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Development and Competition in *Drosophila*: A Tale of Two Densities

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Since *Drosophila* larvae inhabit ephemeral habitats in the wild, faster development has been thought to have been under strong natural selection in wild populations, and adaptation to larval crowding and selection for faster development in *Drosophila* have often implicitly been assumed to have similar evolutionary outcomes. I compare results on direct and correlated responses to selection from two sets of studies on *D. melanogaster* in which selection was imposed for faster development at moderate densities, and for adaptations to high larval density, respectively. I show that the suites of traits that evolve under the two selection regimes are diametrically opposed. Populations adapted to larval crowding evolve increased population growth rates at high density, competitive ability, larval feeding rate, pupation height, larval tolerance to metabolic waste, foraging path length, minimum food required for pupation, lipid content and starvation resistance. When assayed at low larval densities, populations adapted to larval crowding do not differ from controls in egg to eclosion development time and survivorship, or in adult dry weight at eclosion. On the other hand, populations selected for faster development evolve reduced competitive ability, larval feeding rate, pupation height, larval tolerance to metabolic waste, foraging path length, minimum food required for pupation and fecundity. Moreover, unlike populations adapted to high larval density, these populations undergo a large reorganization of the temporal pattern of development, with reductions in the duration of the first and third larval instars, and of the pupal stage. Overall, these results clearly illustrate that the density at which selection occurs can greatly affect the evolution of life history traits. Selection for faster development imposed through food limitation at high density, and direct selection for faster development in moderate density, food rich conditions lead to the evolution of entirely different suites of traits. Hence, larval and adult densities need to be controlled when performing selection experiments, and some knowledge of density is required when speculating about possible selection pressures in wild populations.

Key Words: Adaptation; Density-dependent selection; Competition; Development time; Urea tolerance; Feeding rate; Minimum food requirement; Efficiency; Population growth; *Drosophila melanogaster.*

Introduction

Evolutionary biology, taken in the broadest sense, is today a vast field encompassing many different areas, and utilizing many different methodologies, and the use of selection in the laboratory on model species has proven very fruitful in evolutionary genetics studies wherein the major interest is in the dynamics of the evolutionary process, especially understanding how the force of selection and the genetic architecture of populations interact to give rise to evolutionary trajectories (Rose et al. 1996, Joshi 1997,2000, Mueller 1997). The evolutionary trajectory of a population is a resolution of the force of natural selection acting on it, the genetic structure of the population, its past selection history and ancestry, and chance in the form of genetic drift (Joshi 2000). In this context, the approach used in the studies I will be summarizing is to work with well characterized laboratory systems where one can simplify and control the selection pressures, allow for and quantify historical effects, and circumvent the problems of chance by working with replicated populations. Such studies, no doubt, do not address the issue of what really happens in any particular wild population. To understand the specific constellation of selective forces acting on a particular population would require a detailed study of the ecology of that population. What laboratory selection

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studies permit is a deeper understanding of the genetic architecture of fitness in a population, something that can rarely be obtained from field studies. Ideally, of course, a multi-pronged approach involving theory, laboratory work and field studies is the one that is likely to yield the deepest understanding of the evolutionary process.

Laboratory Studies with *Drosophila*

The fruit-fly *Drosophila melanogaster* is a good model system for studying questions in life-history evolution and has been extensively used in laboratory studies of the evolution of developmental rates, ageing, stress resistance, and temperature adaptation. It has also been used extensively for studies in population dynamics and race formation. It has a short life-cycle and can turn over a generation from egg to egg in about 10 days at 25°C. As most of our discussion is going to be about studies on *Drosophila*, I first briefly describe the life-cycle and relevant laboratory ecology of *D. melanogaster* at 25°C (for detailed review, see Mueller 1985, Mueller & Joshi 2000). Eggs hatch in about 18 hours and the larvae go through three instars over the next 4 days or so, at least in the case of populations typically maintained on a 2 or 3 week discrete generation cycle. If larvae are crowded into a fixed volume with a constant level of resource, survival and adult size and fecundity decrease with increasing larval density. Interestingly, adult size reaches its maximum value at food levels far above that needed for maximum survival. This is thought to be a reflection of the fact that a larva must reach a critical minimum size before it can successfully pupate, even though larvae typically pupate at sizes much larger than this minimum. The critical minimum size is typically attained early in the third instar, but larvae continue to feed beyond this point if food is not limiting. In crowded cultures, in addition to reduced food levels, larvae will also face increased levels of nitrogenous metabolic waste products that are toxic and reduce survival (Shiotsugu et al. 1997, Borash et al. 1998). Thus, the primary stresses placed on a *Drosophila* larva in a crowded culture are shortage of food and accumulation of nitrogenous waste, both of which tend to intensify with time.

After the larvae have completed growth, they form their pupal case, usually on the surface of the food or on the sides of the vials at some distance from the food surface, and the survival of a pupa may be affected by its location (Joshi & Mueller 1993). Under crowded conditions, the food is very soft and pupae close to the surface can get trampled and drowned by feeding larvae. The pupal stage also lasts about 4 days. Adult *Drosophila* do not grow much, as compared to larvae, and differences in size at eclosion can become permanent limitations on maximum female fecundity. Typically, adults will mate within about 15 hours of eclosion and females will lay fertile eggs shortly thereafter. Fecundity tends to be high during the early days of adult life and then declines, although the distribution of lifetime fecundity can be quite different in populations maintained on discrete versus overlapping generations. In cultures where adults are not segregated from growing larvae, larval density can also have indirect effects on female fecundity through increased levels of nitrogenous wastes (Aiken & Gibo 1979, Joshi et al. 1996,1998). There are also direct effects of presence of larvae on fecundity. Food medium with larvae at low densities is preferred as a substrate for oviposition by female *Drosophila*, compared to food without any larvae (Del Solar & Palomino 1966), whereas high larval densities inhibit fecundity (Chiang & Hodson 1950).

Confounding Faster Development and Adaptations to Crowding

Two of the important selection pressures operating on insects whose larvae inhabit ephemeral habitats in the wild are likely to be due to overcrowding and the necessity to complete pre-adult development relatively fast. Larval growth rates in *Drosophila* are thought to be partly shaped by a trade-off between faster development and adult size (Santos et al. 1988, Partridge & Fowler 1993), and this trade-off has been extensively studied. In the ecological and evolutionary literature on *Drosophila*, adaptation to larval crowding and selection for faster development in *Drosophila* have often implicitly been assumed to have similar evolutionary outcomes (Tantawy & El-Helw 1970, Wilkinson 1987, Santos et al. 1988, Prout & Barker 1989, Partridge & Fowler 1993, Borash et al. 2000). Since *Drosophila* larvae inhabit ephemeral habitats (such as rotting fruits) in the wild, faster

development has been thought to have been under strong natural selection in wild populations (Clarke et al. 1961, Robertson 1963, Partridge & Fowler 1992). However, adaptation to ephemeral habitats will typically involve a need to deal with high larval density in addition to the need to complete development fast (Nunney 1990), and perhaps this confounding of selection pressures in nature is partly responsible for shaping views of workers in the field. Of course, one of the major consequences of larval crowding in *Drosophila* cultures is that food runs out well before most individuals have attained the critical minimum size, thereby placing a heavy fitness premium on rapid development. Yet, as I will try to show by reviewing experimental work on the themes of crowding and fast development done on *Drosophila* populations over the past 14 years, the suites of traits that evolve in response to selection for fast development and for adaptation to crowding are actually strikingly different.

Adaptation to Crowding in *Drosophila*

Two sets of selection studies on laboratory populations of *D. melanogaster* carried out over the last 20 years or so have yielded considerable insight into the mechanisms by which populations evolve so as to be better adapted to high density conditions, as reflected in their ability to sustain a higher rate of population growth at high density, relative to control ancestral populations. Several recent reviews of these studies are available in the literature (Joshi 1997, Joshi & Mueller 1996, Mueller 1995,1997), and, consequently, I restrict myself to a brief summary of the traits seen to repeatedly evolve in *Drosophila* under density-dependent selection. In a *Drosophila* culture with very high larval density, selection tends to favour the ability to develop fast under crowded conditions, and also to be able to withstand fairly toxic levels of wastes such as ammonia and urea. Adaptive evolution in response to both these selection pressures seems to occur in *Drosophila* populations.

Compared to control populations maintained over generations under uncrowded conditions, populations subjected to many generations of crowding evolve higher carrying capacity, population growth rates at high densities (Mueller & Ayala 1981, Mueller et al. 1991), and competitive abilities when competed against a common marked strain (Mueller 1988). Other traits seen to evolve in the populations maintained at high density are higher larval feeding rate (Joshi & Mueller 1988,1996), pupation at relatively greater heights above the food medium (Mueller & Sweet 1986, Joshi & Mueller 1993, 1996), greater larval tolerance to toxic levels of urea and ammonia (Shiotsugu et al. 1997, Borash et al. 1998), greater foraging path length (Sokolowski et al. 1997) and higher minimum food requirement for pupation (Mueller 1990, Joshi & Mueller 1996). Although the crowding adapted populations have shorter egg to eclosion development time, and higher pre-adult survivorship than controls at high larval density, they do not differ from controls in development time, survivorship, or size at eclosion when assayed at low larval density (Santos et al. 1997).

Thus, it appears that *Drosophila* populations subjected to larval crowding for many generations evolve enhanced competitive ability primarily by becoming better at acquiring food fast and by being better able to withstand relatively high levels of metabolic waste, even though this ability comes at the cost of decreased efficiency at converting food to biomass (Mueller 1990, Joshi & Mueller 1996, Borash & Shimada, *unpubl. data*), perhaps partly offset by greater efficiency at assimilating lipids (Borash, *pers. comm.*).

Selection for Faster Development in *Drosophila*

Early attempts to select for faster development in *Drosophila* (Clarke at al. 1961, Robertson 1963) were not successful, further strengthening the view that developmental rates had already been optimized by selection for faster development in the wild. Later attempts to select for faster development in large outbred laboratory populations of *D. melanogaster* by choosing only the first 20% or so of pupating or eclosing individuals to breed each generation were quite successful at reducing development time by upto 16 hours or more in a few generations (Zwaan et al. 1995, Nunney 1996). In a longer term selection study, Chippindale et al (1997) achieved a 30 hour reduction in development time (~ 17%) over about 100 generations of selection. In all these studies, correlated decreases in adult size at eclosion were consistently observed, and Chippindale et al. (1997) also reported a survivorship cost to faster

development that became apparent only after about 50 generations of selection. The reduction of adult size as a correlated response to selection for faster development is consistent with other reports of selection for larger size in *D. melanogaster* yielding correlated increases in development time (Partridge & Fowler 1993, Partridge et al. 1999), and positive correlations observed between development time and adult size in *D. buzzatii* (Betran et al. 1998). In all these studies, however, larval behaviours, especially those related to food acquisition and utilization were not studied.

In ongoing selection studies in our laboratory, we have successfully selected for faster development in four populations of *Drosophila*, reporting an unprecedented 40 hour reduction in development time over the course of about 100 generations of selection (Prasad et al. 2000, 2001). In these populations, truncation selection for fast development is applied by choosing only the first 20% or so of eclosing individuals to breed each generation. We have confirmed previous reports of reduction in adult size and pre-adult survivorship as correlated responses to selection for rapid development (Prasad et al. 2000), and have also shown that the reduction in development time is restricted to the first and third larval instars and the pupal stage, whereas the reduction in pre-adult survivorship is entirely due to increased mortality during the larval phase (Prasad et al. 2001). Fluctuating asymmetry, often considered a measure of developmental instability, is not significantly different in the selected and control populations (Shakarad et al. 2001), suggesting that, contrary to a commonly held view, faster development need not necessarily lead to destabilization of the ontogenetic processes.

We have also studied the evolution of various traits relevant to competition, in an attempt to test the notion that faster development should result in enhanced competitive ability. We find that, in stark contrast to the pattern of traits that evolve in populations subjected to very high density, faster developing populations evolve reduced larval feeding rate, foraging path length, digging propensity, pupation height, larval growth rate, and minimum food requirement for pupation, relative to ancestral control populations (Prasad et al. 2000, 2001). Moreover, these populations have lower urea tolerance and early life fecundity than controls, but when reared in monotypic cultures can sustain higher growth rates at relatively high densities, suggesting an increased carrying capacity relative to controls (Joshi et al. 2001)

Conclusions

A comparison of results from density-dependent selection experiments and experiments where shorter development time was selected for, makes it clear that the evolutionary outcomes of these two types of selection regime are very different. Indeed, diametrically opposite traits evolve under the two kinds of regime (table 1). Selection for faster development and for adapting to larval crowding share some superficial similarity in that individuals failing to eclose before a certain point in time die, either because food runs out, or because the experimenter does not include them in the pool of breeding adults. However, there one fundamental aspect in which these selection regimes differ. At high larval densities there is a clear environmental signal, in the form of food running out, available to the larvae such that they

Table 1 *Patterns of correlated responses to selection seen in laboratory studies on Drosophila melanogaster in which either adaptation to crowding or faster development were selected for. Entries refer to the change seen in mean trait values across replicate selected populations, relative to ancestral control populations, over the course of multi-generation experiments*

Trait	Response in populations selected for adaptations to crowding	Response in populations selected for faster developmen
Development time at low density	No change	Decrease
Development time at high density	Decrease	Decrease
Pre-adult viability at low density	No change	Decrease
Pre-adult viability at high density	Increase	Decrease
Urea tolerance	Increase	Decrease
Minimum food requirement for successful pupation and eclosion	Increase	Decrease
Larval feeding rate	Increase	Decrease
Larval foraging path length	Increase	Decrease
Larval digging propensity	Increase	Decrease
Pupation height	Increase	Decrease
Larval competitive ability	Increase	Decrease
Adult size at eclosion	No change	Decrease
Adult weight at eclosion	No change	Decrease
Adult fecundity in early life	No change	Decrease

can make the switch from feeding to pupation. Thus, rather than speeding up development in real time, it is probably more important for larvae to acquire food faster than others, such that they attain the critical size for pupation before food runs out (Joshi & Mueller 1996). Under truncation selection for faster development, however, there is no external signal available to larvae indicating that they need to switch from feeding to pupation. In this context a speeding up of the developmental processes, such that an internal signal for pupation is triggered earlier in real time, is of crucial importance (Prasad et al. 2001).

Since competition is typically defined as a mutual inhibition of population growth rates by the two or more competing genotypes it is possible to think of two components of competitive ability: the ability to inhibit the other group (henceforth 'effectiveness') and the ability to withstand inhibition by the other group (henceforth 'tolerance') (Eggleston 1985, Joshi & Thompson 1995). We have also suggested that a view of density-dependent selection that explicitly recognizes that competition coefficients will often be the primary the targets of such selection (α-selection) allows us to clarify some of the confusion regarding evolution of developmental rates and of adaptations to crowding in *Drosophila* (Joshi et al. 2001). For example, a phenomenon like the evolution of faster feeding rate in *Drosophila* populations adapting to crowding cannot be incorporated into the classical formulation of density-dependent selection at all. Yet, this type of effect is exactly what is seen to happen in the *Drosophila* experiments: faster feeding, in itself, affects neither maximal growth rate or carrying capacity (in fact it increases the minimum food necessary for pupation, implying reduced efficiency). When in competition with relatively slower feeders, however, faster feeders have a clear competitive edge: they can greatly inhibit the population growth rate of slower feeders, and this inhibition is independent of their own sensitivity to their own density and therefore of fundamental growth rate parameters such as r and K.

If we examine the observed evolutionary responses to selection under crowding in *Drosophila* in the context of effectiveness and tolerance, faster feeding will result in increased competitive ability through increased inter- but not intra-genotypic effectiveness, whereas increased ability to withstand metabolic waste is likely to increase tolerance, both inter- and intra-genotypic. Moreover, increased efficiency reduces effectiveness while increasing tolerance and can, therefore, have a net negative effect on competitive ability, especially in situations where the genotype with greater efficiency of food utilization is competing against a genotype with greater efficiency of food acquisition. Exactly this sort of tradeoff has been experimentally observed in *Drosophila* populations adapted to crowding (Mueller 1990, Joshi & Mueller 1996). If we look at the traits that evolved in populations subjected to selection for faster development at low densities (Prasad *et al.* 2000, 2001), we find these populations to have reduced effectiveness due to slower feeding rates and lower adult weight and minimum food requirement for pupation, perhaps partly offset by increased tolerance due to the latter, as well as higher K due to the reduced adult size and minimum food requirement (Joshi et al. 2001). It, thus, becomes apparent that, contrary to many earlier expectations, selection for faster development at low density is unlikely to lead to the evolution of greater competitive ability; we have seen that, in fact, the faster developing populations are much poorer competitors than the control populations (Shakarad et al., *unpubl. ms.*).

Overall, the results summarized here clearly illustrate that the density at which selection occurs can greatly affect the evolution of life-history traits. Selection for faster development imposed through food limitation at high density, and direct selection for faster development in moderate density, food rich conditions lead to the evolution of entirely different suites of traits. Hence, larval and adult densities need to be controlled when performing selection experiments, and some knowledge of density is required when speculating about possible selection pressures in wild populations. I hope that this review also serves to highlight the power and rigour that well replicated laboratory selection experiments can bring to bear on questions in evolutionary genetics.

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