RESEARCH NOTE

Reduced larval feeding rate is a strong evolutionary correlate of rapid development in *Drosophila melanogaster*

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Introduction

To answer the question, are preadult development time and larval feeding rate positively genetically correlated in *Drosophila melanogaster*, we selected four outbred populations of *D. melanogaster* for faster preadult development and late reproduction in the laboratory. We assayed larval feeding rates of individuals from selected populations and their ancestral control populations after 10 and 32 generations of selection, respectively. The mean larval feeding rate of individuals from selected populations was significantly lower than that in controls, by about 10 bites (cephalopharyngeal sclerite retractions) per minute. From the results we conclude that larval feeding rate and preadult development time in *D. melanogaster* are positively genetically correlated.

Increased larval feeding rate — number of cephalopharyngeal sclerite retractions per minute while feeding — is often thought to be an evolutionary correlate of rapid preadult development in *Drosophila* (Burnet *et al*. 1977; Borash *et al*. 2000). This notion is one facet of a broadly held view that adaptation to larval crowding and selection for faster development in *Drosophila* should yield similar evolutionary outcomes (Partridge and Fowler 1993; Borash *et al*. 2000; Krijger *et al*. 2001), a view shaped by the observation that (a) *Drosophila* larvae typically occupy ephemeral habitats, such as rotting fruits, in the wild, thought to result in selection for rapid development (Clarke *et al*. 1961), and (b) *Drosophila* larvae often face high densities and competition for limited food in these ephemeral habitats (Atkinson 1979; Nunney 1990).

This broad view was challenged by studies in our laboratory showing that the suites of traits that evolve under selection for adaptation to larval crowding versus selection for faster development in laboratory populations of *D. melanogaster* are not just different but opposite (Joshi *et al*. 2001; Prasad *et al*. 2001). Among our findings was the observation that populations selected for rapid development showed a substantially lower larval feeding rate than ancestral control populations after 65 generations of selection (Prasad *et al*. 2001). This observation, together with the earlier observed positive correlation of larval feeding rate with preadult competitive ability in *D. melanogaster* populations selected for adaptation to extreme larval crowding (Joshi and Mueller 1988, 1996), was a major reason for our prediction that larvae from our faster developing populations should be poorer competitors than those from control populations, based on differences in feeding rate (Joshi *et al*. 2001), a prediction that was subsequently verified experimentally (Shakarad *et al*. 2005).

If larval feeding rate and development time in *Drosophila* are positively correlated genetically, as the evolution of reduced feeding rate in our faster developing populations would suggest, then we need to seriously revise the commonly held view that competitive ability, adaptation to larval

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crowding and rapid development in *Drosophila* are fundamentally intertwined (Burnet *et al*. 1977; Partridge and Fowler 1993; Borash *et al*. 2000; Krijger *et al*. 2001). However, it is also possible that larval feeding rate is not directly correlated with development time in *Drosophila*, at least in typical cultures not consciously subject to directional selection for some life-history attribute. In the course of prolonged directional selection, genetic correlations between life-history-related traits in *Drosophila* have been seen to undergo even changes in sign (Archer *et al*. 2003; Chippindale *et al*. 2003). Over the course of 60 generations of selection, our faster developing populations underwent mean declines of 29.7 h (15%) in female development time and of 0.124 mg (35%) in female dry weight at eclosion (Prasad *et al*. 2000). However, a survivorship cost to rapid development became apparent only after 50 generations of selection, at which point the mean reduction in preadult survivorship of the faster developing populations was only about 14%. Thereafter, the mean reduction in preadult survivorship of the selected populations increased rapidly, becoming 17% in generation 60, and almost 22% in generation 70 of selection (Prasad *et al*. 2000). Together, these observations suggest that it is possible for *D. melanogaster* populations to undergo a marked reduction in body weight and development time without incurring a severe fitness cost for at least a few tens of generations. Thus, it may be that larval feeding rate in the faster developing populations became lower than in controls only at some point after the first 30–40 generations of selection, perhaps due to a general decline in vigour occurring as a correlated response to a large reduction in development time and/or body size.

Here, we report a study of larval feeding rates carried out on a new set of *D. melanogaster* populations during relatively early stages of selection (generations 10 and 32) for rapid development and extended lifespan. If larval feeding rates were seen to decline during the first 30 generations of selection for rapid development, it would clearly indicate that feeding rate and development time are positively genetically correlated, even in our control populations that have not consciously been subjected to directional selection for any life-history attribute over several hundred generations of laboratory rearing.

Materials and methods

Experimental populations

We used eight populations of *D. melanogaster*, of which four were controls ($JB_{1...4}$) and four subjected to selection for faster development and extended adult lifespan ($FLJ_{1...4}$). The control populations were the same as the controls in our earlier studies on selection for faster development and early reproduction (Prasad *et al*. 2000, 2001; Joshi *et al*. 2001; Shakarad *et al*. 2005), and are maintained on a 21-day discrete-generation cycle at 25°C, about 90% relative humidity and constant light, on banana–jaggery food medium. The egg density is regulated at about 60–80 eggs on about 6 ml of food per vial (9 cm height × 2.4 cm diameter). The number of breeding adults is about 1800 per population, and the adults are maintained in Plexiglas cages (25 cm × 20 cm × 15 cm) with abundant food.

The four FLJ populations are maintained in a manner similar to the JB populations, except that only the fastest developing and longest lived individuals contribute to the next generation. One hundred and sixty vials each containing 60–80 eggs on 6 ml food are collected per population and monitored closely for eclosion once the pupae start darkening. The first 15 or so flies that eclose in each vial are collected into Plexiglas cages with abundant food. The freshly eclosed adult population of about 2400 flies is subdivided randomly into two cages with about 1200 flies in each to avoid adult crowding. The population cages are then monitored for mortality till about 50% of the flies die. The surviving adults are given fresh food with a generous smear of live yeast acetic acid paste for about 60 hours. Typically, the breeding adult number is about 1000–1200 per population (~500–600 per cage). Eggs are collected from these flies on the third day after yeasting by placing fresh food plates into these cages for 1 h. The eggs from the twin cages of a given selection line (example FLJ_1) are intermixed so as to avoid independent evolution in the two cages. The eggs are then dispensed into 160 vials at a density of 60–80 eggs per vial. The selection intensity for faster development in FLJs is the same as that for FEJs used in the studies of Prasad *et al*. (2000, 2001). The FLJs differ from the FEJs only in the time of their reproduction, in that the FEJs were selected for reproduction on day 3 post-eclosion (early reproduction); while there is no fixed day for collecting eggs to start the next generation in case of FLJs it is usually at least 21 days post-eclosion. Each FLJ_i population was derived from a corresponding JB_i population. Thus, selected and control populations with names bearing identical numerical subscripts are genetically closer to each other than to other populations with which they share a selection regime (JB_i and FLJ_i are more closely related than JB_i and JB_j or FLJ_i and FLJ_j; $i, j = 1...4$). Consequently, control and selected populations with names bearing identical subscripts were treated as blocks in the statistical analysis.

To minimize nongenetic parental effects caused by different maintenance regimes, the selected and control populations were maintained under common rearing conditions for one complete generation prior to assaying for larval feeding rate. From running cultures of each selected and control population, 20 vials (10 each from the two population cages in case of FLJs) with about 60–80 eggs each were incubated at 25°C for 12 days. All adults that eclosed (hereafter, standardized flies) were transferred to individual population cages and given yeast enriched food for two full days before collecting eggs for assays. The progeny from standardized flies were used in the assays, which were done at generations 10 and 32 of FLJ selection.

Feeding rate assay

We measured larval feeding rate at physiologically matched larval ages for the selected and control populations following the procedure of Prasad *et al*. (2001). The physiological ages were matched by delaying egg collection from the FLJ standardized flies by the appropriate development time difference. Twentyfive newly hatched larvae were transferred to an agar plate containing 3 ml of 42.5% yeast suspension. Four such plates were set up for each population. The larvae were allowed to feed till they reached the early third instar and larval feeding rate was assayed at this stage. A total of 25 larvae from each population were assayed for feeding rate. Individual larvae were shifted to an assay plate of 5 cm diameter containing a thin layer of 10% yeast suspension on agar, and allowed 15 s for acclimation, and their feeding rate was measured as the mean number of sclerite retractions in two consecutive 1-min intervals.

Statistical analysis

Statistical analysis was implemented using STATISTICA™ for Windows Release 5.0B (StatSoft 1995). Mean larval feeding rate was subjected to mixed-model analysis of variance (ANOVA) in which the four ancestral lineages were treated as random blocks, crossed with selection regime and assay generation. As our objective was to assess differences between the selection regimes across replicated populations, we used population mean values as input data for the analysis.

Results

The mean feeding rate of FLJ larvae was significantly lower than that of the JB larvae, with the ANOVA showing significant effects of selection regime and assay generation, but no significant selection × generation interaction (table 1). The reduction was about 6.52% and 7.53% at generations 10 and 32 respectively (figure 1).

Discussion

Our results clearly show that a reduced larval feeding rate is a strong evolutionary correlate of faster development in *Drosophila*. The feeding rate difference of ~10 bites per minute between the FLJs and the JB controls was significant even after just 10 generations of selection (figure 1), a point at which mean reductions in female development time and dry weight at eclosion in the FLJs were only ~6 h (3%) and 0.023 mg (7%), respectively (M. Rajamani, N. Raghavendra, N. G. Prasad, S. Dey, A. Joshi and M. Shakarad, unpublished data), reductions similar in magnitude to those seen after 10 generations of FEJ selection (Prasad *et al*. 2000). The feeding rate difference between the FLJs and controls appears to have stabilized by 10 generations of selection, as it was also ~10 bites per minute in the generation 32 assay. The decline in larval feeding rate over the first 10 generations of FLJ selection shows that larval feeding rate and development time are positively genetically correlated in the control JBs that have not been under strong directional selection for either trait for several hundred generations. This observation, thus, rules out the possibility that the earlier noticed decline in larval feeding rate in the FEJs selected for faster development and early reproduction (Prasad *et al*. 2001) was an artefact of extreme directional selection for rapid development that led to changes in the correlational structure of development time, larval feeding rate, dry weight at eclosion, and preadult survivorship. A positive genetic correlation between larval feeding rate and development time in the control populations strongly suggests that rapid development is likely to be associated with a loss of competitive ability, at least in typical laboratory populations of *Drosophila*. The genetic architecture of traits like development time and larval feeding rate may, of course, be different in natural populations that presumably face a very different set of selection pressures than laboratory populations.

Table 1. Mixed-model ANOVA on mean larval feeding rate in the selected (FLJ) and control (JB) populations at generations 10 and 32 of FLJ selection. In this design, the main effects of block and interactions involving block cannot be tested for significance and have therefore been omitted for brevity.

Effect	d.f.	MS	*F*	*P*
Selection	1	379.08	90.09	0.0038
Generation	1	756.80	50.64	0.0120
Selection × generation	1	0.20	0.44	0.8446

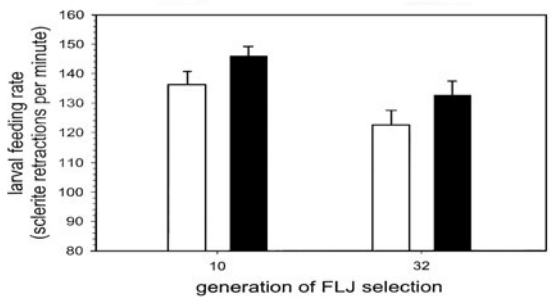

Figure 1. Mean (± s.e.) feeding rate measured as number of cephalopharyngeal sclerite retractions per minute of larvae from the four selected (FLJ: white bars) and four control (JB: black bars) populations at generations 10 and 32 of FLJ selection.

There was a decline in the absolute feeding rates of both selected and control populations from generation 10 assay to generation 32 assay. Such among-assay variation in absolute values of life-history traits is quite common as these traits are notoriously sensitive to even seemingly trivial environmental changes. Consequently, the important observable in such selection studies is the difference between selected and control population means, and its temporal dynamics.

The feeding rate reduction in the FLJ populations (~10 bites per minute) relative to controls was smaller in magnitude than that seen earlier in the FEJ populations (~30 bites

per minute) (Prasad *et al*. 2001), suggesting some constraint on the correlated evolution of feeding rate in the FLJs that was not present in the FEJs. Faster-feeding *Drosophila* larvae are known to assimilate greater lipid reserves than slower feeders (Borash and Ho 2001; Foley and Luckinbill 2001). Since fitness in the FLJs depends not only on developing fast but also on their living long as adults, it is possible that larval provisioning may be acting as a constraint on further reductions of feeding rate in the FLJs. However, there is no clear empirical evidence at this point as to what the constraint might be.

The symmetry of the relationship between feeding rate and development time in *Drosophila* is hard to assess, owing to paucity of studies in which larval feeding rate was the target of selection, and development time at moderate density was assayed. Burnet *et al*. (1977) found that populations selected for faster feeding had a shorter development time at high (competitive) density than those selected for slow feeding, a finding also supported by the observation that faster-feeding (Joshi and Mueller 1996) populations adapted to extreme larval crowding exhibit faster development than controls at high but not low densities (Borash and Ho 2001; but see also Bierbaum *et al*. 1989). Unfortunately, Burnet *et al*. (1977) did not measure development time of their faster-feeding and slower-feeding populations at low or moderate larval density. In another study in which larval feeding rate was the target of selection, development time was not assayed at all (Foley and Luckinbill 2001). Given that larval feeding rate is a polygenic trait with considerable dominance and epistasis (Burnet *et al*. 1977), it is certainly possible that the relationship between development time and larval feeding rate may be asymmetrical, with different patterns of correlated responses to selection on the two traits. However, this study clearly suggests that even small reductions in development time are accompanied by reduced larval feeding rate, making it very unlikely for faster development to confer increased competitive ability in laboratory populations of *D. melanogaster*.

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