population variation to be larger in the r populations than the K-populations for traits not subject to natural selection. The among population variation can be estimated from equations (3) by ignoring the block index and instead labeling each female by a unique population code, for example, r_1 was population 1, r_2 population 2, ..., K_1 population 5, ..., KF_1 population 8. If the index m now indicates

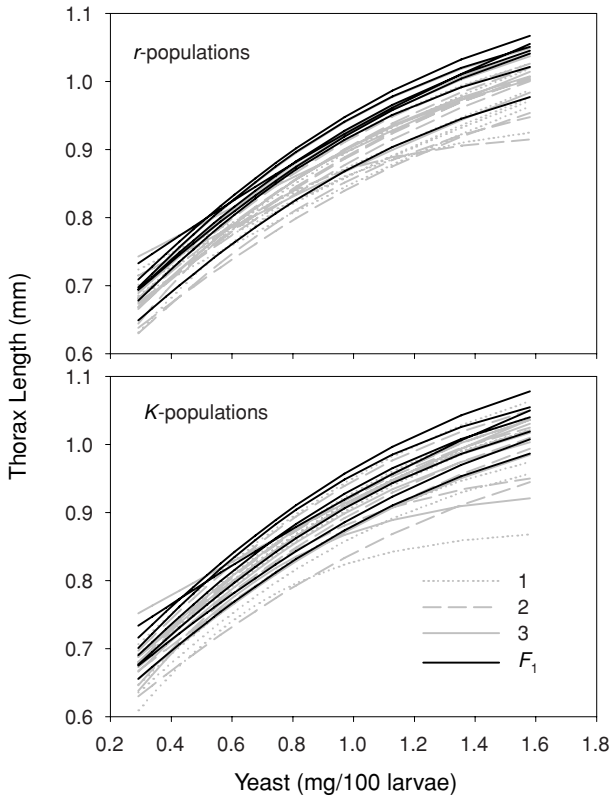

Figure 1. The predictions from equation (1) for each population/block combination of *r* and *K* populations. The legend refers to both figures so the legend for "1" corresponds to r_1 and K_1, etc.

population then the random term in equation (3), d_m, can now be used as an estimate of between population variation.

Results

SELECTION EFFECTS

The individual predictions from equation (1) for each population-block combination are shown in Figure 1. These results illustrate that there is significant variation from block to block. Although there is some crossing of these lines reminiscent of the genotype by environment interactions discussed by Bergland et al. (2008), it is not extensive. The comparison of the three *r* and three *K* populations reveals no significant effects of selection or competition on the parameters of the size model, equation (1) (Table 1). The parameters γ_1 and γ_2 measure the effects of the *r*-selection regime on the parameters *a* and *b* of equation (1), respectively. Neither of these is significantly different from zero (Table 1). It is worth noting that these tests could have detected an effect of selection as small as 2.8% of the value of *a* and 9.9% of the value of *b*. In a similar fashion neither level of competition resulted in a significant change in the parameters of equation (1).

An important biological question is what range of fitness effects does 2.8% variation in *a* and 9.9% variation in *b* correspond to? If this represents a very large fitness range then this study is unlikely to uncover the action of natural selection on body size. As shown first by Chiang and Hodson (1950) and more recently by Bergland et al. (2008), female body size is correlated with fecundity. We have previously published results on the fecundity of various size females from the eight populations studied here during their first week of adult life (Mueller and Joshi 2000, chapter 6).

These data can be used to develop a predictive model of female fecundity. Using the natural log of the total number of eggs plus 1 laid during the first week and using simple linear regression we deduced the following relationship: $fecundity = \exp(2.883 + 2.853 \times size) - 1$. We then used the previously mentioned minimum detectable range to compute female size across a range of yeast levels. These sizes were then converted to female fecundity estimates and normalized to the fecundity of a female at the expected size of a *K* female for each yeast level. These results (Fig. 2) show that if there were fitness differences in the range of 3–8% or greater due to size differences in the *r* and *K* environments our techniques should have been able to detect them.

Table 1. The parameter estimates from the nonlinear mixed effects model (eq. 3) for the three *r* and three *K* populations.

	Value	SE	df	*t*-value	*P*-value
a parameter effects					
α_1 (intercept)	1.08	0.0128	6319	84.6	<0.00001
γ_1 (selection)	0.0103	0.0149	6319	0.692	0.49
φ_1 (even competition)	0.0388	0.0242	6319	1.60	0.11
π_1 (uneven competition)	0.0160	0.0222	6319	0.723	0.47
b parameter effects					
α_2 (intercept)	1.62	0.0577	6319	28.0	<0.00001
γ_2.(selection)	−0.144	0.0800	6319	−1.80	0.072
φ_2 (even competition)	−0.121	0.105	6319	−1.15	0.25
π_2 (uneven competition)	−0.0889	0.0956	6319	−0.929	0.35

σ_1 = 0.0540, 95% confidence interval (0.0531, 0.0550).

σ_1 = 0.0260, 95% confidence interval (0.0191, 0.0353).

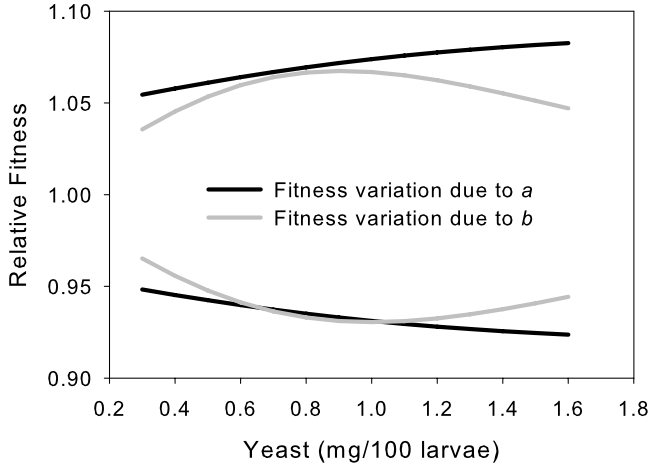

Figure 2. The minimum detectable fitness differences arising from differences in female size. These fitness differences are measured relative to the predicted one week total fecundity of *K* females whose size is predicted from the parameters in Table 1. The relationship between size and fecundity is given in the text.

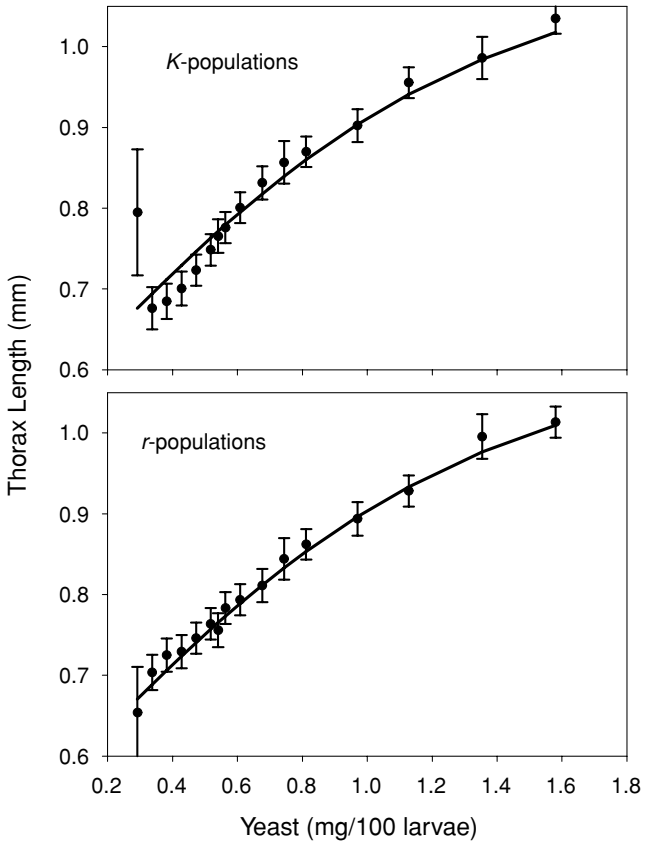

Figure 3. The observed and predicted female thorax lengths for all *r* and *K* populations. The bars are 95% confidence intervals on the mean for each yeast level.

The primary reason for making measurements on the F_1 populations was to check for the possibility of inbreeding depression as a cause of any observed differences between the *r* and *K* populations. Although there were no differences, we report these results in table 2. These results are entirely consistent with the previous observations in that they show no significant effects of selection history or competition. Additionally the F_1 populations give very similar estimates of the error and block variances.

We next pooled all the data and then estimated parameters from equation (3) but with the competition terms removed. The estimates of female size for the *r* and *K* populations are shown along with the mean sizes in Figure 3. It is clear that the basic model (eq. 1) does a reasonably good job summarizing the relationship between yeast level and adult size. To estimate the confidence intervals for these pooled samples we used a linear mixed effects model, which treated each yeast level as a separate fixed treatment. It appears that at three of the lowest yeast levels (0.34–0.43 mg/100 larvae) the *r* females may in fact be slightly larger than the *K* females. We used this linear model to specifically test for size differences at each of those three yeast levels and none of the differences were statistically significant.

Table 2. The parameter estimates from the nonlinear mixed effects model (eq. 3) for the rF_1 and KF_1 populations.

a parameter effects	Value	SE	df	*t*-value	*P*-value
α_1 (intercept)	1.16	0.0249	2609	46.5	<0.00001
γ_1 (selection)	−0.0123	0.0242	2609	−0.507	0.61
φ_1 (even competition)	−0.0230	0.0392	2609	−0.588	0.56
π_1 (uneven competition)	−0.0606	0.0322	2609	−1.88	0.06
b parameter effects					
α_2 (Intercept)	1.49	0.116	2609	12.9	<0.00001
γ_2.(selection)	0.117	0.132	2609	0.880	0.38
φ_2. (even competition)	−0.110	0.180	2609	−0.613	0.54
π_2 (uneven competition)	0.0612	0.153	2609	0.401	0.69

σ_1 = 0.0580, 95% confidence interval (0.0564, 0.0596).

σ_1 = 0.0204, 95% confidence interval (0.0119, 0.0352).

Table 3. **The between population standard deviation in the parameter *a* of equation (1) for the three *r* populations and the three *K* populations. Approximate 95% confidence intervals on the standard deviation were calculated by the methods described in Pinheiro and Bates (2000, page 93).**

Population	Lower	Estimate	Upper
K	0.00200	0.00516	0.0133
r	0.00964	0.0217	0.0489

DRIFT EFFECTS

We separated the three *r* populations from the remaining populations and estimated the standard deviation in the *a* parameter over populations. The same analysis was carried out for the three *K* populations and we found the variation in the *r* population to be much greater (Table 3). Is the difference in the variance estimates consistent with what we know about the population size differences in the *r* and *K* populations? We can get a rough answer to this question by first noting that the among population variation of an additive genetic trait should increase over time according to the relationship $2\sigma_g^2[1 - \left(1 - \frac{1}{2N}\right)^t]$. (Wright 1951), where σ_g^2 is the initial within population variance and t is the number of generations of drift. Although we do not know the initial variation for *a* in these populations since all populations were derived from a common source population we assume σ_g^2 was equal in the *r* and *K* populations. Thus, we can calculate the ratio of the variance in the *r* populations to the variance in the *K* population and $2\sigma_g^2$ will cancel.

If we set $t = 125$, and use 50 for N in the *r* populations and 1000 for N in the *K* populations the square root of the ratio of the variances is predicted to be 3.44. Using the observed standard deviations in Table 3, we can calculate an observed ratio of 4.21, which is very close to the predicted value. Using the methods in Pinheiro and Bates (2000, page 93), we can also put an approximate 95% confidence interval on the observed ratio which is (1.2, 14.7). We note that since the lower limit of the confidence interval is greater than 1 we conclude that the standard deviation in the *r* populations is significantly greater than in the *K* populations. Since, the predicted ratio of the standard deviations, 3.44, is well within the 95% confidence interval of the observed ratio we conclude that the increased variation between *r* populations is consistent with the effects of genetic drift.

To show the effects of population and block variation on the size of females, we have used these estimates to put a confidence interval on the *a* parameter of equation (1) and then use equation (1) to predict the upper and lower female size we would expect from the different sources of variation (Fig. 4). Since the *r* and *K* populations were tested in the exact same set of blocks, we would expect very similar estimates of block variation, which is in fact observed (Fig. 4). However, the population variation is greatly reduced in the *K* populations due to their larger population size and hence smaller drifts effects.

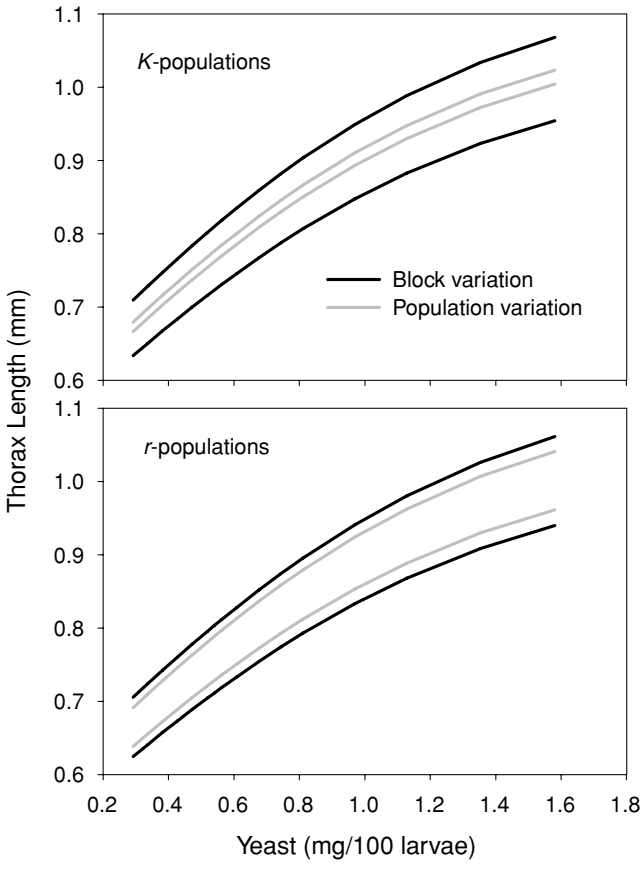

Figure 4. **The variation in female size due to random variation in *a* (± two standard deviations) from either blocks or populations in the *r* and *K* populations. The estimated variation from blocks is almost identical in the *r* and *K* populations whereas the size variation due to populations is much greater in the *r* populations due to the effects of random genetic drift.**

Discussion

This study has shown that extreme density environments do not result in an evolutionary change in the plasticity of adult size versus larval food levels. However, this study has shown that among population variation in this trait does increase due to random genetic drift as if it were a classic neutral character. This result seems at odds with the observations of Bergland et al. (2008), which had suggested that extensive G × E interactions would have important effects on the evolution of adult size plasticity in different larval food environments. We suggest and then address the following possible explanations for these observations. (1) Selection was not of sufficient strength or duration to cause observable phenotypic differences. (2) There were no G × E interactions among the

genotypes in these populations that would lead to the evolution of different phenotypes in these environments. (3) Experimental artifacts and differences might account for the observations.

We consider the first possibility that selection was inadequate to produce changes. If we look at the size variation in the different blocks and populations (Fig. 1), we can ask what variation in fitness does this represent. Although the answer varies with food level, at low food levels the difference between the largest and smallest block represents a threefold difference in predicted first week fecundity. Of course the data in Figure 1 include environmental variation. Our analysis of the sensitivity of our test to detect selection suggested that if there were a "*r*-genotype" whose fitness was 3–8% less than a *K* genotype at high density then we should have been able to detect this difference. We know from other tests of these populations that there has been substantial phenotypic evolution. This suggests that there was very strong selection for traits that affect survival and reproduction in these environments. Thus, if genetic variation existed to create even small increases in adult size at low food levels, we would have expected these to increase due to selection in these experiments.

Mueller and Joshi (2000) do report a temporal decline in average adult size in populations that adapt to high-density regimes. However, this observation was interpreted as a consequence of an increase in the number of surviving larvae, which in turn lowered the average amount of food available for each larva. In fact most of the documented phenotypic changes in the *r* and *K* populations like larval competitive ability and pupation height are believed to act primarily through their effects on larval or pupal survival (Mueller 1988; Joshi and Mueller 1993). We conclude that it is therefore unlikely that selection was not strong enough to produce phenotypic evolution. Of course this conclusion assumes that there was the requisite genetic variation, which we consider next.

It could be that our *Drosophila* populations were different from all others and thus the observed failure for adult size to evolve was an anomaly. We consider this unlikely since, as mentioned previously, many other traits did evolve in response to these environmental extremes and therefore must have harbored genetic variability. Even though the *r* populations may have been subject to some loss of genetic variability over the 125 generations, this is much less likely to have happened in the *K* populations and the crowded environment was the novel and extreme environment compared to normal *Drosophila* cultures. Accordingly, we expect the most evolution to occur in the *K* populations.

This leads to our second possible explanation which is that the genetic characterization of the G × E interactions for the size and food level relationship described by Bergland et al. (2008) is not characteristic of the *r* and *K* populations studied here. An important difference between Bergland et al. and the present study is that the lines studied by Bergland et al. were highly inbred whereas the populations used in this study were outbred and genetically variable. Thus, the genetic variability described by Bergland et al. may be mostly due to the expression of rare deleterious genetic variants that have been made homozygous and that have broad and large negative impacts on multiple components of fitness (Tracey and Ayala 1974; Mackay 1986; Miller and Hedrick 1993).

There is some support for this explanation. The broad fitness consequences of making *Drosophila* homozygous at many loci is well characterized (Spassky et al. 1960; Dobzhansky and Spassky 1963; Temin 1966; Marinkovic 1967a,b; Sved and Ayala 1970; Sved, J.A. 1971, 1975; Tracey and Ayala 1974; Mackay 1986; Partridge et al. 1985; Miller and Hedrick 1993). The *r* and *K* populations used in this study were created by crossing 25 different populations made homozygous for whole second chromosomes. In those original chromosomal homozygous lines, there was a strong positive correlation between a population's growth rate at low and high densities (Mueller and Ayala 1981c). This would suggest that there ought to be some best genotype that grows fastest at both low and high density. However, upon crossing these populations and letting evolution work on the genetically variable populations it was shown that the predominant genotypes in the *r* populations had higher growth rates at low density compared to the *K* populations but lower growth rates at high density and vice versa (Mueller and Ayala 1981a; Mueller et al. 1991b). Thus, for these fitness-related traits inferences about evolution in outbred populations made from inbred populations were misleading (Rose and Charlesworth 1981; Johnson and Wood 1982; Rose 1984, 1991; Rose and Service 1985; Wayne et al. 1997, 2001; Khazaeli et al. 2005; Swindell and Bouzat 2006).

What we do know about evolution at low food levels is that an important response in food-limited environments is to increase larval competitive ability for food (Mueller 1988). This is accomplished by increasing the rate at which larvae feed (Joshi and Mueller 1988). Thus, rather than becoming more efficient at utilizing food and thus possibly larger the larvae evolve an ability to get more food than their slow feeding competitors. Evidence suggests that the fast feeding, highly competitive larvae are in fact less efficient at turning food into biomass (Mueller 1990; Joshi and Mueller 1996).

The final explanation is that the differences between our observations and the expectations based on the Bergland et al. (2008) is a consequence of laboratory artifacts. One such artifact is density versus food level. The study of Bergland et al. (2008) dealt only with the effects of larval food level on adult size. Although the crowded *K* populations will restrict the amount of food available for each larva it changes other properties of the populations in addition to food availability. For instance, levels of nitrogen waste products like ammonia are higher (Borash et al. 1998). Pupation sites are also limited in the *K* populations (Joshi and Mueller 1993). These factors could also impact the evolution

of the plasticity of size versus adult size. There were also differences in the measurements of plasticity in our study and Bergland et al. (2008). We added a yeast paste directly to the surface of the food whereas Bergland et al. varied the concentration of yeast directly in the food. Although it is hard to imagine that these issues alone could be responsible for the lack of evolved differences only further research could address these problems concretely.

This study has shown that the plasticity of adult size and larval food level does not evolve in response to evolution at very high and low population density. We suggest that conclusions from studies of the G × E interactions in inbred *Drosophila* populations are not robust. Our results may be part of a more general problem: insights about fitness-related traits in normally outbred populations are difficult if not impossible to make with highly inbred populations.

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