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Faster development does not lead to correlated evolution of greater pre-adult competitive ability in *Drosophila melanogaster*

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In comparisons across *Drosophila* species, faster pre-adult development is phenotypically correlated with increased pre-adult competitive ability, suggesting that these two traits may also be evolutionary correlates of one another. However, correlations between traits within- and among-species can differ, and in most cases it is the within-species genetic correlations that are likely to act as constraints on adaptive evolution. Moreover, laboratory studies on *Drosophila melanogaster* have shown that the suite of traits that evolves in populations subjected to selection for faster development is the opposite of the traits that evolve in populations selected for increased pre-adult competitive ability. This observation led us to propose that, despite having a higher carrying capacity and a reduced minimum food requirement for completing development than controls, *D. melanogaster* populations subjected to selection for faster development should have lower competitive ability than controls owing to their reduced larval feeding rates and urea tolerance. Here, we describe results from pre-adult competition experiments that clearly show that the faster developing populations are substantially poorer competitors than controls when reared at high density in competition with a marked mutant strain. We briefly discuss these results in the context of different formulations of density-dependent selection theory.

Keywords: life-history evolution; development time; K-selection; α-selection; competition; *Drosophila melanogaster*

1. INTRODUCTION

Across *Drosophila* species, pre-adult development time is negatively correlated with competitive ability, with faster developing species being superior competitors (Krijger *et al.* 2001). In the wild, *Drosophila* larvae typically occupy ephemeral habitats, such as rotting fruit, which is thought to result in selection for rapid development (Clarke *et al.* 1961). Moreover, *Drosophila* larvae also often face high densities and competition for limited food in these ephemeral habitats (Atkinson 1979; Nunney 1990). These observations have shaped a view that adaptation to larval crowding and selection for faster development in *Drosophila* should yield similar evolutionary outcomes (Partridge & Fowler 1993; Borash, Teótonio *et al.* 2000; Krijger *et al.* 2001). The pattern of correlations between traits within- and among- species, however, is known to differ for many sets of traits (Schnebel & Grossfield 1988; Fischer *et al.* 2002; Sharmila Bharathi *et al.* 2003). Tight causal links between traits that can influence the trajectory of their joint evolution under a given selection regime will be manifested as genetic correlations among individuals within-species. Consequently, within-species correlations are more likely to act as constraints shaping adaptive evolutionary responses to selection than among-species correlations that represent a pattern resulting from the outcome of evolutionary changes within species. Traits that are evolutionary correlates of one another are, therefore, expected to exhibit within-species genetic correlations between them.

Unfortunately, studies examining the within-species relationship between development time and competitive ability in *Drosophila melanogaster* have yielded unclear results. Bakker (1969) assayed competitive ability in populations selected for fast and slow larval development, and found that the fast developing population was a better competitor than the slow developing one. However, the lack of population level replication and the finding of no difference in competitive ability between the fast developing population and the ancestral control population (Bakker 1969) suggest that the low competitive ability of the slow developing population may have been a consequence of drift and/or selection for poor fitness. In a complementary study, Roper *et al.* (1996) observed that populations maintained at higher larval densities evolved reduced development time, compared with controls. However, the high density used was actually rather moderate (150 larvae per vial) and the high density populations did not evolve greater competitive ability than controls (Roper *et al.* 1996), making it difficult to infer any correlation between competitive ability and development time.

Correlated responses to selection for faster development and for adaptation to larval crowding in *D. melanogaster* have been extensively studied (reviewed by Prasad & Joshi 2003). The evolution of faster development is accompanied by a reduction in body size and fractional lipid content, pre-adult survivorship, larval feeding rate, larval foraging path length, larval urea tolerance, pupation height and minimum food requirement for pupation, when

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assayed at moderate larval densities. Faster developing populations also appear to have greater carrying capacities than controls, possibly owing to their lower minimum food requirement. Adaptation to larval crowding, on the other hand, is accompanied by increased fractional lipid content, larval feeding rate, larval foraging path length, larval urea and ammonia tolerance, pupation height and minimum food requirement for pupation, whereas body size and pre-adult survivorship do not appear to increase in selected populations, at least at moderate assay densities (reviewed by Prasad & Joshi 2003).

The lower minimum food requirement and higher carrying capacity of faster developing populations is consistent with a prediction of higher competitive ability in these populations because these traits are correlates of fitness (per capita population growth rates) at high density, at least under the canonical formulation of density-dependent selection (MacArthur & Wilson 1967; Roughgarden 1971). Nevertheless, given the importance of larval feeding rate and urea/ammonia tolerance for competitive ability in *D. melanogaster* (Mueller 1997), we earlier predicted that our *D. melanogaster* populations would evolve reduced pre-adult competitive ability as a correlated response to selection for fast development, despite having a lower minimum food requirement and higher carrying capacity than their ancestral controls (Joshi *et al.* 2001). Here, we report results from an assay of pre-adult competitive ability in our fast developing and control populations.

2. MATERIALS AND METHODS

(a) *Experimental populations*

This study used eight laboratory populations of *D. melanogaster* previously described in detail by Prasad *et al.* (2001). Four of these populations (FEJ_{1-4}; faster development, early reproduction, JB derived, henceforth referred to as 'fast' populations) had been subjected to selection for faster pre-adult development and early reproduction for about 120 generations at the time of this study, whereas the other four populations (JB_{1-4}) were ancestral controls. Each FEJ population was derived from one JB population and therefore JB_i and FEJ_i were treated as random blocks in the analysis. At this time, the development time difference between the fast and control populations was about 42 h. The controls had been maintained in the laboratory for over 400 generations on a 21 day discrete generation cycle at large population size ($N \sim 1800$) and a moderate larval density of 60–80 larvae per vial containing about 6 ml of food medium. The fast populations were maintained in a manner similar to controls, except that only the first 20% of the flies that eclosed in each vial were to form the breeding population, and eggs were to be collected on the third day after eclosion. The number of breeding adults in the fast populations was about 1400.

(b) *Competition assay*

Eggs collected from fast and control population flies that had undergone one complete generation of common rearing without selection on development time were placed in vials containing 2 ml of food, at specific total densities (30 and 300 eggs per vial), either by themselves (monotypic culture), or with an equal number of eggs from a yellow body mutant strain used as a standard competitor (bitypic culture: 15 or 150 eggs each of control or fast and yellow body populations). For both monotypic and bitypic competition assays, five vials per population were set up at each of the two densities. Since the assays were staggered by block (FEJ_i, JB_i), a separate set of five vials from the yellow body strain was run with each block in the monotypic cultures. The number of adults eclosing in each vial, and their body colour phenotypes, were recorded and used to calculate pre-adult survival of individuals from fast and control populations in each vial at the two densities in both monotypic and bitypic cultures.

(c) *Statistical analyses*

All analyses were implemented using STATISTICA™ for Windows Release 5.0B (StatSoft Inc. 1995). Mean pre-adult survivorship in fast and control populations at the two densities in both monotypic and bitypic (having competition against yellow body flies) cultures was subjected to mixed model analysis of variance (ANOVA) in which block (ancestral lineage) was treated as a random factor crossed with selection regime, density and type of culture (monotypic or bitypic). As our objective was to assess differences between selection regimes, all analyses used population mean values as input data. Untransformed and arcsine square root transformed data yielded qualitatively similar results; hence, all results presented are for untransformed data. Multiple comparisons were restricted to a small subset decided upon *a priori*, and used Fisher's LSD test.

3. RESULTS

At a low density of 30 eggs per vial, mean pre-adult survivorship of the fast populations was significantly less than that of the controls, and mean survivorship did not differ between monotypic and bitypic cultures for either fast or control populations (figure 1). Overall, mean pre-adult survivorship was lower in the 300 eggs per vial cultures, but the fast or control populations were not differentially affected by the increased density, as evidenced by the non-significant selection × density interaction (table 1). At the higher density of 300 eggs per vial, however, mean pre-adult survivorship of the fast populations was significantly lower (by 38%) in bitypic cultures than that in monotypic cultures, whereas mean pre-adult survivorship of controls was significantly higher (by 19%) in

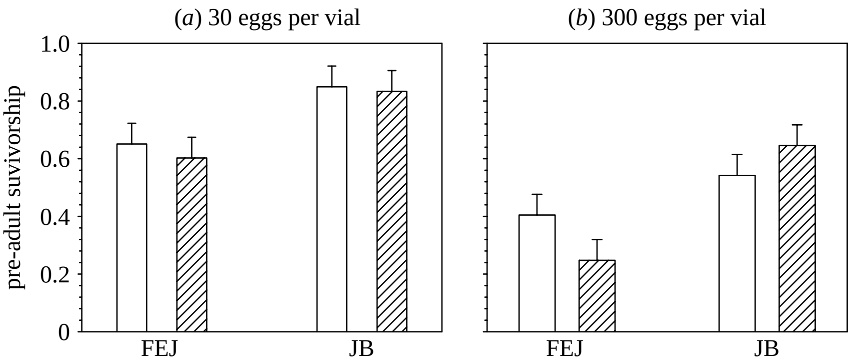

Figure 1. Mean pre-adult survivorship in the fast (FEJ) and control (JB) populations at two different densities, when reared by themselves (monotypic cultures: open bars), and in competition with a marked strain (bitypic cultures: hatched bars). Q1 Error bars are 95% confidence intervals based on the MS (block × selection × culture × density) term in the ANOVA.

Table 1. Mixed model ANOVA on mean pre-adult survivorship in the fast and control populations (selection) in monotypic and bitypic cultures (culture) at densities of 30 and 300 eggs per vial. (In this design, the main effect 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	0.465 452	90.09	0.0025
culture	1	0.006 942	2.35	0.2231
density	1	0.600 791	50.64	0.0057
selection × culture	1	0.042 876	80.75	0.0029
selection × density	1	0.005 671	0.44	0.5539
culture × density	1	0.000 055	0.01	0.9112
selection × culture × density	1	0.025 954	25.37	0.0151

bitypic rather than in monotypic cultures (figure 1), a pattern reflected in the significant selection × culture interaction in the ANOVA (table 1). A comparison with data from monotypic cultures at 300 eggs per vial confirmed that the mean pre-adult survivorship of yellow body flies in competition with fast populations was higher than when reared in monotypic culture by themselves, whereas when in competition with the controls, the yellow body flies had lower mean pre-adult survivorship than that in monotypic culture (data not shown).

4. DISCUSSION

The results show that the fast populations are substantially poorer competitors than the controls (figure 1). Moreover, the reduced competitive ability of the fast populations is not merely because their pre-adult survivorship is generally lower than controls at all densities. Were that the case, the survivorship of the fast populations at high density would not have been lower when cultured with yellow body individuals (the bitypic assay) than when cultured alone (the monotypic assay; figure 1). It was shown earlier that the fast populations have a lower minimum food requirement for pupation (Prasad *et al.* 2001) and a higher carrying capacity (Joshi *et al.* 2001) than the controls. These earlier observations, taken together with the present results, contradict predictions from canonical density-dependent selection theory (MacArthur & Wilson 1967; Roughgarden 1971), as well as the expectation that faster development confers a competitive advantage (Bakker 1969; Borash, Teótonio *et al.* 2000; Krijger *et al.* 2001). A closer examination of the reasons for this apparent contradiction underscores the subtlety of the evolutionary process and the need to be very circumspect in making broad generalizations about what kinds of trait may be expected to evolve under particular selection pressures.

Models of pure density-dependent selection (e.g. Roughgarden 1971) predict that higher carrying capacity is positively correlated with competitive ability and fitness at high density. Increased efficiency, in the sense of being able to sustain development on smaller amounts of food, is also predicted to lead to superior competitive ability (MacArthur & Wilson 1967; Bell 1997). Increased efficiency will, of course, also translate into a higher carrying capacity because the development of a larger number of individuals can be sustained on a given amount of food. These predictions are artefacts of the logistic formulation of density-dependent population growth rates, wherein the only way a genotype can be fitter than others at high density is for it to have a higher carrying capacity, K (Joshi *et al.* 2001). On the other hand, models incorporating arbitrary inter- and intra-genotypic competition coefficients (e.g. Asmussen 1983) suggest that density-dependent selection need not necessarily lead to increased carrying capacity or greater efficiency of conversion of food to biomass. We have previously shown that examining the results of studies on adaptations to crowding and on selection for faster development in *Drosophila* in the light of a broader conception of density-dependent selection that includes the notions of α-selection (selection for increased competitive ability *per se*, as opposed to selection for increased carrying capacity, K) and the effectiveness and tolerance components of competitive ability, leads to the prediction that faster developing populations will have lower competitive ability than controls, even though they possess some of the attributes of a K-selected species, such as greater efficiency, carrying capacity and population growth rates at high density, as compared with controls (Joshi *et al.* 2001). The present results clearly support this view.

Certainly, faster development may be expected to translate into increased competitive ability, all else being equal. However, all else is clearly not equal in these *Drosophila* populations. Most importantly, the fast populations have evolved lower larval feeding rates and urea tolerance than controls, apparently as part of a syndrome of reduced energy expenditure (Joshi *et al.* 2001; Prasad *et al.* 2001). Higher larval feeding rates are a strong correlate of competitive ability in *D. melanogaster*, owing to the necessity of ingesting sufficient food to sustain development before it runs out in a crowded culture (Joshi & Mueller 1996). In crowded cultures, levels of nitrogenous waste (especially ammonia) also rapidly build up to toxic levels (Borash *et al.* 1998). Consequently, tolerance to nitrogenous wastes is also seen to evolve in *D. melanogaster* populations maintained at high larval density (Borash *et al.* 1998). Thus, higher larval feeding rates and a greater tolerance to ammonia, which is genetically correlated with urea tolerance (Borash, Pierce *et al.* 2000), appear to be characteristic adaptations to larval crowding in *D. melanogaster*

(Prasad & Joshi 2003). The lower minimum food requirement of the fast populations is evidently insufficient to offset the reduction in competitive ability owing to lower larval feeding rate and urea tolerance, supporting the notion that, at least in organisms showing primarily scramble competition, the efficiency of food acquisition may be a far more important determinant of competitive ability than the efficiency of conversion of food to biomass (Joshi & Mueller 1996). Overall, our results suggest that, the among-species correlation between fast development and competitive ability notwithstanding, microevolutionary reductions in development time are unlikely to result in the correlated evolution of enhanced competitive ability in *Drosophila* species. This conclusion is strengthened by a recent observation that significant reductions in larval feeding rate are apparent even after just 10 generations of selection for faster development (M. Shakarad *et al.* unpublished data), suggesting that even moderate reductions in development time may not yield a benefit in terms of competitive ability. We stress, however, that faster development leading to reduced competitive ability as a correlated response to selection does not necessarily imply that fast development may not be a good indicator of competitive ability in interspecific *Drosophila* comparisons.

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