The contribution of ancestry, chance, and past and ongoing selection to adaptive evolution

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Abstract

The relative contributions of ancestry, chance, and past and ongoing selection to variation in one adaptive (larval feeding rate) and one seemingly nonadaptive (pupation height) trait were determined in populations of *Drosophila melanogaster* adapting to either low or high larval densities in the laboratory. Larval feeding rates increased rapidly in response to high density, and the effects of ancestry, past selection and chance were ameliorated by ongoing selection within 15–20 generations. Similarly, in populations previously kept at high larval density, and then switched to low larval density, the decline of larval feeding rate to ancestral levels was rapid (15–20 generations) and complete, providing support for a previously stated hypothesis regarding the costs of faster feeding in *Drosophila* larvae. Variation among individuals was the major contributor to variation in pupation height, a trait that would superficially appear to be nonadaptive in the environmental context of the populations used in this study because it did not diverge between sets of populations kept at low versus high larval density for many generations. However, the degree of divergence among populations (F_{ST}) for pupation height was significantly less than expected for a selectively neutral trait, and we integrate results from previous studies to suggest that the variation for pupation height among populations is constrained by stabilizing selection, with a flat, plateau-like fitness function that, consequently, allows for substantial phenotypic variation within populations. Our results support the view that the genetic imprints of history (ancestry and past selection) in outbreeding sexual populations are typically likely to be transient in the face of ongoing selection and recombination. The results also illustrate the heuristic point that different forms of selection—for example directional versus stabilizing selection—acting on a trait in different populations may often not be due to differently shaped fitness functions, but rather due to differences in how the fitness function maps onto the actual distribution of phenotypes in a given population. We discuss these results in the light of previous work on reverse evolution, and the role of ancestry, chance, and past and ongoing selection in adaptive evolution.

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Introduction

The genetic structure of a population at any given time is a reflection of the combined effects of many factors such as past selection, ongoing selection, ancestry (lineage) and chance (random divergence among lineages derived from a common ancestor) (Kimura 1968; King and Jukes 1969; Gould and Lewontin 1979; Mayr 1983; Parker and Maynard Smith 1990; Williams 1992). Moreover, the genetic variation present in a population at any given time not only constitutes the raw material for agents of evolutionary change to act upon, but also reflects the outcome of past evolutionary change (Lloyd and Gould 1993). Thus, the evolutionary trajectory of a population represents the

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outcome of the resolution of three types of forces: (i) the deterministic force of current natural selection, (ii) the stochastic force of genetic drift, and (iii) the inertial effect due to history, which encompasses effects of ancestry and past selection. Recently, the importance of historical factors, such as ancestry and past selection, as well as of chance, to adaptation at the microevolutionary level has been explicitly considered in the design and interpretation of laboratory experiments on the evolutionary genetics of life histories (Rose *et al*. 1990; Mueller 1995; Joshi and Mueller 1996; Teotónio and Rose 2001), the coevolution of interspecific competitors (Joshi and Thompson 1995, 1996), and the evolution of ecological specialization (Joshi and Thompson 1997). Similarly, the importance of history and chance in determining patterns of within-species diversity in nature has also been receiving increasing attention from ecologists (Thompson 1994). There have also been attempts to experimentally assess the relative contributions of ongoing selection, history and chance to adaptive evolution in populations of the bacterium *Escherichia coli* adapting to novel nutritional and temperature regimes (Travisano *et al*. 1995). The major findings of this study were that variation in fitness was largely explained by adaptation (ongoing selection), although the roles of history and chance were not negligible, whereas in the case of a trait only loosely related with fitness, history and chance together contributed as much as ongoing selection to phenotypic variation even after 1000 generations of ongoing selection in the novel environment (Travisano *et al*. 1995). More recent studies of *Drosophila melanogaster* populations that had adapted to a variety of selection regimes and were then subjected to reverse selection in the ancestral regime showed that the trajectories of reverse evolution could be varied, ranging from full reversion of phenotypes to the ancestral state to partial reversion to no change at all over 50 generations of reverse selection (Teotónio and Rose 2000; Teotónio *et al*. 2002). Although it is very likely that the dynamics of adaptive evolution, and the contribution of history to those dynamics, will differ substantially between asexual and sexual species (Teotónio and Rose 2001), these *Drosophila* studies did not attempt to quantify the contribution of history (ancestry and past selection), chance and ongoing selection. The results, however, did suggest that for outbreeding sexual species like *D. melanogaster*, the genetic imprint of history tends to be transient in the face of selection and the genomic reshuffling accomplished by recombination, especially for traits closely related to fitness (Teotónio and Rose 2000; Teotónio *et al*. 2002). In this paper, we report results from a study which complements those of Travisano *et al*. (1995), Teotónio and Rose (2000), and Teotónio *et al*. (2002). We quantified the contributions of past selection, ongoing selection, ancestry and chance to phenotypic variation in larval feeding rate (closely related to fitness) and pupation height (seemingly not strongly correlated with fitness) at various time points during adaptation to different larval densities in 20 populations of *D. melanogaster* in order to not only assess the roles of these various factors in adaptive evolution in an outbreeding sexual species, but also to obtain a feel for the time course of changes in the relative contributions of these factors to variation within and among the populations undergoing adaptive evolution.

Materials and methods

***Study populations*:** We used a set of 20 laboratory populations of *D. melanogaster*—the CU-HL, CU-LH, UU-HL and UU-LH populations (C, crowded; U, uncrowded; H, high food level; L, low food level; first letter of pair, larval stage; second letter, adult stage) described by Mueller *et al*. (2000)—whose ancestry, and past selection regime in the specific context of larval density, were known for over 500 generations (figure 1). The 20 experimental populations were set up by deriving two populations from each of a set of 10 populations that had been subjected to either low ($UU_{1...5}$) or high ($CU_{1...5}$) larval density for the preceding 65 generations (derivation and maintenance of CU and UU populations is described in detail by Joshi and Mueller (1996)). The CU and UU populations were themselves derived from the five B populations of Rose (1984), which had been maintained in the laboratory for ~360 generations since their derivation from the IV population (figure 1), an outbred laboratory population derived, in turn, from the wild South Amherst, Massachusetts, USA, population of *D. melanogaster* (Ives 1970). The IV population was, thus, the ultimate ancestor of all populations used in the present study. The IV and B populations were maintained on a 14-day discrete-generation cycle, at 25°C and continuous light, at controlled and moderate larval densities of ~80 larvae per 8-dram (9 cm × 2.4 cm) vial containing about 6 ml food (Rose 1984). From each B population, one CU and one UU population were derived (Joshi and Mueller 1996). The CU populations were subjected to extreme larval crowding, and eventually (after about 45 generations of selection) maintained at densities of ~1500 larvae per 6-dram vial containing about 4 ml food. The UU populations served as controls and were kept at densities of ~80 larvae per 8-dram vial containing about 6 ml food (Joshi and Mueller 1996). All the CU and UU populations were maintained on a 21-day discrete-generation cycle, at 25°C and continuous light, with adults housed in plexiglas cages (25 cm × 20 cm × 14.4 cm), with typical breeding adult numbers of 1500–2000. All populations were maintained on a banana–molasses medium.

After 65 generations of CU and UU maintenance, one HL and one LH population were derived from each of the CU and UU populations. These populations were part of a long-term study of the possible evolution of stable dyna-

mics, and their maintenance has been described in detail elsewhere (Mueller *et al*. 2000). Consequently, we shall restrict ourselves here to the details of their rearing that are pertinent to the present paper. The LH populations were given low levels of food (20 ml food medium per half-pint milk bottle) during the larval phase, and high levels of food during the adult phase (i.e. food medium supplemented with a generous dab of live yeast paste). The HL populations, on the other hand, were given high levels of food (40 ml food medium per half-pint milk bottle) during the larval phase, and low levels of food during the adult phase (i.e. food medium supplemented with 1.5 ml of a 1.5% yeast solution added to the surface of the food). Each individual population consisted of eight half-pint bottles from which eclosing adults were collected daily and dumped into a plexiglas cage. To initiate a new generation, all eggs laid on food in a Petri dish over an 18-h time window on day 21 from previous egg lay were collected and equally distributed among the eight culture bottles making up the population. Although larval density was not directly manipulated in these populations, the HL populations experienced relatively moderate larval densities compared to the LH populations. Evidence of this difference in larval density was obtained from the dry weights of adults. Over the 20 generations of this experiment the average dry weight per fly of the HL adults was 0.25 mg (± 0.05, 95% confidence interval) while the LH adults were almost half that size, with an average dry weight per fly of 0.14 mg (± 0.02, 95% c.i.). Population sizes were always above 1000 breeding adults, and all HL and LH populations were maintained on a 21-day discrete-generation cycle, at 25°C, under continuous light. Thus, these 20 experimental populations (CU-HL_i, CU-LH_i, UU-HL_i, UU-LH_i; i = 1...5) represented five replicates each of all four combinations of past (CU vs UU) versus ongoing (LH vs HL) selection at high and low larval densities, respectively. Larval feeding rate and pupation height were assayed at generations 3, 6, 9, 12, 15 and 20 of HL and LH selection on all 20 populations. In addition, larval feeding rate was also assayed on the 10 CU and UU populations at generations 0, 3, 6, 9, 12 and 15 of HL and LH selection.

***Phenotypes assayed*:** To provide a contrast, we chose to assay two phenotypes that were, respectively, divergent and similar between the CU and UU populations. In the course of 65 generations of evolution under crowded larval conditions, the CU populations had evolved a higher larval feeding rate compared to their UU controls (Joshi and Mueller 1996). Larval feeding rate in *D. melanogaster* is known to be strongly correlated with competitive ability when food is limiting (Sewell *et al*. 1975; Joshi and Mueller 1988; Guo *et al*. 1991; Mueller *et al*. 1991). Pupation height, the height above the food surface that larvae pupate, was not significantly different in the CU and UU populations at the time of initiating the experiments reported here (Joshi and Mueller 1996), although this trait had earlier been seen to diverge between crowded and control populations of *D. melanogaster* kept in different food environments than those used in the present experiments (Mueller and Sweet 1986). In this study, we wanted to examine the contribution of various factors like selection, ancestry and chance to variation in these two traits. Prior to doing the assays described below, pro-

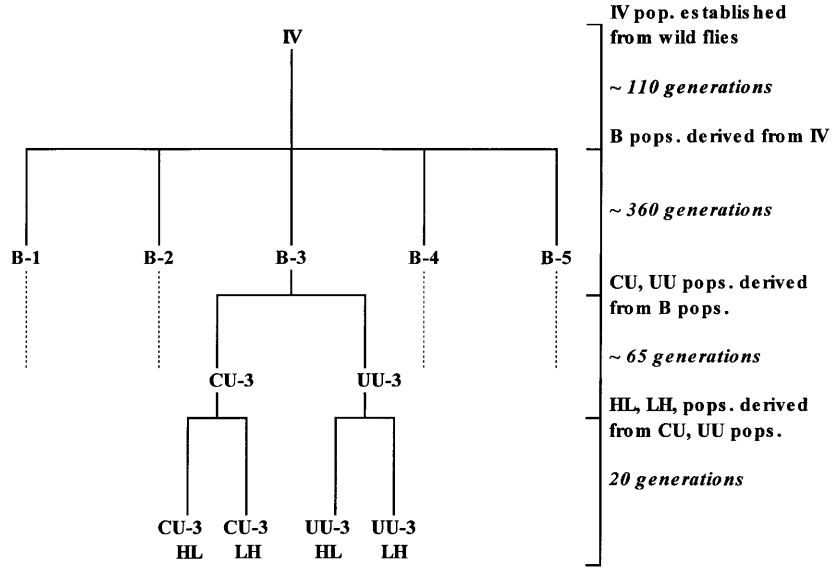

Figure 1. Derivation of the 20 populations of *D. melanogaster* used in this study (only the derivation of one block out of five is shown in detail).

geny from all test populations were passed through a full generation of identical rearing in low-density (UU type) conditions to eliminate any phenotypic differences between selected and control populations due to nongenetic maternal effects.

***Larval feeding rate*:** The rates of cephalopharyngeal contractions of 48-h-old larvae were measured by techniques described in Joshi and Mueller (1996). Briefly, larvae raised on an excess of live yeast were placed on a Petri dish filled with agar overlaid with a dilute solution of yeast. Under a dissecting microscope, the number of times the mouth parts (cephalopharyngeal sclerites) of the larvae moved back and forth in one minute was recorded as a measure of the feeding rate. Twenty larvae (sometimes 25) were assayed from each population at each sample interval.

***Pupation height*:** The height above the food that a larva pupates was determined by techniques described in Joshi and Mueller (1996). Briefly, standard food vials were seeded with 50 newly hatched larvae. Seven days after the first adult had eclosed, the height above the food of each pupa was measured to the nearest millimetre. Five replicate vials were started for each population at each assay period.

***Statistical analyses*:** All analyses were implemented on STATISTICA™ for Windows Release 5.0 B (StatSoft, Inc. 1995). Data on larval feeding rate in the 10 CU and UU populations at generations 0, 3, 6, 9, 12 and 15 of HL and LH selection (corresponding to generations 65, 68, 71, 74, 77 and 80 since the derivation of the CUs and UUs from the Bs) were subjected to a three-way mixed-model analysis of variance (ANOVA). Because CU_i and UU_i were more closely related to each other than either of them was to other populations subjected to the same selection regime (i = 1...5), pairs of CU and UU populations, matched by subscripted indices, were treated as random blocks in the ANOVA. Selection regime (CU versus UU) and generation were treated as fixed factors crossed with block. Data on UU and CU feeding rates from each generation were also subjected to separate two-way ANOVAs with block and selection regime as the factors. Variance components were estimated from the ANOVA mean squares, as outlined in appendix 1. For each set of five CU or five UU populations at each generation, expected F_{ST} values (a measure of genetic divergence among populations, Wright (1951)) under the null hypothesis of selective neutrality, and observed F_{ST} values were also estimated, as outlined in appendix 2.

Similarly, feeding-rate data from the 20 CU-HL, CU-LH, UU-HL and UU-LH populations at each generation were subjected to three-way ANOVA with past selection (CU and UU) and ongoing selection (HL and LH) treated as fixed factors crossed with each other and with random blocks. In the case of pupation-height data, the ANOVA model contained an additional factor (vial) nested within the three-way interaction between block, past selection and ongoing selection. Variance components were estimated from the ANOVA mean squares, as outlined in appendix 1. For each set of replicate populations within a selection regime at each generation, expected F_{ST} values under the null hypothesis of selective neutrality, and observed F_{ST} values were also estimated, as outlined in appendix 2. Any negative variance component estimates were treated as zero. The contribution of ancestry was computed as the fraction of total variation ascribable to the main effect of blocks in the variance components analysis, since each block identifies a set of two (CU/UU) or four (CU-HL/CU-LH/UU-HL/UU-LH) populations derived from the same B population. The chance contribution was computed as the sum of the fractional variance components due to block × past selection, block × ongoing selection and block × past selection × ongoing selection. Thus the chance contribution represents effects that arise in specific combinations of blocks and past selection, for instance, that are most likely due to unique events that occurred in the evolution of these populations. Among these unique events would be sampling events either during reproduction or during experimental assays. It should be pointed out that, in the case of feeding rate, assays were done on one block at a time, staggered by a day. Consequently, the variance due to block would include a potential contribution of day-to-day variation in assay environment, in addition to the contribution of ancestry. In the pupation height assays, all blocks were run simultaneously and randomized with regard to placement in incubators and other such possible sources of microenvironmental variation.

Results

CU and UU feeding rates

Mean feeding rates of CU larvae were significantly greater than those of their UU counterparts at all six generations assayed (figure 2, tables 1, 2). Although there was a significant ANOVA effect of generation (table 1), there was no temporal trend in the difference between the mean larval feeding rates of the CU and UU populations, which, averaged across the six assay generations, was 14.76 bites per minute (± 95% c.i. of 1.95). Thus, the feeding rate difference between the UU and CU populations appears to have stabilized by 65 generations of selection. In most replicate populations at every assay generation (56 out of 60), the distribution of feeding rate, scaled by the mean and expressed in units of standard deviation based on the values for the UU population for that replicate pair, did not differ significantly from a

normal distribution (Shapiro–Wilks's W test, $P > 0.05$). Over the six assay generations, the average difference between the scaled mean feeding rates of the CU and UU populations was 1.88 standard deviations (± 95% c.i. of 0.83); a representative distribution is shown in figure 3. The coefficient of variation of feeding rate varied significantly across generations (ANOVA main effect $F_{5,20} = 15.46$; $P < 0.001$), but there was no significant effect of selection ($F_{1,4} = 0.04$; $P = 0.84$) or of the generation × selection regime interaction ($F_{5,20} = 1.27$; $P = 0.31$). In general, coefficients of variation were low, ranging between 0.04 and 0.09 in the CU and UU populations. Observed F_{ST} values among the five CU and five UU populations at each assay generation were computed using equation A2 (see appendix 2). The observed F_{ST} values did not differ significantly between selection regimes (t test, $P > 0.05$) when averaged over the six assay generations, and neither was any temporal trend seen in the observed F_{ST} values (table 3). Observed F_{ST} values in the CU and UU populations were significantly (an order of magnitude) lower than the value expected (expected $F_{ST} = 0.191$ and 0.198 at generations 0 and 15, respectively, of HL and LH selection) if feeding rate were selectively neutral, at least among the five replicate populations within each selection regime (expected F_{ST} values were computed using equation A1 (appendix 2), using the number of generations t from the details in figure 1). All these observations suggest that (a) adaptation to high larval density in the CU populations led to the evolution of a mean feeding rate stabilized at about two standard deviations above that of the control UU populations; (b) feeding rates in both UU and CU populations, at least after 65 generations of CU selection, are under stabilizing selection, as replicate populations within selection regime have not appreciably diverged from one another; and (c) the strength of stabilizing selection on the UU populations is probably similar to that on the CU populations, and the fitness functions acting on the two

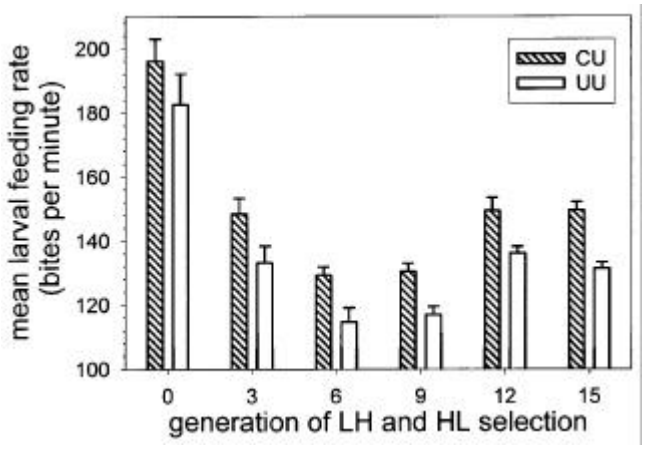

Figure 2. Mean larval feeding rate in the CU and UU populations during the first 15 generations of LH and HL selection. The error bars represent 95% confidence intervals based on the variation among the means of the five replicate populations in each selection regime.

Table 1. Summary of ANOVA results on the combined analysis of feeding-rate data from the CU and UU populations over generations 0, 3, 6, 9, 12 and 15 of HL and LH selection.

Effect	F	P	Fractional contribution to variance
Generation	296	< 0.001	0.743
Block	5.5	< 0.001	0.002
Selection	457	< 0.001	0.120
Generation × block	4.3	< 0.001	0.009
Generation × selection	1.6	0.193	0.001
Block × selection	1.3	0.249	0.001
Generation × block × selection	0.9	0.529	0.000
Error			0.124

The fractional contribution of each effect and interaction to variation in feeding rate is given in the last column (contributions less than 0.001 are listed as 0).

Table 2. Summary of ANOVA results on the generationwise analysis of feeding-rate data from the CU and UU populations over generations 0, 3, 6, 9, 12 and 15 of HL and LH selection.

Generation	Effect	F	P	Fractional contribution to variance
Gen. 0	Block	6.9	< 0.001	0.083
	Selection	62.8	0.0013	0.216
	Block × selection	0.6	0.648	0.000
	Error			0.701
Gen. 3	Block	6.2	< 0.001	0.055
	Selection	124	< 0.001	0.523
	Block × selection	1.0	0.408	0.000
	Error			0.422
Gen. 6	Block	2.2	0.071	0.011
	Selection	58.7	0.002	0.517
	Block × selection	2.5	0.043	0.027
	Error			0.445
Gen. 9	Block	0.97	0.424	0.000
	Selection	87.7	< 0.001	0.564
	Block × selection	1.5	0.194	0.011
	Error			0.425
Gen. 12	Block	4.3	0.003	0.027
	Selection	118	< 0.001	0.623
	Block × selection	1.6	0.188	0.009
	Error			0.341
Gen. 15	Block	2.2	0.066	0.006
	Selection	512	< 0.001	0.794
	Block × selection	0.78	0.540	0.000
	Error			0.200

The fractional contribution of each effect and interaction to variation in feeding rate is given in the last column (contributions less than 0.001 are listed as 0).

types of populations are of similar shape, as there are no significant differences between selection regimes in either F_{ST} values or coefficients of variation for feeding rate, and the shapes of the feeding-rate distributions in the CU and UU populations are quite similar.

Feeding rates under LH and HL selection

During the course of 20 generations of HL and LH selection, larval feeding rates underwent substantial evolution (figure 4). At generations 3, 6 and 9 of HL and LH selection, larvae from CU-derived populations (CU-HL and CU-LH) were significantly faster feeders than those from UU-derived populations (figure 4). By generation 9, the UU-LH populations, which were undergoing extreme larval crowding, had evolved significantly higher feeding rates than the UU-HL populations. Moreover, by generation 12, the feeding rates of CU-HL populations, which were essentially under reversed selection by virtue of being kept at moderate larval densities after having first evolved for 65 generations at high larval densities, had become significantly less than those of the CU-LH populations, although they were still faster than those of the UU-LH populations (figure 4). This trend of UU-LH and CU-HL populations evolving higher and lower feeding rates, respectively, continued thereafter, and by generation 20 of ongoing selection, the feeding rates of CU-LH and UU-LH populations were not significantly different from each other, and were significantly higher than those of either the UU-HL or CU-HL populations (figure 4).

These evolutionary trends in feeding-rate changes were mirrored in the ANOVA results, which are summarized in table 4. Over the first six generations of HL and LH selection, the only significant fixed effect was due to past selection (CU versus UU). By generation 9, there was also a significant past selection × ongoing selection interaction, and from generation 12 onwards the effect of ongoing selection (HL versus LH) also became significant (table 4). By generation 20, the past selection × ongoing selection interaction was no longer significant (table 4), paralleling the pattern of change in the mean feeding rates of the CU-HL and UU-LH populations, relative to the CU-LH and UU-HL populations, respectively (figure 4). Although there was still a significant effect of past selection at generation 20 (table 4), largely due to a very small block × past selection term, the magnitude of the feeding-rate difference between the means for all CU-derived and all UU-derived populations was small enough—1.49 bites per minute—to be rather meaningless biologically. By

Table 3. Observed F_{ST} for feeding-rate data among the five replicate populations within the CU and UU selection regimes over generations 0, 3, 6, 9, 12 and 15 of HL and LH selection.

Generation	CU	UU
Gen. 0	0.0303	0.0733
Gen. 3	0.0485	0.0745
Gen. 6	0.0000	0.0843
Gen. 9	0.0057	0.0068
Gen. 12	0.0726	0.0093
Gen. 15	0.0130	0.0122
Mean	0.0283	0.0434
95% c.i.	0.0294	0.0393

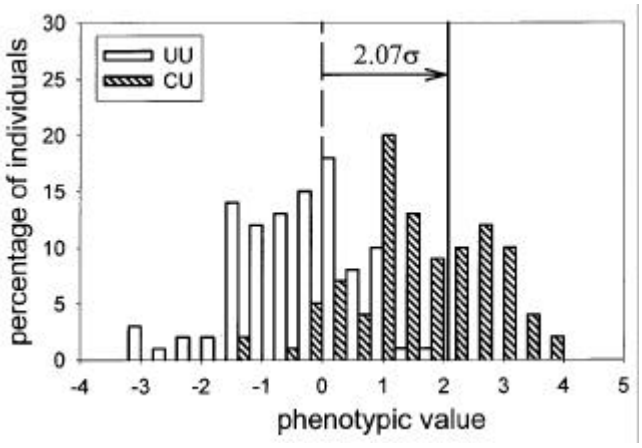

Figure 3. Distribution of feeding rate (expressed for each population CU_i, UU_i as a deviation from the mean value in population UU_i, in units of standard deviation) in the CU and UU populations at generation 12 of HL and LH selection. Data depicted are pooled across replicate populations (n = 100 individuals per selection regime). The dashed and solid vertical lines indicate the UU and CU means, respectively.

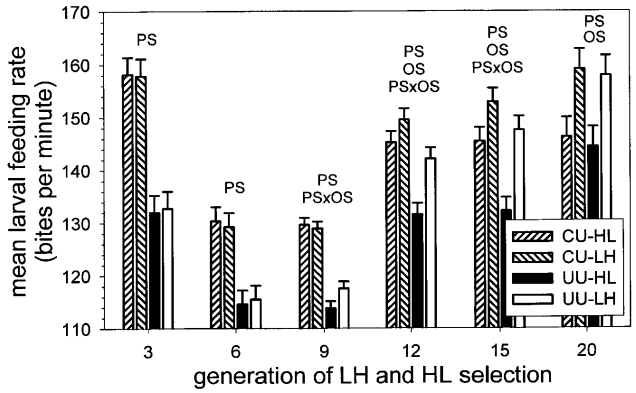

Figure 4. The evolution of larval feeding rate during 20 generations of HL and LH selection. The bars represent mean values, averaged across all five replicate populations of a selection regime, and the error bars are 95% confidence intervals based on least squares estimates of cell means in the randomized block ANOVAs. The course of evolution is indicated by listing which of the relevant fixed ANOVA effects were significant ($P < 0.05$) at each generation (PS, past selection; OS, ongoing selection; PS × OS, interaction between past and ongoing selection).

Table 4. Summary of ANOVA results on the feeding-rate data from the CU-HL, CU-LH, UU-HL and UU-LH populations over generations 3, 6, 9, 12, 15 and 20 of HL and LH selection.

Effect	Gen. 3	Gen. 6	Gen. 9	Gen. 12	Gen. 15	Gen. 20
B	32.2*	35.9*	29.6*	15.3*	0.80	13.7*
PS	97.1*	35.4*	33.8*	29.9*	73.3*	34.7*
OS	0.16	0.01	1.30	58.4*	67.5*	653*
B × PS	3.53*	10.0*	10.0*	3.15*	1.48	0.05
B × OS	0.17	1.95	3.29	0.79	2.45*	0.25
PS × OS	0.19	1.17	21.7*	16.5*	18.1*	0.04
B × PS × OS	0.72	1.47	0.42	0.49	1.10	1.68

Entries represent F values for each effect, and asterisks denote significance at the 0.05 level (B, block; PS, past selection; OS, ongoing selection).

contrast, for example, the difference between the means for all LH and all HL populations was 13.14 bites per minute. Coefficients of variation for feeding rate in the 20 populations were similar in magnitude to those seen in the CU and UU populations, ranging between 0.05 and 0.10. As in the case of the CU and UU populations, the distribution of feeding rates in the CU-HL, CU-LH, UU-HL and UU-LH populations was typically normal.

Analysis of the variance components (figure 5) clearly shows that the major contributions to variation in feeding rate were (a) variation among larvae within replicate populations (mean fractional contribution 0.39 ± 95% c.i. of 0.11); (b) variation due to past selection, which declined from about 0.50 over the first nine generations to less than 0.01 at generation 20; and (c) variation due to ongoing selection, which increased from less than 0.01 over the first nine generations to 0.41 at generation 20. The mean fractional contribution of block—representing ancestry and also day-to-day variation in assay conditions—over the 20 generations of HL and LH selection was 0.09 (± 95% c.i. of 0.05). The fractional contribution of chance to variation in feeding rate was minuscule, averaging only 0.04 (± 95% c.i. of 0.03). The past selection × ongoing selection interaction made no contribution to variation in feeding rate during generations 3, 6 and 20, briefly making a mean fractional contribution of 0.05 (± 95% c.i. of 0.05) during generations 9 through 15, a time when feeding rates in the CU-HL and UU-LH populations were changing (figures 4, 5).

Under the null assumption of selective neutrality of feeding rate among the five replicate populations within each selection regime, expected values of F_{ST} would be 0.192 and 0.197 at generations 3 and 20, respectively, of HL and LH selection (appendix 2, equation A1). Observed mean F_{ST} values among the CU-LH and CU-HL populations over the 20 generations of selection were significantly less (t test, $P < 0.05$) than the null expectation (table 5). However, mean F_{ST} values among the UU-LH and UU-HL populations over the same period were 0.184 (± 95% c.i. of 0.14) and 0.148 (± 95% c.i. of 0.12), respectively, and did not significantly differ from the null expectation (t test, $P > 0.05$). Nevertheless, F_{ST} values among the UU-derived populations were much lower at generations 15 and 20 of HL and HL selection (table 5), perhaps indicating that the UU-derived populations underwent feeding-rate increases for some time even

Table 5. Observed F_{ST} for feeding-rate data among the five replicate populations within the CU-LH, CU-HL, UU-LH and UU-HL selection regimes over generations 3, 6, 9, 12, 15 and 20 of HL and LH selection.

Generation	CU-LH	CU-HL	UU-LH	UU-HL
Gen. 3	0.1244	0.1967	0.2066	0.1466
Gen. 6	0.0885	0.0297	0.0336	0.2758
Gen. 9	0.1143	0.0000	0.3191	0.2819
Gen. 12	0.0488	0.0544	0.1912	0.0841
Gen. 15	0.0171	0.0000	0.0270	0.0129
Gen. 20	0.1144	0.0280	0.0247	0.0881
Mean	0.0846	0.0515	0.1841	0.1482
95% c.i.	0.0450	0.0777	0.1423	0.1152

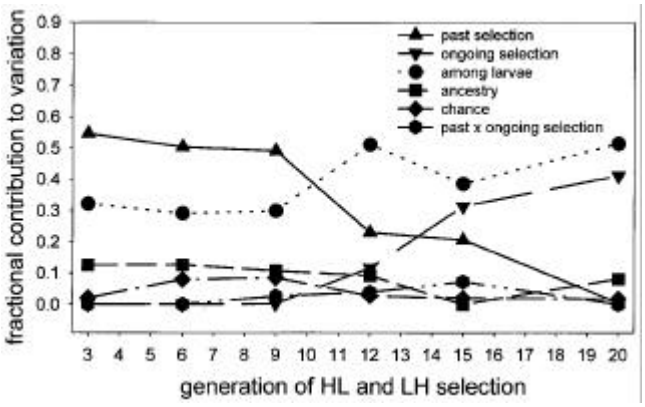

Figure 5. The relative contributions of past selection, ongoing selection, ancestry, chance and variation among larvae within populations to variation in larval feeding rate in the set of 20 experimental populations during the course of the 20 generations of HL versus LH selection.

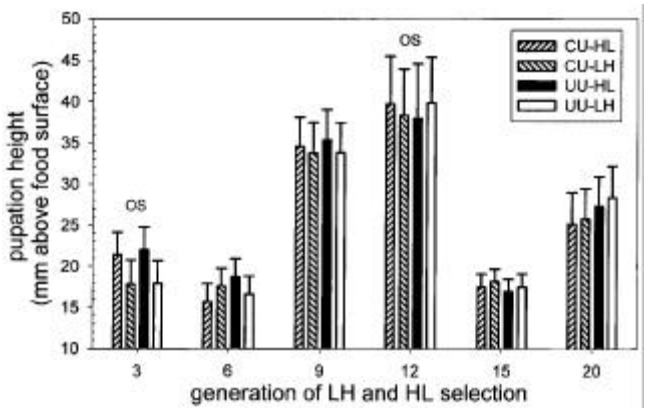

Figure 6. Mean pupation height in the experimental populations under different selection regimes during 20 generations of HL and LH selection. The bars represent mean values, averaged across all five replicate populations of a selection regime, and the error bars are 95% confidence intervals based on least squares estimates of cell means in the randomized block ANOVAs. Relevant fixed ANOVA effects (OS, ongoing selection) that were significant ($P < 0.05$) at each generation are indicated.

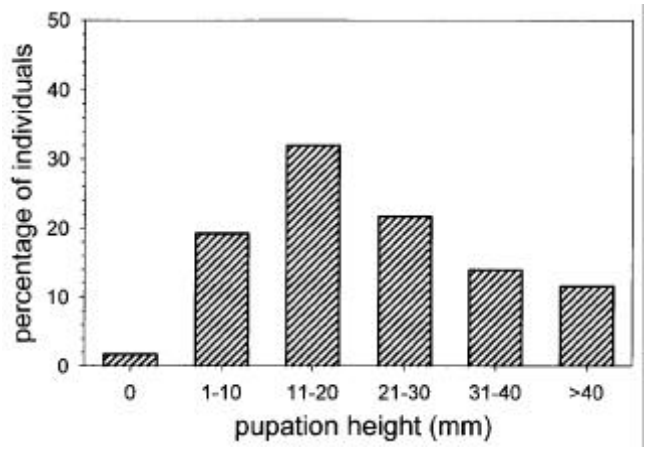

Figure 7. Distribution of pupation height in the 20 experimental populations (pooled) at generation 3 of HL and LH selection.

after being shifted to the HL regime which is likely to have had higher larval density than the ancestral UU regime, even though much less than the density experienced by populations in LH conditions.

Pupation heights under LH and HL selection

Although mean pupation height varied considerably across different assay generations, there was no consistent trend across selection regimes (figure 6). The ANOVA results at different assay generations reveal the only consistent significant effects to be those of block and vial (table 6). At various generations, different interactions involving block and past or ongoing selection, or both, were also significant, but, once again, there was no clear temporal pattern to these effects (table 6). The only significant fixed factor was ongoing selection at generations 3 and 9, and in both cases the mean of the LH populations was less than that of the HL populations (figure 6, table 6). There was, however, no pattern to the relative magnitude of mean pupation height in LH and HL regimes over time (figure 6). The coefficient of variation within populations for pupation height did not exhibit any temporal pattern, but the magnitude of the coefficients of variation tended to be higher than that seen for feeding rate, ranging from 0.46 to 0.53. Distributions of pupation height in these populations also tended to significantly differ from normality, particularly in kurtosis, being flatter and more spread out than a normal distribution (figure 7).

The major contribution to variation in pupation height, ranging from 0.86 to 0.94, was variation among individuals within vials (figure 8). The contributions of past selection, ongoing selection and past selection × ongoing selection interaction to variation in pupation height were negligible, their combined fractional contribution averaging only 0.015 (± 95% c.i. of 0.022). The mean (± 95% c.i.) fractional contributions of chance, vial and ancestry, respectively, were 0.037 (± 0.019), 0.030 (± 0.016) and 0.016 (± 0.012). Observed mean F_{ST} values among the CU-LH, CU-HL, UU-LH and UU-HL populations over the

Table 6. Summary of ANOVA results on the pupation-height data from the CU-HL, CU-LH, UU-HL and UU-LH populations over generations 3, 6, 9, 12, 15 and 20 of HL and LH selection.

Effect	Gen. 3	Gen. 6	Gen. 9	Gen. 12	Gen. 15	Gen. 20
B	2.68*	5.18*	19.9*	3.67*	8.69*	5.54*
PS	0.33	4.37	0.15	0.00	0.30	3.39
OS	20.0*	0.01	30.4*	0.08	0.68	0.17
Vial	1.84*	3.70*	1.81*	3.25*	2.21*	2.05*
B × PS	1.57	0.83	2.68*	1.18	4.11*	4.02*
B × OS	2.81*	3.00*	0.10	2.74*	1.93	1.81
PS × OS	0.07	6.40	0.08	0.26	0.00	0.02
B × PS × OS	3.84*	2.12	3.60*	4.44*	1.01	4.38*

Entries represent *F* values for each effect, and asterisks denote significance at the 0.05 level (B, block; PS, past selection; OS, ongoing selection).

20 generations of selection were significantly less (*t* test, $P < 0.05$) than the null expectation by an order of magnitude (table 7). Thus the overall picture that emerges of pupation height is that of a trait under stabilizing selection, at similar phenotypic values in CU and UU populations at the start of HL and LH selection, that does not undergo much alteration either in terms of variation within or among populations or in terms of mean values. This is a picture consistent with a trait under stabilizing selection where the fitness function has a broad, flattened plateau, which falls off rather rapidly on both sides as trait values approach the tails of the phenotypic distribution, an issue we will discuss further in the next section.

Discussion

Forward and reverse evolution of feeding rate

The evolutionary trajectories of traits under reverse selection can vary considerably, exhibiting rapid reversion to ancestral values, incomplete reversion, reversion to a value beyond ancestral values, or no change (Teotónio and Rose 2000, 2001). Larval feeding rate is a trait that has repeatedly been seen to undergo evolutionary increases under conditions of high larval density (Joshi and Mueller 1988, 1996; Guo *et al*. 1991). Independent sets of populations that evolved increased competitive ability and higher feeding rates under high larval densities were also seen to be less efficient at utilizing food to successfully complete development (Mueller 1990; Joshi and Mueller 1996), leading to the hypothesis that faster feeding may exact a fitness cost under low-density conditions, resulting in a tradeoff between larval food acquisition and utilization (Joshi and Mueller 1996). Some support for this hypothesis comes from the observation that *D. melanogaster* populations subject to strong truncation selection for rapid preadult development quickly evolve reduced larval feeding rate, possibly as part of an adaptive syndrome of reducing energy expenditure in a selection regime where time for putting on mass is severely limiting (Prasad *et al*. 2001). Our present results on the course of feeding-rate evolution in the CU-HL populations provide clear support for the notion that faster feeding carries a high fitness cost under low-density conditions. The rapid (within 20 generations) reversion of feeding rate in the CU-HL populations to more or less ancestral (UU) level suggests reverse evolution of a trait closely related to fitness, governed by strong antagonistic pleiotropy, and, moreover, one in which the original (forward) evolution was not characterized by major contributions of epistasis or gene × environment interactions (Teotónio and Rose 2000, 2001; Teotónio *et al*. 2002).

The fact that feeding rate rapidly increases or decreases when UU-type populations (moderate larval density and no strong selection on preadult development time) are subjected to selection for adaptation to crowding (Joshi and Mueller 1996; UU-LH populations in this study) or selection for faster development (Prasad *et al*. 2001), respectively, suggests abundant additive genetic variation for this trait in UU-type populations. After 65 generations of selection for adaptation to high larval density in the CU populations, larval feeding rate appeared to have stabilized at a rate about of 14 bites per minute greater than that in the UU populations (figure 2). Since feeding rate in the CU-HL populations, essentially under reverse selection for adapting to low larval density, decreased rapidly to levels similar to that in the UU populations, it is clear that the plateau in the selection response of the CU feeding rates by generation 65 was not a consequence of additive genetic variation being depleted. The alternative is that once the CU populations had diverged from the UUs by about two standard deviations, feeding rate in these populations was under stabilizing selection with further increases in feeding rate no longer yielding a net fitness benefit to the individual. Given the similarity bet-

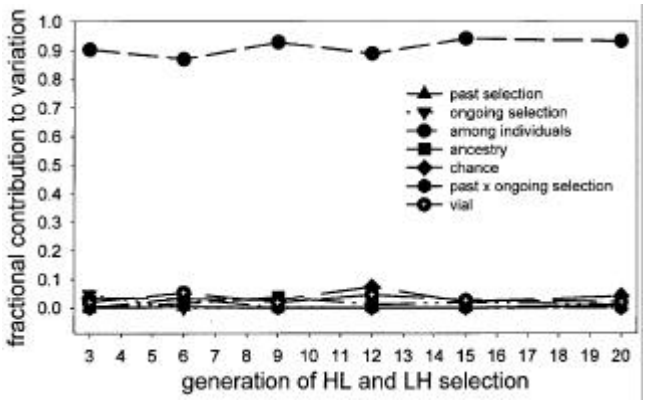

Figure 8. The relative contributions of past selection, ongoing selection, ancestry, chance, and variation among vials within population and among larvae within vials to variation in pupation height in the set of 20 experimental populations during the course of the 20 generations of HL versus LH selection.

Table 7. Observed F_{ST} for pupation-height data among the five replicate populations within the CU-LH, CU-HL, UU-LH and UU-HL selection regimes over generations 3, 6, 9, 12, 15 and 20 of HL and LH selection.

Generation	CU-LH	CU-HL	UU-LH	UU-HL
Gen. 3	0.0021	0.0018	0.0211	0.0130
Gen. 6	0.0177	0.0048	0.0135	0.0209
Gen. 9	0.0263	0.0286	0.0680	0.0126
Gen. 12	0.0158	0.0000	0.0031	0.0177
Gen. 15	0.0053	0.0288	0.0120	0.0112
Gen. 20	0.0129	0.0291	0.0084	0.0013
Mean	0.0133	0.0155	0.0224	0.0128
95% c.i.	0.0092	0.0154	0.0246	0.0070

ween the shapes of the distribution (close to normal) of feeding rates in CU and UU populations, the relatively low coefficients of variation, and the low F_{ST} values among replicate populations within selection regime, it appears that at the start of the HL and LH selection feeding rate in both the UU and CU populations was under fairly strong stabilizing selection, with relatively steeply humped fitness functions.

Contributions of selection, ancestry and chance

In the case of the evolution of larval feeding rate in the course of HL and LH selection, it is clear that the effect of past selection, though strong initially, was transient. By generation 20, the fractional contribution of past selection to variation in feeding rate had dropped from 0.54 to almost 0, while that of ongoing selection had risen from 0 to 0.41 (figure 5). Ancestry—in this case confounded with day-to-day variation in assay conditions, but independent of past selection history—made an average contribution of about 0.09, while chance played practically no role in determining variation in feeding rate in this experimental system. Variation within populations and divergence among populations also did not appear to be affected by any factor other than ongoing selection by the end of the 20 generations of HL and LH selection. Thus, neither history (past selection and ancestry) nor chance seem to play much role in affecting the distribution of feeding-rate variation within and among populations adapting to different larval densities.

In experimental studies of adaptive evolution in *E. coli*, divergence of both fitness and morphological traits loosely related to fitness has been observed among initially identical populations, especially over the first few thousand generations of selection in a new environment (Lenski and Travisano 1994; Travisano *et al*. 1995). However, these bacterial populations had no initial standing genetic variation and reproduced asexually. Any new variation, therefore, had to arise *de novo* via mutation, and it is thought that differences among populations in the sequence in which beneficial mutations arose could explain the among-population divergence in fitness (Lenski and Travisano 1994). Another finding of these studies with *E. coli* was that the effects of history and chance on variation for cell size, a trait only loosely associated with fitness in the conditions of that experiment, were considerable, and similar or greater in magnitude than that of ongoing selection, after 1000 generations of adaptation to new nutritional or thermal environments (Travisano *et al*. 1995). In the case of competitive fitness, however, the contribution of ongoing selection to variation after 1000 generations of adaptation was considerably greater than those of history and chance, although the contributions of the latter two factors were not entirely negligible (Travisano *et al*. 1995).

However, in populations of sexually reproducing organisms, unlike bacteria, there is usually considerable standing genetic variation and this variation is, moreover, reshuffled extensively each generation by recombination. In addition, the maintenance of gametic phase disequilibrium among loci through epistasis for fitness requires exceptionally strong selection pressure, or fortuitously favourable patterns of assortative mating with regard to the traits encoded by those loci. Genomic imprints of history are, therefore, likely to be obliterated rapidly in even moderately sized outbreeding sexual populations, unless we are dealing with phylogenetic constraints at the macroevolutionary level. Consequently, at least on microevolutionary time scales, one major mechanism by which past selection or ancestry can continue to affect patterns of variation among and within populations in the face of new and different ongoing selection pressures is if the past selection gave rise to particular differing patterns of genotype × environment (G × E) interactions (Teotónio and Rose 2000, 2001). Not surprisingly, perhaps, microbial studies of reverse evolution suggest that epistasis often plays a major role, whereas the main factor preventing full reversion to ancestral phenotypes in reverse evolution studies on *Drosophila* appears to be G × E interaction (reviewed by Teotónio and Rose 2001). It also appears that adaptive evolution in uniform environments can result in convergent evolution of fitness, or traits that are strongly correlated with it, albeit through different routes and involving varied changes in subsidiary phenotypes or at the genetic level (Cohan and Hoffmann 1989; Joshi and Thompson 1995; Travisano and Lenski 1996; Rainey and Travisano 1998; Bürger and Gimelfarb 1999; Teotónio *et al*. 2002). Clearly, G × E interactions do not appear to have played a role in the original evolution of the feeding-rate differences between our CU and UU populations, given the rapid and complete reversion of feeding rates in the CU-HL populations to ancestral levels. Indeed, it is possible to speculate that, in general, the evolution of traits closely linked to fitness in model systems with relatively uniform and simple environments will be more or less fully reversible if the selection pressures were to be reversed. In natural conditions, with more complex and variable environments, there may be more opportunities for the evolution of specific G × E interactions which could then result in a lower degree of reversibility of adaptive evolutionary trajectories.

The case of pupation height, a trait that had similar phenotypic distributions among the CU and UU populations at the start of HL and LH selection, is very different from that of larval feeding rate, a trait for which the CU and UU populations had already diverged at the beginning of this study. In terms of the role of history versus selection, selection regimes in which larval density was high or low appear to have played practically no role in determining patterns of variation in pupation height in these experimental populations (figures 6 and 8, table 6). Although ancestry did make an average fractional contri-

bution of 0.02 to variation in pupation height, the possible role of history was limited because past selection had not contributed to differences between CU and UU populations to start with. Overall, in any assay generation, 0.91 of the variation in pupation height was, on average, due to variation among individuals within vials, and another 0.04 due to variation among vials nested within each population (figure 8). In the absence of any knowledge of the trajectory of pupation height in the course of the derivation of the UU and CU populations from the B populations of Rose (1984) (figure 1), the simplest inference to be drawn from the lack of pupation-height divergence between the CU and UU populations would be that the trait was uncorrelated with fitness in the context of these two selection regimes. However, the low F_{ST} values among replicate populations—significantly lower by an order of magnitude from those expected if the trait were selectively neutral—within each of the four selection regimes over 20 generations of HL and LH selection (table 7) suggest that pupation height was under stabilizing selection for similar values in both the UU and CU populations, as well as during HL and LH selection. Moreover, the flattened distributions of pupation height in the populations used in this study (figure 7) also suggest that the fitness function is likely to have a fairly wide plateau coinciding with the range of pupation heights that accounts for the majority of individuals in these populations.

In the case of pupation height in the CU and UU populations, it is actually possible to piece together the outline of events, and speculate about causal mechanisms, giving rise to the pattern of among-population and within-population variation seen in the experimental populations in this study. This is partly owing to information we have about the selection acting on pupation height in another set of *D. melanogaster* populations subjected to maintenance at low or high densities (Mueller and Sweet 1986; Joshi and Mueller 1993). Mean pupation height in three crowded *K* populations was seen to evolve to become greater than that in three uncrowded *r* populations that acted as controls (Mueller and Sweet 1986). Yet, a study in which pupation height and pupal viability were assayed on the *r* and *K* populations at low and high assay densities (20 versus 150 larvae per 8-dram vial with about 6 ml food) revealed clear evidence for stabilizing selection on pupation height with a fitness function that had a wide plateau and fell off rapidly at both extremes (figure 9; Joshi and Mueller 1993). The shape of the fitness function suggested that even the *r* populations should have been under selection for increased pupation height, because, even at low density, pupae on the food surface had poor viability, leading Joshi and Mueller (1996) to speculate that perhaps the *r* populations in their maintenance regime escaped selection for pupation height by pupating on the paper tissue provided in the culture bottles, a substrate not available in the vials in which pupation height was actually assayed by Mueller and Sweet (1986) and Joshi and Mueller (1993).

When the CU populations were first derived from the B populations of Rose (1984), they were maintained at a density of ~500 larvae per 8-dram vial with about 6 ml food for the first 12 generations and, during this period, neither feeding rate nor pupation height in the CUs diverged significantly from those in controls (Mueller *et al.* 1993). At that point, larval density in the CU populations was increased to ~1000 larvae per 6-dram vial with about 4 ml food, and by generation 15 of selection, both pupation height and larval feeding rate in the CU populations were greater than in controls, although the difference between CU populations and controls in pupation height was not always significant in assays repeated at generations 17 and 18 of CU selection (Mueller *et al.* 1993). Both feeding rate and pupation height were consistently significantly greater in CUs than in controls by generation 25 of selection (Mueller *et al.* 1993). The mean feeding-rate difference between the CUs and controls remained consistent at about 14 bites per minute from generation 15 (Mueller *et al.* 1993) through around generation 60 (Joshi and Mueller 1996), and up until the duration of the present study (figure 2). Pupation height, on the other hand, did not significantly differ between the CU populations and the UU controls in the assay carried out at around generation 60 of selection (Joshi and Mueller 1996). It appears, therefore, that not only the CUs but also the UUs were under selection for increased pupation height after their derivation from the B populations of Rose (1984), even though the food medium and larval density of the

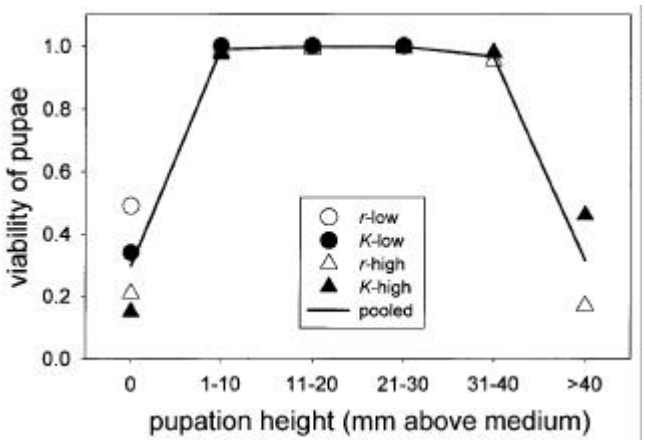

Figure 9. The fitness function for pupation height in the *r* and *K* populations of Joshi and Mueller (1993). Symbols are mean viabilities of all pupae falling within a given pupation-height interval, averaged over the three replicate populations of the *r* (low density) or *K* (high density) selection regimes, assayed at low (20 larvae per vial) or high (150 larvae per vial) density (redrawn from data first presented in Joshi and Mueller 1993).

UU and B populations were the same. However, the B populations were maintained on a two-week discrete-generation cycle whereas the UU populations were maintained on a three-week discrete-generation cycle. It is known that relaxation of the generation cycle of B populations by just four days, to 18 days rather than 14, results in a rapid increase in preadult development time (Chippindale *et al*. 1997), and also that (a) selection for faster development in *Drosophila* results in a correlated reduction in pupation height (Prasad *et al*. 2001), and (b) selection for reduced pupation height leads to the correlated evolution of faster development (Markow 1979). Thus, it appears that the UU populations, despite being maintained at moderate density, underwent an evolutionary increase in pupation height, albeit at a slower rate than the high-density CU populations, as a consequence of being released from the B-regime selection pressure for faster development. Eventually, as mean pupation height in the UU populations reached levels similar to that of the CU populations, they also began to undergo stabilizing selection on pupation height (figure 10). The distributions of pupation heights, assayed at a density of 50 larvae per vial, in the CU-derived and UU-derived populations (figure 7) are quite similar to those seen in the *r* and *K* populations at high (150 larvae per vial) assay density (Joshi and Mueller 1993). This may be partly due to the fact that the banana–molasses food used in CU and UU maintenance is softer and more moist at low larval densities than the cornmeal food used in the *r* and *K* maintenance regimes; pupation height is known to increase in response to many environmental factors, including the moisture content of the medium (Sameoto and Miller 1968). It is therefore not unreasonable to conclude that the CU-derived and UU-derived populations by the start of this study did in fact experience stabilizing selection, with a flattened fitness function similar to that estimated for the *r* and *K* populations (figure 9) studied by Joshi and Mueller (1993).

Overall, our results clearly show that in these large outbreeding populations of *D. melanogaster*, the genetic imprints of historical (past selection/ancestry) and chance events on traits closely related to fitness are very labile, and rapidly disappear in the face of ongoing selection. This is consistent with other observations on *D. melanogaster* in studies dealing with many more types of populations and traits (Teotónio and Rose 2000; Teotónio *et al*. 2002), and suggests that historical constraints on adaptive microevolutionary changes in sexual outbreeding populations are likely to operate mostly in situations where past selection shapes the evolution of different G × E interactions among populations. Moreover, the primary determinant of historical effects on the distribution of genetic variation within and among such populations is likely to be past selection in different environments, rather than ancestry per se. This is in contrast to the situation in asexual microbes in which adaptive evolution is greatly dependent on the serial *de novo* occurrence of new genetic variation by mutation.

One other point we would like to make is that most traits related even very indirectly to the life history or functional biology in the prereproductive phase of life

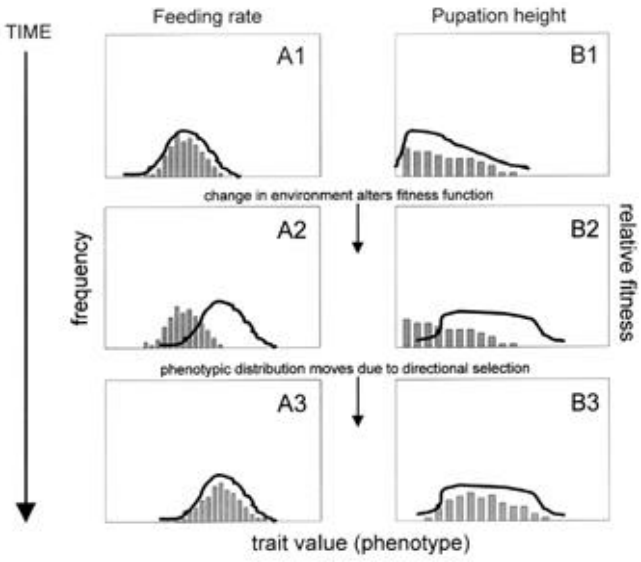

Figure 10. Schematic depiction of a speculative interpretation of the course of evolution of feeding rate in the CU populations (A1...A3) and pupation height in the CU and UU populations (B1...B3), illustrating how a humped fitness function can yield either stabilizing or directional selection depending on how it maps onto the phenotypic distribution. The bars represent frequencies of different phenotypes, and the solid curve indicates the fitness function acting on the phenotypic distribution. (A1) Phenotypic distribution of feeding rate in the UUs with a relatively steeply humped fitness function resulting in stabilizing selection. (A2) Owing to imposition of the CU selection regime, the fitness function moves to the right on the phenotypic axis, although its shape does not change appreciably, resulting in directional selection for increased feeding rate. (A3) Owing to the directional selection, the phenotypic distribution also moves to the right along the phenotypic axis, its mode once again becoming coincident with the hump of the fitness function, eventually resulting in stabilizing selection for feeding rate on the CUs, albeit at a higher mean value than in the UUs. (B1) Phenotypic distribution of pupation height in the Bs with a humped and skewed fitness function resulting in stabilizing selection for relatively low values of pupation height owing to the selection pressure for relatively rapid development. (B2) Owing to the shift to a three-week cycle, relaxing the selection for fast development, the fitness function loses its skew, and acquires a wide plateau rather than a hump, because a wide range of intermediate pupation heights yield similar fitness (see figure 9). Yet, due to its right-shifted position on the phenotypic axis, this fitness function results in directional selection for increased pupation height in both the CU and UU populations, albeit at different rates. (B3) Due to the directional selection, the phenotypic distribution also moves to the right along the phenotypic axis, and changes shape, eventually becoming coincident with the plateau of the fitness function, resulting in stabilizing selection for pupation height in the CUs and UUs.

are likely to be under selection of a broadly stabilizing kind, as opposed to being selectively neutral, if they are not undergoing directional change in the populations being studied. This is basically a consequence of the integration of various traits into the phenotype at the organismal level, reflected at the genetic level by widespread networks of pleiotropic effects and interactions. Estimating F_{ST} among such populations, and comparing its value either to an expectation based on neutrality (if the history of the populations is known, as in this study), or to the F_{ST} values observed among the same populations for traits expected to be neutral (Prout and Barker 1993; Long and Singh 1995), are likely to be a useful way of qualitatively assessing the relative magnitude of such stabilizing selection. As in the case of pupation height in this study, we believe that the fitness functions in the case of such traits will often be characterized by fairly wide plateaus, such that over a reasonably large range of phenotypes fitness and phenotypic value will tend to be uncorrelated.

Fitness functions, trait distributions and the form of selection

The fitness function, which embodies natural selection, arises as a consequence of the interaction of phenotype and environment (Wade and Kalisz 1990). Thus, in principle, the relationship between fitness and different phenotypic values can be defined over a whole expected range of phenotypic values in a particular environment. However, in reality, the fitness function will exert its influence on the realized distribution of phenotypic values in any given combination of population and environment. A heuristic point that we wish to make in this final section is that, at least in a reasonably homogeneous environment such as often exists in laboratory studies, the form of selection acting on life-history-related traits that are typically studied is not so much due to the shape of the fitness function, but is rather due to the way in which the fitness function is juxtaposed on the realized phenotypic distribution along the trait axis. We believe that very often the typical form of fitness functions on life-history-related traits will be humped, because, in any given environment, there will be a range of phenotypic values that yield reasonably high fitness, and phenotypes above or below this range will tend to yield lower fitness due to tradeoffs with other life-history-related traits. It should be noted, however, that a humped fitness function defined on the phenotypic axis need not necessarily have the same shape when defined on an axis of breeding values (Bürger and Gimelfarb 1999), depending on how the breeding-value and phenotypic-value distributions map onto each other. The mapping of the phenotypic distribution onto the breeding-value distribution, in turn, will strongly affect how a particular fitness function shapes the phenotypic distribution over generations.

The notion that the form of selection is determined by the juxtaposition of the fitness function on the realized trait distribution on the phenotypic axis is exemplified by the repeated observation in laboratory studies that responses to directional (truncation) selection plateau out reasonably fast (20–50 generations), unless the truncation point keeps moving over the course of selection as the population mean shifts towards lower or higher trait values. Yet, populations at the response plateau typically still harbour significant additive genetic variation for the trait that has stabilized because they tend to respond readily to both increases in selection intensity, and to reverse selection (Prasad *et al*. 2001; Teotónio and Rose 2000; Teotónio *et al*. 2002). Thus, over generations, directional selection becomes stabilizing selection as the phenotypic distribution moves along the trait axis, a scenario reflected in the results for feeding rate and pupation height in this study (figure 10). We believe that the shape of the fitness function for many life-history-related traits probably does not change drastically when the environment (selection regime) changes, especially in typical laboratory studies. What changes is the location of the fitness function on the realized trait distribution, as a result of the different fitness weighting of trait values. When the environmental change results in the hump of the fitness function no longer being largely coincident with that of the realized trait distribution, directional selection ensues, and once the trait distribution has moved in response to the directional selection such that it is once again largely coincident with the hump of the fitness function, the form of selection reverts to being stabilizing (figure 10).

If the above scenario is, in fact, fairly common, then it follows that the form of selection acting on life-history-related traits much of the time is of the stabilizing type. Classical quantitative-genetics models involving multiple loci with small individual effects generally suggest that it is difficult to maintain genetic variation under stabilizing selection (reviewed by Bürger and Gimelfarb 1999), yet life-history-related traits under stabilizing selection typically retain substantial additive genetic variation (Prasad *et al*. 2001; Teotónio and Rose 2000; Teotónio *et al*. 2002). Some possible mechanisms that can result in the maintenance of genetic variation in the face of stabilizing selection are pleiotropy (Rose 1982), epistasis (Gimelfarb 1989), and the presence of major loci with heterozygote superiority (Bürger and Gimelfarb 1999). Both pleiotropy (Prasad and Joshi 2003) and epistasis (Leips and Mackay 2000; Vieira *et al*. 2000) are now known to be major factors contributing to the genetic architecture of life-history traits. Indeed, these factors may be contributing to the hump-shaped fitness functions that we believe are fairly common for life-history-related traits. Given that present-day genetic technologies are beginning to give us unprecedented insight into the functional and genetic architecture of life histories (White *et al*. 1999; Pletcher *et al*. 2002), our results underscore the importance of more detailed genetic investigations, both theoretical and experimental,

into the causes, consequences and interrelationships of stabilizing and directional selection in the course of adaptive evolution.

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Appendix 1

We list here the formulae used for computing variance components due to different effects in a four-way mixed-model ANOVA with factors A (random blocks, *a* levels), B (fixed, *b* levels), C (fixed, *c* levels) and D (random, nested within A × B × C, *d* levels), following methods described in Neter *et al*. (1990). For the time being, we assume *n* replicate individual observations in each A × B × C × D combination. The fixed factors B and C are crossed with themselves and with block. Thus the ANOVA model for this design is:

$$Y_{ijklm} = a_i + b_j + g_k + d_{l(ijk)} + ab_{ij} + ag_{ik} + bg_{jk} + abg_{ijk} + e_{m(ijkl)},$$

where $i = 1...a$, $j = 1...b$, $k = 1...c$, $l = 1...d$, and $m = 1...n$.

The expected mean squares for the different effects and interactions in this design are given by:

$$E[\text{MSA}] = bcdn\,s_a^2 + ns_d^2 + s_e^2$$

$$E[\text{MSB}] = \frac{acdn\sum_j b_j^2}{b-1} + cdn\,s_{ab}^2 + ns_d^2 + s_e^2$$

$$E[\text{MSC}] = \frac{abdn\sum_k g_k^2}{c-1} + bdn\,s_{ag}^2 + ns_d^2 + s_e^2$$

$$E[\text{MSD} \sim (\text{A}\times\text{B}\times\text{C})] = ns_d^2 + s_e^2$$

$$E[\text{MS(A}\times\text{B)}] = cdn\,s_{ab}^2 + ns_d^2 + s_e^2$$

$$E[\text{MS(A}\times\text{C)}] = bdn\,s_{ag}^2 + ns_d^2 + s_e^2$$

$$E[\text{MS(B}\times\text{C)}] = \frac{adn\sum_j\sum_k (bg)^2}{(b-1)(c-1)} + dn\,s_{abg}^2 + ns_d^2 + s_e^2$$

$$E[\text{MS(A}\times\text{B}\times\text{C)}] = dn\,s_{abg}^2 + ns_d^2 + s_e^2$$

$$E[\text{MSError})] = s_e^2.$$

Consequently, the variance components for this model can be derived from the expected mean square formulae as follows.

Block A: $s_a^2 = \dfrac{\text{MSBlock} - \text{MSFactorD}}{b\times c\times d\times n}$

Factor B: $\sum_j b_j^2$

$$= \frac{(\text{MSFactorB} - \text{MS(Block}\times\text{FactorB))}\times(b-1)}{a\times c\times d\times n}$$

Factor C: $\sum_k g_k^2$

$$= \frac{(\text{MSFactorC} - \text{MS(Block}\times\text{FactorC))}\times(c-1)}{a\times b\times d\times n}$$

Factor D ~ (A × B × C): s_d^2

$$= \frac{\text{MSFactorD} - \text{MSError}}{n}$$

Interaction (A × B): s_{ab}^2

$$= \frac{(\text{MS(Block}\times\text{FactorB)} - \text{MS(FactorD))}}{c\times d\times n}$$

Interaction (A × C): s_{ag}^2

$$= \frac{(\text{MS(Block}\times\text{FactorC)} - \text{MS(FactorD))}}{b\times d\times n}$$

Interaction (B × C): $\sum_k\sum_j (b_j g_k)^2$

$$= \frac{\text{MS(FactorB}\times\text{FactorC)}\times(b-1)\times(\text{c -1})}{a\times d\times n}$$

$$- \frac{\text{MS(Block}\times\text{FactorB}\times\text{FactorC)}\times(b-1)\times(\text{c}-1)}{a\times d\times n}$$

Interaction (A × B × C): s_{abg}^2

$$= \frac{(\text{MS(Block}\times\text{FactorB}\times\text{FactorC)} - \text{MS(FactorD))}}{d\times n}$$

Error: $s_e^2 = \text{MSError}$.

If the numbers of replicate observations per cell (A × B × C × D combination) vary, as they do for the pupation-height data, we can substitute n' for n in the above formulae, where n' is given by

$$n' = \frac{1}{(abcd)-1}\left[\sum_{i=1}^{abcd} n_i - \frac{\sum_{i=1}^{abcd} n_i^2}{\sum_{i=1}^{abcd} n_i}\right].$$

In this expression, n_i is the number of replicate observations in the ith cell of the design. From the variance components estimated as described above, we obtained the fractional contribution of each factor and interaction as the variance component of that factor or interaction divided by the sum of variance components of all factors / interactions.

The pupation-height data for the CU-HL, CU-LH, UU-HL and UU-LH populations conform to the ANOVA model described above, with past selection and ongoing selection crossed with themselves and with random blocks representing ancestry, and with vial nested within the block × past selection × ongoing selection interaction. The feeding-rate data for the CU-HL, CU-LH, UU-HL and UU-LH populations follow a three-way ANOVA model, similar to the one described above, but without the nested vial term. Similarly, the CU and UU feeding-rate data of all six generations together follow a three-way ANOVA model, with selection regime and generation crossed with each other and with block, whereas the CU and UU feeding-rate data from any one generation follow a two-way ANOVA model with selection regime crossed with block. Variance components for all of these models can be easily obtained from the formulae described above, with appropriate terms and coefficients deleted.

Appendix 2

To assess the role of random genetic drift on phenotypic variation in feeding rate and pupation height in our study populations, we computed expected and observed F_{ST} values among the five replicate populations within each selection regime at the various assay generations. Using standard population-genetics theory (e.g. Hartl and Clark 1989), the expected F_{ST} values at generation t were computed as

$$F_{ST(t)} = \left[1 - \left(1 - \frac{1}{2N_e}\right)^t\right], \qquad [A1]$$

with t representing the number of generations elapsed since the five B populations of Rose (1984) were derived from a common ancestral population ($F_{ST(0)} = 0$). Information on the number of generations elapsed between the derivation of the five B populations from the IV population and the various assay generations of HL and LH selection is summarized in figure 1. The effective population size of the CU and UU populations is on the order of 1000 individuals (L. D. Mueller, unpublished data), and for the CU-HL, CU-LH, UU-HL and UU-LH populations we can make a crude but conservative estimate of $N_e = 2000$.

Observed F_{ST} values among the five replicate populations within each selection regime were estimated following Prout and Barker (1993) and Long and Singh (1995), based on table 15.1 in Falconer (1981). The F_{ST} value for a set of five replicate populations is obtained from the variance components (see appendix 1) following a one-way (in the case of feeding rate) or two-way (in the case of pupation height) ANOVA, as

$$F_{ST} = \frac{s^2_{rep}}{2s^2_e + s^2_{rep}}, \qquad [A2]$$

where s^2_{rep} and s^2_e are estimates of the phenotypic variance between replicate population means, and variance among individuals within a replicate population, respectively. In the case of pupation height, s^2_e estimates the variance among individuals within replicate vials nested within population. Although this formulation is derived for additive genetic variances (Falconer 1981), it can be used with phenotypic variances in the case of traits for which variation is known to be largely additive (Prout and Barker 1993; Long and Singh 1995). Genetic variation for pupation height in *D. melanogaster* is known to be largely additive (Bauer and Sokolowski 1985; Sokolowski and Bauer 1989), whereas the results for larval feeding rate vary, with some studies suggesting it is largely additive (Sokolowski 1980) and others suggesting that the role of dominance and epistasis, at least in determining between-line variation in an artificial selection experiment on feeding rate, is quite large (Burnet *et al*. 1977). However, the rapidity with which feeding rate responds to both direct artificial selection (Sewell *et al*. 1975) and indirect selection through (a) rearing at high density (Guo *et al*. 1991; Mueller *et al*. 1993), or (b) selection for faster development (Prasad *et al*. 2001) suggests a substantial additive genetic component to the within-population genetic variance. In the present study, especially given the homogeneity of environments experienced by all five replicate populations in a selection regime, we believe that the above formulation for F_{ST} can be used, with the caveat that it may tend to be somewhat of an underestimate if a substantial part of the genetic variation among individuals is nonadditive.

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