REVIEW ARTICLE

What have two decades of laboratory life-history evolution studies on *Drosophila melanogaster* taught us?

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Abstract

A series of laboratory selection experiments on *Drosophila melanogaster* over the past two decades has provided insights into the specifics of life-history tradeoffs in the species and greatly refined our understanding of how ecology and genetics interact in life-history evolution. Much of what has been learnt from these studies about the subtlety of the microevolutionary process also has significant implications for experimental design and inference in organismal biology beyond life-history evolution, as well as for studies of evolution in the wild. Here we review work on the ecology and evolution of life-histories in laboratory populations of *D. melanogaster*, emphasizing how environmental effects on life-history-related traits can influence evolutionary change. We discuss life-history tradeoffs—many unexpected—revealed by selection experiments, and also highlight recent work that underscores the importance to life-history evolution of cross-generation and cross-life-stage effects and interactions, sexual antagonism and sexual dimorphism, population dynamics, and the possible role of biological clocks in timing life-history events. Finally, we discuss some of the limitations of typical selection experiments, and how these limitations might be transcended in the future by a combination of more elaborate and realistic selection experiments, developmental evolutionary biology, and the emerging discipline of phenomics.

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Introduction

Ze baad aamadi, raft khwaahi be gard
Che daani ke ba tu che khwaahand kard

(As you came from the breeze, into dust you will go,
What occurs in between, you will strive hard to know!)

(Ferdowsi)

Between the discrete events of birth and death lies the life-history of an organism—the schedule of reproduction and mortality over its lifetime (Roff 1992; Stearns 1992). It is the life-history that constitutes the interface between a phenotype and its Darwinian fitness (Charlesworth 1994), and the life-history itself results from the interaction of the evolutionary history, functional biology and genetics of the organism (Rose 1983; Partridge and Sibly 1991; Reznick and Travis 1996; Rose and Bradley 1998). Various adaptive facets of the phenotype must be filtered through the life-history before being encashed in the currency of fitness, and this is why life-history evolution is central to evolutionary biology. Studying life-history evolution requires understanding how various morphological, behavioural and physiological traits give rise to a particular schedule of survival and reproduction in a given ecological scenario, as well as how these traits are genetically correlated and respond to the selection pressures placed on them by a particular ecology. This is clearly not a trivial task, and most empirical studies of life-history evolution in different organisms have tended to focus on the life-history itself, and how it varies across environments, rather than studying how life-histories

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actually evolved, or going into the details of the underlying physiology or genetics.

Laboratory cultures of *Drosophila melanogaster* constitute a powerful model system that has been and continues to be extensively used to study life-history evolution empirically. The strength of the *D. melanogaster* system lies in the ability of experimenters to manipulate the laboratory ecology and probe its effects on life-histories through phenotypic manipulation experiments, to do long-term selection experiments, and follow them up with behavioural, physiological and genetic studies of the mechanisms underlying evolved changes in the life-history (Partridge and Barton 1993a; Rose *et al*. 1996; Joshi 1997; Mueller 1997; Gibbs 1999; Zwaan 1999). Much of this work has centred around life-history tradeoffs, some of which have proven to be fairly robust across studies, whereas other combinations of traits trade off in some studies but not in others (Harshman and Hoffmann 2000; Ackermann *et al*. 2001).

Recently, concerns have been raised about the possibility that patterns of correlated responses to selection seen in laboratory populations of *Drosophila* are artifacts of unnatural laboratory environments (Harshman and Hoffmann 2000; Sgrò and Partridge 2000; Hoffmann *et al*. 2001b; Linnen *et al*. 2001). On the other hand, much of our understanding of exactly how environment and selection can interact to produce specific patterns of correlated responses to selection has been made possible by laboratory experiments in which experimenters can control a simplified environment (Roper *et al*. 1993; Joshi and Mueller 1996; Rose *et al*. 1996; Harshman and Hoffmann 2000; Joshi *et al*. 2001; Prasad *et al*. 2001). We believe that more creative laboratory experiments are likely to yield a better understanding of *Drosophila* life-history evolution than attempts to shift the focus to studying only wild populations: we shall return to this theme towards the end.

Overall, the work done on life-history evolution in *D. melanogaster* over the past twenty years or so warrants taking stock of current understanding and critically examining experimental results, with an intent to stitch together disparate observations into a meaningful, though necessarily incomplete, tapestry. Here we review the important features of the life-cycle and laboratory ecology of *D. melanogaster*, and then discuss life-history tradeoffs, at least within the context of the typical laboratory environment. We also discuss maternal effects, sexual dimorphism, and the possible role of biological clocks in timing life-history events, issues that we feel are likely to be important in studies of life-history evolution in *Drosophila* in the future. There are many lessons to be learnt from the past two decades of selection experiments in *D. melanogaster*, even for those not working with this species, or on life-history evolution, and we shall briefly dwell upon how our experience with *D. melanogaster* life-history evolution studies can help point out some of the pitfalls of experimental biology that are all too often not taken seriously enough. We conclude by discussing the need for, and possible experimental approaches to, a better integration of the information we get from selection experiments and phenotypic manipulations with some understanding of the ontogeny of the genotype × genotype (G × G) and genotype × environment (G × E) interactions that, in our view, ultimately shape the genetic architecture and phenotypic expression of the life-history.

A model microcosm: life-cycle and laboratory ecology of *D. melanogaster*

Guftand jahaan-e-ma aaya be tu mi saazad
Guftam ke nami saazad, guftand ke barham zan

(God asked me if I did find his universe to my liking and taste
When I said no, then he replied, create your own and lay this waste)

(Sheikh Mohammad Iqbal)

While poets and mathematicians have the luxury of creating alternative universes, biologists must make do with model systems. Among the species used as model systems in biological research, *Drosophila melanogaster* occupies pride of place for studies in areas ranging from molecular development to community dynamics. Not surprisingly, then, *D. melanogaster* has also been the major model system used to study life-history evolution experimentally (Rose *et al*. 1996). In this section, we discuss key aspects of the life-cycle and ecology of *D. melanogaster* in typical laboratory cultures, especially focussing on issues relevant to life-history evolution. Nutritional requirements, density-dependent regulatory mechanisms and population dynamics of *D. melanogaster* cultures have been discussed in detail elsewhere (Robertson and Sang 1944; Chiang and Hodson 1950; Sang 1950, 1956; Mueller 1988a; Mueller *et al*. 1991; Mueller and Huynh 1994; Mueller and Joshi 2000), and we will not spend time on these issues here.

D. melanogaster undergoes complete metamorphosis, and can cycle from egg to egg in about ten days on nutritious food medium at about 25°C. Eggs typically hatch 18–24 h after laying, although if oviposition substrates are not available females can retain eggs, which are then laid at a more advanced developmental stage and hatch in a shorter time after laying. The larvae are the major feeding life-stage, and pass through three instars in about four days. The pupal duration is another four days, and adults live about 35–40 days, although adult lifespan varies considerably among individuals. Typically, females can begin to lay eggs within 24–48 h of eclosion, although peak fecundity is usually not attained for a couple of days after commencement of egg laying.

Preadult stages

The larval stage is important to the life-history because the size of the adults is largely fixed by the size at which third instar larvae undergo pupariation, although increases in dry body weight do occur after eclosion, especially in females. The first two larval instars are about 24 h each in duration whereas the third instar lasts about 48 h (Bakker 1959). Very early in the third instar, the larvae attain a critical developmental stage marked by a small ecdysone pulse, and a commitment to metamorphosis is made at this point (Berreur *et al*. 1979). The attainment of this critical developmental stage of 'no return' appears to be correlated with the attainment of a certain critical size/weight in many insects (de Moed *et al*. 1999; Davidowitz *et al*. 2003), including *D. melanogaster*, in which the critical size is about half of the final size of a well-fed larva prior to pupation (Bakker 1959; Robertson 1963). In *D. melanogaster*, it is difficult to alter the duration from the attainment of the critical size/weight till pupariation by changing the nutritional environment, whereas the time taken from hatching to attainment of the critical size/weight is markedly sensitive to nutritional levels and can be lengthened greatly by feeding larvae a suboptimal diet (Bakker 1959; Robertson 1963). Late in the third instar, a large ecdysone pulse sets the stage for pupariation, which occurs about 5 h after the pulse; another ecdysone pulse about 10 h after pupariation finally sets into motion a cascade of events leading to pupa formation (White *et al*. 1997, 1999). Studies on lepidopterans indicate that the timing of the prepupariation hormonal pulse is determined by the clearing of juvenile hormone from the haemolymph, and further subjected to circadian gating, yielding a circadian rhythm in pupariation (Davidowitz *et al*. 2003), but it is not clear if this is so in *D. melanogaster*, although pupariation seems to be rhythmic in at least some *Drosophila* species (Bakker and Nelissen 1963; Pittendrigh and Skopik 1970).

The time course of larval growth in *D. melanogaster* is a roughly S-shaped curve, with rapid increase in the rate of weight gain (henceforth, larval growth rate) during the mid-to-late second instar and the early third instar, before a levelling off late in the third instar (Bakker 1959; Partridge *et al*. 1994a; Santos *et al*. 1997; de Moed *et al*. 1999). The rate of larval cephalopharyngeal sclerite retraction (henceforth, larval feeding rate) increases rapidly during the first instar and then the rate of increase lessens, and the feeding rate finally levels off in early-to-middle third instar, and declines towards the end of the third instar (Santos *et al*. 1997; N. G. Prasad, M. Shakarad and A. Joshi, unpublished data). The larval feeding rate is also lower around the time of moults. Dry weight reaches its maximum about 84 h after hatching, 10–12 h after the attainment of the wet-weight maximum (Bakker 1959; Santos *et al*. 1997). Once larvae have stopped feeding, the weight tends to drop through the 'wandering' larval phase, and over the pupal phase, such that the size of eclosing adults is less than the maximum weight attained by the late third instar larva (Bakker 1959; Santos *et al*. 1997). Exponential or power functions fit typical data on *D. melanogaster* larval weight gain up till the attainment of maximal weight very well, and thereafter weight declines almost linearly (Bakker 1959; A. Joshi, unpublished data). Female larvae have a greater growth rate than males, and are heavier at the time of pupariation (Partridge *et al*. 1994a, 1999b), and females eventually eclose as larger and heavier adults than males. When third instar larvae are removed from food at different points in time and then starved, however, a clear weight difference between eclosing males and females is seen only when larvae are allowed to feed for about 70 h or more (Santos *et al*. 1997), which is consistent with the observation that the male–female difference in larval weight becomes apparent only in mid-third instar (Partridge *et al*. 1994a). The duration of the larval stage does not differ between the sexes, but pupal duration in males is about 6 h more than in females (Bakker and Nelissen 1963; Nunney 1983), and it is speculated that the longer male pupal duration is due to some aspects of sperm maturation (Nunney 1996).

The division of the larval stage into precritical and postcritical size phases has important implications for the relationship between larval development time and adult size at eclosion, both of which are important life-history characters (Bakker 1959; Robertson 1960, 1963; van der Have and de Jong 1996; de Moed *et al*. 1999). It is useful to consider the larval stage as consisting of two distinct processes occurring over time: growth (increase in biomass) and development (a complex series of steps involving hormone-mediated changes in gene expression patterns leading to the differentiation of cell types). A simple model of the temperature dependence of growth and developmental rates has been shown to yield results consistent with observed reaction norms for size at eclosion versus temperature in *D. melanogaster* (van der Have and de Jong 1996). Indeed, if growth and developmental rates are at least partly under independent genetic control, then a whole variety of correlated responses to selection for body size or development time would be possible. For example, body size could, in principle, be altered either by changing critical size, thereby leading to a change in development time, or by altering the growth rate in the postcritical size period, which would not alter the development time (Robertson 1963). Whether critical size or growth rate is affected more by selection on development time or body size has been shown to depend partly on the nutritional environment (Robertson 1963).

Adult stage

Like in many other insects, adult eclosion from the pupa in *D. melanogaster* is under control of a circadian clock (Pittendrigh and Skopik 1970; Qiu and Hardin 1996;

Sheeba *et al*. 1999a), and in most wild-type strains peak eclosion occurs shortly after the dark-to-light transition. After eclosion, males and females can begin mating in 8–10 h, although mean time to first mating is usually between 12 and 20 h post-eclosion. Females typically start laying within 1–2 days after eclosion. Mating (Sakai and Ishida 2001; Tauber *et al*. 2003), vitellogenesis (Allemand 1976) and oviposition (McCabe and Birley 1998; Sheeba *et al*. 2001) all exhibit circadian rhythms in *D. melanogaster*, with the typical pattern of phasing being peak mating activity around the dark-to-light transition and a subsidiary peak around the light-to-dark transition (Partridge *et al*. 1987c; Sakai and Ishida 2001), and peak oviposition shortly after the light-to-dark transition (Allemand 1976; Sheeba *et al*. 2001). There is also some evidence that sperm production or release from the testes may be under circadian control (Beaver *et al*. 2002). Multiple mating by females is common, and there is evidence of sperm competition (Civetta 1999; Price *et al*. 1999) mediated by accessory fluids (Xue and Noll 2000) as well as female genotype (Clark *et al*. 1999). Female fecundity is greatly affected by nutritional status with the difference in daily fecundity during peak egg laying between poorly fed and well-fed females spanning about an order of magnitude (Chippindale *et al*. 1993). Females starved after eclosion can lay eggs for about six days by utilizing resource reserves built up during the larval stage, but then need to replenish resources by feeding in order to continue laying eggs (Robertson and Sang 1944).

The typical time course of fecundity in *D. melanogaster* females, especially from wild-caught populations or those maintained in the laboratory with overlapping generations, is a rapid increase in daily fecundity over the first 2–4 days after eclosion, a plateau at maximal fecundity lasting for 2–20 days, and a gradual decline thereafter (Robertson and Sang 1944; Rose 1984; Novoseltsev *et al*. 2002). However, populations maintained on a three-week discrete generation cycle for a few hundred generations appear to evolve a subsidiary peak in daily fecundity 10–12 days after eclosion, which corresponds to the period of egg collection for initiating the next generation (Sheeba *et al*. 2000; M. Shakarad, N. G. Prasad and A. Joshi, unpublished data). These populations, however, do retain the first major peak of daily fecundity 2–4 days after eclosion, even though fecundity at that early age does not contribute to fitness in a three-week discrete generation culture.

Typical adult lifespan of *D. melanogaster* kept on a two-week or three-week discrete generation cycle in the laboratory is on the order of 30–40 days for females, with males usually living about 5–10 days longer than females, although the lifespan of individuals varies considerably, ranging between 10 and 80 days (Rose 1984; Rose *et al*. 1992; Chippindale *et al*. 1993; Joshi *et al*. 1996b; Partridge *et al*. 1999a). Virgin males and females tend to live 10–20 days longer than their reproducing counterparts, supporting the notion of a tradeoff between lifespan and reproductive activity (Partridge *et al*. 1986; Partridge and Fowler 1992; Sheeba *et al*. 2000), although virgin females do lay eggs and their lifetime egg production can often be at par with that of mated females (Partridge *et al*. 1986). Reproduction and lifespan trade off in *D. melanogaster* (Luckinbill *et al*. 1984; Rose 1984, 1989; Chippindale *et al*. 1993; Partridge *et al*. 1999a), in part owing to the necessity of allocating energy reserves to either egg production or somatic maintenance (Service *et al*. 1985; Service 1987; Chippindale *et al*. 1993; Simmons and Bradley 1997), although the energetic tradeoff is not quantitatively exact (Rose and Bradley 1998). The allocation of reserves to reproduction versus somatic maintenance appears to be mediated by lipid level, with a low lipid content triggering off an increased relative allocation to somatic maintenance (Leroi *et al*. 1994c), and there is now evidence suggesting that this resource allocation tradeoff is mediated by the response to nutritional levels of a signalling pathway involving insulin-like growth factor (Partridge and Gems 2002). In addition to the cost of producing eggs, the cost of reproduction in female *D. melanogaster* includes a fitness cost due to mating (Partridge *et al*. 1987b) that is attributable to accessory gland proteins in the male ejaculate (Chapman *et al*. 1995; Chapman 2001), as well as a cost of exposure to males without mating (Partridge and Fowler 1991). Males also incur costs of courtship (Cordts and Partridge 1996) and reproduction in terms of lifespan (Partridge and Andrews 1985), and lipid reserves as reflected in starvation resistance (Chippindale *et al*. 1997b). The cost of mating in terms of elevated mortality rates, however, appears to be transient in both males and females (Partridge and Andrews 1985; Sgrò and Partridge 1999).

Other than fecundity and lifespan, two adult traits that have been extensively studied in *D. melanogaster* are starvation and desiccation resistance. This is partly because they are correlated with lipid and glycogen content, respectively, and are thus related to the survival–reproduction tradeoff, being indirect measures of the resource reserves of individual flies (Service *et al*. 1985; Service 1987; Hoffmann and Parsons 1989; Leroi *et al*. 1994c; Chippindale *et al*. 1996, 1997b, 1998; Harshman *et al*. 1999). It is also thought that starvation and desiccation are major sources of mortality in wild populations of *Drosophila*, and thus the study of these traits has also been undertaken in the context of understanding the role of environmental stress resistance in ecological adaptation (David *et al*. 1983; Gibbs *et al*. 1997; Hoffmann and Harshman 1999; Hoffmann *et al*. 2001a). Over adult life, female weight increases a little in the early days after eclosion, although the rate of early adult life weight gain can be altered by certain selection regimes (Joshi *et al*.

1998a), especially those placing importance on accumulation of resources for the long term, such as selection for late-life fecundity and elongated lifespan (Chippindale *et al*. 1994; Djawdan *et al*. 1996; M. Shakarad, N. G. Prasad and A. Joshi, unpublished manuscript). Males typically lose weight for a few days after eclosion and their weight then stabilizes (Djawdan *et al*. 1996). In males, lipid content (Djawdan *et al*. 1996), starvation resistance (Chippindale *et al*. 1994, 1997b; but see also Service *et al*. 1985) and desiccation resistance (Service *et al*. 1985; Chippindale *et al*. 1998) tend to decrease after eclosion, although carbohydrate content increases for a few days after eclosion and then decreases to a lower level (Djawdan *et al*. 1996; Chippindale *et al*. 1998). The pattern of changes in carbohydrate content and desiccation resistance with age in females is similar to that in males (Service *et al*. 1985; Djawdan *et al*. 1996; Chippindale *et al*. 1998). Lipid content in females, however, tends to increase for about 10–20 days after eclosion (Djawdan *et al*. 1996; M. Shakarad, N. G. Prasad and A. Joshi, unpublished data), but starvation resistance dips in the first 3–4 days after eclosion (Chippindale *et al*. 1994, 1997b) and then increases (M. Shakarad, N. G. Prasad and A. Joshi, unpublished data), reaching a plateau at about 20 days of adult age (Service *et al*. 1985). The dip in starvation resistance at 3–4 days post-eclosion, and its subsequent rise, despite a continuous increase of lipid content in the first 10–20 days after eclosion, is likely to be due to the fecundity peak around 3–4 days of adult age resulting in a major investment of lipid into egg production.

Environmental effects

It must be stressed that the life-cycle outlined above is for typical 2–3-week discrete generation *D. melanogaster* cultures, raised on rich food at about 25°C, under either constant light (LL) or a light : dark (LD) cycle reasonably close to 12 : 12 h. Most aspects of the *D. melanogaster* life-cycle are affected phenotypically by environmental factors such as nutrition (Sang 1950, 1956; Robertson 1960), larval density (Joshi 1997; Mueller 1997), temperature (David *et al*. 1983; de Moed *et al*. 1998, 1999; Partridge *et al*. 1994a) and the light : dark regime (Sheeba 2002). More to the point, some of these environmental variables can differentially amplify the phenotypic expression of genetic variation (Luckinbill and Clare 1986; Hoffmann and Merilä 1999; Imasheva *et al*. 1999; Hoffmann *et al*. 2003), which then opens up the possibility of varying patterns of direct and correlated responses to selection on the same life-history traits in slightly different environments (Robertson 1963; Graves and Mueller 1993; Pérez and Garcia 2002). For example, adult body size in *Drosophila*, which is largely fixed by larval size at the time of pupariation, is often seen to be positively correlated with both male (Partridge *et al*. 1987a; Bangham *et al*. 2002 and references therein) and female (Mueller 1985; Zwaan *et al*. 1995a; Houle and Rowe 2003) reproductive success; indeed it has been suggested that the evolution of body size in *Drosophila* is constrained by a tradeoff between adult reproductive fitness and the fitness costs of increasing larval growth rates (Partridge and Fowler 1993). Yet, the correlations between female size and fecundity, size and ovariole number, and ovariole number and fecundity in *D. melanogaster* are known to be affected strongly by G × E interactions, when the environmental variable is nutrition (Robertson 1957a,b). Similar G × E interactions involving temperature have been observed for the relationship between female size and fecundity (McCabe and Partridge 1997), whereas wing length is affected by a larval density × genotype interaction (Wilkinson *et al*. 1987). In *Drosophila* males too, the association between body size and mating success depends critically both on the causes of the size variation (e.g. temperature, density or nutrition) and on the genetic composition of the population (Santos *et al*. 1994; Zamudio *et al*. 1995; Santos 1996; Joshi *et al*. 1999; da Silva and Valente 2001). It appears that under moderate density and rich nutrition conditions in the laboratory, the size variation among flies is not necessarily correlated with fitness, even though the greater size variation seen in wild populations and higher density cultures, or large and small flies from different selection regimes, does seem to yield a positive correlation between male and female size and fitness (Joshi *et al*. 1999).

Temperature and density both have major phenotypic effects on life-history traits in *D. melanogaster*. Compared to individuals reared at the standard temperature of about 25°C, rearing at a colder temperature (16–17°C) results in increased egg size (Azevedo *et al*. 1996), as well as increased larval and pupal duration, mature larval size, and adult size at eclosion; larger wing size in cold-reared flies is due to increased cell size, rather then number (French *et al*. 1998, and references therein). There is some evidence suggesting that cold-reared larvae have an increased critical weight (de Moed *et al*. 1999) and reduced efficiency of conversion of food to biomass (Neat *et al*. 1995), but there are also some observations contradicting these findings, albeit with different sets of flies (Partridge *et al*. 1994a; Robinson and Partridge 2001). In adults, rearing at colder temperature increases lifespan, as well as lifetime fecundity and progeny production, but reduces daily fecundity (Partridge *et al*. 1995).

Increased larval crowding in laboratory cultures results in a decrease in food available over time, and an increase in metabolic waste levels, especially ammonia (Borash *et al*. 1998). The major phenotypic effects of rearing larvae at high (several hundred per vial) versus moderate (50–100 per vial) density are increased larval and pupal mortality, larval development time, pupation height and

adult lifespan, as well as reduced adult size and, therefore, fecundity (Joshi 1997; Mueller 1997; Mueller and Joshi 2000; Borash and Ho 2001). The higher pupal mortality in crowded cultures is likely to be a consequence of higher metabolic waste levels (Shiotsugu *et al*. 1997). The effects of high larval density on fecundity and lifespan are likely to be compounded by the effects of food deprivation, which, within limits, tends to increase lifespan while decreasing fecundity (Chippindale *et al*. 1993), and exposure to metabolic waste as larvae, which tends to decrease both lifespan and fecundity of adults (Shiotsugu *et al*. 1997). In a study of 15 laboratory populations of *D. melanogaster*, dry weight, total lipid content, and starvation resistance at eclosion were all seen to be reduced as a consequence of high larval density, even though fractional lipid content was higher in flies reared as larvae at high density (Borash and Ho 2001; but see also Zwaan *et al*. 1991). An inspection of the data in Borash and Ho (2001) suggests that starvation resistance per unit mass of lipid was also higher in the flies reared at high larval density, a result consistent with the notion that low total lipid content influences greater allocation of reserves towards somatic maintenance rather than reproduction.

The effects of adult crowding on the life-history of *D. melanogaster* have not been as extensively studied as those of larval crowding. A few days of crowding early in adult life can reduce subsequent fecundity (Joshi *et al*. 1998a) and lifespan (Graves and Mueller 1993; Joshi and Mueller 1997) even without continued crowding. Female lifespan is reduced more than that of males following an early-life episode of adult crowding (Joshi and Mueller 1997). Fecundity is also markedly reduced by adult density at the time of assay, and this effect can be reduced but not altogether eliminated by supplying the females with yeast (Mueller and Joshi 2000; Mueller *et al*. 2000). Episodes of adult crowding result in increased mortality during the period of high adult density (Joshi *et al*. 1998a). In overlapping generation cultures, the presence of larvae can reduce fecundity directly (Aiken and Gibo 1979), as well as indirectly through the buildup of metabolic wastes, with the latter effect causing a concomitant increase in adult lifespan (Joshi *et al*. 1996b, 1998b).

The observation of heterogeneity among experiments in correlated responses to selection on life-history traits in *D. melanogaster* has been the focus of considerable discussion (Roper *et al*. 1993; Chippindale *et al*. 1994; Partridge *et al*. 1999b; Harshman and Hoffmann 2000; Ackermann *et al*. 2001). Most of the inconsistent results among experiments have been related to selection for starvation or desiccation resistance, or selection for late-life fecundity and elongated lifespan (reviewed by Harshman and Hoffmann 2000). Some of the inconsistencies can be explained by inadvertent selection for traits later assayed as correlated responses to selection, especially in studies where larval density was not explicitly regulated (Roper *et al*. 1993). For example, females from early-reproduced lines are typically more fecund early in life than those from late-reproduced ones, and if egg laying is over a fixed time window in both types of line, early-reproduced lines will experience higher larval densities than late-reproduced ones, unless egg density is explicitly regulated. The higher larval density, in turn, is expected to lengthen development time and, consequently, early-reproduced lines may be subjected to inadvertent selection for faster development (Roper *et al*. 1993). Even in the absence of such a density-mediated effect, lines reproduced on a 14-day discrete generation cycle are typically under selection for rapid development, as evidenced by a rapid increase of development time in 14-day populations shifted to an 18-day rearing cycle (Chippindale *et al*. 1997a).

The point we would like to stress is that environmental differences between experiments that impinge upon the growth rates of larvae are likely to exacerbate the problem of inadvertent selection in *Drosophila* life-history experiments, especially because growth rate during the precritical and postcritical stages of larvae affects the size and the lipid and carbohydrate reserves of the eclosed adult. While the importance of explicitly regulating larval density has at least been recognized (Roper *et al*. 1993), if not always followed, there are many other aspects of this problem that have perhaps not received the attention they merit. Even if density is controlled, larval density and food media may both vary among laboratories, and this can have important and unpredictable effects on growth rates, especially since density and food are likely to interact in their effect on growth rate. Even more neglected is the issue of light : dark regime. Laboratory studies with *D. melanogaster* have been variously conducted under LL, LD 12 : 12 h, LD 16 : 8 h, and sometimes even under fluctuating LD regimes wherein the timing of lights on and lights off is a function of when people enter or leave the laboratory. Often, *Drosophila* life-history evolution studies do not even mention the light : dark regime used! Light regime affects most life-history traits in *D. melanogaster*, including preadult development time (Sheeba *et al*. 1999b) and, possibly, larval growth rate (Sheeba 2002). Development time in LL is shorter than in LD 12 : 12, although flies in both light regimes eclose at the same sex-specific dry weight (Sheeba 2002). Fecundity in the first few days of life and in mid-life (20–30 days post eclosion) is also higher in flies kept in LL rather than LD 12 : 12 (Sheeba *et al*. 2000; Sheeba 2002). It is worth noting in this context that many of the inconsistencies in observed correlated responses to selection on age-specific fecundity and lifespan are between laboratories using LL and LD 12 : 12, respectively, as the rearing light regime. If nothing else, the lengthening of development time under LD 12 : 12 is likely to have strengthened the inadvertent selection for faster development in the

early-reproducing lines discussed by Roper *et al*. (1993). There may well be other ways in which light regime interacts with selection in mediating correlated responses of which we are yet unaware, and this is an area that, in our opinion, deserves closer attention than it has hitherto received.

Tradeoffs in *D. melanogaster* life-history evolution

Yaaron baaham gunthe hue hain kaayanaat ke bikhre tukde
Ek phool ko jumbish doge to ik taara kaanp utthega

(Thus are all things intertwined, that if you make a flower quiver
In some corner of the world, somewhere else a star will shiver)

(Raghupati Sahai 'Firaaq' Gorakhpuri)

Tradeoffs are central to life-history evolution, because in the absence of constraints upon the joint distributions of multiple fitness-related traits, all components of the life-history could be separately optimized by natural selection and, consequently, all organisms would be expected to live forever, attain reproductive competence upon birth, and produce an infinite number of offspring, and this clearly has not happened. What tradeoffs are, how they differ from constraints, and how they should be studied have been the subject of much debate (Service and Rose 1985; Rose *et al*. 1987, 1996; Wagner 1989; Charlesworth 1990; de Laguerie *et al*. 1991; Houle 1991, 2001; Partridge and Sibly 1991; Price and Schluter 1991; Partridge and Barton 1993b; Joshi and Thompson 1995a; Worley *et al*. 2003), and we will not go into these contentious issues here. For our purposes, a tradeoff will refer to a negative additive genetic correlation between traits, and we will focus more on the traits involved in life-history-related tradeoffs in *Drosophila*, and what we know about the underlying physiology of these tradeoffs. Ultimately, different life-history traits are rooted in a common underlying physiological and metabolic network and, in many cases, compete for the same resources. It is therefore not surprising that many of these traits are negatively correlated at the phenotypic level, and that many of these phenotypic relationships are reflected in underlying genetic correlations. Although putative tradeoffs have been identified between many life-history-related traits in *D. melanogaster*, it is often not clear as to how labile these tradeoffs are in the face of environmental variation, or how conserved they are evolutionarily across taxa and ecologies, or even how much of a constraint they pose to joint responses to direct multivariate selection pressures. Some progress has been made in addressing some of these issues, and we foresee much more empirical work on these lines in the *Drosophila* system. In this section, we review what has been learnt about the genetic, environmental and physiological basis of life-history-related tradeoffs in *D. melanogaster*.

Tradeoffs related to adult lifespan

Empirical studies on the evolution of ageing in *D. melanogaster* have come a long way since the time when ageing was claimed by some to be a nongenetic process (Lints 1978). Since then, several selection studies have amply demonstrated that specific patterns of ageing can indeed evolve (Luckinbill *et al*. 1984; Rose 1984; Partridge and Fowler 1992; Zwaan *et al*. 1995b; Partridge *et al*. 1999a), theory has been developed to link rates of ageing to first principles of population genetics (Mueller and Rose 1996; Rose 1997), evidence has been found for the role of both antagonistic pleiotropy (Williams 1957) and mutation accumulation (Medawar 1952) in ageing in laboratory populations of *Drosophila* (Mueller 1987; Service *et al*. 1988; Rose 1989; Zwaan 1999; Gasser *et al*. 2000), and a combination of quantitative and molecular-genetic investigations on selected lines, QTL mapping and genomics techniques have helped identify genes affecting longevity in *D. melanogaster*, as well as highlight the numerous G × G and G × E interactions that play a role in shaping lifespan (Luckinbill *et al*. 1988; Hutchinson and Rose 1991; Hutchinson *et al*. 1991; Arking *et al*. 1993, 2000; Buck *et al*. 1993; Tyler *et al*. 1993; Deckert-Cruz *et al*. 1997; Nuzhdin *et al*. 1997; Tatar 1999; Leips and Mackay 2000; Tower 2000; Vieira *et al*. 2000; Partridge and Gems 2002; Pletcher *et al*. 2002; Sun *et al*. 2002). The developmental theory of ageing (Lints 1978, 1988), which holds that developmental rates and rates of ageing are causally correlated, being part of the same ontogenetic program, has been conclusively refuted (Chippindale *et al*. 1994; Zwaan *et al*. 1995a), although a fallacious belief persists in some circles that since cells have programmed ageing and death, so too must organisms.

Many studies on the evolution of lifespan have focussed on the tradeoff between lifespan and early-life fecundity, a tradeoff predicted by the antagonistic pleiotropy hypothesis for the evolution of ageing (Williams 1957). The results have been somewhat inconsistent, with some studies not finding a tradeoff (Partridge and Fowler 1992) while others reported a tradeoff between lifespan and early fecundity (Rose 1984; Luckinbill and Clare 1985). The results concerning late-life fecundity have also been inconsistent, with some studies reporting increased late-life fecundity (Rose 1984; Partridge and Fowler 1992), whereas in other studies no change in late-life fecundity was seen in populations selected for increased lifespan (Partridge *et al*. 1999a; Gasser *et al*. 2000). A possible explanation is that the early-reproduced populations of Rose (1984) and Partridge and Fowler (1992), unlike those of Partridge *et al*. (1999a) and Gasser *et al*. (2000), were under selection for high early fecundity because of the nature of their maintenance regime. If late-life and early-life fecundity trade off, then this could result in the early-reproducing lines of Rose (1984) and Partridge and Fowler (1992) having reduced late fecundity, which in turn could give

rise to an artifactual fecundity difference late in life between early-reproducing and late-reproducing populations. Some of the discrepancies between the observations of Partridge and Fowler (1992) and the results of other studies can also be explained in part by inadvertent selection on larval crowding coupled with inbreeding depression in the study by Partridge and Fowler (1992) (discussed in Roper *et al*. 1993; Chippindale *et al*. 1994). To what extent differences in initial genetic composition, maintenance environment and assay environment also play a role in the different correlated responses to selection is not clear, although these effects can be important (Ackermann *et al*. 2001). Overall, however, there is reasonably good evidence for a cost of reproduction in terms of increased mortality and, hence, decreased lifespan in *D. melanogaster* (Rose 1984; Partridge and Andrews 1985; Partridge *et al*. 1987b, 1999a; Partridge and Fowler 1991; Chippindale *et al*. 1993, 1997b; Cordts and Partridge 1996; Sgrò and Partridge 1999; Chapman 2001).

One potential problem with most studies on the evolution of senescence is that elongated lifespan is typically selected for indirectly, by selecting for late-life fecundity (e.g. Luckinbill *et al*. 1984; Rose 1984; Partridge and Fowler 1992; Partridge *et al*. 1999a). Hence, all else being equal, an individual with higher fecundity at late age has a huge fitness advantage in such selection regimes. Under such circumstances, lower early-life fecundity can evolve if it is negatively correlated with late-life fecundity, even if early-life fecundity and lifespan are not correlated genetically. This issue was elegantly addressed by Zwaan *et al*. (1995b), using family selection to select for increased lifespan but not increased late-life fecundity. After six generations of selection, adult lifespan in the selected populations had increased by about 30% relative to controls, whereas fecundity in the selected populations was lower than that in controls throughout their life. This study also supports the view that there is indeed a tradeoff between longevity and fecundity.

The tradeoff between longevity and early fecundity results in part from sharing of a resource (probably lipid) by the two traits (Service 1987; Graves *et al*. 1992; Chippindale *et al*. 1993; Zwaan *et al*. 1995b) and approximates a simple Y-model of resource allocation (van Noordwijk and de Jong 1986), albeit imperfectly (Djawdan *et al*. 1997). Flies from late-reproducing populations forego early reproduction but build up metabolic reserves in the form of increased lipid and glycogen content (Service 1987; Graves *et al*. 1992), but their metabolic rates are not different from that of young flies (Djawdan *et al*. 1997), and the metabolic reserves built up by the old flies are quantitatively lower in energy content than the additional eggs produced by flies from early-reproducing populations (Djawdan *et al*. 1997). Further work on the energetics of the reproduction versus somatic maintenance tradeoff is clearly needed.

It has also been suggested that laboratory-adapted populations may not be good material for studying the evolutionary genetics of ageing, especially for discriminating between the effects of mutation accumulation and antagonistic pleiotropy (Sgrò and Partridge 2000), because selection in the laboratory can proceed by reversals of mutations accumulated during laboratory adaptation to a 2-week or 3-week discrete generation culture. This argument is supported by the finding that *D. melanogaster* populations evolved high early-life fecundity and lower late-life fecundity and longevity in the course of laboratory adaptation (Sgrò and Partridge 2000). Moreover, some wild-caught populations have been shown to live as long as the late-reproduced populations of Rose (1984) that were under selection for increased lifespan for nearly 15 years (Linnen *et al*. 2001). However, results from sib analysis (Rose and Charlesworth 1981) and selection studies on freshly caught wild flies (Luckinbill and Clare 1985) do support the notion of an antagonistic pleiotropy-based tradeoff between early-age and late-age fitness.

The relationship between preadult development time and adult lifespan is of interest since it can be used to test the developmental theory of ageing (Lints 1978, 1988). Two sets of populations subjected to selection for extended adult lifespan via late-life fecundity exhibited a correlated increase in development time (Partridge and Fowler 1992; Chippindale *et al*. 1994), a result seemingly consistent with the developmental theory of ageing. However, the detailed study of a number of demographically selected sets of populations provided no evidence for a causal relationship between lifespan on the one hand and development time, preadult viability and adult size on the other (Chippindale *et al*. 1994). The main finding of this study was that faster development traded off with preadult viability, a result confirmed by later studies in which selection for faster development was carried out (Chippindale *et al*. 1997a; Prasad *et al*. 2000). The observed development time difference between extended lifespan populations and controls (Partridge and Fowler 1992; Chippindale *et al*. 1994) appeared to be due to inadvertent selection for faster development in control populations, as a consequence of a premium being placed on reproduction rather early in life (Roper *et al*. 1993; Chippindale *et al*. 1994). Selection on lifespan alone (Zwaan 1995b), or on late-life fecundity without concomitant selection for very early fecundity in control populations (Partridge *et al*. 1999a), also revealed no correlated response in development time, preadult viability or adult size, confirming the view that preadult development time and adult lifespan are not causally linked.

One study of populations selected for extended lifespan through late-life fecundity yielded correlated decreases in preadult viability, pupation height and adult weight (Buck *et al*. 2000), a pattern of results clearly inconsis-

tent with most other studies selecting for extended lifespan in *D. melanogaster* (Partridge and Fowler 1992; Chippindale *et al*. 1994; Partridge *et al*. 1999a). Although the populations used by Buck *et al*. (2000) do differ from other extended lifespan populations in some of the physiological and genetic correlates of ageing, the lower preadult viability and adult weight in their extended lifespan populations is likely an artifact of insufficiently rigorous maintenance, resulting in inadvertent selection for adaptation to crowding in their control lines. Larval density was not explicitly regulated in the experimental populations of Buck *et al*. (2000), and their control lines would most likely experience higher crowding compared to the extended lifespan lines, because younger flies are far more fecund than older flies. The greater pupation height of their control lines strongly supports this view, as pupation height has not been seen to decrease in other extended lifespan populations (Mueller *et al*. 1993) but is well known to increase in populations maintained at high larval density (Joshi 1997; Mueller 1997). Moreover, a generation of common rearing prior to assay in order to eliminate nongenetic parental effects does not appear to have been used by Buck *et al*. (2000). Given the density differences between selected and control lines in their running cultures, there are likely to have been differences in maternal nutritional status between selected and control lines, and maternal nutritional status is now known to have effects on offspring preadult viability (Prasad *et al*. 2003b). It is therefore difficult to make too much of the findings of Buck *et al*. (2000); indeed, the above-mentioned problems serve to underscore the importance of rigorous control over seemingly trivial aspects of the maintenance and assay regimes when conducting selection experiments (Rose *et al*. 1996).

The relationship between adult lifespan and resistance to starvation, desiccation and oxidative stress has also been studied in *D. melanogaster* populations selected for extended lifespan. The extended lifespan populations of Rose (1984) have greater lipid content and starvation resistance (Service *et al*. 1985; Service 1987), as well as higher glycogen content and desiccation resistance (Graves *et al*. 1992), compared to the early-reproduced controls. These results are seemingly consistent with a simplistic view of a nutritional reserve-mediated tradeoff between early reproduction and somatic maintenance. However, more careful studies revealed that the difference in starvation resistance between the extended lifespan and control populations of Rose (1984) are apparent only four days after eclosion, and appear to be due to a decline in starvation and desiccation resistance in flies from the early-reproduced control populations which have a greater fecundity than those from extended lifespan populations over the first four days of adult life (Chippindale *et al*. 1994, 1996). The extended lifespan populations of Rose (1984) also exhibit high frequency of the high-activity allele of the Cu–Zn superoxide dismutase (SOD) (Tyler *et al*. 1993). Thus the long-lived phenotype of Rose (1984) is positively correlated with increased metabolic storage, and increased resistance to environmental stress and free-radical damage. Contrary to these findings, the extended lifespan lines of Force *et al*. (1995) did not show higher desiccation resistance or glycogen content than controls, although their lipid content and starvation resistance were higher than controls under some but not all assay conditions. Moreover, there was no allozyme differentiation for SOD in these lines, but there was a coordinated upregulation of a number of antioxidant defense system (ADS) genes (Arking *et al*. 1993; Dudas and Arking 1995). These extended lifespan lines also showed increased resistance to paraquat, an exogenous source of free radicals. Thus the long-lived populations of Luckinbill *et al*. (1984) seem to have evolved greater resistance to oxidative stress, although by genetic means partly different from that seen in the extended lifespan populations of Rose (1984), but not resistance to starvation or desiccation.

Overall, despite the various discrepancies in correlated responses to selection for increased lifespan seen in studies from different laboratories, there has been much progress in testing hypotheses about the evolution of ageing, and in elucidating some of the morphological, physiological, biochemical and genetic correlates of postponed senescence in *D. melanogaster*. Given the importance of G × G and G × E interactions (Luckinbill and Clare 1986; Leroi *et al*. 1994a,b,c; Leips and Mackay 2000; Vieira *et al*. 2000), and the interaction of both with gender (Nuzhdin *et al*. 1997), in determining lifespan in *D. melanogaster*, along with the possible role of maternal effects (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished data), it is perhaps not surprising that studies using different base populations and differing in maintenance and assay conditions should yield slightly different responses to selection on lifespan (Ackermann *et al*. 2001). Elucidating the details of such genetic and environmental effects and interactions with life-history traits like lifespan, and the extent to which these effects are phylogenetically conserved among drosophilids, is likely to be an important area of further research in *Drosophila* life-history evolution.

Tradeoffs related to starvation and desiccation resistance

Resistance to environmental stress is a much studied aspect of *Drosophila* ecology, physiology and genetics, and studies have been carried out on the evolutionary genetics of resistance to various stresses such as urea (Joshi *et al*. 1996a; Shiotsugu *et al*. 1997; Borash *et al*. 2000b), ammonia (Borash *et al*. 2000a; Borash and Shimada 2001), ethanol (McKenzie and Parsons 1972; Service *et al*. 1985; Hoffmann and McKechnie 1991; Fry 2001), extreme temperature (Hoffmann *et al*. 2003) and density (Joshi

1997; Mueller 1997) in both larval and adult stages of *D. melanogaster*. Here, we restrict ourselves to a discussion of starvation and desiccation resistance, because these two traits have been shown to be closely related to life-history traits like lifespan and fecundity. Selection for increased starvation and desiccation resistance in *D. melanogaster* has been successfully done in separate studies, and the correlated responses to selection have not always been consistent (Harshman and Hoffmann 2000).

Two sets of selection studies using different initial populations have provided evidence that evolutionary increases in starvation and desiccation resistance in *Drosophila* can occur either by increased storage of specific metabolites (Chippindale *et al*. 1996; Gibbs *et al*. 1997) or by a reduction in metabolic rate (Hoffmann and Parsons 1989, 1993; Harshman *et al*. 1999). Selection for increased starvation or desiccation resistance on populations derived from the extended lifespan and control populations of Rose (1984) yielded correlated increases in preadult development time, larval growth rate and size at eclosion, and a correlated decrease in preadult viability, interpreted as a reflection of a tradeoff between larval growth rate and preadult viability (Chippindale *et al*. 1996, 1998). Populations selected for increased starvation resistance had higher lipid levels than controls, but did not differ from controls in the efficiency with which they utilized their lipid reserves to survive under starvation (Chippindale *et al*. 1996). There was also no difference between starvation-resistant populations and controls in metabolic rate (Djawdan *et al*. 1997). Populations selected for increased desiccation resistance had higher glycogen and bulk water content, and reduced water loss rates, compared to controls, but were not any more tolerant of reduced water content (Gibbs *et al*. 1997; Chippindale *et al*. 1998; Williams *et al*. 1998). Desiccation-resistant populations also evolved differences in gas exchange patterns (Williams *et al*. 1997), although these differences did not appear to be causally involved in reducing water loss rates (Williams and Bradley 1998). In general, stress-resistant populations from the Rose (1984) ancestry tend to show reduced early-life fecundity (Service *et al*. 1988) and increased lifespan (Rose *et al*. 1992). The correlated responses of populations derived from the lines of Rose (1984) to selection for starvation and desiccation resistance thus reveal a strategy of dealing with these stresses by evolving a greater level of metabolite storage, at a slight cost in terms of preadult viability, and perhaps allocating a smaller fraction of these stored metabolites to reproduction.

In other studies on selection for resistance to starvation and desiccation, a correlated increase in development time was seen by Harshman *et al*. (1999), but not by Hoffmann and Parsons (1993). This may, however, be due to the fact that eclosion was scored once a day by Hoffmann and Parsons (1993), and once in 6 h by Chippindale *et al*. (1996, 1998) and Harshman *et al*. (1999). Given that differences in development time between the selected and control populations of Chippindale *et al*. (1996, 1998) and Harshman *et al*. (1999) were of the order of 6 h, the reduced resolution in Hoffmann and Parsons's (1993) assay may well be the reason why they found no significant development time difference between selected and control lines. Alternatively, the differences may reflect different genetic compositions in the initial populations used in the various studies (Sgrò and Partridge 2001). Hoffmann and Parsons (1989, 1993) also found that their stress-resistant populations had reduced early-life fecundity and increased lifespan, as in the case of populations derived from the lines of Rose (1984). The notion that lifespan and starvation and desiccation resistance are linked is strengthened by the observation that extended lifespan populations evolve increased resistance to starvation and desiccation (Service *et al*. 1988; Graves *et al*. 1992).

The major difference between studies was seen with regard to metabolic rates and storage of metabolites. Selection for desiccation resistance resulted in a correlated reduction in metabolic rates, along with reduced fecundity, water loss rates and general activity levels (Hoffmann and Parsons 1989, 1993). These desiccation-resistant populations, unlike those of Chippindale *et al*. (1998), also showed cross resistance to starvation, ethanol fumes and radiation stress. Starvation-resistant populations were similarly reported to have lower metabolic rates, as well as cross resistance to desiccation and acetone fumes (Harshman *et al*. 1999), although they also had increased lipid content, as in the case of the starvation-resistant populations of Chippindale *et al*. (1996). It may be possible that reduced metabolic rate, unlike increased metabolite storage, is an effective means of acquiring generalized stress resistance (Hoffmann and Parsons 1989).

In a recent study of several wild populations of *D. melanogaster*, no correlation was seen between lipid content and starvation resistance (Hoffmann *et al*. 2001a). In a separate study, it was seen that both starvation and desiccation resistance rapidly decline as a result of laboratory adaptation in *D. melanogaster*, while early fecundity increases (Hoffmann *et al*. 2001b). On the basis of these observations, it has been suggested that variation for stress resistance may be lost during laboratory maintenance on a short-generation cycle, and, therefore, much of the response to selection for increased stress resistance in long-term laboratory populations (e.g. those used by Chippindale *et al*. 1996, 1998) may involve returning the populations to levels of stress resistance seen in the wild. If so, patterns of correlated responses might be expected to differ between populations selected for starvation or desiccation resistance starting with laboratory-adapted or wild lines (Harshman and Hoffmann 2000; Hoffmann *et al*. 2001b). Further studies on metabolite storage and

starvation and desiccation resistance in wild and laboratory populations of *Drosophila* species from different sources may be needed to assess how general the phenomenon discussed by Hoffmann *et al*. (2001b) really is. While a few systematic studies of laboratory adaptation have recently been carried out in *D. subobscura* (Matos *et al*. 2000a) and *D. melanogaster* (Sgrò and Partridge 2000; Hoffmann *et al*. 2001b), more detailed studies on a variety of species and a variety of laboratory maintenance regimes are required, and there are still contentious issues of experimental design and interpretation to be ironed out in this regard (Matos *et al*. 2000b; Matos and Avelar 2001; Sgrò and Partridge 2001).

Tradeoffs related to preadult development time

Early attempts to select for reduced preadult development time in *Drosophila* failed to elicit any response (Sang and Clayton 1957; Bakker 1961, 1969; Clarke *et al*. 1961), leading to the view that *Drosophila* populations in the wild were subjected to directional selection for rapid development as a consequence of the larvae inhabiting ephemeral habitats like rotting fruits (Clarke *et al*. 1961; Robertson 1963; Partridge and Fowler 1992). Consequently, larval growth rates in wild *Drosophila* were often thought to be an evolutionary compromise between the need to develop fast and the fact that faster development typically results in smaller size and, hence, presumably reduced fitness (Santos *et al*. 1988; Partridge and Fowler 1993; but see also Santos 1996; Joshi *et al*. 1999). Since larval densities in the wild are often high enough to be suboptimal (Atkinson 1979; Nunney 1990; Thomas 1993), it was also believed that selection for faster development and for adaptation to larval crowding would have similar evolutionary outcomes (Tantawy and El-Helw 1970; Wilkinson 1987; Santos *et al*. 1988; Prout and Barker 1989; Partridge and Fowler 1993; Borash *et al*. 2000b), a view reinforced by the finding that development time is a good indicator of larval competitive ability in studies of interspecific competition among a number of tropical *Drosophila* species (Krijger *et al*. 2001). These long-held views, however, are now called into question by the results from some recent studies on the evolution of development time in *D. melanogaster*, in which large responses to selection for faster development have been seen (Zwaan *et al*. 1995a; Nunney 1996; Chippindale *et al*. 1997a; Prasad *et al*. 2000, 2001). In this section, we focus on the results from these studies pertaining to tradeoffs of development time with other traits, while the differences between selection for faster development and for adaptation to larval crowding are discussed in a subsequent section.

The four studies include two short-term (Zwaan *et al*. 1995a; Nunney 1996) and two long-term (Chippindale *et al*. 1997a; Prasad *et al*. 2000) selection experiments examining correlated responses to selection for faster development at moderate larval densities in *D. melanogaster*. We will not discuss responses to selection for slower development as they are hard to interpret, being in the direction of lowered fitness (Chippindale *et al*. 1997a). Genetically, development time seems to be affected largely by autosomal loci with additive effects, and a small X-linked influence (Nunney 1996). At a gross level, some results of comparable studies have been consistent, with tradeoffs apparent between faster development and body size early in selection in all four studies, and between faster development and preadult survival in the two longer-term studies after ~ 50 generations of selection. The response of fecundity differed between the two short-term studies, and possible causes for this discrepancy have been discussed by Nunney (1996). Adult lifespan did not differ between control and selected lines in the two short-term studies, whereas in the one long-term study in which it was monitored, lifespan first decreased and then increased in the faster-developing lines (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished manuscript). During the first ~ 40 generations of selection, there were large decreases in development time and body size at eclosion relative to controls. During this time, adult lifespan was reduced by about 7 days (20%), and this reduction was not apparent in virgins, suggesting it was due to an increased cost of reproduction. This was corroborated by the observation that, early in life, the faster-developing flies were producing almost as many eggs per day as control flies, despite being smaller. Later on, after about 70 generations of selection had elapsed, the adult lifespans of faster-developing and control flies were repeatedly seen to be similar. Selected-line flies, by then, were about 50% smaller than controls, and also had much lower fecundity, lipid content, and starvation tolerance early in life. However, they tolerated starvation for a longer duration of time per unit lipid than control flies, suggesting that a greater proportion of their lipid reserve was available for use, or used more efficiently, during starvation. Our interpretation of these results is that at some point between generations 40 and 70 of selection, lipid levels in the faster-developing populations fell below a threshold that activates a physiological switch resulting in greater allocation to somatic maintenance versus reproduction. A previous study revealed no difference between faster-developing and control lines for starvation tolerance or lipid fraction (Zwaan *et al*. 1995a), but these measurements were made on flies 21 days after eclosion and are not directly comparable with our result. Our finding of a positive association between lipid content and development time is supported by the observation that flies selected for greater starvation tolerance show increased development time and lipid content relative to controls (Chippindale *et al*. 1996, 1998; Harshman *et al*. 1999).

A more detailed comparison of the long-term studies (Chippindale *et al*. 1997a; Prasad *et al*. 2000, 2001) is

instructive because these two studies were conducted on populations derived from the same ancestors, and with fairly similar maintenance regimes and assay methods. In both studies, only the first 20% or so of eclosing flies were allowed to breed, but Chippindale *et al*. (1997a) collected eggs from the adults as soon as enough eggs were laid, which was less than 24 h post-eclosion after many generations of selection, whereas we kept the adults in cages for about two and a half days prior to collecting eggs (Prasad *et al*. 2000, 2001). We believe this small difference in maintenance regime explains some of the differences seen between the studies in the pattern of reduction in preadult survivorship and development time. Chippindale *et al*. (1997a) observed reductions in both larval and pupal survivorship, but only larval and not pupal duration in selected lines, whereas Prasad *et al*. (2001) observed reductions in both larval and pupal duration, but only in larval survivorship. It is likely that the selection for extremely early fecundity in the study of Chippindale *et al*. (1997a) made it difficult for pupal duration to be reduced, and the requirement of rapid sexual maturation also exacted a cost in pupal mortality. In our populations, it appears that some aspects of reproductive maturation were postponed from the pupal to the early-adult stage, thus permitting a reduction in pupal duration with only a minor mortality cost (Prasad *et al*. 2001).

The other interesting results coming from selection for faster development relate to food acquisition and utilization in larvae, and suggest that, contrary to a widely held view, faster development may not be correlated with greater competitive ability. Our faster-developing populations have reduced larval feeding rate, pupation height, foraging path length and digging propensity compared to the controls, suggesting the evolution of a syndrome of energy conservation in these populations (Prasad *et al*. 2001). Larval feeding rate, a trait strongly correlated with competitive ability, actually declines rapidly within a few generations of selection for faster development (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished manuscript), suggesting that feeding rate and faster development trade off in *Drosophila*. The major reduction in development time in our populations is due to a reduction in the third instar duration, a critical period for weight gain, and the likely explanation for reduced adult size, although there is suggestive evidence that faster-developing lines may also have a reduced critical size (Prasad *et al*. 2001). Overall, it seems that selection for faster development at moderate larval densities does not lead to greater competitive ability; what evolves appears to be major reductions in larval duration, especially the third instar, and energy expenditure in foraging (Prasad *et al*. 2001; Joshi *et al*. 2001).

Another issue that has not been much studied is whether faster development leads to concomitant increase in developmental instability. Our faster-developing populations do show reduced urea tolerance (Joshi *et al*. 2001), and reduced starvation and desiccation resistance (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished data) compared to controls, as well as reduced viability at low, nonstressful density (Prasad *et al*. 2000, 2001). The reduced starvation and desiccation tolerance, of course, need not be due to a general susceptibility to stress due to developmental instability, as they are expected to decline relative to controls simply owing to the reduction in third instar duration, the time when major assimilation of energy reserves occurs. The cause of the reduced urea tolerance and larval viability is not known at this time, and it may well be greater developmental instability. Fluctuating asymmetry has been suggested as a good measure of developmental instability (Leary and Allendorf 1989; Markow 1995; but see also Palmer and Strobeck 2003), but we did not find any evidence for greater fluctuating asymmetry of sternopleural bristle number in our faster-developing populations relative to controls (Shakarad *et al*. 2001). The issue of faster development and developmental stability would therefore appear to require further study.

Adaptation to crowding

Competitive ability—fitness under conditions of resource limitation—is important to adaptive evolution, and is central to the theory of density-dependent selection, one of the first bridges between population ecology and population genetics (Mueller 1997; Joshi *et al*. 2001). There have been two systematic and rigorous sets of long-term studies of laboratory adaptation to crowding in *D. melanogaster* by Larry Mueller and colleagues (reviewed by Joshi 1997; Mueller 1997), and here we briefly summarize the findings from these studies, including those obtained after the reviews in 1997. Maintenance at high larval densities for many generations led to the evolution of greater population growth rates at high density (Mueller and Ayala 1981), competitive ability (Mueller 1988b), larval feeding rates (Joshi and Mueller 1988, 1996), level of locomotor behaviour during foraging (Sokolowski *et al*. 1997), minimum food requirement for pupation (Mueller 1990; Joshi and Mueller 1996), larval growth rate during the postcritical size period and weight loss during the postfeeding period (Santos *et al*. 1997), urea tolerance (Shiotsugu *et al*. 1997; Borash *et al*. 1998), starvation resistance and total and fractional lipid content at eclosion (Borash and Ho 2001), and pupation height (Mueller and Sweet 1986; Joshi and Mueller 1993; but see also Joshi and Mueller 1996). Direct selection on larval feeding rate results in individuals from faster-feeding lines accumulating more lipid prior to eclosion, and this is also correlated with higher early fecundity and reduced lifespan as adults (Foley and Luckinbill 2001). Crowding-

adapted lines, which also evolve higher feeding rates, are known to have greater lipid content at eclosion (Borash and Ho 2001), but these lines do not show major divergence in fecundity patterns or lifespan (Joshi and Mueller 1997; Joshi *et al*. 1998a). The reason for this discrepancy is not clear, but it should be noted that crowding-adapted lines also evolve other traits than higher larval feeding rate, and these may be contributing to the differences between results of Foley and Luckinbill (2001) and those of Mueller and colleagues (Joshi and Mueller 1997; Joshi *et al*. 1998a). Another potential reason could be the fact that different populations of flies were used in the different studies.

One of the major findings of these studies, contrary to theoretical expectations about *K*-selection favouring greater efficiency, was that populations adapted to larval crowding were actually less efficient at converting food to biomass, and that efficiency of food conversion traded off with efficiency at food acquisition through a higher feeding rate and higher levels of foraging-related locomotor behaviour (Mueller 1990; Joshi and Mueller 1996; Santos *et al*. 1997). Although populations adapted to larval crowding show faster development (Borash and Ho 2001), higher preadult survivorship (Mueller *et al*. 1993; but see also Borash and Ho 2001), and greater size at eclosion (Borash and Ho 2001) than control populations when assayed at high densities, there is no difference in preadult survivorship (Mueller *et al*. 1993; Borash and Ho 2001), development time (Santos *et al*. 1997; Borash and Ho 2001), size at eclosion (Santos *et al*. 1997; Borash and Ho 2001), early-life fecundity (Joshi *et al*. 1998a) or adult lifespan (Mueller *et al*. 1993) between selected and control populations when assayed at moderate larval density. These results, together with the observation that selection for rapid preadult development at moderate density leads to almost exactly the opposite suite of traits of that which evolves under larval crowding (Joshi *et al*. 2001; Prasad *et al*. 2001), strongly suggest that the widely held view that faster development and greater competitive ability are positively associated in *Drosophila* (Wilkinson 1987; Santos *et al*. 1988; Partridge and Fowler 1993; Borash *et al*. 2000b) may not hold true at the within-population level, even if development time is a good indicator of competitive ability in interspecific comparisons (Krijger *et al*. 2001). Indeed, we have recently shown that populations successfully selected for rapid preadult development are, in fact, substantially poorer competitors than their controls (M. Shakarad, N. G. Prasad, K. Gokhale, V. Gadagkar, M. Rajamani and A. Joshi, unpublished manuscript). Overall, it seems clear that selection at high larval densities leads to the evolution of faster-feeding and more actively foraging larvae, rather than more efficient larvae and genetically smaller flies. There is one study in which selection at moderate and very low larval densities resulted in the evolution of a development time difference between lines, with low-density lines developing more slowly and eclosing at larger size than high-density lines (Roper *et al*. 1996). It is, however, hard to interpret the results of this study in view of adaptations to crowding because the high density used was actually rather moderate (150 larvae per vial), and no direct response to selection in the form of a difference in competitive ability between the two sets of selected lines was observed.

The evolutionary consequences of maintenance at high adult densities in *D. melanogaster* have not been studied in such detail. Populations maintained at high larval and adult densities have been shown to be less sensitive to the detrimental effects of adult density on lifespan (Mueller *et al*. 1993). Populations maintained at moderate larval densities, and high density during the first several days of adult life, show reduced mortality during 3–5-day episodes of severe adult crowding, and this is partly mediated by behavioural avoidance of the food medium which is extremely mushy during such episodes (Joshi *et al*. 1998a). These populations also exhibit reduced starvation tolerance and lipid content at eclosion (Borash and Ho 2001), and reduced rates of female weight gain over the first few days of adult life (Joshi *et al*. 1998a). There is also suggestive evidence that the populations adapted to adult crowding are less affected than controls by the detrimental effects of adult crowding on fecundity and lifespan (Joshi and Mueller 1997; Joshi *et al*. 1998a), as well as evidence suggesting that adaptations to larval versus adult crowding trade off in *D. melanogaster*, with populations adapted to larval crowding being more susceptible to deleterious effects of adult crowding and vice versa (Joshi *et al*. 1998a; Borash and Ho 2001). This is an area that we think warrants further attention, especially regarding the physiological mechanisms underlying the life-stage density-specific tradeoffs in fitness components.

Thermal adaptation and life-history traits

Thermal adaptation has been extensively studied in *Drosophila* using a combination of ecological, quantitative-genetic and molecular approaches. This body of work has recently been reviewed (Hoffmann *et al*. 2003) and we will restrict ourselves here largely to summarizing the results from one set of detailed studies on the thermal evolution of body size and other life-history traits in *D. melanogaster*, conducted by Linda Partridge and colleagues. Six populations were derived from an outbred stock collected in Brighton, England, after about a year of laboratory rearing at 25°C. Three replicate populations each were then maintained as population cage cultures with overlapping generations at either 16.5°C or 25°C (Huey *et al*. 1991). Relative to the 25°C lines, the 16.5°C lines evolved reduced ability to withstand heat shock

(Huey *et al*. 1991), and a shorter larval development time, greater larval growth rate and efficiency of conversion of food to biomass, and greater wing and thorax length when assayed either at 25°C or 16.5°C (Partridge *et al*. 1994a,b; Neat *et al*. 1995). Preadult survival, daily and lifetime fecundity, and adult lifespan, on the other hand, showed evidence of adaptation to maintenance temperature, with the 16.5°C lines being superior to the 25°C lines when assayed at 16.5°C, and vice versa (Partridge *et al*. 1994a, 1995); evidence of tradeoffs between heat and cold resistance, associated with the *hsr-omega* locus on chromosome 3, has also been found in a study of natural populations of *D. melanogaster* in Australia (Anderson *et al*. 2003). A similar pattern of thermal adaptation was also seen for critical weight, with the 16.5°C lines showing higher critical weights than 25°C lines when assayed at 16.5°C, and vice versa (Partridge *et al*. 1994a; but see also de Moed *et al*. 1999). Surprisingly, lines derived from the 25°C lines and subsequently reared at 29°C did not diverge in development time from the 25°C lines over four years of selection, and the reason for this lack of change was not clear (James and Partridge 1995). Results from another long-term study of laboratory thermal adaptation in *D. melanogaster* (Cavicchi *et al*. 1995) are different in some details from those summarized above, but as that study was conducted with small populations ($N = 80$), and a very different starting stock, it is difficult to make clear and meaningful comparisons. The picture is further complicated by several lines of evidence suggesting that the genetic control of heat or cold shock tolerance in *Drosophila* may be at least partly different across different life stages (Tucic 1979; Loeschcke and Krebs 1996; Hoffmann *et al*. 2003).

Taken together with studies of body size clines, adaptations to crowding and selection on body size, these laboratory studies on thermal adaptation fit into a very interesting, albeit incomplete and hazy, picture of how body size evolution may be shaped in natural populations of *D. melanogaster*. Body size increases with latitude in the southern hemisphere in Australia, South America and Africa, and variation in both cell size and number appears to contribute to clinal variation in wing area (James and Partridge 1995; James *et al*. 1995; Zwaan *et al*. 2000). However, the genetic architecture of the clinal differences in wing size varies among continents (Gilchrist and Partridge 1999), and the contribution of variation in cell size, compared to cell number, is much less in the Australian rather than the South American cline (Zwaan *et al*. 2000). It is not clear exactly why larger size may be adaptive at colder temperatures (Jenkins and Hoffmann 1994; Partridge *et al*. 1994b), but there is some evidence from lines selected for increased and decreased wing area at constant wing cell sizes that large size may increase both male and female fitness at low temperatures (McCabe and Partridge 1997; Reeve *et al*. 2000). In addition to body size, larval and preadult development time (James and Partridge 1995) and food conversion efficiency (Robinson and Partridge 2001) also exhibit clinal variation, with populations from higher latitudes developing faster and being more efficient at converting food to biomass. However, development time and body size are not strongly associated among populations across the cline (James *et al*. 1995).

Overall, larvae from cold-adapted wild and laboratory populations appear to have higher growth rates and food conversion efficiencies than those from populations adapted to relatively warmer temperatures, and there is some suggestive evidence that these traits may be trading off with competitive ability in the cold-adapted populations (James and Partridge 1998), especially in light of suggestions that tropical populations of *Drosophila* in nature face higher levels of intraspecific competition than temperate ones (David and Capy 1982). Given the evidence of a tradeoff between larval feeding rate / competitive ability and efficiency of food conversion (Mueller 1990; Joshi and Mueller 1996; Santos *et al*. 1997), studies on the feeding rates and foraging locomotor behaviour of cold-adapted and warm-adapted populations may help clarify this issue. It is also clear that there are many different paths by which body size can evolve, and these can depend on temperature, density and the nutritional environment (Robertson 1963). For example, reduced body size due to larval crowding or less nutritious food is due to reductions in both cell size and number, but predominantly cell number (Robertson 1959), whereas increased wing area in cold-reared or cold-adapted laboratory lines is due to increased cell size rather than number (Partridge *et al*. 1994b). Direct selection for increased body size yields increased wing size primarily due to increased cell number, whereas selection for smaller body size yields decreased wing size predominantly through a reduction in cell size (Partridge *et al*. 1999b). It seems to us that studies on larval growth rates, critical weights, food conversion efficiency, feeding rates, foraging behaviour, competitive ability and the underlying developmental mechanisms of body size differentiation in natural populations from clines, and laboratory populations differentiated for body size under various selection pressures need to be carried out at a variety of temperature × density × nutrition combinations if we are to better understand the factors affecting the evolution of body size.

Reverse evolution

Reverse evolution has been defined as 'the reacquisition by derived populations of the same character states, including fitness, as those of ancestor populations' (Bull and Charnov 1985, quoted by Teótonio and Rose 2001). It is

generally agreed that, given the many contingent processes involved, long-term evolution in nature is likely to be irreversible. Despite a few studies in *Drosophila* (Service *et al*. 1988; Graves *et al*. 1992; Chippindale *et al*. 1997a; Joshi *et al.* 2003), the question of whether short-term evolution in relatively simple and stable environments is reversible in sexually reproducing diploid organisms, and to what degree, had not been addressed rigorously until a relatively recent set of studies using *D. melanogaster* (Teótonio and Rose 2000, 2001; Teótonio *et al*. 2002). The principal issues addressed by these studies were whether (a) evolution over short periods of time is reversible and, if so, to what degree, (b) fitness and traits related to fitness respond in a similar manner during reverse evolution, and (c) evolutionary history constrains reverse evolution.

In these studies, different sets of *D. melanogaster* populations, derived from a common ancestral stock by the imposition of various selection regimes targeting different life-history and demographic traits, were returned to their ancestral regime of a 14-day discrete generation cycle which imposes selection for increased early fecundity. The evolution of competitive fitness, and of fitness-related traits, in these populations, and in hybrids among the replicate populations within each forward-selection regime, was then assessed after 50 generations since reverting to the ancestral regime (Teótonio and Rose 2000, 2001; Teótonio *et al*. 2002). In this set of studies, traits related to fitness showed four distinct trajectories with regard to their tendency to return to ancestral values (figure 2 in Teótonio and Rose 2000): (i) complete reversal to ancestral trait values, (ii) a tendency to converge towards the ancestral value though convergence was not complete by 50 generations of selection, (iii) rapid convergence initially, followed by a later phase of stasis without full convergence to ancestral values, and (iv) no change throughout the period of the study. Thus, reverse evolution was found to be neither impossible nor inevitable in these experiments, and past evolutionary history was shown to play a role in determining the degree to which convergence to ancestral values occurred (Teótonio and Rose 2000).

The results also shed some light on possible genetic mechanisms involved in the process of trait convergence under reverse selection. A rapid reversal to ancestral trait values under reversed selection implicates antagonistic pleiotropy in the evolution of the trait during forward selection, and such rapid reversion to ancestral values has previously been seen for early-life fecundity at the cost of starvation resistance (Service *et al*. 1988), preadult survivorship at the expense of slower development (Chippindale *et al*. 1997a), and larval feeding rate, which is known to trade off with efficiency of food utilization (Joshi *et al.* 2003). The slow convergence of trait values to ancestral levels seen for many traits can, in principle, be due to a variety of reasons. If a trait that evolved during forward selection is neutral with regard to fitness during reverse selection in the ancestral regime, then it will decay only by mutation accumulation, which takes a large number of generations to give rise to observable effects. For example, desiccation and ethanol resistance in the extended lifespan populations of Rose (1984) converged to ancestral levels only after about 100 generations of reverse selection on an early-reproducing regime (Graves *et al*. 1992). Slow or partial convergence to ancestral trait values during reverse evolution may also be due to either lack of genetic variation or epistatic interactions (Teótonio and Rose 2000). In such situations, hybrids between populations should exhibit greater convergence towards ancestral values than the parental populations, because hybridization tends to restore genetic variation and severely perturbs epistatic patterns. However, Teótonio and Rose (2000) found no difference in the hybrid and parental populations in their tendency to converge to ancestral values, thus ruling out the possibility that the incomplete convergence was due to paucity of genetic variation or epistasis. This conclusion is supported by the finding that direct selection for desiccation resistance on populations derived from the extended lifespan populations of Rose (1984) yields a large and rapid response (Rose *et al*. 1992; Chippindale *et al*. 1998), even though desiccation resistance converged to ancestral values very slowly in reverse-selected O populations (Graves *et al*. 1992). Yet another possible explanation for incomplete convergence to ancestral values under reverse selection is altered G × E interactions. If the relationship of a given trait and fitness, with respect to the ancestral environment, is altered during forward selection, then the trajectory under reverse selection in the ancestral environment may not simply be the reverse of what it was during forward selection (Teótonio and Rose 2000). Although a variety of trajectories of reverse evolution were seen in these experiments for traits related to fitness, early-life male competitive fitness of reverse-selected populations reverted to ancestral levels in all cases (Teótonio *et al*. 2002), while female and populational early-life fitness did not differ among forward-selected, reverse-selected or ancestral populations. The contrast between the degree of convergence for fitness and that for fitness-related traits reinforces the view that it is possible to attain the same level of fitness through different combinations of fitness-related traits, a phenomenon also noticed in the case of the evolution of interspecific competitive ability in *D. melanogaster* and *D. simulans* (Joshi and Thompson 1995b). We believe that the results of reverse selection experiments so far highlight the importance of more theoretical and experimental work on the kinds of genetic changes underlying the diverse trajectories that are seen in the process of reverse evolution, especially for different fitness-related traits.

Some emerging issues in *D. melanogaster* life-history evolution

Zamaana aaya hai be-hijaabi ka a'am deedaar-e-yaar hoga
Sakut tha pardadaar jiska vo raaz ab aashkaar hoga

(The time to lift the veil that hides the face of truth is drawing near
The secret that till now was veiled in silence will at last be clear)

(Sheikh Mohammad Iqbal)

In this section, we discuss a few areas of research that have only recently begun to receive attention from people working on life-history evolution in *Drosophila*. We believe that a better understanding of these topics will result in the refinement of experimental approaches to studying *Drosophila* life-history evolution, and also take us further on the road towards a fuller understanding of life-history evolution in general. Conversely, we also believe that at this time the laboratory *Drosophila* system is perhaps the best model system available for addressing these issues.

Parental effects and life-histories

The possible adaptive significance of nongenetic parental effects has recently been studied in many taxa (Mousseau and Fox 1998), and these studies suggest that parental and offspring environments can often interact to affect the phenotypic expression of parental effects in offspring (Rossiter 1996, 1998). When such interactions occur, they can be major confounding factors in experiments, especially those involving phenotypic manipulation followed by an assay of physiological traits or fitness components (Crill *et al*. 1996; Hercus and Hoffmann 2000). Moreover, parental effects could also influence responses to selection by altering the realized phenotypic distribution among offspring (Watson and Hoffmann 1996). Parental effects on life-history traits in *Drosophila*, however, have not been studied as extensively as in several other taxa. Deleterious effects of increasing parental age on offspring survival have been observed in several species of *Drosophila* (Butz and Hayden 1961; Hercus and Hoffmann 2000), and parental rearing temperature has also been seen to have an effect on offspring fitness components (Zamudio *et al*. 1995; Crill *et al*. 1996; Watson and Hoffmann 1996; Gilchrist and Huey 2001). Interactions between the effects on fitness of maternal and grandmaternal age on the one hand, and maternal and assay environment (stressful versus nonstressful) on the other have also been observed in *D. serrata* (Hercus and Hoffmann 2000), suggesting that interactions between parental effects and environment may be important in *Drosophila* life-history studies.

Parental nutritional status is known to affect offspring fitness, and also to interact with offspring nutritional status, in many invertebrate and vertebrate species (Rossiter 1998). In mammals, negatively correlated maternal and offspring nutritional status can have deleterious effects on offspring metabolism of glucose (Iglesias-Barreira *et al*. 1996) and poor maternal nutrition coupled with better nutrition of offspring is implicated in many cases of diabetes in humans (Ravelli *et al*. 1998). Parental effects and interactions involving nutritional status are of particular relevance to life-history evolution studies because of the focus on tradeoffs surrounding resource acquisition and allocation (van Noordwijk and de Jong 1986; Houle 1991; Partridge and Sibly 1991; de Jong and van Noordwijk 1992; Rose and Bradley 1998; Worley *et al*. 2003). Nutrition-related life-history tradeoffs have been extensively studied in *D. melanogaster* (Partridge *et al*. 1987b; Trevitt *et al*. 1988; Hillesheim and Stearns 1992; Chippindale *et al*. 1993, 1997b, 1998; Leroi *et al*. 1994c; Djawdan *et al*. 1998; Borash and Ho 2001), but these studies have typically not included parental nutritional status as a factor in the experimental design. If phenotypic effects of offspring nutritional status depend in part on the parental nutritional environment in *Drosophila*, as in many other taxa, there is clearly some cause for concern.

We have observed interactions between maternal and larval food levels on larval survivorship such that larval survivorship was highest in individuals from a combination of poor maternal and rich larval food, whereas survivorship did not significantly differ among the other three combinations of rich and poor maternal or larval food (Prasad *et al*. 2003b). Dry weight at eclosion was, however, not affected by maternal food, or any interaction involving maternal food, larval food and sex in this experiment. Similar parental effects and interactions were also seen in a study in which adult flies subjected to eight different treatments were assayed for lifetime fecundity and lifespan: all factorial combinations of rich and poor maternal, larval or adult food levels (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished data). While adult food and sex accounted for most of the variation in lifespan, significant maternal × larval food, and larval × adult food interactions on daily and lifetime fecundity, and adult lifespan were also observed. The low daily and lifetime fecundity of females kept as adults on poor food was, nevertheless, substantially higher if those females had been reared as larvae on rich rather than poor food. In general, maternal, larval and assay food effects on lifespan showed interactions among one another, and with sex and reproductive status, with the presence or absence of significant differences between levels of one factor often depending critically on some specific combination of food levels at various life stages. There was also some evidence suggesting that the pattern of interaction effects varied with adult density (2 versus 8 flies per vial).

While the mechanisms underlying these cross-generation and cross-life-stage effects of nutrition and temperature are not yet known, the existence of such interactions between parental, larval and adult environments on life-history

traits in *D. melanogaster* highlights the importance of explicitly including parental nutritional status as a factor in experiments on nutrition-mediated tradeoffs. Further studies on the physiological and genetic underpinnings of such parental effects and interactions are clearly required. In light of the possibility that temperature, nutrition and density affect not only growth rates, but also patterns of larval and adult resource allocation, cross-generation and cross-life-stage effects of temperature, nutrition and larval and adult density on life-history traits need to be studied together, along with parental age effects. It would also be worthwhile to compare the patterns of such parental effects and interactions in laboratory and wild-caught populations of *D. melanogaster*, as well as in other species of *Drosophila*, to ascertain how conserved these parental effects are, and to assess the extent to which they may be evolved responses to particular nutritional or thermal ecologies, or both.

Sexual antagonism and sexual dimorphism in life-history evolution

In multicellular organisms, the sexes are very often subjected to differing selection pressures, partly because of different reproductive roles and strategies, and partly because their ecologies may differ. Consequently, optimal phenotypes can differ between sexes, setting the stage for sexual antagonism or intersexual ontogenetic conflict (Rice and Chippindale 2001, and references therein). The divergence between sexes of phenotypic distributions of traits expressed in both sexes—the evolution of sexual dimorphism—requires getting around genetic constraints such as positive between-sex correlations among traits (Reeve and Fairbairn 1996; Rhen 2000), and necessitates the action of modifier genes, or sex-limited expression of genes, if males and females are to attain separate phenotypic optima (Rice and Chippindale 2001). A series of elegant experiments, reviewed by Rice and Chippindale (2001, 2002), has revealed genomewide fitness variation that has sexually antagonistic effects in *D. melanogaster*. Moreover, in an experiment in which entire haploid genomes were cloned and expressed in a variety of genetic backgrounds in both male and female individuals, genome-specific preadult survival under competition was positively correlated between sexes, whereas measures of adult fitness were negatively correlated between sexes (Chippindale *et al*. 2001; Rice and Chippindale 2001). The X chromosome has been shown to contribute greatly to sexually antagonistic fitness variation in the adult stage, but not much to fitness variation in the juvenile stage (Gibson *et al*. 2002). These findings indicate that in larvae, where the male and female 'goals' are very similar, genetic correlations between sexes are strongly positive, and that, therefore, dimorphism is likely to be seen only in the adult stages where sexually antagonistic genetic variation for fitness is large.

In *D. melanogaster*, adults are certainly dimorphic for various measures of body size, and female wings have a greater number of larger cells than male wings (French *et al*. 1998). There is also dimorphism for total and fractional lipid content at eclosion. However, since the larval stage plays such a major role in determining adult size and body composition at eclosion, there must be male–female differences underlying traits that show sexual dimorphism in adults. At low larval density, there is sexual dimorphism in preadult development time, because males have a longer pupal duration than females (Bakker and Nelissen 1963; Nunney 1983). However, this male–female difference in development time is ameliorated by even moderately high larval densities (Zwaan *et al*. 1995a; Joshi *et al*. 1999), and this is likely to be due to a density-mediated extension of female larval development time rather than a reduction in the duration of male pupal development. In contrast, the sexual dimorphism in size/weight at eclosion is only slightly reduced even at very high larval density (Borash and Ho 2001; A. Joshi, unpublished data). These observations on density effects on the sexual dimorphism for development time and size at eclosion are consistent with a hypothesis that females have a higher critical weight than males, as this would increase the female larval duration relative to males under larval crowding while not affecting relative size.

Sexual size dimorphism in *D. melanogaster* is thought to be a correlated response to selection on female fecundity, and indeed sexual size dimorphism has been seen to increase in lines selected for greater fecundity (Reeve and Fairbairn 1999). However, sex-specific selection for decreased thorax width only on males, or for increased thorax width only on females, did not yield correlated increases in sexual size dimorphism (Reeve and Fairbairn 1996). Thus, it appears that the sexual size dimorphism in *D. melanogaster* may be due to genes responsible for higher fecundity that have female-limited expression and cause correlated increases in size by affecting aspects of resource acquisition and accumulation in larvae. If there are relatively many more genes, or genes with larger effects, that affect body size in both sexes, this could explain why single-sex selection on size does not increase the degree of dimorphism. However, in a study of five species of *Drosophila*, belonging to the *melanogaster* and *immigrans* species groups, the degree of sexual size dimorphism was not correlated across species with either total fecundity or fecundity per unit body weight (Sharmila Bharathi *et al.* 2003). While across-species and within-species correlations can differ greatly, this result suggests that more studies on the link between fecundity and sexual size dimorphism in *Drosophila* species may be useful. In general, study of sexual dimorphism in *Drosophila* has not really been well integrated into life-history evolution studies. We believe that since sexual dimorphism exists for traits central to the life-history, such as

larval growth rate, larval and adult body size, and lipid content at eclosion, more knowledge of the ontogeny of these sexual dimorphisms and the selection pressures shaping them is important for a better understanding of life-history evolution in *Drosophila*.

Biological clocks and life-histories

Circadian clocks are a fundamental adaptation to life on a rotating planet, and the disruption or alteration of circadian organization by genetic or environmental manipulations affects most aspects of behaviour and physiology, and also various life-history and fitness-related traits (Pittendrigh 1993; Sheeba 2002; Sharma and Joshi 2002). We have earlier discussed the circadian control of key life-history traits, as well as the phenotypic effects of light regime on them. Although there is now preliminary evidence for laboratory evolution of circadian organization in response to maintenance in different light : dark regimes, it is not yet clear what the light-regime-specific life-history correlates of these changes in circadian rhythm parameters are (Sheeba 2002, and references therein). Conversely, it is also not clear whether life-history changes in the course of laboratory evolution are typically accompanied or mediated by changes in circadian organization, although it has been observed that development time was positively correlated with the phase of mating rhythm (longer development line flies mated later in the night), and the freerunning period of the locomotor activity rhythm across populations of the melon fly *Bactrocera cucurbitae* selected for shorter or longer development time (Miyatake 1997, and references therein). In this study, however, selection was successful only for longer development time, and only the longer-development lines diverged in correlated circadian rhythm traits from the ancestral population. There were also major mean phenotype differences between the two replicate longer-development lines, and crosses between shorter-development and longer-development lines showed dominance effects for shorter development (Miyatake 1997). Population sizes were also quite small ($N = 100$): all these facts together make it difficult to rule out inbreeding/genetic drift and selection for generally bad genotypes (as a consequence of selecting for longer development, i.e. for lowered fitness) as an alternative explanation for their results. In a more recent study, lines of *B. cucurbitae* selected for reproduction at early or late ages were seen to diverge in phase of mating rhythm and in period of locomotor activity rhythm, with flies from the early-reproducing lines mating earlier in the day and showing a shorter period of locomotor activity rhythm than flies from the late-reproducing lines (Miyatake 2002). Moreover, eclosion rhythm period mutants at the *per* locus in *Drosophila* show parallel differences in development time with short-period mutants developing faster, and long-period mutants slower, compared to wild-type flies (Kyriakou *et al*. 1990). In Syrian hamsters (*Mesocricetus auratus*), mutants at the *tau* locus with shortened period of the locomotor activity rhythm have been found to differ in metabolic rate, growth rate and lifespan from wild-type individuals (Oklejewicz 2001, and references therein). Thus, several observations now appear to be consistent with the view that circadian organization may play a role in mediating evolutionary change in life-history traits.

The speculation that biological clocks may play a role in life-history evolution is only natural given that life-histories are all about the timing of important ontogenetic events, and circadian biological clocks are the organism's chronometer. Yet, biological clocks by their very nature have to be temperature compensated (Pittendrigh 1960) whereas life-stage duration is markedly affected by temperature in ectotherms, suggesting that, perhaps, the role of biological clocks in timing life-history events may be subtle and indirect (Pittendrigh and Skopik 1970). The most obvious candidate life-history trait for clock-determined/mediated timing in *D. melanogaster* is preadult development time, because eclosion is subject to circadian gating. The developmental state of a pupa is assessed once a day through some unknown circadian-clock-controlled/mediated mechanism, and individuals that have attained a certain developmental state by then will eclose during the next available circadian gate (Qiu and Hardin 1996). In flies that are wild type for *per*, under an LD 12 : 12 h cycle, the circadian gate is several hours long, starting 1–2 h before the dark-to-light transition, and the check on developmental status takes place ~ 10 h prior to the gate's opening (Qiu and Hardin 1996).

Two extreme hypotheses can be framed about the role of circadian clocks in determining development time in *D. melanogaster*, based on whether subjective time (biological clock time) or objective time (external time) is what the development process scales to. In the first scenario, developmental processes are assumed to scale to internal or biological clock time. If so, the total development time for a given population should be a fixed multiple of the period of the biological clock, plus some additional time determined by the phasing of the eclosion gate. In the second scenario, the developmental processes are assumed to be determined by real time (external time based on the earth's rotation). If so, the total development time for a given population should be fixed in calendar days, plus some additional time determined by the phasing of the eclosion gate. Another way of looking at these hypotheses is that in the first it is the biological clock that times eclosion, whereas in the second the biological clock merely determines the time of day at which peak eclosion occurs.

The observation that short-period and long-period mutants have relatively shorter and longer development time, respectively, under constant light (Kyriakou *et al*. 1990) does not permit us to distinguish between these two

hypotheses. However, the period of the eclosion clock can also be altered by changing the total period of the imposed LD cycle, and the eclosion rhythm in *D. melanogaster* populations in our laboratory entrains to 10 : 10 h, 12 : 12 h and 14 : 14 h LD cycles (Paranjpe *et al.* 2003). We measured preadult development time in these populations under five light : dark regimes: constant light (LL) and constant darkness (DD) (in both of which the free-running period of the clock is expressed, although freerunning periods in LL and DD are different), and LD 10 : 10 h, 12 : 12 h and 14 : 14 h. Entrainment implies that in the three LD regimes the period of the biological clock is 20, 24 and 28 h, respectively. From the data on clock period, and phase of eclosion, in these five regimes, expected development times can be derived under both the hypotheses outlined above, and compared to observed data. The observed development times in the five light : dark regimes were not consistent with predictions under either of the two hypotheses, indicating that although the eclosion clock does play a role in determining development time beyond its role in timing the eclosion gate to a specific part of the day, the relationship between clock period and development time is also not as simple as the latter being a multiple of the former (D. A. Paranjpe, D. Anitha, V. K. Sharma and A. Joshi, unpublished data). Thus, the few data available suggest that biological clocks are likely to play a subtle role in mediating the timing of life-history events in *D. melanogaster*, a view further reinforced by the identification of over 100 genes, involved in a variety of functions including detoxification, olfaction, signalling, conveying nutritional information, cuticle formation and immunity, that are transcribed in a circadian manner under the control of the *clk* (clock) locus (McDonald and Rosbash 2001). In another recent study, a peripheral clock in the prothoracic gland has been found to be necessary, in addition to the main clock in the lateral neurons, for the proper expression of the eclosion rhythm in *D. melanogaster* (Myers *et al.* 2003). The need for a better integration of evolutionary biology and chronobiology has recently been discussed in detail (Sharma and Joshi 2002), but we would still like to stress here that life-history evolution studies need to take greater cognizance of the ubiquity of circadian phenomena in living systems, and their sensitivity to the photic environment, in the dual context of experimental design and interpretation.

Life-history evolution and population dynamics

Life-history evolution and population dynamics are fundamentally linked because formal life-history theory developed out of models of population growth in age-structured populations (Cole 1954; Gadgil and Bossert 1970; Stearns 1992; Charlesworth 1994), and, moreover, life-history traits like survivorship and fecundity, and their sensitivity to density, are the major determinants of population dynamics (Cole 1954; Mueller and Joshi 2000). Moreover, the link between population size and life-history evolution was also drawn through the theory of density-dependent selection (reviewed by Mueller 1997; Joshi *et al.* 2001; Reznick *et al.* 2002). In this context, given the detailed understanding of their life-history and ecology, it has been argued that laboratory cultures of *Drosophila* constitute a powerful—perhaps the best—system with which to address questions on the interface of evolutionary genetics and population ecology (Mueller and Joshi 2000). One such question pertains to the mechanism(s) for the evolution of population stability. A variety of theoretical scenarios have been proposed for the evolution of stability, and include group selection acting through long-term persistence of stable populations, individual selection acting directly on demographic parameters, and the evolution of stability as a correlated byproduct of individual selection on life-history traits (reviewed in Mueller and Joshi 2000; Mueller *et al.* 2000).

A problem with the first two views is that stability is favoured by low realized per capita growth rates, and it is hard to envisage the evolution of reduced fecundity or survival through the direct action of natural selection acting among individuals (Mueller and Joshi 2000). An alternative path to greater stability could be the evolution through individual selection of increased sensitivity to density of population growth determining traits like fecundity and survival. This is an issue that needs more empirical study, but so far the one experiment that explicitly looked for such evolutionary change in the sensitivity of fecundity to adult density in *D. melanogaster* populations maintained in a destabilizing environment failed to find evidence of any such changes (Mueller *et al.* 2000). Consequently, it has been argued that the most likely scenario for the evolution of stability would be as a result of the evolution of, say, reduced fecundity, as a correlated response to life-history evolution (Travis and Mueller 1989; Mueller and Joshi 2000). We have recently demonstrated this experimentally. Populations of *D. melanogaster* selected for rapid development at controlled moderate density in our laboratory evolved reduced fecundity and preadult survival as correlated responses (Prasad *et al.* 2000, 2001; Joshi *et al.* 2001), and we have seen that these populations indeed exhibit greater stability of adult census numbers than their ancestral control populations, when maintained in an uncontrolled-density culture (Prasad *et al.* 2003a).

The evolution of population stability is cited here as just one example of the strengths of the *Drosophila* laboratory system for investigating issues on the interface of life-history evolution and population dynamics. In a broader context, what we really need is a better integration of formal life-history theory and the biological minutiae of the *Drosophila* experimental system. Most experimental studies of life-history evolution in *D. melanogaster* are

conducted on populations reared with discrete generations, often with some control over larval or adult densities or both, whereas formal life-history evolution theory has been derived from models of the growth of age-structured populations with overlapping generations (Partridge and Sibly 1991; Partridge and Barton 1993b). On the other hand, heuristic models of the functional architecture of traits involved in life-history tradeoffs have been developed and have proven very helpful in clarifying and focussing debate about life-history tradeoffs (van Noordwijk and de Jong 1986; Houle 1991; de Jong and van Noordwijk 1992; Worley *et al*. 2003). These models, however, cannot yield specific predictions about expected patterns of correlated responses to selection on particular life-history traits in *Drosophila* populations.

A similar situation persisted in population dynamics for quite some time, with the simple heuristic models available collapsing the entire biology of density dependence into a single-humped recursion, whose parameters bore no clear relationship to biological traits. Eventually, the incorporation of biological details of the life-history of model organisms like *Drosophila* and *Tribolium* into mathematical models of population growth has led to tremendous refinement in our understanding of how life-history and ecology interact to generate observed patterns of population dynamic behaviour (reviewed in Mueller and Joshi 2000). We believe that the development of formal life-history evolution models that are specific to discrete generation laboratory cultures of *Drosophila* under various maintenance regimes will not only sharpen our understanding, but also sharpen experimental design, and result in a dynamic interplay between theory and experiment that has so far eluded studies of *Drosophila* life-history evolution. Such models will need to explicitly incorporate the correlations of various life-history traits with fitness under different maintenance regimes; an endeavour that poses a daunting challenge to theorist and experimentalist alike. We also see a complementary need for the development of population growth models for overlapping generation *Drosophila* cultures that include life-stage-specific and age-class-specific life-history details, and also for models predicting life-history evolution in populations with periodic rather than equilibrium dynamics.

We have earlier discussed the insights into the subtleties of life-history evolution gained from studies in which selection pressures were clearly defined, and applied cleanly to specific traits and life-stages. In the context of the development of theory of the sort described above, however, experimental studies of life-history variation in *Drosophila* cultures maintained on an overlapping generation schedule and without explicit control on density are likely to be useful, both for developing the theory and testing and refining it. A couple of studies (Gasser *et al*. 2000; Houle and Rowe 2003) have taken this kind of an approach, with attempts being made to quantify selection pressures and predict responses to selection in laboratory populations maintained in a manner such that the force of selection is a little more natural and less narrowly targeted than in some of the extreme directional selection studies. Theoretical studies are also beginning to address the joint dynamics of population numbers and genetic composition, and results suggest that many interesting outcomes like repeated evolutionary reversals are possible in some situations (Dercole *et al*. 2002), although such studies do not yet explicitly include life-history evolution. To conclude, we hope to see in the future a closer interaction between theory and experiment, and between population dynamics and life-history evolution.

What have laboratory studies taught us about life-history evolution?

Saamne rakhta hoon is daur-e-nishaat afzaa ko mein
Dekhta hoon dosh ke aaine mein fardaa ko main

(The golden age that has gone by, is always in my heart and mind
And in that mirror of the past, I see the future days outlined)

(Sheikh Mohammad Iqbal)

The relative merits and demerits of selection experiments, phenotypic manipulations, and the comparative method as means to probe life-history tradeoffs and to understand the process of adaptive evolution have been discussed at length previously (Partridge and Harvey 1985; Partridge and Sibly 1991; Reznick 1992; Partridge and Barton 1993a,b; Leroi *et al*. 1994c,d; Rose *et al*. 1987, 1990, 1996), and we do not wish to cover the same ground here. We believe that it should be clear from the preceding review that the combination of laboratory selection experiments and phenotypic manipulations and physiological/molecular investigations on selected and control populations have greatly refined our understanding of the ontogenetic and physiological details underlying the life-history of *D. melanogaster*, and how this underlying biology interacts with the environment, and the precise selection pressure applied, to shape the broad contours of life-history tradeoffs and life-history evolution. In this concluding summation, we want to address three broad issues. We will first discuss some of the important implications of what we have learnt from *Drosophila* selection experiments for the manner in which we think about and empirically study the process of adaptive evolution. Next, with a narrower focus on *Drosophila* life-history, we will examine some of the limitations of the kinds of selection experiments hitherto carried out, and what we think will be useful ways to transcend some of those limitations in the future.

Lessons from selection experiments

***G × E interactions are ubiquitous and affect both selection and assay*:** G × E interactions can affect responses to selection, as well as our ability to detect them. The appearance and disappearance of tradeoffs in different assay environments has been termed the 'Cheshire cat' effect, and is discussed at length by Rose *et al*. (1996). Moreover, G × E interactions can also affect the outcome of selection. To give just two examples, selection for faster development at high versus moderate larval densities leads to the evolution of diametrically opposite suites of traits (Joshi *et al*. 2001; Prasad *et al*. 2001), and increased lifespan evolves in response to selection for late-life fecundity at moderate but not very low larval densities (Rose 1984; Luckinbill and Clare 1986). The type of food medium used in the course of selection can affect the pattern of joint response seen when either increased or decreased development time or body size are subjected to selection (Robertson 1963). Although such G × E interactions have not been studied for several other variables, like temperature, we have no reason not to expect their existence. The ubiquity of G × E interaction effects on *Drosophila* life-history evolution in the laboratory suggests that similar effects may be common in wild populations that inhabit an environment of far more biotic and abiotic complexity. It also suggests that broad generalizations about what is or is not adaptive in the wild are likely to be wrong more often than not, even when the generalization is being made across populations of the same species, because the specific details of the environment of the local populations, and the extent of gene flow among populations, will play a major role in shaping the life-history of any given population.

***Trait contributions to fitness are highly context specific*:** Fitness is a multifaceted thing, and the relative contributions of different traits to fitness vary in different environments and contexts. For example, the correlation of lifetime fecundity with fitness is clearly much higher in an overlapping generation versus a 14-day discrete generation culture. This may seem like a statement of the obvious, but this point is often not fully appreciated, especially outside the *Drosophila* literature. Selection experiments with *Drosophila* exemplify the context specificity of fitness and underscore how seemingly small changes in the environmental context can have large evolutionary consequences. As discussed in Prasad *et al*. (2001), a difference of about 30–35 h in the time eggs are collected to initiate the next generation can lead to different patterns of reduction in larval and pupal durations, and in the correlated changes in larval and pupal mortality and time to sexual maturity, in populations selected for faster preadult development and early reproduction. Conversely, as discussed in Nunney (1996), the correlated response of lifetime reproductive success differs between populations selected for faster larval versus faster preadult development. One set of *D. melanogaster* populations adapted to high larval density evolved to become polymorphic for two strategies of coping with life in a crowded deteriorating environment: to be faster feeding, and rapidly developing, though less urea tolerant and efficient, versus being slow to develop but more urea tolerant, to be able to complete development during later stages in the vials (Borash *et al*. 1998). This polymorphism appears to be sustained by a quirk of the maintenance regime that inadvertently imposed assortative mating of early-eclosing and late-eclosing flies in the culture vials (Borash *et al*. 1998), and is unlikely to have been seen in populations with a slightly different maintenance regime but subjected to the same major selection pressure.

Essentially, the life-history in an equilibrium population, which long-term laboratory-adapted populations seem to be, can be viewed as being a multiarmed seesaw with the arms representing various life-history-related traits. The arms are weighted by the trait correlations to fitness, and are connected to each other in a complex many-to-many relationship, reflecting the network of genetic variances and covariances (the **G** matrix). The balance of the seesaw can change in a complex way if the weighting of even one arm is altered, and, moreover, the effect of a given change in weighting will be different for different seesaws. In selection experiments, one has the ability to investigate, and ultimately piece together the causes of, a particular nonintuitive response to selection (Rose *et al*. 1996). In the majority of wild populations, the full context of subsidiary selection on parts of the life-history other than the one being studied is likely to be poorly known, rendering evolutionary predictions shaky at best and, more important, rendering it very difficult to understand why exactly a predicted response was not seen.

***Unity in ends, diversity in means*:** Very often in evolution, populations subjected to the same overall selection pressure can evolve in different ways to achieve higher fitness in the new environment, especially when adaptation to the biotic environment is also taken into account (Joshi and Thompson 1995b, 1996). However, even in single-species experiments, when a set of differentiated populations is subjected to identical selection regimes, responses to selection can be significantly affected by past selection history, and a closer examination of results from reverse-selection experiments reveals that often traits underlying fitness evolve differently across populations, even as fitness measures converge (reviewed in Teótonio and Rose 2001; Teótonio *et al*. 2002). Multiple genetic and ontogenetic pathways can be explored in the process of adaptive evolution, and thus environment, genetics and history all affect evolutionary trajectories. These myriad effects can actually be teased apart in model systems like *Drosophila*, and this is one of its main strengths.

***What you expect is not always what you get*:** Intuitive common-sense expectations of what traits should evolve under a given scenario have often proven to be wrong. Needless to say, figuring out why they were wrong has led to a clearer and more detailed understanding of the subtlety of adaptive evolution. For example, as discussed by Joshi and Mueller (1996), the long-held notion that selection at high density would result in the evolution of greater efficiency of food conversion turned out not to hold in *Drosophila* cultures. What evolved instead was a combination of faster feeding and increased tolerance to metabolic waste, one or both of which actually traded off with food conversion efficiency, resulting in crowding-adapted populations that were actually less efficient at food conversion than controls. Conversely, populations that were fast developing, more efficient at converting food to biomass, and had a higher carrying capacity than controls, were actually poorer competitors because of lower feeding rate and urea tolerance (Joshi *et al*. 2001; M. Shakarad, N. G. Prasad, K. Gokhale, V. Gadagkar, M. Rajamani and A. Joshi, unpublished manuscript). This is just one example in which detailed study of populations subjected to laboratory selection not only revealed new tradeoffs, but also showed that the dominant theory precluded such tradeoffs from being considered because the possible evolutionary options in the face of crowding were limited by the logistic formulation of density-dependent selection (Joshi *et al*. 2001), highlighting the danger that models, while aiding our thinking about a problem, can also often constrain it.

While in a broad sense adaptive evolution is certainly an optimization process, the use of optimality approaches in life-history evolution has been controversial because optimality arguments tend to ignore genetic constraints, and have often been built around knowledge of phenotypic tradeoffs gained from manipulative experiments that do not necessarily mirror evolutionary tradeoffs (e.g. see Chippindale *et al*. 1993, 1994; Leroi *et al*. 1994c). A host of empirical evidence from *Drosophila* studies further suggests that simplistic notions of optimal life-histories are likely to be of little more than heuristic value. Populations selected for faster development and early reproduction evolve a smaller rather than greater larval growth rate compared to controls (Prasad *et al*. 2000), even though a higher growth rate would clearly be favoured by selection on optimality arguments. Populations maintained for several hundred generations on a three-week discrete generation cycle, wherein only eggs laid on day 12 of adult life contribute to fitness, do evolve a small peak in fecundity around that critical day. However, the high peak of fecundity around day 4 of adult life is not reduced in these populations, even though it would be clearly advantageous to save resources for egg production at day 12 (Sheeba *et al*. 2000; M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished data). Populations routinely maintained in a manner such that living beyond the first week of adult life brings no fitness return still have mean adult lifespan in excess of three weeks, suggesting that fitness components cannot be 'switched' on and off in optimal ways, a phenomenon termed pleiotropic echo by Nusbaum *et al*. (1996). Widespread sexually antagonistic genetic variation for fitness suggests that it is not likely that sex-specific optimal phenotypes are easily attained (Rice and Chippindale 2001), as do tradeoffs within and between larval and adult stages for life-stage-specific optimal phenotypes (Chippindale *et al*. 1994; Borash *et al*. 1998). In populations that have had over 600 generations to adapt to a maintenance regime in which development needs to be completed before a 14-day deadline imposed by transfer to a new bottle, a substantial number of individuals take longer than 14 days to develop, and eclose at sizes larger than the minimum size for successful development, whereas it would be advantageous for them to eclose at a smaller size and obtain representation in the breeding pool by meeting the 14-day deadline (Houle and Rowe 2003).

Clearly, even in simple situations devoid of fluctuations in the environment or selection pressures, and in the absence of competitors, predators or parasites, life-histories that are seen to evolve over hundreds of generations in *Drosophila* populations are typically not those that would have been predicted on the basis of simple optimality arguments. The reasons for this discrepancy are manifold, and include the multifaceted nature of fitness, the problems of G × E interactions, past selection history, and pleiotropic echoes. Past selection history will often influence not just trait evolution, but also the evolution of specific patterns of plasticity, epistasis, G × E interactions, and cross-generational effects/interactions which may then constrain future responses to changed selection pressures. Our inability to correctly predict clean optimal life-histories in the *Drosophila* model system, with all the detailed understanding we have of its genetics, physiology, and laboratory ecology and history, should sound a strong cautionary note to those who routinely make such predictions about wild populations.

Limitations of selection experiments

Typical *Drosophila* selection experiments involve discrete generation populations subject to strong, consistent, directional truncation selection, often with the truncation point moving as the phenotypic distributions shift in response to selection imposed on large, long-term laboratory-adapted populations (e.g. Rose 1984; Chippindale *et al*. 1996, 1997a, 1998; Prasad *et al*. 2000, 2001). In some cases, where selection is more 'natural', the environment in the form of density or temperature is defined and the population then allowed to evolve in that setting,

with subsidiary selection pressures on early-life fecundity or development time being determined by the maintenance regime (Partridge *et al*. 1994a; Joshi and Mueller 1996). The distinction between 'artificial' and 'natural' selection experiments is sometimes made (e.g. Rose *et al*. 1996; Scheiner 2002), but we believe it to be largely semantic; the consequential distinction is between selection experiments involving large versus small populations (most 'artificial selection' experiments use small populations), because selection on small populations often yields misleading results owing to inbreeding or lack of genetic variation, as discussed by Chippindale *et al*. (1997a). Often in laboratory experiments, direct selection is applied on one (e.g. development time) or a couple of related life-history traits (e.g. late-life fecundity and lifespan), although most other traits do remain subject to natural selection based on maintenance regime (Rose *et al*. 1996). Moreover, typical selection experiments are conducted in a constant environment, on rich food, and in the absence of interspecific competitors, parasitoids and other antagonists.

Partly as a result of the insight gained from selection experiments, it is now becoming clear that the framework of the typical selection experiment outlined above also delineates its limitations. Populations kept in the laboratory for a long time adapt to their culture regime and conditions, and some of these changes are increased competitive ability and early fecundity, along with reduced lifespan, and starvation and desiccation tolerance, relative to wild populations from the same collection site (Sgrò and Partridge 2000, 2001; Hoffmann *et al*. 2001b; but see also Matos and Avelar 2001). It has been suggested that some observed responses to selection for increased lifespan or stress resistance in long-term laboratory populations may, therefore, be artifacts of prior laboratory adaptation (Harshman and Hoffmann 2000; Sgrò and Partridge 2000; Hoffmann *et al*. 2001b; Linnen *et al*. 2001). Unfortunately, selection experiments on populations from the wild are also not artifact free, as the correlational structure of the life-history can be affected by the shift to a new laboratory environment (Service and Rose 1985). Moreover, the dichotomy between wild and laboratory populations is a somewhat simplistic one. Laboratory studies have shown how sensitive to small environmental differences responses to selection and the ability to detect them can be (e.g. Leroi *et al*. 1994a,b; Ackermann *et al*. 2001). Wild populations from different sources are likely to differ greatly among themselves in the genetic architecture of life-history, and at present we do not have a good feel for the degree of this variation. For example, unlike in some previous studies (Sgrò and Partridge 2000; Hoffmann *et al*. 2001; Linnen *et al*. 2001), we have found that our laboratory-adapted *D. melanogaster* populations have vastly higher fecundity, lifespan, and starvation and desiccation tolerance, compared to wild populations of four other species of the *melanogaster* and *immigrans* groups (Sharmila Bharathi *et al*. 2003). One of the problems in assessing the degree of disconnect between laboratory and wild populations is, of course, the relative lack of knowledge about the field ecology of many *Drosophila* species, including *D. melanogaster*. Data on typical densities or mortality rates experienced in the wild, for example, are extremely sketchy and often contradictory. A better knowledge of the nature of selection acting on *Drosophila* species will be required if there is to be some hope of resolving this problem.

One major aspect in which we believe wild and laboratory populations will tend to differ is in the degree of canalization of selection responses. Selection pressures, and the environmental context in which they act, are unlikely to be constant for long time spans in the wild (Harshman and Hoffmann 2000). Selection experiments have revealed that the response of different traits to selection may be canalized to varying degrees. For example, populations adapted to high larval density show faster development than controls at high but not low density (Borash and Ho 2001), whereas pupation height differences are seen at both low and high density (Joshi and Mueller 1993). Similarly, cold-adapted populations have faster development, greater larval growth rate and efficiency of food conversion, and greater body size than controls maintained at 25°C regardless of assay temperature (Partridge *et al*. 1994a,b; Neat *et al*. 1995). However, when preadult survival, fecundity and adult lifespan were assayed at 25°C and 16.5°C, the cold-adapted lines were superior when assayed at 16.5°C, and vice versa (Partridge *et al*. 1994a, 1995). Lifespan differences between lines selected for late-life fecundity and their controls are apparent over a range of adult densities (Graves and Mueller 1993), whereas early-life fecundity differences depend on assay conditions (Leroi *et al*. 1994a,b). However, so far selection responses have been compared in different environments only in a few cases, and the reaction norms of selection responses across environmental variables other than the one forming the axis of selection have not been examined. For example, how differences in larval growth rate between cold-adapted and warm-adapted lines show up across a range of larval densities is not known. We suspect that selection responses in typical laboratory conditions will not be as canalized as they may be in nature. As interest increases in the study of phenotypic plasticity as an adaptive phenomenon in its own right (Via *et al*. 1995; Schlichting and Pigliucci 1998), and as theory linking life-history evolution to the evolution of phenotypic plasticity (e.g. Kindlmann *et al*. 2001; de Jong and Behera 2002) is refined, selection experiments will become a valuable tool with which to understand the genetic architecture and evolutionary dynamics of phenotypic plasticity (Scheiner 2002).

The long-term dynamics of correlated responses to intense directional selection in typical selection experiments can also be fairly convoluted, as we have discussed earlier in the case of the 'up and down' correlated evolution of adult lifespan in populations selected for faster development and early reproduction (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished manuscript). Evolutionary correlations and tradeoffs can break down over a couple of hundred generations of selection, even in the absence of direct selection for the amelioration of a tradeoff (Phelan *et al*. 2003). In other cases, tradeoffs may become apparent only after many generations of continuing intense selection. The preadult survival cost to faster development is seen only after many generations of selection have elapsed, and size and development time have already undergone substantial reduction (Chippindale *et al*. 1997a; Prasad *et al*. 2000). Yet, a tradeoff between larval feeding rate and faster development becomes apparent within 10 generations of selection (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished manuscript). We believe that traits and tradeoffs that appear as correlated responses relatively early in selection experiments are likely to be more relevant to evolution in natural populations, compared to those that become apparent only after prolonged and intense directional selection.

Drosophila life-history evolution and selection experiments in the future

Khol kar aankhein mere aaina-e-guftaar mein
Aane waale daur ki dhundhli si ik tasveer dekh

(Behold in the mirror of my words and rhymes
A shadowy picture of the coming times)

(Sheikh Mohammad Iqbal)

Although there are clearly limitations to the usefulness of selection experiments as they have been carried out in the past, we believe that innovative and more realistic selection experiments will be extremely useful to life-history evolution studies in the post-genomics era. There are several ways in which selection experiments can be improved in light of what we have already learnt. We have identified many life-history tradeoffs, but we do not have a good feel yet for how easy or difficult it is to break such tradeoffs. Multiple-trait selection experiments could provide an empirical backdrop here to complement theoretical ideas of correlational selection (Sinervo and Svensson 2002), and comparative quantitative genetics: the study of **G**-matrix evolution (Steppan *et al*. 2002). A recent experiment in which *D. melanogaster* populations were subjected simultaneously to selection for faster development and late-life fecundity suggests that the inverse relationship between faster development and increased lifespan usually seen when these traits are selected for individually (Chippindale *et al*. 1994) can be easily overridden by selection, at least in the short term: in the first 10 generations of selection, development time was reduced by ~ 5 h whereas lifespan went up by ~ 5 days (M. Shakarad, N. G. Prasad, M. Rajamani and A. Joshi, unpublished manuscript). More such experiments, with followup studies on the underlying physiological mechanisms, will help address the issue of the stability/lability of various life-history tradeoffs.

Though logistically daunting, we believe that multifactorial selection experiments, where individual selection regimes are combinations of different levels of environmental factors like temperature, nutrition and density, will be very useful, especially in elucidating the evolution of larval growth rates and body size. Selection experiments in fluctuating environments are likely to be a useful framework for addressing issues about the evolution of cross-generational effects and interactions, canalization and phenotypic plasticity, as are more detailed studies of the reaction norms of direct and correlated responses to selection in single-factor selection experiments. Such experiments will also help provide a framework for integrating the vast body of information on genetic variation and phenotypic plasticity for morphological and stress-resistance traits in wild populations of *Drosophila* (e.g. Hoffmann and Parsons 1989; Hoffmann *et al*. 2003; Moreteau *et al*. 2003) with our understanding of *Drosophila* life-history evolution. Selection experiments on populations with overlapping generations, and equilibrium versus cycling dynamics will help understand the relationship between population dynamics and life-history evolution. In all such experiments, attention must also be paid to identifying and minimizing, as far as possible, inadvertent selection. A better understanding of selection pressures in the wild would clearly complement such studies, and assays of life-history traits as well as selection experiments in quasinatural settings may be very useful in this context. One other major dimension that needs to be added to selection experiments is the presence of antagonistic species. For example, resistance to hymenopteran parasitoids is known to exact a fitness cost in *Drosophila* under some conditions and to trade off with traits important in life-history evolution, such as larval feeding rate, adult size and starvation and desiccation tolerance (Fellows *et al*. 1999; Kraaijeveld *et al*. 2001; Hoang 2002). Similarly, the evolution of fitness in *Drosophila* competition experiments can often be competitor specific (Joshi and Thompson 1996). Life-history responses to various selection pressures are, therefore, likely to be very different in experiments with and without the presence of antagonistic species.

Developmental genetics has made great strides recently, and a new field of evolutionary developmental biology (evo-devo) has emerged. However, our knowledge of the genetic control of the timing of major events in develop-

ment, especially those relevant to life-history, is meagre compared to our understanding of pattern formation and organ development. Not surprisingly, a major thrust of evo-devo is the comparative study of developmental pathways and their genetic control, with an aim to understand the evolution of ontogenies (Arthur 2002). From the point of view of *Drosophila* life-history evolution, however, what we really need to understand is the ontogeny of life-history traits (Lewontin 2000), particularly the genetic control of the timing of key events like the duration of the postcritical-size period of larval growth: devo-evo, rather than evo-devo. We also need to know more about the plasticity/reaction norms of the ontogenies of life-history traits in response to important environmental variables like density, nutrition and temperature. Given the high extent of sequence homology seen among related, and even not so related species, one may ask in what level of biological organization species differences reside. We believe that the answer may be that many important differences between species reside in their functional architecture, and specific patterns of G × G and G × E interactions. To what extent the genetic architecture of life-histories is conserved in related species is not clear. Selection experiments on other species of *Drosophila*, similar to those done on *D. melanogaster*, may be a useful first approach to this issue, but will have to be followed up with studies on the ontogeny of life-history differences. Differences in the timing of gene expression and its sensitivity to environmental cues can, in principle, generate differences in G × G and G × E interactions among genomes similar at the sequence level. Clock genes may have a role to play here, although it is likely to be an indirect and subtle one.

Today we are crossing the threshold of the age of phenomics (Houle 2001), with an increasing ability to elucidate the structure and primary function of genomes, and a nascent but growing ability to merge these technologies with classical phenotypic approaches like quantitative genetics, and with developmental biology, physiology and ecology. We believe that this merger will lead to a more holistic approach to understanding life-history evolution. Some elements of this approach have already been used (Nuzhdin *et al*. 1997; Shiotsugu *et al*. 1997; White *et al*. 1997, 1999; French *et al*. 1998; Fellowes *et al*. 1999; Santos *et al*. 1999; Leips and Mackay 2000; Vieira *et al*. 2000; Ackermann *et al*. 2001; Jin *et al*. 2001; Pletcher *et al*. 2002; Toma *et al*. 2002; Hoffmann *et al*. 2003; Houle and Rowe 2003), and incipient integration of these elements and the development of the necessary theory is now in sight (Wagner and Mezey 2000; Houle 2001; Rice 2002; Sinervo and Svensson 2002; Steppan *et al*. 2002). It seems clear to us that selection experiments of the type discussed above will continue to be central in this new integrated approach, and *Drosophila* will remain the ideal model system to address emerging issues in this exciting coming phase of life-history evolution studies.

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