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Life-history Evolution in Laboratory Populations of *Drosophila melanogaster* Subjected to Selection for Faster Development and Early Reproduction

A Thesis

Submitted for the Degree of

Doctor of Philosophy

By

Prasad N. G.

Evolutionary and Organismal Biology Unit

Jawaharlal Nehru Centre for Advanced Scientific Research (A Deemed University)

Bangalore – 560 064 (INDIA)

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Dedicated to

My Family,

Teachers and Students

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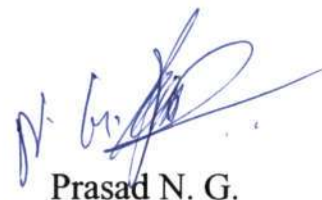
DECLARATION

I declare that the matter presented in my thesis entitled " Life-history Evolution in Laboratory Populations of *Drosophila melanogaster* Subjected to Selection for Faster Development and Early Reproduction" is the result of studies carried out by me at the Evolutionary and Organismal Biology Unit of the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India, under the supervision of Prof. Amitabh Joshi and that this work has not been submitted elsewhere for any other degree.

In keeping with the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described has been based on the findings of other investigators. Any omission, which might have occurred by oversight or error of misjudgment, is regretted.



Prasad N. G.

Place: Bangalore

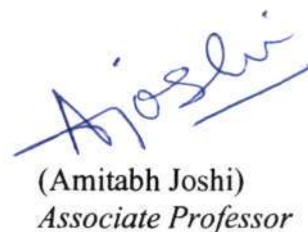
Date: September 20, 2003

Evolutionary & Organismal Biology Unit
JAWAHARLAL NEHRU CENTRE FOR ADVANCED SCIENTIFIC RESEARCH
P. O. BOX 6436, JAKKUR, BANGALORE, 560 064, INDIA

September 22, 2003

CERTIFICATE

This is to certify that the work described in the thesis entitled " **LIFE-HISTORY EVOLUTION IN LABORATORY POPULATIONS OF *DROSOPHILA MELANOGASTER* SUBJECTED TO SELECTION FOR FASTER DEVELOPMENT AND EARLY REPRODUCTION**" is the result of investigations carried out by Mr. Prasad N. G. in the Evolutionary and Organismal Biology Unit of the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India, under my supervision, and that the results presented in this thesis have not previously formed the basis for the award of any other diploma, degree or fellowship.



(Amitabh Joshi)
Associate Professor

Evolutionary & Organismal Biology Unit
JAWAHARLAL NEHRU CENTRE FOR ADVANCED SCIENTIFIC RESEARCH
P. O. BOX 6436, JAKKUR, BANGALORE, 560 064, INDIA

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(Amitabh Joshi)
Associate Professor

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List of publications

1. Prasad N. G., Shakarad M., Gohil V. M., Sheeba V., Rajamani M. and Joshi A. 2000 Evolution of reduced pre-adult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time. *Genet. Res. Camb.* **76,** 249-259.
2. Shakarad M., Prasad N. G., Rajamani M. and Joshi A. 2001 Evolution of faster development does not lead to greater fluctuating asymmetry of sternopleural bristle number in *Drosophila. J. Genet.* **80,** 1- 7.
3. Prasad N. G., Shakarad M., Anitha D., Rajamani M. and Joshi A. 2001. Correlated responses to selection on faster development and early reproduction in *Drosophila*: the evolution of larval traits. *Evolution* **55,** 1363- 1372.
4. Joshi A., Prasad N. G. and Shakarad M. 2001 *K*-selection, *a* -selection, effectiveness, and tolerance in competition: density-dependent selection revisited. *J. Genet.* **80,** 63- 75.
5. Prasad N. G., Dey S., Shakarad M. and Joshi A. 2003 The evolution of population stability as a by-product of life-history evolution. *Biol. Lett.* 03bl0037: S1-S3; DOI: 10.1098/rsbl.2003.0020.

6. Prasad N. G., Shakarad M., Rajamani M. and Joshi A. 2003 Interaction between the effects of maternal and larval nutritional levels on pre-adult survival in *Drosophila melanogaster*. *Evol. Ecol. Res.* **5**, 903-911.

7. Prasad N. G. and Joshi A. 2003 What have two decades of laboratory life-history evolution studies on *Drosophila melanogaster* taught us? *J. Genet.* 82:45-76

Summary

The life-history of an organism — the schedule of reproduction and mortality over its lifespan — is central to understanding evolution because the life-history is the interface at which a phenotype realizes its Darwinian fitness. Although offspring production occurs only in the adult life-stage, reproduction is conditional upon survival to adulthood. Consequently, the duration of, and survival during, the pre-adult stage(s) of life also have a major impact on fitness. Moreover, in many organisms, adult body size and energy reserves are largely dependent on pre-adult food acquisition and assimilation, resulting in a cascading ontogenetic and physiological linkage between pre-adult and adult components of fitness. In this thesis, I present results from a 200 generation laboratory study in which I studied direct and correlated responses to selection in four replicate populations of *Drosophila melanogaster* (FEJ populations) subjected to selection for rapid development and early (third day post eclosion) reproduction, relative to four ancestral control populations (JB populations) that were routinely maintained on a 21 day discrete generation cycle with no conscious selection on development time. The results of this study led to the identification of new tradeoffs between development time and larval behaviours related to food acquisition and utilization. Knowledge of these tradeoffs, in turn, allowed the development and experimental testing of new hypotheses about the relationship among development time, adaptations to crowding, and competitive ability in *Drosophila*, considerably altering the traditional view in this regard. Moreover, the correlated responses to selection of various adult life-history related traits observed in the FEJ populations provided novel insights into the subtlety of the process of adaptive evolution through selection, highlighting how incomplete

knowledge of genetic architecture of fitness and its behavioural and physiological correlates can severely constrain our ability to make meaningful evolutionary predictions as to the suites of traits expected to evolve under a given ecological scenario.

Over 90 generations of selection, development time in the FEJs decreased by ~ 40 hours, a 20% decline relative to the JB controls, and then did not change significantly over the next 110 generations of selection. The difference in male and female development time was also reduced in the FEJs, providing the first evidence that the development time dimorphism in *D. melanogaster* can respond to selection. FEJ flies also evolved to become substantially lighter and smaller than JB flies at eclosion, indicating a reduction in larval food acquisition and assimilation as a consequence of reduced development time. For the first 40 generation of selection, pre-adult viability did not differ between the FEJs and JBs, but thereafter the FEJs suffered an increasing viability cost to faster development became apparent, with FEJ viability being ~30% less than JBs by generation 100 of selection. Detailed examination of pre-adult traits revealed that the FEJs evolved reduced average larval growth rate (dry weight at eclosion/development time), larval feeding rate, foraging path length, digging propensity, pupation height, urea tolerance and minimum food requirement for pupation, compared to the JBs, and that reductions in the duration of the first and third larval instars and the pupal stage contributed to the overall reduction in development time, whereas ~ 90% of the increased pre-adult mortality in the FEJs was due to larval mortality. These last results differ from observations made in a similar study on populations that share a common ancestry with the FEJs and JBs. In that study, faster developing populations did not differ in pupal duration from controls, and the mortality cost of rapid development was spread over the

larval and pupal stages. I suggest that these differences are due to selection in that study being for extremely early reproduction, within 24 hours of eclosion, and postulate that in the FEJs, some aspects of sexual maturation are postponed till after eclosion, allowing a reduction in pupal duration and an easing of the pupal mortality cost. This hypothesis is supported by the observation that the time from eclosion to first mating in the FEJs evolved to become greater than that in the JBs.

Simple optimization arguments would predict that the FEJs should evolve higher larval growth rates to partly compensate for their reduced size at eclosion, given that they are also under selection for early reproduction, relative to controls. However, data on larval weight gain over ages in the FEJs and JBs revealed that the FEJ rates of weight gain during the third instar were significantly lower than JBs, suggesting some constraint on growth rate increase in these populations. Comparison of the FEJ larval traits with those earlier seen in populations adapted to larval crowding revealed that the suites of traits that evolve under selection for faster development and selection at high larval density are opposite, rather than similar as was widely believed among *Drosophila* workers. The evolution of lower larval feeding rate and urea tolerance led us to make a novel prediction that faster development should lead to the evolution of reduced competitive ability in *Drosophila*, and a competition experiment showed that the FEJs were in fact markedly less competitive than JBs.

Given the selection for faster development and reproduction at day three post eclosion, the optimal life-history for the FEJs would be to develop fast and then raise early life fecundity, even at the expense of longevity which is uncorrelated with fitness after three days of adult life in the FEJ maintenance regime. Study of adult traits revealed

that the FEJs did evolve toward this optimal life-history during the first 30 generations of selection. In this phase of selection, FEJ females produced more eggs per unit body weight early in life than the JBs, thus partly compensating for their reduced size, and also had lower longevity than the JBs, reflecting the cost of increased allocation of energy reserves to reproduction early in life. By generation 70 of selection, however, the FEJs appeared to have paradoxically evolved back toward a maladaptive life-history, as FEJ females produced less eggs than the JBs early in life, but lived as long, even though lifespan beyond the third day post eclosion is irrelevant to FEJ fitness. I postulated the existence of a physiological switch that alters relative allocation of reserves to reproduction versus somatic maintenance, and is sensitive to lipid content. Such a switch would be adaptive in a fluctuating nutritional environment and could have evolved in the wild in the ancestors of these flies. If such a switch exists, then it is possible that between generations 30 and 70 of selection, lipid content in the FEJs fell below a threshold, triggering an allocation bias toward somatic maintenance rather than reproduction. I found that the FEJs at generation 125 had lower absolute and fractional lipid content than JBs, but showed greater starvation resistance per unit lipid, which is consistent with the hypothesis of the lipid switch becoming a maladaptive constraint to the attainment of the optimal life-history in the FEJs.

The results on FEJ life-history traits, thus, highlight the subtlety of the evolutionary process, and the potential pitfalls of making evolutionary predictions without a knowledge of the genetic architecture of fitness related traits in a given ecological scenario. The thesis also discusses results from studies on the FEJs and JBs that test hypotheses about directional selection and developmental instability, the

evolution of population stability as a by-product of life-history evolution, and the possible impact of maternal nutritional environment on the effect of offspring nutritional levels on their fitness.

Chapter 1: Introduction

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 & Sibly, 1991; Reznick & Travis, 1996; Rose & 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, behavioral 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 with one another, and how they 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 actually evolve, or going into the details of the underlying physiology or genetics.

All else being equal, the fittest life-history would be one in which an organism has an infinitesimally small pre-adult stage, followed by an infinitely large reproductive output over an infinitely long life span. The fact that such life-histories are not seen in nature suggests that there are constrains on the evolution of life-histories. It has been suggested that for any given organism, time and resources are limiting, and the functions

of growth, maintenance, and reproduction compete for these limited resources (Gadgil & Bossert, 1970). Increased investment of resources in any one of these functions would, consequently, lead to a reduced investment in the other functions. Thus, the finiteness of time and resources are expected to lead to trade-offs between various components of the life-history and traits related to them. Over the last thirty years, a large number of life-history related trade-offs have been identified (Stearns, 1992), and most of these concern present reproduction versus future reproduction or survival. In holometabolous insects like *Drosophila*, the trade-offs can span different life stages (Chippindale *et al.* 1996, 1998), thereby creating a complex web of correlations such that selection acting on any one part of the life-history is likely to impinge upon many traits in other life-stages as well.

Pre-adult duration, the time spent prior to the attainment of reproductive maturity, and survival during this stage are important life-history traits (Stearns, 1992). The importance to fitness of survival till the attainment of reproductive maturity is obvious. The principal benefit of a shorter development time (earlier age at reproductive maturity), on the other hand, comes from a consideration of population growth in age-structured populations. Short pre-adult duration is associated with higher fitness simply because it results in a greater turnover rate of generations due to greater value of the Malthusian parameter r. All else being equal, an organism with a faster turnover of generations has higher fitness compared to organisms with a slower turnover of generations. An additional benefit of shorter development is due to the fact that faster developing organisms have a greater probability of survival to maturity (Bell, 1980). There are also at least two major predicted costs to faster development.

1. In the ecological literature, faster pre-adult development has been associated with greater foraging, which can increase the risk of predation, thereby reducing juvenile survival (Rowe & Ludwig, 1991; Werner & Anholt, 1993; Abrams *et al.* 1996).
2. Many mathematical models assume a positive correlation between development time and adult size at the attainment of reproductive maturity (Werner, 1986; Rowe & Ludwig, 1991; Sibly *et al.* 1991), and between adult size at maturity and early life fecundity (Stearns, 1992). Hence faster development may reduce body size and early life adult fecundity. If the benefits of increased early life fecundity are large enough to offset the losses due a slightly longer generation time, a longer pre-adult duration can evolve under natural selection. Similarly, if development time is positively correlated with life-time reproductive output, then, faster development would entail a long term fitness cost.

Given the myriad possible costs and benefits associated with development time, it is not surprising that organisms exhibit a wide variation in the pre-adult duration.

Complex life cycles such as those found in holometabolous insects like *Drosophila* exhibit an interesting orchestration of life-history traits. Since insects form the majority of all living organisms and pterygotes form a majority of all insects, complex life cycles are extremely common in nature. One important feature of complex life cycles is the abrupt shift in the ecology or way of life in different life-stages. For example, in *Drosophila*, the larvae are found burrowing in food and are essentially semi-aquatic whereas the adults are free-flying. These two stages are separated by a pupal stage that is sessile and relatively well insulated from the environment within a puparium. Hence, the organisms with complex life cycles face the problem of adapting to different

environments at each stage of the life cycle. The pre-adult duration in holometabolous insects mainly consists of the larval and pupal stages and the duration between the eclosion of an adult from the puparium and its attaining sexual maturity is relatively short. Again, the variation in the pre-adult duration across taxa of insects is very high as exemplified by the cicada which takes 17 years to mature, and the fruit fly which can complete development within ten days. It has been already stated that trade-offs in insects can span different life-stages. Trade-offs can also be expected between fitness components within a given life-stage. Hence, pre-adult duration in insects probably evolves as a resolution of these various trade-offs, both within and across life-stages.

Fitness costs to faster development may also be intrinsic to the organism, in addition to costs mediated through ecological factors like predation. For example, Chippindale *et al.* (1997a) selected replicate populations of *Drosophila melanogaster* for faster development and observed decreased pre-adult survivorship in these populations. Similarly, in a much larger study involving demographically selected populations, development time and pre-adult growth rate were found to be negatively correlated with pre-adult survivorship (Chippindale *et al.* 1994). Though the exact causes of such intrinsic fitness costs to faster development are not known yet, the fact that such costs exist is significant.

Pre-adult duration is also claimed to be correlated with adult size and hence adult fitness. In holometabolous insects, adults are post mitotic they do not grow in size after eclosion, although changes in weight can occur. Adult size in insects is, thus, largely determined by larval resource acquisition. In some studies in *Drosophila*, adult size has been found to be positively correlated with adult fitness. Larger female flies have greater early

fecundity compared to flies selected for smaller body size, even though lifetime egg production does not differ between the two (Hillesheim & Stearns, 1992). Larger male flies are reported to mate more often in the wild (Partridge *et al.* 1987 a; Santos *et al.* 1988), and male mating success is seen to be positively correlated to body size in some laboratory studies as well (Partridge *et al.* 1987b). Female fecundity is also seen to be correlated with larger body size in *Drosophila* (Robertson, 1957; Mueller, 1985).

However, the relationship between adult size and fitness in *Drosophila* is not all that unequivocal. A large number of studies have failed to find a correlation between adult size and fitness (Santos *et al.* 1994; Zamudio *et al.* 1995; Santos, 1996; Joshi *et al.* 1999; da Silva & Valente, 2001), and it has been suggested that the degree of size variation in a population affects the correlation between size and fitness traits (Joshi *et al.* 1999; da Silva & Valente, 2001; Prasad & Joshi, 2003). Moreover, the correlation between development time and adult size itself is not clean. Some recent studies in which faster development in *D. melanogaster* was directly selected for did yield a correlated decrease in adult size (Zwaan *et al.* 1995a; Nunney, 1996). However, in populations of *D. melanogaster* selected for different ages at reproduction, development time evolves as a correlated response without accompanying changes in adult size (Chippindale *et al.* 1994). It has also been found that the correlation between development time and adult size is at least partly mediated through diet (Robertson, 1963). One likely cause for differing results about the relationship between development time and size is the division of the larval stage of *Drosophila* into a pre-critical size and post-critical size stages. Increased duration of pre-critical stages of growth can increase the development time without a correlated change in size, whereas increased duration of post critical stages

leads to concomitant changes in adult size. Changes in the larval growth rate in the final stage of growth can also increase adult size without increasing development time (Robertson, 1963), but need not necessarily do so (Santos *et al.* 1997).

Irrespective of whether development time and adult size are correlated or not, the importance of larval resource acquisition for adult fitness cannot be denied. Certain insects like silk moths and may-flies completely lack adult feeding, and all the energetic resources required for reproduction have to be sequestered during the larval stage. Similarly, a study of blow-flies selected for the ability to lay fertile eggs in the absence of protein diet for the adults revealed that adult reproduction was bolstered by resources carried over from the larval stage (Nicholson, 1957). Even in *Drosophila*, the evolution of adult starvation and desiccation resistance is known to critically depend upon larval resource acquisition. Larvae of populations selected for starvation resistance assimilate lipids at a higher rate while the larvae of populations selected for desiccation resistance assimilate glycogen at a higher rate, and these larvae also show higher larval growth rates and development time (Chippindale *et al.* 1996, 1998). Similarly, populations adapted to larval crowding are known to have evolved higher rates of lipid assimilation than controls (Borash & Ho, 2001).

Evolution of development time in *Drosophila* has also been of interest for a long time because of its ecology. Many *Drosophila* species in the wild inhabit ephemeral habitats like rotting fruits. Moreover, *Drosophila* larvae in the wild often live at sub-optimal, relatively high densities (Atkinson, 1979; Nunney 1990). It was suggested that both high density and the ephemeral nature of their habitat, favour the evolution of faster development in *Drosophila* species (Bakker & Nelissen, 1963). This view was further

bolstered by the observation that a number of earlier attempts to select for decreased development time in *Drosophila* failed to elicit any response (Sang & Clayton, 1957; Clarke *et al.* 1961; Sang, 1962). Hence it was hypothesized that due to a long history of directional selection for faster development in the wild, additive genetic variance for development time had been exhausted in *Drosophila*. This view of the evolution of development time in *Drosophila* also led to a general belief that the evolutionary outcomes of selection for faster development and adaptation to larval crowding should be largely similar (Tantawy & El-Helw, 1970; Wilkinson, 1987; Santos *et al.* 1988; Prout & Barker, 1989; Partridge & Fowler, 1993; Borash *et al.* 2000).

Given the foregoing discussion, it seems clear that there are sound theoretical and empirical grounds for treating pre-adult duration as a very important life-history trait in *Drosophila*. The studies reported in this thesis were conducted in an attempt to understand the evolution of the life-history of *D. melanogaster* populations in response to directional selection for faster pre-adult development and early reproduction, a selection pressure favouring a rapid turnover of generations. Not only do the results from the experiments reported here shed light on how various pre-adult and adult traits evolve in response to such selection, they also illustrate many conceptual issues relating to the subtler intricacies of the process of adaptive evolution that are of general interest in evolutionary biology.

Chapter 2: The Experimental System

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 the effects of such manipulations on life-histories through either phenotypic manipulations, or long-term selection experiments. Moreover, such experiments can then be followed up with behavioral, physiological and genetic studies of the mechanisms underlying observed changes in the life-history, whether phenotypic or evolved (Partridge & Barton, 1993; Rose *et al.* 1996; Joshi, 1997a; Mueller, 1997; Gibbs, 1999; Zwaan, 1999; Prasad & Joshi, 2003). In this chapter, I briefly describe the life-cycle of *D. melanogaster* in our laboratory at 25°C temperature, 90% relative humidity and constant light. I also discuss the strengths of laboratory selection experiments and then give a detailed account of the maintenance protocol used in our laboratory for the populations on which the experiments described in this thesis were carried out. *D. melanogaster* is a holometabolous insect and hence has distinct life stages such as egg, larva, pupa and adult (imago). Females lay eggs when suitable substrates are provided to them. The eggs hatch about 18 hours after laying, though in the absence of suitable substrates for oviposition, the females are capable of laying eggs in more advanced stages of development, which then hatch much earlier. The larval stage consists of three instars. The first and the second instar are about 24 hours each in duration, whereas the third instar lasts for nearly 48 hours. During the early third instar stage, the larvae attain a certain minimum size called the critical minimum size at which point a commitment is

made to pupation, although the time lag between attainment of minimum size and pupariation is considerably large. The pre-critical period of growth in *Drosophila* can be extended by manipulating the nutritional environment, but the duration of the post critical stage of growth seems to be fixed for a given strain of flies (Robertson, 1963). The critical minimum size is about half the final size that a well-fed larva can attain and to a large extent determines the adult size at eclosion. At some point, late third instar larvae stop feeding, emerge out of the food and wander around prior to pupariation. On finding a suitable place, the larva becomes quiescent, empties the gut of its contents, everses the anterior spiracles and finally forms a puparium. The pupal stage is a major developmental stage and forms the transitional stage between a semi-aquatic larva and a free-flying adult. The pupal stage lasts for about four days, after which the adult ecloses out of the puparium. The young adult then takes about 8-10 hours to start sexual activity. Males and females can start mating within 10 hours of eclosion. The females can start laying fertile eggs within one day of eclosion. The peak of fecundity is typically reached around 3 - 4 days after eclosion and declines after remaining at that level for a few days. The adult life span of *D. melanogaster* in our laboratory is 30 - 35 days.

LABORATORY SELECTION EXPERIMENTS

Laboratory selection experiments have been defined as "..experiments in which populations are cultured in the laboratory so that selection proceeds under defined, reproducible conditions.." (Rose *et al.* 1996). The advantages of laboratory selection experiments are many and have been exhaustively discussed earlier (Rose *et al.* 1996; Joshi, 1997a; Prasad & Joshi, 2003) and I merely summarize some of them here. A great degree of control over environmental factors is possible in

laboratory experiments, and the ecology of laboratory cultures can be greatly simplified, permitting the experimenter to impose a specific selection pressure of his or her choice, and to a great extent avoid the confounding effects of multiple selection pressures that one is likely to encounter if one were to study evolution in the wild. This, however, does not mean that laboratory selection experiments are free of such problems, but only that the experimenter has relatively more freedom in deciding the exact selection pressures that he wants to apply and that with some creativity and careful forethought, this objective can be largely achieved. However, this benefit also leads to the artifact that most laboratory selection experiments yield highly environment-specific responses, and the canalisation of selection responses is, perhaps, much lower than it would be in populations evolving in more complex environments (Prasad & Joshi, 2003).

Population size is an important consideration in any study of evolution. Populations of small effective size are susceptible to inbreeding depression and artifactual genetic correlations (Rose *et al.* 1996). Selection studies done with small sample sizes, consequently, often fail to elicit a response to selection, and can give rise to inferences about the genetic architecture of fitness that are not really valid for large outbreeding populations. Large, random mating populations that are typically used in laboratory selection experiments can partly overcome this problem. Genetic drift, the random changes in allele frequencies due to sampling errors, can also potentially lead to spurious or artifactual correlations among fitness related traits. The problem of drift can be overcome in laboratory selection experiments in two ways: by using large populations and replicating these large populations. The magnitude of random genetic drift in large

populations is low, hence the chance of finding a spurious correlation among fitness related traits is low. Moreover, by looking for consistent patterns in the evolutionary trajectory of each of the replicate populations, one can reasonably rule out the chances of drift being responsible for the observed response to selection.

One of the great advantages of laboratory evolution is the possibility of having matched controls (or freezing the ancestral populations, if one works with bacteria). Maintaining matched controls to the selected lines which are maintained under similar conditions as selected lines, except for the specific selection pressure applied, is of vital importance to the interpretation of results. Such ideal controls can usually be hoped for only in laboratory studies. Maintenance of replicate populations and matched controls also allows for rigorous statistical analysis of the data.

Moving flies to new environments, either for starting a selection experiment or to assay traits, can give rise to novel environment effects (Service & Rose, 1985; Rose *et al.* 1996). Loci that were not under selection in the previous environment may now experience selection and hence the genetic equilibrium can be disturbed. Starting a selection experiment from flies adapted to a given environment seems to be a good solution, although one needs to be aware that evolutionary results of the same selection regimes on flies previously adapted to a different environment could be quite different (Harshman & Hoffmann, 2000). Laboratory experiments also permit the careful matching of assay and selection environments, which is important to reducing the likelihood of the evolved responses to selection getting obscured during assays due to G×E interactions (Leroi, 1994a,b).

EXPERIMENTAL POPULATIONS

The study reported in this thesis was done on eight populations of *D. melanogaster*, of which four served as ancestral controls and four were subjected to selection for faster development and early reproduction, relative to the controls. The control populations employed here were the four populations (JB_{1-4}) first described by Sheeba *et al.* (1998). The JB populations are maintained in incubators on a 21 day discrete generation cycle at 25 ± 1 °C, about 90% relative humidity, and constant light, on banana-jaggery food (Table 2.1). The larval density is regulated at about 60-80 larvae per 8 dram vial (9 cm height × 2.4 cm diameter) with 6 ml of food. The number of breeding adults is about 1800 per population and the adults are maintained in Plexiglas cages (25 cm × 20 cm × 15 cm) with abundant food provided in a petri plate. Eggs are collected from these flies by placing fresh petri plates with food into these cages for 18 hours. The eggs collected off these food plates are then dispensed into 40 vials at a density of 60-80 eggs per vial. On the 12th, 14th and the 16th day after egg collection the eclosed flies are transferred into fresh food vials and on the 18th day after egg collection all the eclosed flies are collected into Plexiglas cages containing a petri plate of food to which a generous smear of yeast-acetic acid paste has been applied. Three days later eggs are collected for the next generation. The four JB populations are ultimately descended from a single population of *D. melanogaster* (about 450 generations prior to setting up the study described here) — the IV population described by Ives (1970). The immediate ancestors of JBs are the UU populations described by Joshi and Mueller (1996), that had been maintained for over 100 generations on a 21 day discrete

generation cycle at 25°C and constant light. The four JB populations, therefore, had been independent evolutionary entities for over 450 generations and had been on a three week cycle for over 100 generations at the time the present study was initiated.

The four populations selected for faster development and early reproduction were derived from the four JB populations and are designated as FEJ_{1-4} (F- faster development; E- early reproduction; J- JB derived) (Prasad *et al.* 2000). Each FEJ population was derived from one JB population; thus, selected and control populations bearing identical numerical subscripts are more closely related to each other, than to other populations with which they share a selection regime (JB_i and FEJ_i are more closely related than JB_i and JB_j or FEJ_i and FEJ_j; i, j = 1-4). Consequently, control and selected populations with identical subscripts were treated as random blocks in all statistical analyses.

The selected populations are maintained on a regime similar to the JB populations except that 80 vials of 60-80 larvae are collected per population and monitored closely for eclosions once the pupae begin to darken. The first 15 or so flies that eclose in each vial are collected into Plexiglas cages with abundant food and a generous smear of live yeast-acetic acid paste. Typically, the breeding adult number is about 1000-1200 per population. Eggs are collected from these flies on the 3rd day after eclosion by placing fresh petri plates into these cages for 1 hour. The eggs are then dispensed into 80 vials at a density of 60-80 eggs per vial. Thus, selection is essentially on (a) the total egg to eclosion development time and (b) on fecundity at an adult age of about two and a half days post-eclosion. The major differences between the FEJ and JB populations are that (a) eggs are collected from

the FEJs the third day post-eclosion to initiate the next generation, whereas in JBs the eggs are collected nine to ten days post-eclosion, (b) the egg lay window for FEJ is one hour whereas for JB it is 18 hours, (c) in FEJs only the first 25% of the flies to eclose have an opportunity of contributing to the next generation, whereas in JBs all the flies that eclose by the 12th day after egg collection, by which time all viable individuals would have eclosed at the moderate density used, can contribute to the next generation, and (d) the number of breeding adults in FEJs is about 1000-1200 whereas in JBs it is about 1800.

COLLECTION OF FLIES FOR ASSAYS

Imposition of different maintenance regimes in laboratory selection experiments can induce non-genetic parental effects. Consequently, all selected and control populations were maintained under common rearing conditions for one complete generation prior to assaying different phenotypic traits, in order to eliminate all such non-genetic effects. Eggs were collected from the running cultures and dispensed into vials with about 6 ml of food at a density of 60-80 eggs per vial. On the 12th day after egg collection, by which time all normally developing individuals would have eclosed, the flies were collected into Plexiglas cages with abundant food. The adult numbers were usually around 1200-1800 per population. They were supplied with live yeast-acetic acid paste for two days prior to egg collection for assays. The progeny of these flies, hereafter referred to as standardized flies, were used for the various assays described in this thesis. Eclosion of the assay flies from the selected and control standardized populations was synchronized by staggering the egg collections from standardized flies by the developmental time difference between the selected and control populations, in order to

be able to set up adult trait assays of FEJs and JBs on the same day with freshly eclosed flies.

Table 2.1. The composition of 1 litre of regular banana-jaggery food (used in the maintenance of selected FEJ and control JB populations).

Ingredient	Amount
Banana (g)	205
Barley flour (g)	25
Jaggery (unrefined cane sugar) (g)	35
Yeast (g)	36
Agar (g)	12.4
Ethanol (ml)	45
Water (ml)	180
p-Hydroxymethyl benzoate (g)	2.4

Chapter 3: Evolution of Pre-adult Traits

In recent years, much empirical work on life-history evolution has focused upon the elucidation of trade-offs between components of fitness, especially those generated by antagonistic pleiotropy, and the bulk of this work has been done on *Drosophila* species (Rose *et al.* 1987, 1996; Joshi, 1997a; Prasad & Joshi, 2003). There is now clear evidence for multiple trade-offs between components of adult fitness in *Drosophila*, for example negative effects of early reproduction upon later reproduction and adult survival/longevity (Rose, 1984; Service *et al.* 1985, 1988; Roper *et al.* 1993; Zwaan, 1993; Leroi *et al.* 1994a,b,c; Joshi *et al.* 1996), as well as between larval components of fitness, such as rate of food acquisition and the efficiency of its utilization (Mueller, 1990; Joshi & Mueller, 1996; Santos *et al.* 1997), or the rate of food acquisition and survival to eclosion, especially in the presence of nitrogenous metabolic wastes (Borash *et al.* 1998). Trade-offs between larval and adult fitness components, however, have not received as much attention, even though selection on juvenile stages in organisms with a complex life-cycle, such as holometabolous insects, can have profound effects on traits directly relevant to adult fitness (Chippindale *et al.* 1997a; Santos *et al.* 1997).

Most work on trade-offs linking larval and adult fitness components in *Drosophila* has centred around the relationship between development time, adult size and adult lifespan, and, unfortunately, studies in different laboratories have tended to yield somewhat discordant results (*e.g.* see discussion in Chippindale *et al.* 1994). Adult size at eclosion is an important fitness trait in holometabolous insects like *Drosophila*, being at the junction of the pre-adult and adult life-stages.

Adult size at eclosion is, thus, a good example of a trait that is determined largely by resource acquisition and utilization during the larval stage but exerts its effects on fitness through adult life-history components such as fecundity and lifespan. Large body size in *Drosophila* tends to be positively correlated with both male mating success (Partridge *et al.* 1987) and female fecundity (Mueller, 1985), although this correlation may hold only for relatively large size differences (Joshi *et al.* 1999). Consequently, it has been thought that there is a trade-off between faster development and adult size, and that this trade-off, in part, has shaped the evolution of larval growth rates in nature (Partridge & Fowler, 1993; Santos *et al.* 1988). In different studies on *Drosophila*, direct selection for fast development has been seen to yield correlated decreases in adult weight (Zwaan *et al.* 1995a; Nunney, 1996; Chippindale *et al.* 1997a). Selection for larger flies has also been seen to result in correlated increases in development time, but selection for smaller flies did not result in the evolution of decreased development time (Partridge & Fowler, 1993). The notion of a trade-off between fast development and adult size is also supported by quantitative genetic studies of fitness effects of chromosome inversions in *D. buzzatii* (Betran *et al.* 1998). However, subjecting *Drosophila* populations to extreme larval crowding, a scenario in which faster development is also under indirect selection because food runs out well before most larvae have finished development, does not result in the evolution of smaller body size (Santos *et al.* 1997). It is likely, therefore, that even this fairly consistently seen trade-off between fast development and adult size may be susceptible to environmental effects, especially density.

There is also some evidence of a trade-off between faster development and pre-adult survivorship. In a survey of laboratory populations subjected to varying demographic maintenance regimes, Chippindale *et al.* (1994) observed a negative correlation between development time and pre-adult survivorship. Similarly, selection for faster development was seen to result in decreased pre-adult survivorship (Chippindale *et al.* 1997a). However, in a study using flies from a different ancestry than those used by Chippindale *et al.* (1994, 1997a), no significant differences were observed in pre-adult survivorship between control lines and those selected for faster development (Zwaan *et al.* 1995a).

Given the importance of large body size due to the correlation of size and fecundity, and the trade-off between faster development and adult body size, it is reasonable to expect that, all else being equal, selection for faster pre-adult development will result in the evolution of a greater larval growth rate (*i.e.* the rate at which weight is put on during development). If such selection does not result in the evolution of a higher larval growth rate, it would indicate the existence of some hitherto unexplored constraints on the optimization of growth rate and body size. Due to the impact of body size on adult components of fitness, such constraints, if they exist, would be of considerable significance in shaping the evolution of life-histories under scenarios where faster development is selectively favoured.

It has been argued that larval crowding is one scenario where faster development is selectively favoured, and that two of the important selection pressures operating on insects whose larvae inhabit ephemeral habitats in the wild are likely to be due to overcrowding and the necessity to complete pre-adult

development relatively fast (Bakker & Nelissen, 1963). The evolution of *Drosophila* life-history traits in response to larval and adult crowding has already been studied extensively (reviewed by Joshi, 1997a; Mueller, 1997). When populations of *Drosophila* are kept at very high larval density for many generations, larval feeding rates, and the minimum food required for larvae to complete development, increase relative to controls (Mueller, 1990; Joshi & Mueller, 1988, 1996). Yet, populations maintained at high larval density do not exhibit faster development, increased larval growth rate or increased adult weight at eclosion, when assayed at moderate densities (Santos *et al.* 1997). Selection for faster development at moderate density on the other hand, results in decreased adult weight at eclosion (Nunney, 1996; Chippindale *et al.* 1997a). Unlike in the case of adaptation to larval crowding, however, not much is known about larval behaviours related to food acquisition in populations subjected to selection for faster development.

Since selection for faster development directly impinges on the pre-adult life stages, one may expect that the genetic correlations among the various traits expressed in the juvenile stages may play a major role in moulding the outcome of selection. The genetic control of larval instar duration in *Drosophila* is not, however, well understood, although it is known that the durations of the first and the second instar and the early part of the third instar can be environmentally manipulated, whereas the duration of the third instar after attainment of the minimum critical size for pupation does not easily respond to environmental manipulation (Bakker, 1961). Reduction in the duration of the larval stage in *Drosophila* is also constrained by the necessity of early third instar larvae attaining

a 'critical size' required to successfully pupate and eclose. The critical size is known to be environmentally (de Moed *et al.* 1999) and genetically (Robertson, 1963) alterable, and flies with a larger adult body size are known to often take longer to attain the minimum critical size (Robertson, 1963). Therefore, in a population where shorter development time, smaller adult body size, and lower growth rates have evolved, one may expect the evolution of shorter time to the attainment of minimum critical size and/or smaller minimum critical size itself.

Many energy costly larval behaviours connected with resource acquisition are known to have an impact on larval growth, minimum food requirement for pupation and pre-adult fitness (Joshi & Mueller, 1996; Joshi, 1997a). Consequently, the optimal levels of expression of such behaviours may be expected to depend upon the selection regime, with the relative importance to fitness of adult size (and therefore fecundity) and development time shifting the optimum in one or the other direction. Larval feeding rates (Sewell *et al.* 1975; Joshi & Mueller, 1988, 1996), the height above the medium that larvae pupate (Mueller & Sweet, 1986; Joshi & Mueller, 1993), and foraging path length (Sokolowski *et al.* 1997) are known to increase as an adaptation to larval crowding. Similarly, the propensity of larvae to dig deep into the medium is a trait that has been shown to possess ample additive genetic variance (Godoy-Herrera, 1994) and is, therefore, likely to respond to selection in laboratory.

At this time, little is known about the correlated responses of larval behaviours, or of the pre-adult life stage specific mortality rates, to selection for faster development. In one study, larvae from faster developing populations were

observed to pay a mortality cost for their rapid development after about 40 generations of selection (Chippindale *et al.* 1997a). In the only study to examine larval and pupal development time and mortality separately, Chippindale *et al.* (1997a) found that pre-adult mortality in the accelerated development time populations was evenly distributed over the larval and pupal life stages, whereas the bulk of the reduction in egg to eclosion development time was due to a shortening of the larval life stage. Larval feeding rates in the accelerated development time populations did not differ significantly from the controls after 50 generations of selection (Chippindale *et al.* 1997a), although Borash *et al.* (2000) reported increased feeding rates in these selected populations after 100 generations of moderate relaxation of selection. Pupation height in the selected populations were significantly lower than in control populations after about 50 generations of selection (Chippindale *et al.* 1997a).

In this chapter, I report results from the first 115 generations of FEJ selection, comparing the selected FEJ populations and their JB controls for egg to eclosion development time and survivorship, dry weight at eclosion, and larval growth rate. Several larval behaviours related to food acquisition and pupation were also studied. The major questions I attempt to address with these data on pre-adult traits in the FEJ and JB populations are:

(1) What is the relative contribution of the three larval instars and the pupal stage to the observed reduction in pre-adult development time and survivorship?

(2) Has the minimum critical size necessary for completing development evolved in the selected FEJ populations?

(3) Have larval feeding rates, larval digging behaviour, larval foraging path lengths and pupation height evolved in the selected FEJ populations?

Repetition of studies of correlated responses to selection is desirable because observed patterns of correlations among life-history traits are often affected by seemingly small differences in either the genetic composition of the populations used, or in laboratory protocols (Joshi & Mueller, 1996; Rose *et al.* 1996; Reznick & Ghalambor, 1999; Harshman & Hoffmann, 2000; Ackermann *et al.* 2001). The populations used by us share common ancestry with the populations used by Chippindale *et al.* (1997a) in their studies on evolution of faster development, as well as with those used for many of the studies on adaptation to larval crowding (Mueller, 1990; Joshi & Mueller, 1996). Hence, my results are amenable to comparison with these previous studies and can be used to investigate parallels between the evolutionary consequences of larval overcrowding and selection for faster development.

MATERIALS AND METHODS

Development time and survivorship assays

These assays were conducted every 10 generations during the course of selection till generation 115. Standardised flies of each JB and FEJ population were supplied with yeasted agar plates in the cages for 1 hour. Eggs were collected off these plates with the help of a moistened brush and placed in vials containing 5 ml banana food at a density of exactly 30 eggs per vial. Sixteen such vials were set up per population (8 for the generation 10 and 20 assays). Once the pupae darkened, the vials were checked every 4 hour and any eclosed adults were removed, sexed

and the time of their eclosion recorded. These 4 hourly checks were continued until three consecutive days passed with no eclosion recorded from any vial.

Life-stage specific development time and survivorship assays

After 56 generations of selection had elapsed, the contribution of different pre-adult life stages to the overall egg to eclosion development time and survivorship was assessed. Eggs of approximately identical age were collected from the standardized flies by placing a fresh food plate in the cage for 1 hour. The plate was then replaced by another food plate. After 1 hour this plate, too, was discarded and a third food plate was kept in the cage for 30 minutes. Eggs for the assay were collected from the last food plate and dispensed into vials with 5 ml of food at a density of 30 eggs per vial and incubated. Eighty five such vials were set up per population. Thirty-six hours after the mid point of the 30 minute egg collection window, five vials from each population were removed from the incubator and the larvae killed by immersion in hot water. The number of the first, second and third instar larvae in each vial was determined by looking at their mouth hooks (Ashburner, 1989). This procedure was repeated at 2 hour intervals, until 66 hours had elapsed from the mid point of the 30 minute egg collection window. From these data, median times of each molt were obtained by extrapolation. The difference between the median hatching time and the median time of first molt was taken as the duration of first instar, and so on. The five vials that were left over were used to determine pupation and development times. After the first pupa (P1 pupa, as described by Ashburner, 1989) was observed, the vials were checked regularly at 2 hour intervals. Any new pupae that had formed were scored and marked using a color pen. The two hourly observations were continued till no new pupae were formed in any of the vials for two consecutive days.

The vials were then monitored for the first eclosion. Thereafter, the vials were checked regularly at 2 hour intervals and the number of eclosing males and females recorded. These 2 hourly observations were continued until no flies eclosed for 3 consecutive days in any of the vials. From these data, I obtained mean egg to pupation development time, mean egg to eclosion development time and larval and pupal survivorship for each vial.

Dry weight assays

These assays were conducted every 10 generations, from generation 20 onward till generation 70. Freshly eclosed adults (< 2 hours post eclosion), originating from eggs laid by standardized flies, were collected, killed by freezing, dried for 18 hour at ~ 70°C and weighed in batches of 5 males or 5 females. The flies collected for the assays were reared at a larval density of 30 eggs per vial, and all flies eclosing during the eclosion peak (~ 6 hours) for each vial were collected and frozen. From these frozen flies, six batches each of males and females were chosen haphazardly and weighed for each FEJ and JB population. Data on dry weight at eclosion and egg to eclosion development time were also used to estimate mean larval growth rates for each FEJ and JB population by dividing population mean dry weight at eclosion by the mean development time.

At generation 40 of FEJ selection, the dry weight of third instar larvae, pupae and adults from selected and control lines was assayed. Eggs were collected at a density of 50 eggs per vial and 12 such vials were set up per population, of which four vials each were used to weigh third instar larvae, pupae and adults at eclosion. The third instar larvae were picked up with a moist paintbrush and were immediately frozen. They were later grouped into batches of five, without sexing, and were placed into previously weighed

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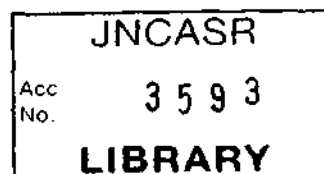
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aluminium foils, which in turn were placed in clean dry vials. Twelve such replicate vials were set up for each population. After drying at 70°C for 36 hours these were cooled and immediately weighed along with the aluminium foil. The difference in the initial and final weights of the foil yielded the cumulative dry weight of five larvae. The procedure for determining the weights of pupae was essentially the same, except that the P1 pupae were picked off the walls of the vial.

Larval weight gain profile

I assayed the larval weight gain over time (larval age) after 100 generations of FEJ selection. Eggs from standardized flies were collected off agar plates and transferred on to a petri plate containing non-nutritive agar. Twenty five freshly hatched larvae from the plates were transferred to a petri plate containing a thin layer of non-nutritive agar overlaid with 3 ml of the banana-jaggery food, prepared without adding agar to retain it in liquid form, and further diluted by 50%. Ninety such petri plates were set up per population and were randomly distributed within the incubator. Later, at intervals of six hours, four plates of each population were pulled out at random, and a total of 30 larvae from these plates were removed from the food, gently washed in water, dried on a paper towel and weighed to the nearest 0.1mg. The larvae were weighed starting from hatching till they reached the P1 pupa stage. Between 36 and 54 hours after hatching, weights were taken every three hours instead of every six hours. Only two of the four blocks were used for this assay.

Critical minimum feeding time

I assayed the minimum time of larval feeding required for individuals to successfully complete development (henceforth, critical minimum feeding time) after 48

generations of FEJ selection. Eggs from standardized flies were collected off agar plates and transferred on to a petri plate containing non-nutritive agar. Twenty five freshly hatched larvae from these plates were transferred to a petri plate containing a thin layer of non-nutritive agar overlaid with 3 ml of 42.5% yeast suspension. Thirty such petri plates were set up per population and were randomly distributed within the incubator. Later, at intervals of four hours, 10 plates of each population were pulled out at random, and a total of 150 larvae from these plates were removed from the food, gently washed in water and transferred into 10 vials containing 5 ml of non-nutritive agar at a density of 15 larvae per vial. These transfers were done at 46, 50 and 54 hours after hatching. Each vial was then monitored for pupation and eclosion.

Larval feeding rate

After 65 generations of selection, the feeding rates of FEJ and JB larvae were measured at physiologically equalized ages. This was done by collecting eggs off agar from the standardized FEJ flies 6 hours later than the JB flies. Thus, at the time of assay, FEJ larvae were 42 hours old while JB larvae were 48 hours old and consequently were in the same relative phase of their larval development. Twenty five newly hatched larvae were transferred into petri plates containing a thin layer of non-nutritive agar over laid with 3 ml of 42.5% yeast suspension. Four such petri plates were set up per population. The larvae were allowed to feed for 42 (FEJ) or 48 (JB) hours, by which time they were in the early third instar. At this point, 25 larvae from each population were assayed for feeding rates, following the procedure of Joshi and Mueller (1996), by placing them individually in a small petri plate (5 cm diameter) containing a layer of agar overlaid with a thin layer of 10% yeast suspension. After allowing for a 15 second acclimation period,

feeding rates were measured as the number of cephalopharyngeal sclerite retractions in two consecutive 1 minute intervals. Selected and control populations, matched by the subscripted indices, were assayed together, with one larva from the selected population and one from the control population being assayed alternately.

Pupation height

Pupation heights were measured after 65 generations of selection. Thirty eggs collected from standardized flies on banana-jaggery food were placed in vials (20 cm height × 2.5 cm diameter) containing 5 ml food. Once all the individuals had pupated, the pupation heights were measured, following Mueller and Sweet (1986), as the distance from the surface of the medium to the point between the anterior spiracles of the pupae. Any pupae on the surface of the food were given a pupation height of zero.

Foraging path length

After 65 generations of selection had elapsed, eggs laid by standardized flies during a 1 hour window were collected on banana jaggery food. Eggs from FEJs were collected 6 hours later than the JBs to equalize their physiological ages at the time of assay. Twenty five newly hatched larvae were transferred into petri plates containing a thin layer of non-nutritive agar over laid with 3 ml of 42.5% yeast suspension; six such plates were set up per population. Forty eight hours after transfer of JB larvae and 42 hours after transfer of FEJ larvae, the foraging path lengths were measured. A single larva was placed in the center of a petri plate containing a thin layer of agar overlaid with a very thin layer of 50% yeast suspension. A 15 second duration was allowed for acclimation. Five minutes later, the larva was removed from the petri plate and the path made by it on the yeast

suspension was traced onto a transparent plastic sheet. The lengths of these paths were later measured. Thirty larvae were assayed per population.

Larval digging behaviour

Larval digging behaviour was measured after 65 generations of selection, following the method of Godoy-Herrera (1994) with some modifications. Eggs laid over a 1 hour window were collected from standardized flies on banana-jaggery food, and 30 eggs were then placed into a vial containing 5 ml of charcoal-banana-jaggery medium overlaid with 3 ml of regular banana-jaggery medium. Ten such vials were set up per population. After 90% of the larval duration had elapsed for FEJ and JB larvae, the larvae were fixed by pouring hot water into the vials and were then taken out of the food and observed under the microscope. Larvae with charcoal stained guts were scored as 'diggers', and the fraction of diggers was calculated for each vial.

Statistical analyses

Data from all the assays were subjected to separate mixed model analyses of variance (ANOVA), treating block (ancestry of replicate population) as a random factor and selection as a fixed factor crossed with block. For pre-adult development time and survivorship, adult weight at eclosion and larval growth rate, completely randomized mixed model ANOVAs were carried out treating time (generation of assay), sex (except in the case of survivorship) and selection regime as fixed factors, crossed amongst themselves and with random blocks based on ancestry. For the critical minimum feeding time assay, the duration for which the larvae were allowed to feed before being transferred to agar vials was treated as a fixed factor crossed with selection regime and

block. All the fractional data (survivorship and digging behaviour) were arcsine square root transformed (Freeman & Tukey, 1950) before analysis. In all cases, the population means were used as the units of analysis, as the main interest in selection studies is in examining the robustness of differences over replicate populations within each maintenance regime (Rose *et al.* 1996). To the data on larval weight gain over age, I fitted the exponential equation

$$W_x = A\,e^{\alpha x},$$

where W_x is the weight of the larva at age x, A is the intercept, and α reflects the rate of increase of growth rate. The estimates of the slope parameter (α) from FEJ and JB populations were examined for differences using a one way ANOVA. All statistical analyses were implemented on STATISTICA™ for Windows Release 5.0 B (StatSoft Inc., 1995).

RESULTS

Egg to eclosion development time

I observed a strong and consistent direct response to selection on egg to eclosion development time, with the mean difference between FEJ and JB populations increasing from ~ 6 hours at generation 10 to ~ 40 hours (a 20% reduction, relative to controls) at generation 115 of selection (Fig. 3.1). Although the absolute values of development time changed considerably from assay to assay, the difference in development time between the selected and control lines underwent an almost linear increase over time, before plateauing off after about 100 generations of selection. The ANOVA revealed significant main effects of time, sex

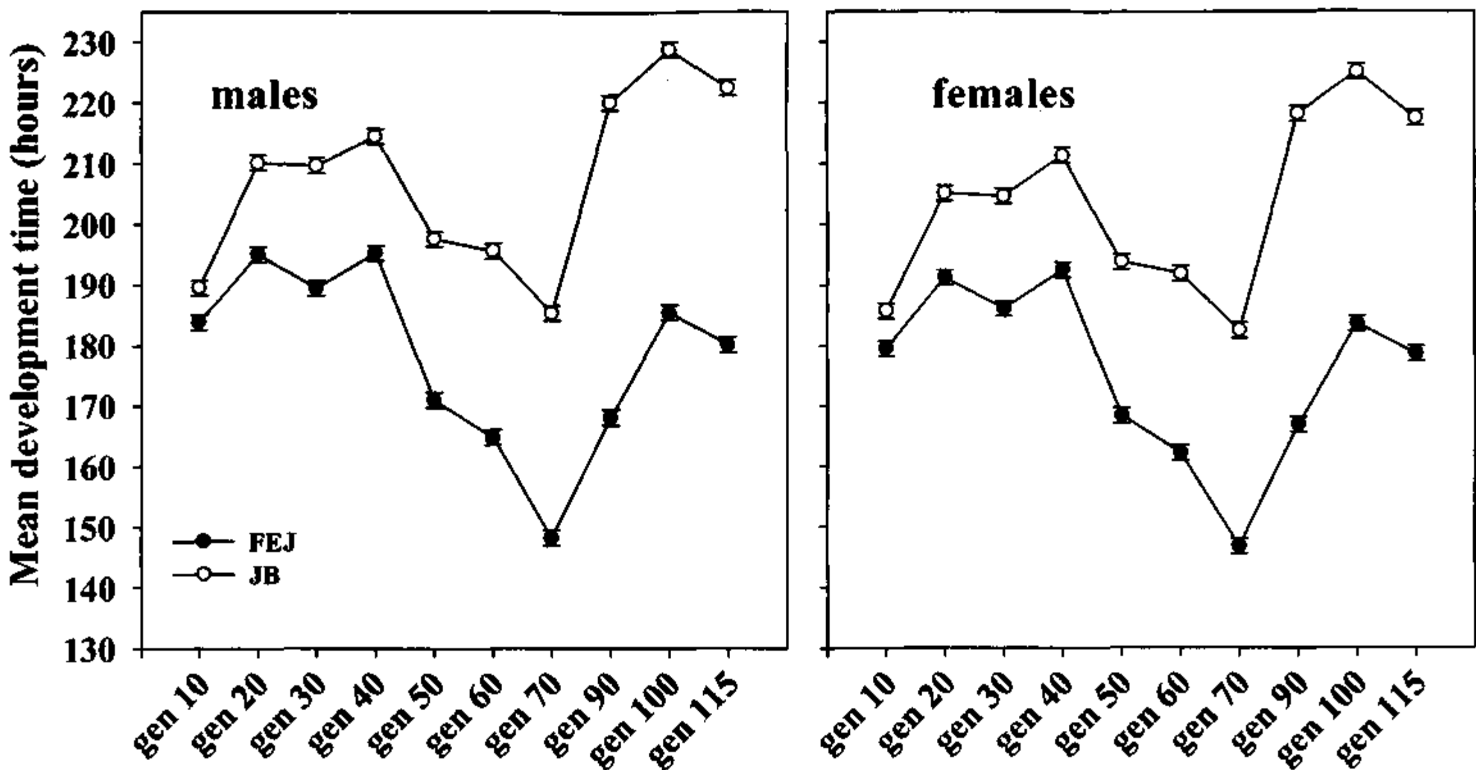

Figure 3.1. Mean egg to eclosion development time of males and females from the selected (FEJ) and control (JB) populations over the course of 115 generations of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomised block ANOVA, and can, therefore, be used for visual hypothesis testing.

(males took longer to develop than females) and selection regime, as well as significant time × sex and time × selection regime interactions (Table 3.1).

As selection proceeded, the difference between male and female mean development time was consistently reduced in the FEJ populations, declining from 4.5 hours at generation 10 to 1.6 hours at generation 115 (Fig. 3.2). Regressing the difference between males and females over time in generations revealed a significantly negative slope in the FEJ populations (slope = -0.789; $p < 10^{-5}$; R^2 = 0.62), whereas the slope in the JB populations did not differ significantly from zero (slope = -0.202; p = 0.21; R^2 = 0.041). The mean difference between males and females, averaged across selection regimes, also declined with time as a consequence of the decline in the FEJ difference, giving rise to the significant time × sex interaction in the ANOVA (Table 3.1), although the time × sex × selection regime interaction was not significant. Overall, the difference between male and female development times was less in the FEJ populations, compared to the JB controls at every generation except the tenth and the differences observed at generations 30, 70, 100 and 115 were statistically significant (Fig. 3.2).

Pre-adult survivorship

Egg to adult survivorship did not differ significantly between the FEJ and JB populations for the first 40 generations of selection, even though survivorship in the FEJ populations was consistently lower than the JB populations by ~ 0.03 (Fig. 3.3). After the fortieth generation of selection, an increasing survivorship cost to faster development became apparent, with the FEJ populations showing significantly reduced survivorship compared to the JB controls. Between generations 40 and 100,

Table 3.1. Results of analysis of variance (ANOVA) on mean egg to eclosion development time in the FEJ and JB populations. The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table. Time refers to generation of assay.

Effect	*df*	MS	*F*	*P*
Time	9	2639.79	20.7976	< 0.001
Sex	1	426.322	850.388	< 0.001
Selection regime (Sel)	1	32821.3	785.866	< 0.001
Time × Sex	9	3.42497	8.45697	< 0.001
Time × Sel	9	811.057	49.9417	< 0.001
Sex × Sel	1	15.5158	63.806	0.004
Time × Sex × Sel	9	1.06963	1.42418	0.227

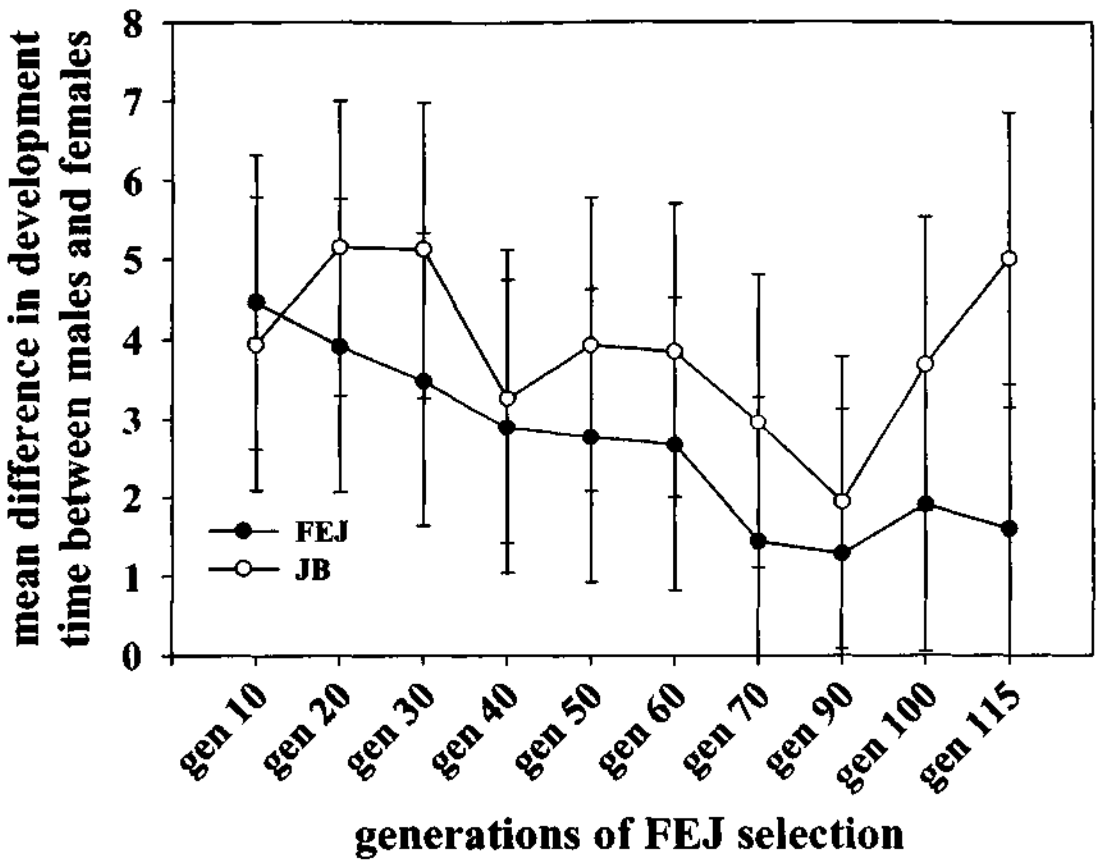

Figure 3.2. Mean difference between males and females (in hours) in egg to eclosion development time in the selected (FEJ) and control (JB) populations over the course of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomised block ANOVA, and can, therefore, be used for visual hypothesis testing.

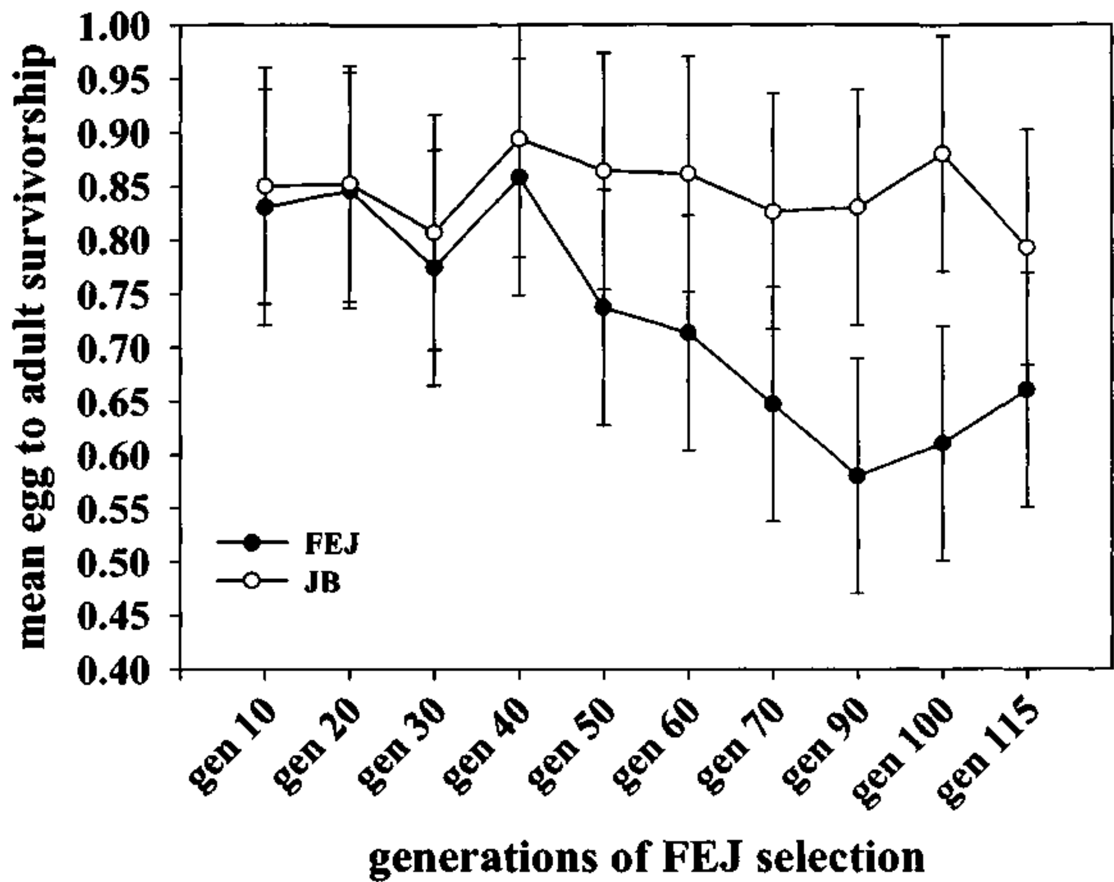

Figure 3.3. Mean egg to eclosion survivorship in the selected (FEJ) and control (JB) populations over the course of 115 generations of selection selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomised block ANOVA, and can, therefore, be used for visual hypothesis testing.

the mean difference in survivorship of the FEJ and JB populations increased from 0.035 to 0.27 (Fig. 3.3), yielding a 30% reduction in FEJ survivorship at generation 100. Pre-adult survivorship in the JB populations remained within the range 0.8 - 0.9 throughout the 115 generations of selection, which is the typical range of pre-adult survivorship in these populations and their ancestors. The ANOVA revealed significant main effects of time and selection regime, and time × selection regime interaction (Table 3.2). Regressions of pre-adult survivorship on time in generations revealed a significantly negative slope in the FEJ populations (slope = -0.69; $p < 0.0001$; $R^2 = 0.47$), whereas the slope in the JB populations did not differ significantly from zero (slope = -0.18; $p = 0.26$; $R^2 = 0.033$) (Fig. 3.3).

Life-stage specific development time and survivorship

By 50 generations of selection, overall mean egg to eclosion development time in FEJ populations had been seen to be 26 hours less than that in JB populations. The duration of the first and third larval instars, and of the pupal stage, were significantly shorter in the FEJ populations, relative to the JB controls (Fig. 3.4, Table 3.3). The duration of the second instar, however, did not differ significantly between FEJ and JB populations (Fig. 3.4, Table 3.3). The overall larval and pupal durations in the FEJ populations were shorter than in the JB populations by about16 hours (a reduction of ~15%), and 10 hours (a reduction of ~11%), respectively (Fig. 3.4). Almost 90% of the difference between FEJ and JB populations in egg to eclosion survivorship was accounted for by reduced larval survivorship in the FEJ populations (Fig. 3.5). Separate ANOVAs done on the larval and pupal survivorship data revealed a significant main effect of selection on larval, but not on pupal, survivorship (Table 3.4).

Table 3.2. Results of analysis of variance (ANOVA) on mean egg to eclosion survivorship in the FEJ and JB populations. The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table. Time refers to generation of FEJ selection.

Effect	*df*	MS	*F*	*P*
Time	9	0.03744	8.36893	< 0.000
Selection regime (Sel)	1	0.40477	121.891	0.00159
Time × Sel	9	0.02379	4.14742	0.00192

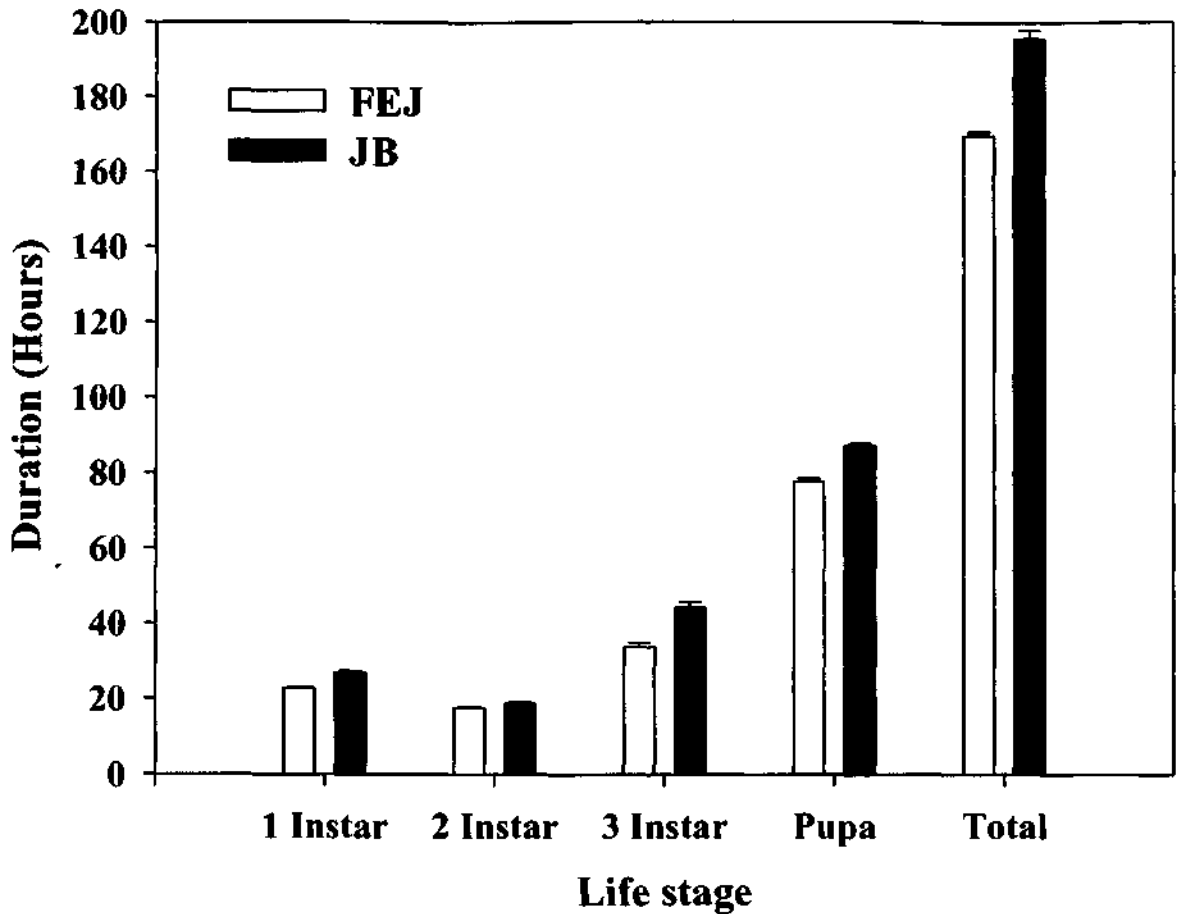

Figure 3.4. Mean duration of different pre-adult life stages in FEJ and JB populations at generation 50 of FEJ selection. The error bars represent standard errors around the mean, constructed using the variation among replicate population means within selection regimes. Total: egg to eclosion development time.

Table 3.3. Summary of results of separate ANOVAs on mean life stage specific development time. In these two-way mixed model ANOVAs, selection regime was treated as a fixed factor crossed with random blocks. In this design, only the main effect of selection regime can be tested for significance.

Stage	Effect	*df*	MS	*F*	*P*
First instar	Selection	1	32	96	0.002
Second instar	Selection	1	2	2	0.252
Third instar	Selection	1	218.052	81.192	0.003
Pupa	Selection	1	184.599	141.216	0.001
Total	Selection	1	1361.133	290.747	< 0.001

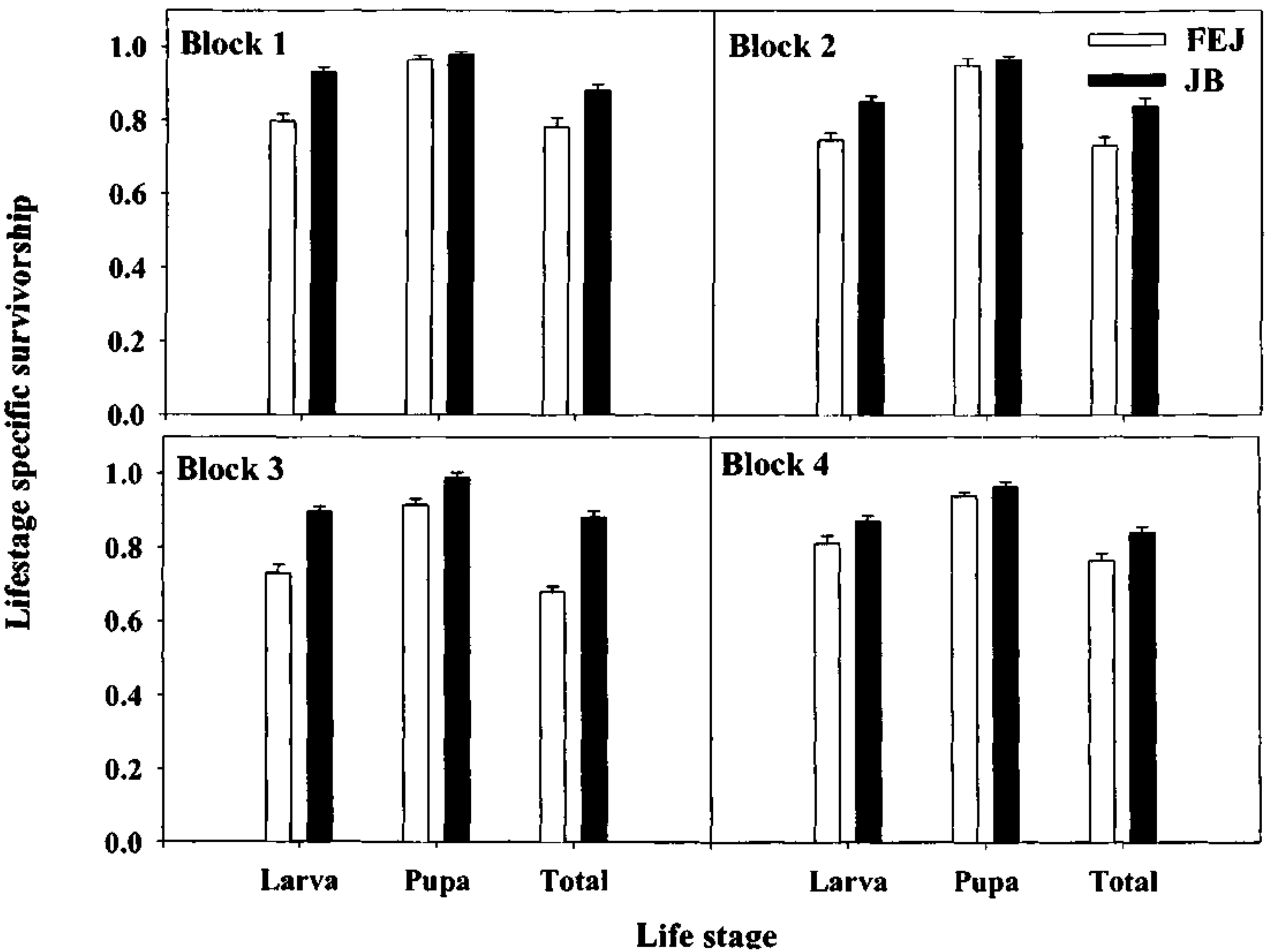

Figure 3.5. Mean life stage specific pre-adult survivorship in FEJ and JB populations at generation 50 of FEJ selection. The error bars represent standard errors around the mean, constructed using the variation among replicate vials within populations. Total: egg to eclosion survivorship.

Table 3.4. Summary of results of separate ANOVAs on mean pre-adult life stage specific survivorship. In these two-way mixed model ANOVAs, selection regime was treated as a fixed factor crossed with random blocks and, consequently, only the main effect of selection regime can be tested for significance.

Stage	Effect	*df*	MS	*F*	*P*
Larva	Selection	1	0.051	23.730	0.017
Pupa	Selection	1	0.015	5.571	0.099

Dry weight at eclosion

Dry weight at eclosion of both males and females in the FEJ populations was significantly lower than their JB counterparts from the twentieth generation of selection onward, and continued to decrease as selection proceeded (Fig. 3.6). Over the 70 generations of selection, dry weight at eclosion of FEJ flies underwent a reduction of ~ 45% in males and ~ 39% in females, relative to the JB controls. The slope of the regression of dry weight at eclosion on time in generations was significantly negative for FEJ males and females, but not significantly different from zero for JB males and females (Table 3.5). Consistent with these observations, the ANOVA revealed significant main effects of time, sex (females heavier than males) and selection regime (JB heavier than FEJ), as well as significant time × selection regime, sex × selection regime, and time × sex × selection regime interactions (Table 3.6).

Life-stage specific dry weight

Third instar larvae, pupae and freshly eclosed adults (averaged across sexes), of the FEJ populations had significantly lower dry weight than their JB counterparts (Fig. 3.7). The difference in dry weight was apparent in the third instar larvae and remained relatively unchanged through the pupal duration, even though absolute dry weights of both FEJ and JB populations changed with life stage assayed (Fig. 3.7, Table 3.7).

Larval growth rate

Interestingly, the mean larval growth rate (dry weight at eclosion divided by egg to eclosion development time) in the FEJ populations decreased relative to the JB controls as selection proceeded, with FEJ individuals putting on less weight per

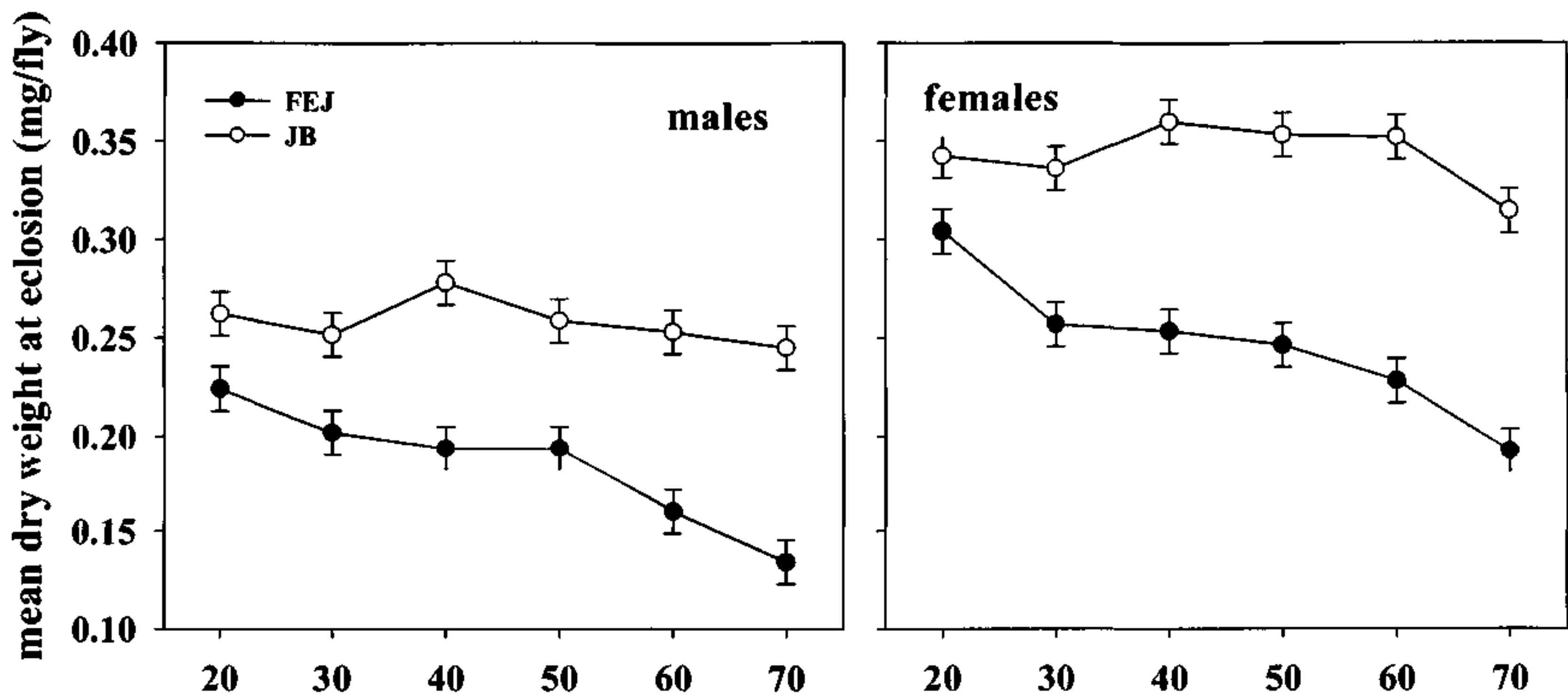

Figure 3.6. Mean dry weight at eclosion of males and females from the selected (FEJ) and control (JB) populations over the course of 70 generations of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomised block ANOVA, and can, therefore, be used for visual hypothesis testing.

Table 3.5. Summary of the results of linear regressions of mean trait value over time in generations for dry weight at eclosion (in mg) and larval growth rate (in μg/hr). Entries are the slopes of the regressions, followed by *P* values and coefficients of determination (R^2), respectively, in parentheses.

Trait	Selection Regime			
	FEJ		JB	
	males	females	males	females
Dry weight at eclosion	-0.0017	-0.0019	-0.0003	-0.0003
	(4.4×10^{-11}; 0.87)	(6.6×10^{-9}; 0.79)	(0.1357; 0.10)	(0.2388; 0.06)
Larval growth rate	-0.0039	-0.0034	+0.0019	+0.0027
	(8.7×10^{-5}; 0.51)	(0.0126; 0.25)	(0.0418; 0.18)	(0.0216; 0.22)

Table 3.6. Results of analysis of variance (ANOVA) on mean dry weight at eclosion in the FEJ and JB populations. The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table. Time refers to generation of FEJ selection.

Effect	*df*	MS	*F*	*P*
Time	5	0.00733	18.52	< 0.001
Sex	1	0.12961	1925.96	< 0.001
Selection (Sel)	1	0.17281	626.96	< 0.001
Time × Sex	5	0.00021	2.42	0.085
Time × Sel	5	0.00343	26.57	< 0.001
Sex × Sel	1	0.00312	35.11	0.01
Time × Sex × Sel	5	0.00022	4.01	0.017

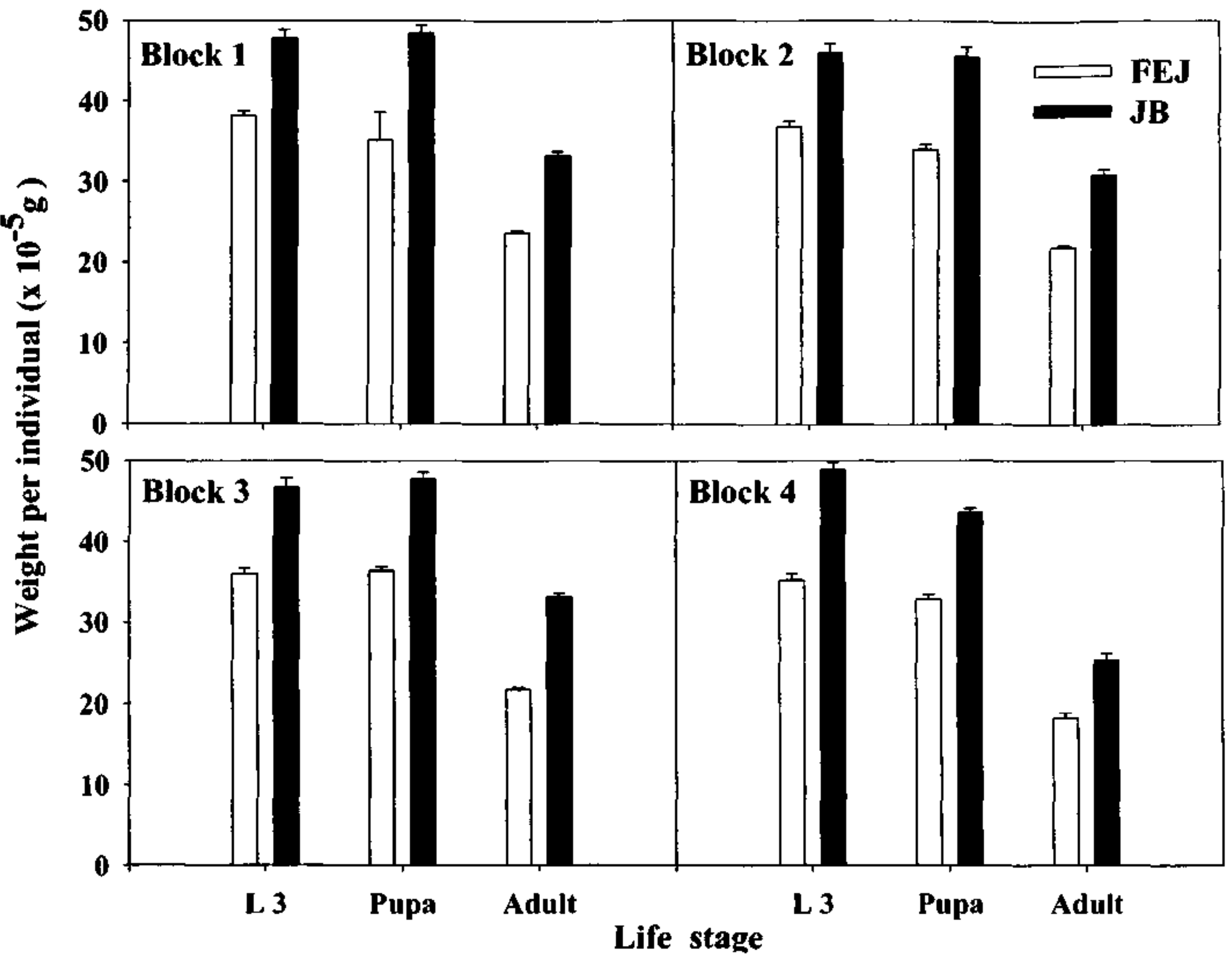

Figure 3.7. Mean dry weight of third instar larvae, pupae and freshly eclosed adults of FEJ and JB populations. The data for adults have been averaged over sexes. The error bars represent standard errors around the mean, constructed using the variation among replicate batches of five individuals within populations. L3: third instar.

Table 3.7. Summary of ANOVA results for mean life-stage specific dry weights. In this three-way mixed model ANOVA, selection regime and life-stage (third instar larva, pupa and freshly eclosed adults) were treated as fixed factors crossed with random blocks and, consequently, only fixed main effects and interactions can be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	645.922	1278.327	< 0.001
Life-stage	2	543.461	235.295	< 0.001
Selection × Life-stage	2	0.992	0.724	0.523

unit time during pre-adult development than their JB counterparts (Fig. 3.8). FEJ males had significantly lower larval growth rates than JB males from generation 40 onward, whereas difference between FEJ and JB females was significant from generation 30 onward (Fig. 3.8). Regressions of larval growth rate on time in generations revealed that the increased divergence between selected and control populations was due to declining growth rate in the FEJ populations, coupled with an increase in growth rate of the JB populations over the 70 generations of selection (Table 3.5). As in the case of dry weight at eclosion, the overall relative reduction of larval growth rate in the FEJ populations was greater for males (~32%) than females (~24%). All fixed main effects and interactions in the ANOVA were significant (Table 3.8).

Larval weight gain profile

An exponential model (weight = $A\ e^{\alpha x}$; x = age) fit data on larval wet weight at different ages up to cessation of feeding very well for both FEJ and JB populations (Fig 3.9), with R^2 values for all the populations being around 0.96. The mean (± s.e.) slope parameter (α) of the FEJ populations (0.051 ± 0.0009) was lower than that of the JB populations (0.058 ± 0.0015) and this difference was marginally significant ($F_{1,1}$ = 123.9, p = 0.057). The mean (± s.e.) intercepts of FEJs (2.59 ± 0.155) and JBs (2.37 ± 0.28) were not significantly different from one another.

Critical minimum feeding time

After feeding for only 46 hours, very few larvae survived in either FEJ or JB populations, whereas mean survivorship rose to about 0.3-0.4 for both FEJ and JB populations when larvae were allowed to feed for 54 hours (Fig. 3.10). The ANOVA

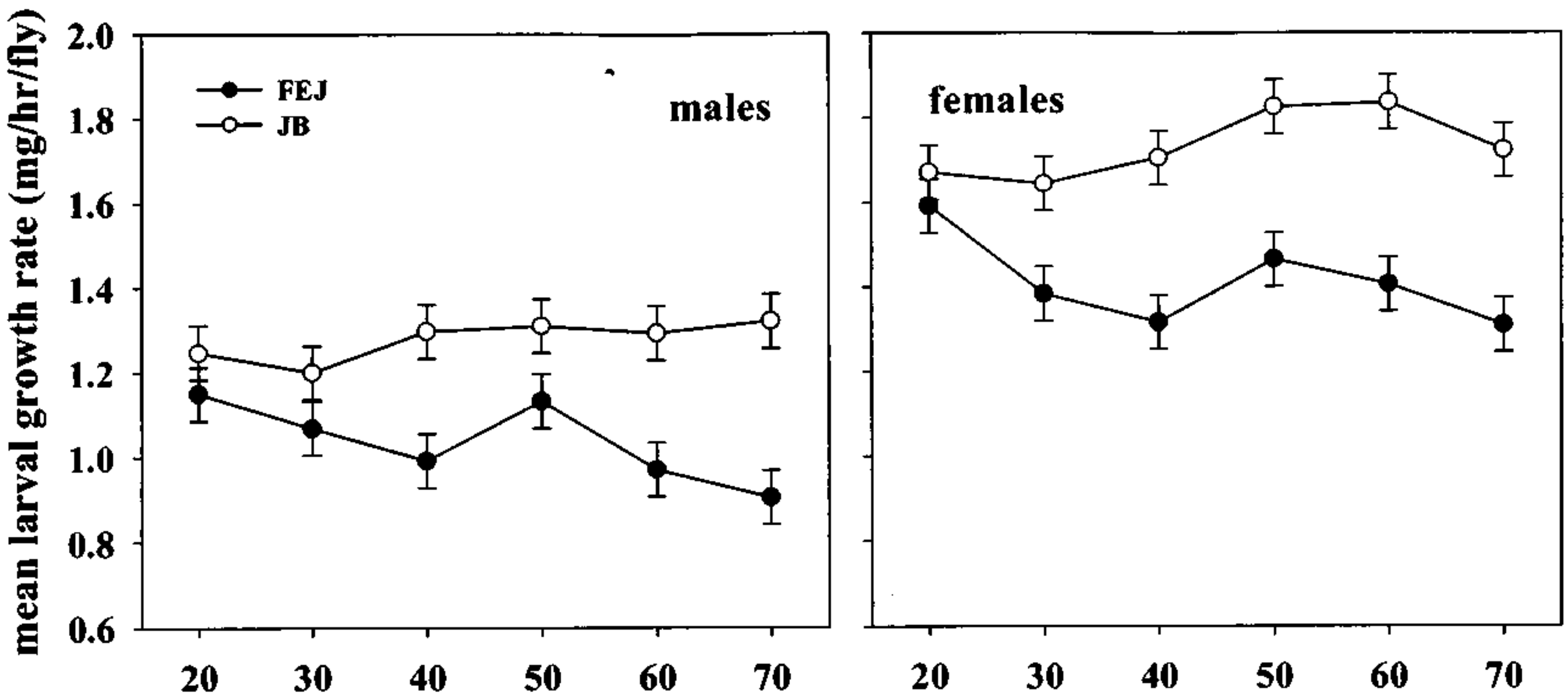

Figure 3.8. Mean larval growth rate of males and females from the selected (FEJ) and control (JB) populations over the course of 70 generations of selection for faster development and early reproduction. The error bars represent 95% confidence intervals about the mean of the four replicate populations in each selection regime, calculated using least squares estimates of the standard errors of the appropriate cell means in the randomised block ANOVA, and can, therefore, be used for visual hypothesis testing.

Table 3.8. Results of analysis of variance (ANOVA) on mean larval growth rate in the FEJ and JB populations. The effects of block and interactions involving block cannot be tested for significance in the randomized block design and have, therefore, been omitted from the table. Time refers to generation of FEJ selection.

Effect	*df*	MS	*F*	*P*
Time	5	0.04114	3.73	0.022
Sex	1	4.20059	1429.07	< 0.001
Selection (Sel)	1	1.94124	229.54	< 0.001
Time × Sex	5	0.00819	4.29	0.013
Time × Sel	5	0.0615	11.9	< 0.001
Sex × Sel	1	0.03905	13.87	0.034
Time × Sex × Sel	5	0.00613	3.41	0.03

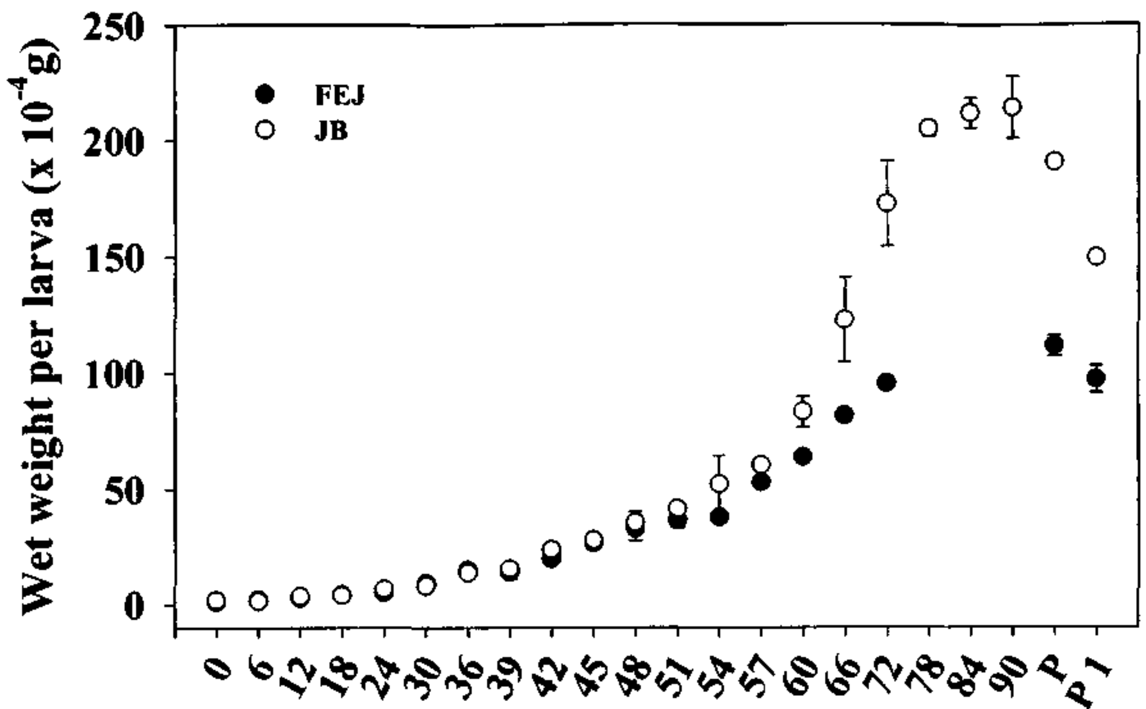

Figure 3.9. Mean wet weight of the larvae from the selected and control populations at different ages. The error bars represent standard errors around the mean, constructed using the variation among replicate populations with in selection regimes. P: Non-motile larvae about to pupate; P 1: Pupa in P 1 stage. Since the age at which larvae in FEJs pupate is less than that of JBs, the time axis is not to scale for P & P1.

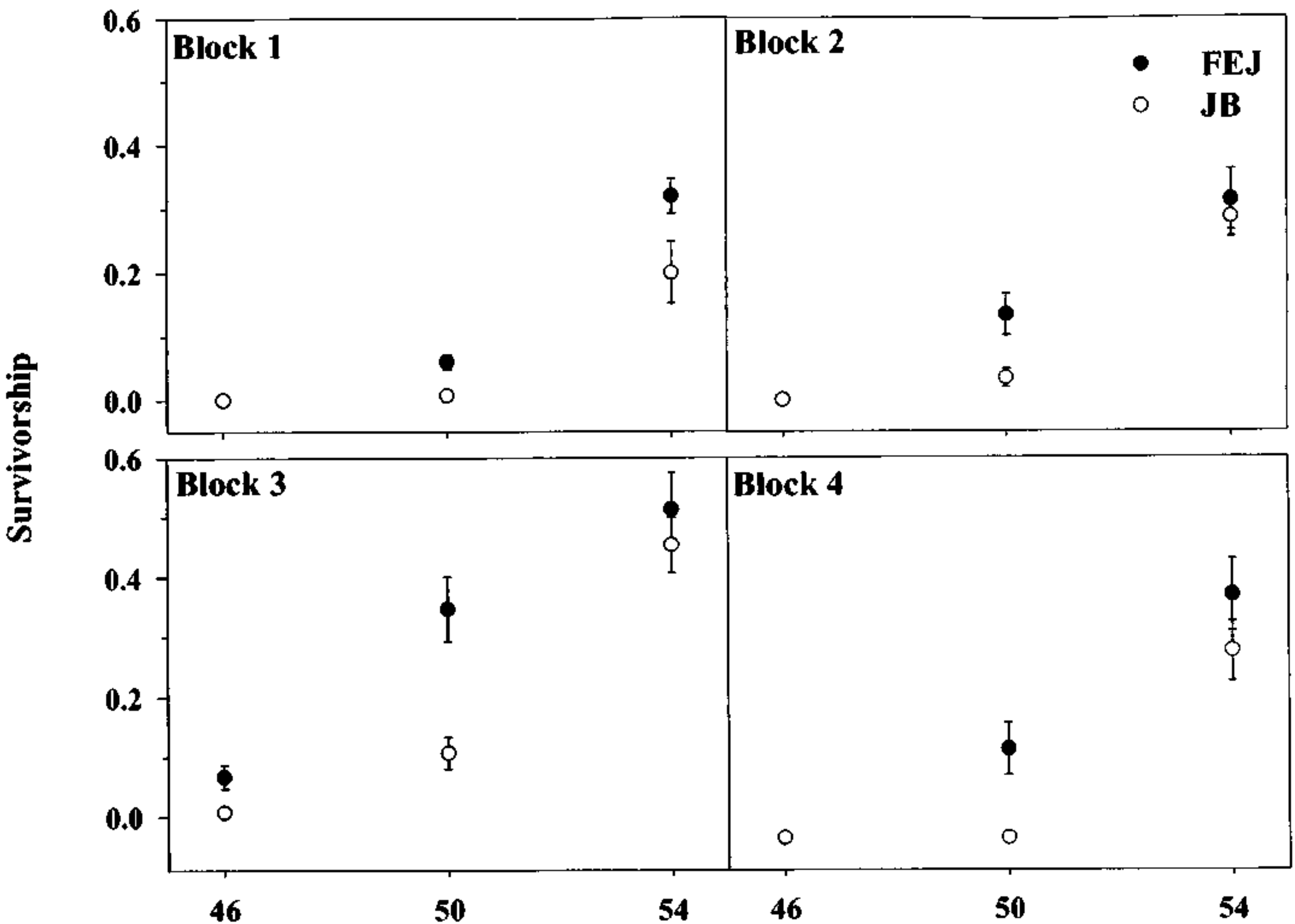

Figure 3.10. Mean survivorship of the larvae of FEJ and JB populations after feeding for different periods of time. The error bars represent standard errors around the mean, constructed using the variation among replicate vials within populations.

revealed significant effects of selection, feeding time, and selection × feeding time interaction (Table 3.9). Multiple comparisons revealed no significant difference in mean survivorship of FEJ and JB larvae after 46 ($t = 0.58$, $df = 6$, $p > 0.05$) and 54 hours ($t = 1.3$, $df = 6$, $p > 0.05$) of feeding, whereas after 50 hours of feeding, FEJ larvae had significantly greater mean survivorship than JB larvae ($t = 4.79$, $df = 6$, $p < 0.005$) (Fig. 3.10).

Larval behaviours

FEJ larvae had a significantly lower mean feeding rate, pupation height, and foraging path length than the JB larvae (Table 3.10). The fraction of 'diggers' (larvae digging > 5 mm into the medium during feeding) in the FEJ populations was also significantly less than in the JB controls (Table 3.10).

DISCUSSION

Successful selection for faster development has been achieved in *Drosophila* in several studies in the past decade, and a correlated decrease in adult size/weight has consistently been observed (Zwaan *et al.* 1995a; Nunney, 1996; Chippindale *et al.* 1997a, 2003a). Other lines of work have also supported the notion of a trade-off between development time and adult size in *Drosophila* (Partridge & Fowler, 1993; Betran *et al.* 1998), and results from the present study are consistent with such a trade-off (Figs. 3.1, 3.4). Indeed, this trade-off is often seen in studies on other insects as well (Miyatake, 1995, 1998; Tucic *et al.* 1997).

The magnitude of the direct response to selection that was observed in this study is greater than even that seen by Chippindale *et al.* (1997a), who recorded a 17% decrease in development time after 125 generations of selection for fast

Table 3.9. Summary of ANOVA results for mean survivorship in the critical minimum feeding time assay. In this three-way mixed model ANOVA, selection regime and time for which the larvae were allowed to feed were treated as fixed factors crossed with random blocks and, consequently, only fixed effects and interactions can be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	0.098	27.045	0.014
Time	2	0.685	199.998	< 0.001
Selection × Time	2	0.026	8.084	0.020

Table 3.10. Population means (± s.e.) of larval behavioural traits in the selected (FEJ) and control (JB) populations. All mean differences between selection regimes were significant in one-way ANOVAs (p values for the F tests are shown in parentheses below each trait).

Selection	Population	Feeding rate (bites per minute) ($P = 0.002$)	Pupation height (cm) ($P < 0.001$)	Path length (cm) ($P = 0.01$)	Fraction of 'diggers' ($P = 0.011$)
FEJ	1	157.48 ± (5.720)	1.81 ± (0.211)	6.90 ± (0.736)	0.010 ± (0.007)
	2	142.60 ± (6.411)	1.29 ± (0.393)	3.60 ± (0.785)	0.000 ± (0.000)
	3	172.80 ± (6.276)	1.91 ± (0.254)	3.70 ± (0.602)	0.081 ± (0.031)
	4	125.48 ± (9.105)	0.96 ± (0.167)	3.90 ± (0.643)	0.031 ± (0.013)
JB	1	188.84 ± (5.413)	4.96 ± (0.245)	8.85 ± (1.135)	0.479 ± (0.039)
	2	186.92 ± (6.778)	4.55 ± (0.292)	7.55 ± (1.019)	0.304 ± (0.031)
	3	205.96 ± (5.840)	5.45 ± (0.495)	6.58 ± (0.822)	0.282 ± (0.046)
	4	153.68 ± (11.553)	4.18 ± (0.090)	5.93 ± (0.834)	0.310 ± (0.056)

development and early reproduction. The study by Chippindale *et al.* (1997a, 2003a) is the only previous study on *Drosophila* where selection for faster development was continued for a large number of generations; other studies have been of about 15 generations in duration (Zwaan *et al.* 1995a; Nunney, 1996). Moreover, the populations I used share common ancestry with the populations used by Chippindale *et al.* (1997a, 2003a), making a detailed comparison of my results and theirs all the more meaningful.

Our observation of a correlated decrease in pre-adult survivorship in the FEJ populations is consistent with the negative correlation between development time and pre-adult survivorship seen in a survey of populations maintained under varying demographic regimes (Chippindale *et al.* 1994), as well as with the results of a study in which faster development was directly selected for (Chippindale *et al.* 1997a). As also noticed by Chippindale *et al.* (1997a), the survivorship cost of faster development became apparent in my study only after 50 generations of selection had elapsed (Fig. 3.3). Yet, by this time, differences in development time (Fig. 3.1) and dry weight at eclosion (Fig. 3.6) between selected and control lines were already considerable, indicating that it is possible to reduce development considerably, at the expense of putting on weight, without seriously compromising pre-adult survivorship. This is also a possible explanation for why no trade-off between development time and pre-adult survivorship was observed by Zwaan *et al.* (1995a) in a selection study lasting only 16 generations, although the possibility of a different genetic architecture of traits related to development and survival in their

populations cannot be altogether discounted (Chippindale *et al.* 1997a; Harshman & Hoffmann, 2000).

The amelioration of the difference in male and female development times in my study (Fig. 3.2) is counter to observations made by Zwaan *et al.* (1995a) and Chippindale *et al.* (1997a). Even though females in *Drosophila* cultures typically eclose earlier than males and, therefore, males should experience stronger selection in a regime selecting for faster development, male and female development times have not previously been seen to differentially respond to selection. Possible explanations for this apparent paradox have been that the difference in male and female development times can be ameliorated by either high (Zwaan *et al.* 1995a) or variable (Joshi *et al.* 1999) larval densities in the culture vials, or that sex-specific expression of heritable variation for development time is lacking in these populations (Chippindale *et al.* 1997a), or that the sexual dimorphism in development time is subject to strong canalizing influences (Chippindale *et al.* 1997a). While one or more of these explanations may, in fact, be operating to ameliorate the selection differential between males and females in a culture subjected to truncation selection for fast development, my results clearly indicate that the sexual dimorphism in development time in *Drosophila* can, nevertheless, respond to selection in the manner expected, with males gradually narrowing the development time gap with females.

As in the case of the trade-off between development time and pre-adult survivorship, it is possible to argue that a reduction of the male-female difference in development time was not observed by Zwaan *et al.* (1995a) because of the short

duration of their study, or perhaps because of differences in the genetic composition of the base populations used. It is not clear, however, why such a reduction was not seen in the study of Chippindale *et al.* (1997a), given the similarities between my study and theirs: they continued selection for 125 generations, and their flies and ours share common ancestry. There are, however, two major differences between our selection protocol and that used by Chippindale *et al.* (1997a). In the FEJ populations, flies had over 48 hours after eclosion before eggs were collected for initiating the next generation, whereas in the selected lines of Chippindale *et al.* (1997a) eggs were collected as soon as enough were available, typically within 24 hours of eclosion. It is possible that FEJ males, not being under as strong selection for early sexual maturity as males in the populations of Chippindale *et al.* (1997a), were able to undergo a reduction in the duration of some phase of pupal development related to the reproductive system that could be compensated for after eclosion. It is known that male and female *D. melanogaster* differ only in pupal and not larval duration, and it is postulated that longer pupal duration in males is due to some aspect of sperm maturation (Nunney, 1996). The selection intensity in the present study was also somewhat greater than that of Chippindale *et al.* (1997a), as evidenced by the more rapid response to selection, and population numbers in the FEJ populations were also greater (80 vials of 60-80 eggs *versus* 50 vials). It is, therefore, also possible that such a reduction in the male-female difference in development time may have been seen by Chippindale *et al.* (1997a) had they continued selection for a longer duration.

Life-stage specific development time and mortality

Selection for faster development in the FEJ populations has resulted in large changes in the temporal organization of pre-adult development. After 56 generations of selection, the pupal duration was substantially reduced, and accounted for almost 33% of the total reduction in egg to eclosion development time (Fig. 3.4). This is a novel finding and is in contrast to a previous observation that pupal duration did not change over 36 generations of selection in populations successfully selected for faster development (Chippindale *et al.* 1997a), even though substantial additive genetic variation for pupal duration in *Drosophila* has previously been demonstrated (Tantawy & El-Helw, 1970). Since our flies share a common ancestry with those used by Chippindale *et al.* (1997a), the lack of reduction in pupal duration in their study is seemingly somewhat surprising. However, as noted in the previous section, the difference in the timing of egg collection between our selection protocol and that followed by Chippindale *et al.* (1997a) may explain this apparent discrepancy. It is possible that our FEJ flies postpone or compensate for some aspect of development related to reproduction (*e.g.* ovary and ovariole maturation, sperm maturation) until after eclosion, thereby making a reduction of pupal duration evolutionarily possible. Indeed, the time taken from eclosion to first copulation is significantly greater in FEJ than in JB populations (Fig. 5.1 of this thesis), which is opposite of what was seen by Chippindale *et al.* (1997a).

The reduction in the duration of the different instars in the FEJ populations was not symmetrical, with only the first and third instar duration being reduced after 56 generations of selection (Fig. 3.4). I have also observed that there are no

significant differences between the egg hatching time and egg hatchability in the FEJ and JB populations (N. G. Prasad *pers. obs.*). This is consistent with the observations of Chippindale *et al.* (1997a), and indicates that the difference between the larval duration in the FEJ and JB populations is almost entirely due to reduced duration of the first and third larval instars in the FEJ populations. It is not clear at this time why the duration of the second larval instar did not respond to selection. Possibly, the first and third larval instars are predominantly feeding stages and a reduction in their duration, therefore, does not impose a strong mortality cost, at least early in the selection response. Yet, studies on populations related to ours but selected for adaptations to larval crowding indicate that second instar larvae put on weight at a higher rate and have higher feeding rates than first instar larvae (Santos *et al.* 1997). Of course, it is also possible that the duration of the second instar has already been optimized by selection on the ancestors of these populations in the wild.

The pre-adult mortality cost to faster development that I observed in the FEJ populations was almost entirely due to larval mortality (Fig. 3.5), whereas the difference in pre-adult mortality between the selected and control populations of Chippindale *et al.* (1997a) was evenly distributed over the larval and pupal stages, although there was no significant reduction in pupal duration. Chippindale *et al.* (1997a) speculated that increased pupal mortality in their selected populations was due to decreased larval resource provisioning that affected some aspect(s) of pupal metabolism. My results suggest an alternative possibility that the increased pupal mortality seen by Chippindale *et al.* (1997a) was, in fact, due to some aspect(s) of

selection in the adult stage. Possibly, selection for reduced duration from eclosion to egg-laying in the protocol of Chippindale *et al.* (1997a) exacted a cost in pupal mortality, whereas in our FEJ populations the two and a half day holding period in cages before egg collection is buffering pupal survivorship. I suspect that this is a more likely explanation of the increased pupal mortality seen by Chippindale *et al.* (1997a), because our FEJ populations have greatly reduced larval feeding rates (Table 3.10) compared to JB populations and third instar FEJ larvae are substantially lighter than their JB counterparts (Fig. 3.7). If pupal mortality were causally related to reduced larval provisioning, the FEJ populations would also be expected to show higher pupal mortality than the JB controls. The exact reasons and specific underlying mechanisms for increased larval and pupal mortality in populations that have evolved rapid development under selection are, however, not known at this time.

Larval growth rates

One of the interesting results in the present study of larval traits in FEJ populations is that mean larval growth rate actually decreased in the FEJ populations that were selected for shorter development time. All else being equal, it is not unreasonable to expect that the FEJ populations would have evolved a higher larval growth rate, being under selection for both a shorter development time, and fecundity on the third day of adult life. Yet, larval growth rate, the average rate of dry weight gain over the course of development from egg to eclosion, clearly decreased in the FEJ populations as selection proceeded (Fig. 3.8). In the case of both dry weight at eclosion and larval growth rate, the fractional reduction, relative

to controls, in FEJ males was greater than that seen in FEJ females. This is not altogether surprising, given that weight at eclosion is clearly important to females due to its relationship to early life fecundity (Mueller, 1985), whereas male size is not strongly related to reproductive success in laboratory cultures maintained at low larval densities (Joshi *et al.* 1999). Part of the decrease in growth rate of the FEJ populations relative to the JB controls was actually due to an increase in JB growth rates as selection proceeded (Fig. 3.8, Table 3.2). I suspect that this may be an expression of the JB populations adapting to some novel aspect of rearing in our laboratory, perhaps the banana-jaggery food which is slightly different from the food on which their ancestors were reared.

Although previous studies in which faster development was selected for (Zwaan *et al.* 1995a; Nunney, 1996; Chippindale *et al.* 1997a) did not explicitly address the issue of larval growth rate, as opposed to development time, some data from other studies are consistent with my observation that shorter development time is accompanied by a slower larval growth rate. Nunney (1996) selected for shorter egg hatch to pupation time, and if I divide the mean dry weight of eclosing adults in his control and selected populations by the larval development time, the average larval growth rates obtained are 2.44 and 3.13 μg/hr for control males and females, respectively, and 2.28 and 2.81 μg/hr for males and females from the selected lines. In another study, larval growth rates of female *D. melanogaster* from two geographically distinct populations were found to vary with development time (Azevedo *et al.* 1997). A population from Ecuador had mean development time of 208.5 hr and mean larval growth rate of 1.394 μg/hr, whereas a North Carolina

population had mean development time and larval growth rate of 215.6 hr and 1.449 μg/hr, respectively. Similarly, the average larval growth rates obtained by Tucic *et al.* (1997) in a study of density-dependent selection on the bean weevil *Acanthoscelides obtectus* were 0.156 and 0.186 mg/day for males and females from slow developing lines, and 0.155 and 0.183 mg/day for males and females from faster developing lines, respectively. Although none of these data from the literature permit testing for statistical significance, the trend observed is consistent with my finding of a slower larval growth rate in the FEJ populations.

The reason for the reduction in overall larval growth rate in FEJ populations becomes apparent upon examining the pattern of larval weight gain over time (Fig. 3.9) in the FEJ and JB populations. The weight gain curve is a sigmoid curve with an exponential increase followed by a terminal fall upon cessation of feeding later in the third instar. Until about 42 hours post hatching, the weight gain patterns in the FEJ and JB populations are almost indistinguishable from each other. But beyond 45 hours post hatching (by which time the larvae are in the third instar), the JB populations gain weight at a much higher rate and for a much longer period than the FEJ populations (Fig. 3.9). Selection has not only reduced the third instar duration in the FEJs, but has also caused a related change in the shape of the terminal part of the larval weight gain curve. Essentially, the shape of the weight gain curve implies that a small reduction in third instar duration will result in a disproportionately large reduction in weight at eclosion, relative to the mean growth rate, thereby explaining why mean growth rate tends to decrease with faster development.

Larval behaviour and minimum critical size

The observation of reduced larval feeding rate, foraging path length, pupation height and the fraction of 'diggers' in the FEJ populations (Table 3.10) is consistent with a scenario of the evolution of reduced energy expenditure, and with the observation by Chippindale *et al.* (1997a) of reduced pupation height in their accelerated development populations. This suite of evolved behaviours in the FEJ populations is also consistent with earlier observations that 'rover' phenotypes (which have greater foraging path length) have significantly higher pupation heights than 'sitter' phenotypes (Sokolowski & Hansell, 1983), and that populations that have evolved higher larval feeding rates under density dependent selection show a greater frequency of 'rovers' (Sokolowski *et al.* 1997).

However, Borash *et al.* (2000) have reported increased larval feeeding rates in the faster developing ACO and ACB populations of Chippindale *et al.* (1997a), relative to their controls. Borash *et al.* (2000) interpret this result in terms of an earlier reported (Borash *et al.* 1998) polymorphism in populations of *D. melanogaster* adapted to very high larval density. In that study, the CU populations described by Joshi and Mueller (1996) were seen to consist of individuals falling into at least two categories. Individuals eclosing early from crowded larval cultures had high feeding rates and relatively poor egg to adult viability and tolerance to nitrogenous metabolic wastes, whereas individuals eclosing later had lower feeding rates, but higher viability and tolerance to metabolic wastes (Borash *et al.* 1998). Consequently, Borash *et al.* (2000) interpret the faster feeding rate of ACO and

ACB populations of Chippindale *et al.* (1997a) as reflecting a direct relationship between faster feeding and faster development.

Yet, other studies indicate that faster feeding does not result in faster development at low densities, such as those at which the ACO and ACB populations were reared. Neither the CU populations (Santos *et al.* 1997) nor the progeny of early eclosing flies from crowded CU populations (D. J. Borash *pers. comm.*) exhibit faster development than controls, when assayed at low density. In fact, I suspect the reason for the faster feeding rate of the faster developing ACO and ACB populations observed by Borash *et al.* (2000) is because they did not assay larvae of physiologically matched ages (the first authors of these papers also agree that this is a likely explanation: D. J. Borash *pers. comm.*, A. K. Chippindale *pers. comm.*). After 48 hours from egg hatching, larvae from their accelerated populations would have been in mid-to-late third instar, whereas the control larvae were probably caught in very early third instar, right after moulting, at which point feeding rates are low.

My results show that the FEJ populations have evolved a smaller critical minimum feeding time, thereby attaining the critical size earlier than the JB controls (Fig. 3.10). The reduction in minimum feeding time, however, is only about two hours and my data do not allow any direct inference about the evolution of minimum critical size in the FEJ populations to be drawn. The results from the larval behavioural assays, however, do tend to rule out a simplistic explanation that the FEJ populations achieve the same critical size as the JB populations, but earlier, by simply feeding faster. The evolution of lower larval feeding rates (Table 3.10),

along with lower larval growth rates and lower weight of FEJ third instar larvae (Fig. 3.7), suggests that the reduction in critical minimum feeding time is likely to reflect reduced critical size in the FEJ populations, compared to the JB controls.

Density dependent selection and selection for faster development

Selection for faster development and for adapting to larval crowding share some superficial similarity in that individuals failing to eclose before a certain point in time die, either because food runs out, or because the experimenter does not include them in the pool of breeding adults. A comparison of results from density-dependent selection experiments and experiments where shorter development time was selected for, however, makes it clear that the evolutionary outcomes of these two types of selection regime are very different. *Drosophila* populations maintained at very high larval densities evolve increased population growth rates at high density (Mueller & Ayala, 1981), competitive ability (Mueller, 1988a), larval feeding rate (Joshi & Mueller, 1988, 1996), pupation height (Mueller & Sweet, 1986; Joshi & Mueller, 1993, 1996), larval tolerance to metabolic waste (Shiotsugu *et al.* 1997; Borash *et al.* 1998), foraging path length (Sokolowski *et al.* 1997) and minimum food required for pupation (Mueller 1990; Joshi & Mueller, 1996). When assayed at low larval densities, populations adapted to larval crowding do not differ from controls in egg to eclosion development time and survivorship (Santos *et al.* 1997). Although crowding adapted populations did not differ from their controls in adult dry weight at eclosion, they showed greater rate of weight gain in the post-critical stage of larval development (Santos *et al.* 1997), greater fecundity, lipid content and starvation resistance (Borash & Ho, 2001).

Thus, *Drosophila* populations evolve enhanced competitive ability, when evolving at high larval density, primarily by becoming better at acquiring food fast, even though this ability comes at the cost of decreased efficiency at converting food to biomass (Mueller 1990; Joshi & Mueller, 1996), perhaps partly offset by greater efficiency at assimilating lipids (Borash & Ho, 2001). Larvae in such populations are also better able to withstand relatively high levels of metabolic waste, another aspect of life in crowded *Drosophila* cultures (Shiotsugu *et al.* 1997; Borash *et al.* 1998). In contrast, the evolution of reduced pre-adult development time in our FEJ populations is accompanied by increased pre-adult mortality (Fig. 3.2) and reduced larval feeding rate, pupation height, foraging path length, digging propensity (Table 3.10), minimum food required for completion of development (Fig. 3.10), and rates of larval weight gain in the post-critical size stage of development (Fig. 3.9).

The differences in the suite of traits that evolve under high larval density and under selection for fast development can be understood in terms of one fundamental aspect in which these selection regimes differ. At high larval densities there is a clear environmental signal, in the form of food running out, available to the larvae such that they can make the switch from feeding to pupation. Therefore, it is not necessary, in principle, for larvae to speed up the developmental process in terms of real time. What is probably more important in this context is for the larvae to acquire food faster than others, such that they attain the critical size for pupation before food runs out. Under truncation selection for faster development, however, there is no external signal available to larvae indicating that they need to switch from feeding to pupation. In this context a speeding up of the developmental

processes, such that an internal signal for pupation is triggered earlier in real time, is of crucial importance. I speculate that this is a likely explanation for why the overall intrinsic timing of developmental events is unchanged in populations adapted to larval crowding (Santos *et al.* 1997), whereas the FEJ populations exhibit large changes in the temporal organization of pre-adult development.

Overall, my results clearly suggest that there is more to the evolution of faster development than merely a reduction in development time. It appears likely that pre-adult development in *Drosophila* consists of distinct phases during which either weight gain or developmental processes take precedence, respectively. If so, it may be that the fitness cost of reduction in periods of weight gain is less than that of reduction in periods when key developmental processes are occurring, and the duration of periods of weight gain is the first to be reduced in response to selection for faster development. The nature of change in the larval weight gain profile of FEJ populations leading to a slowing down of larval growth rate and the relatively late observation of reduced pre-adult survivorship as selection proceeds are consistent with this scenario. Possibly, if the selection regime was such that both shorter development and larger adult size (perhaps through longer adult lifespan) were at a premium, larval growth rates would actually increase during selection. There is some evidence from the lepidopteran *Epirrita autumnata*, that short development time and larger adult size can evolve simultaneously (Kause *et al.* 1999), and studies on the melon fly *Bactrocera cucurbitae* suggest that short development and higher early life fecundity can also be successfully selected for simultaneously (Miyatake, 1998).

Moreover, my results also clearly illustrate a more general point that the density at which selection occurs can greatly affect the evolution of life-history traits. Selection for faster development imposed through food limitation at high density, and direct selection for faster development in moderate density, food rich, conditions lead to the evolution of entirely different suites of traits. Hence, larval and adult densities need to be controlled when performing selection experiments, and some knowledge of density is required when speculating about possible selection pressures in wild populations. It is also apparent that a relaxation of selection pressures on adult life-history traits can greatly affect the direct response of pre-adult traits to selection. Thus, relatively relaxed selection on reproduction very early in adult life in the FEJ populations appears to have permitted the evolution of a substantially reduced pupal duration in contrast to the faster developing populations of Chippindale *et al.* (1997a). Thus, even when selection pressures acting on development time are similar, differences in early adult life expectancy could yield different responses to selection. In organisms undergoing complete metamorphosis, where larval provisioning is a major determinant of adult resource reserves, it is intuitively obvious that selection acting on pre-adult life stages can profoundly affect the responses of adult life-history traits to selection acting upon them, and there is substantial empirical evidence for such genetic constraints on life-history evolution that exert their effects in the direction of the unfolding of the ontogeny (Partridge & Fowler, 1992; Roper *et al.* 1993; Zwaan *et al.* 1995a; Chippindale *et al.* 1994, 1996, 1997a). My results suggest that such constraints on life-history evolution can also exert their influence against the

direction of the unfolding of the ontogeny, often leading to unexpected and counter intuitive correlated responses to selection.

Chapter 4: Evolution of Competitive Ability

The purpose of this chapter is three-fold. Drawing upon a combination of previously published theoretical and experimental studies, and some new theory and experimental data, it is suggested that:

(a) The theory of density-dependent selection for single populations is better thought of in terms of selection in crowded environments favouring increase in competition coefficients, α, rather than in carrying capacity, K. It is important in this context to realize that intra- and inter-genotypic competition coefficients need not necessarily be correlated.

(b) Specific considerations of 'aggression' and 'response' (*sensu* Eggleston, 1985) or 'effectiveness' and tolerance' (*sensu* Joshi & Thompson, 1995) are useful in thinking about evolution in crowded environments, and about the correlated effects of selection on development time on the evolution of competitive ability.

(c) Contrary to a fairly widespread belief among *Drosophila* workers, evolution of faster development *per se* should not confer enhanced competitive ability; this prediction arises from a consideration of (a) and (b) above, and is put forward as an empirically testable proposition in the first section of this chapter, while its experimental testing is described in the subsequent section.

Most of the theoretical and experimental elements that are woven together into an argument here were originally presented in diverse contexts and, in some cases, seem to have escaped the attention of several subsequent workers in the field. In this chapter, these various elements are put together to make a focused case for looking at density-dependent selection from the point of view of competition coefficients rather than

carrying capacities, and it is argued that this point of view leads to predictions about the relationship between development time and competitive ability in *Drosophila* that are at odds with a fairly widely accepted notion that faster development confers greater competitive ability (Krijger *et al.* 2001).

Section A: Faster development should lead to decreased competitive ability

In this section, results from various theoretical and experimental studies of density-dependent selection are reviewed. These findings are then drawn together into what I believe is a more useful way of looking at density-dependent selection, and some experimental results on the FEJs and JBs are presented.

DENSITY-DEPENDENT SELECTION THEORY

The theory of density-dependent selection was one of the first attempts to unite population ecology and population genetics, by explicitly considering population growth in genetic models of evolution, and suggesting that the fitness of different genotypes could be a function of the population density. Though it was first developed largely as a verbal theory by Dobzhansky (1950), and MacArthur and Wilson (1967), and extended in its verbal form to explain diverse life-history patterns (Pianka, 1970), formal mathematical treatments of density-dependent selection were soon available (Gadgil & Bossert, 1970; Roughgarden, 1971; Clarke, 1972; Matessi & Jayakar, 1976; Asmussen, 1983; Anderson & Arnold, 1983). Density-dependent selection theory, and its use in ecology have been extensively reviewed several times (Stearns, 1977; Parry, 1981; Boyce, 1984; Mueller, 1995, 1997) and I do not wish to rework that material. It seems

clear that the verbal theory of density-dependent selection is now clearly discredited as being over ambitious and muddled (Joshi & Mueller, 1996; Mueller, 1997).

The basic premise of density-dependent selection theory is that genotypic fitnesses are a function of population density. The first formal models of density-dependent selection (Anderson, 1971; Charlesworth, 1971; Roughgarden, 1971) were framed in the context of the logistic model of population growth with the fitness of a single-locus genotype $A_i A_j$ being represented by

$$w_{ij} = 1 + r_{ij}\left[1 - \frac{N}{K_{ij}}\right] \quad (4.1).$$

In this formulation, N represents the total population size, regardless of genotype, whereas the r and K terms are genotype specific; consequently, these models have often been referred to as pure density-dependent selection models because there are no frequency-dependent inter-genotypic interactions. At the time these models were developed, a major concern in population genetics was to understand forces that may maintain genetic polymorphism in populations, and one of the main results from these models was that the condition for maintenance of genetic polymorphism at a bi-allelic locus under density-dependent selection was overdominance of carrying capacity ($K_{ij} > K_{ii}, K_{jj};\ i,j = 1,2$). There is, however, an interesting problem in these models which becomes apparent if we formulate them in terms of coefficients of competition reflecting the sensitivity of realized per capita growth rate to the addition of one more individual to the population. In the logistic model, the sensitivity of per capita growth rate to density is given by $\alpha = r/K$, the slope of the linear decline in realized growth rate with increasing population size (Fig. 4.1), such that $N_{t+1} = N_t[1 + (r - \alpha N_t)]$. Thus, according to the

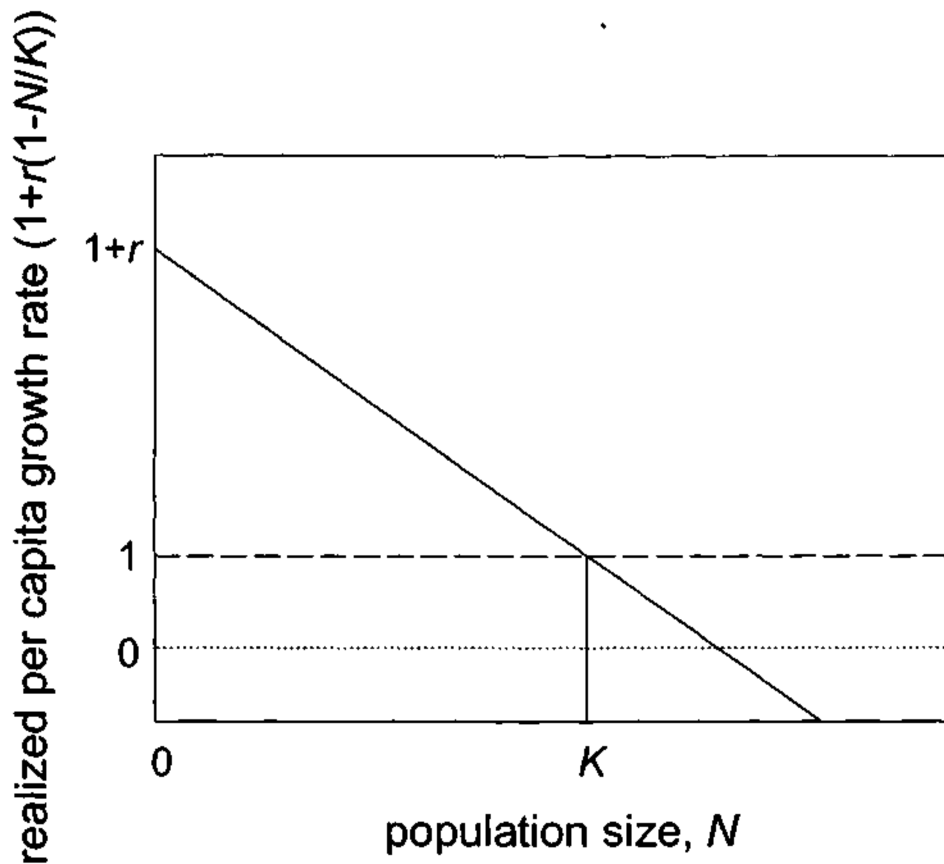

Figure. 4.1. The linear density-dependence of realized per capita population growth rate assumed in the logistic model. When $N = K$, the realized per capita growth rate is 1 and thus K is the equilibrium population size, as well as the saturation capacity of the environment. The slope of the line (r/K) is the coefficient of competition α in this formulation.

formulation in Eqn. 4.1, the impact of all individuals of all genotypes on the realized per capita growth rate of the target genotype $A_i A_j$ is mediated through the sensitivity of growth rate of this genotype to the addition of one more individual of its own genotype. Another way of putting this is to say that in this formulation intra- and inter-genotypic competition coefficients are assumed to be the same. Several modified versions of the logistic model were subsequently proposed (e.g. Hairston *et al.* 1970; Gilpin *et al.* 1976; Hallam & Clark, 1981), but all of these formulations make the assumption about intra- and inter-genotypic competition coefficients being the same.

Hairston *et al.* (1970) argue against the formulation of the *r-K* spectrum in terms of profligacy versus efficiency ascribed to MacArthur and Wilson (1967), and make a case for formulating density-dependent selection around birth and death rates, rather than realized growth rates which are, after all, a function of the birth and death rates. Although some aspects of their argument are unclear (e.g. see Pianka, 1972), Hairston *et al.* (1970) make the point that the logistic equation was derived in an ecological context and it should, therefore, not be forced into an evolutionary frame of reference because its parameters are not designed to capture the essence of evolutionary processes. This is an important point but, in my opinion, Hairston *et al.* (1970) do not follow this argument up. Instead they eventually suggest that given a tradeoff between birth rate (b) and death rate (d), such that b cannot be increased while simultaneously reducing d, selection at low densities will favour higher b even at a cost of higher d, and at high densities lowered d will be favoured even though this implies lowered b as well. Intra- and inter-genotypic competition coefficients do not explicitly enter into their framework, which remains that of the logistic equation.

Another alteration to the logistic framework for density-dependent selection was proposed by Hallam and Clark (1981), and elaborated upon by Clark (1983) in the context of the expected *r-K* tradeoff in density dependent selection. Their point of departure is the observation that in the traditional form of the logistic model, the parameter K incorporates in it the three distinct notions of environmental carrying capacity, equilibrium population size, and sensitivity of growth rate to density. In a way, their formulation may be viewed as an attempt to incorporate some of the realism introduced by models with an arbitrary matrix of competition coefficients (as in Eqn. 4.4) into a heuristically useful "logistic equation like" framework. Thus, they introduce two new parameters reflecting sensitivity to density(c) and saturation capacity of the environment (B), respectively, such that the recursion for population numbers becomes

$$N_{t+1} = N_t\left[1+\left(r-\tfrac{c}{B}N_t\right)\right] \quad (4.2).$$

In this formulation, the slope parameter α is now given by c/B and both c and B can be manipulated independently. This is an interesting formulation, but the crucial point is that here, too, the linear framework of density-dependent effects posited by the logistic equation is preserved, and sensitivity to density is still affected by K, albeit now scaled by the ratio of c and B (Fig. 4.2).

Gilpin *et al.* (1976) suggest an alternative formulation which does away with the assumption of linear density-dependence, a change that certainly makes more sense biologically than modifications retaining linearity of density-dependence. They introduce an additional parameter θ which governs the way in which realized per-capita growth rate varies with density (Fig. 4.3) such that, in discrete time, one would get the following expression for the fitness of genotype A_iA_j.

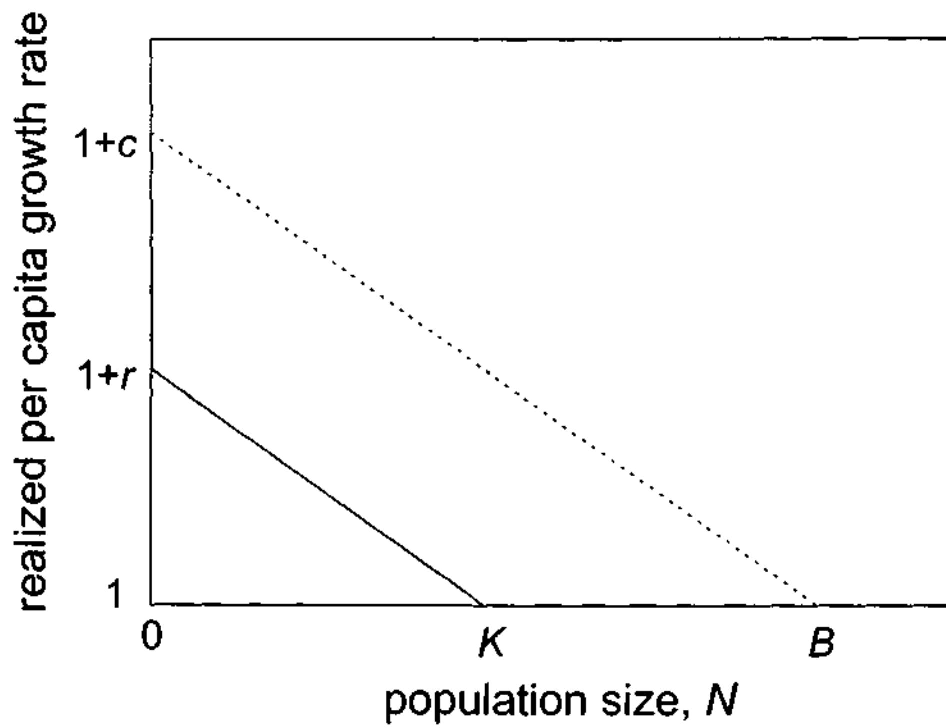

Figure. 4.2. The linear density-dependence of realized per capita population growth rate assumed in the model of Hallam and Clark (1981). Here the coefficient of competition $\alpha = c/B$, and this slope determines the equilibrium population size K.

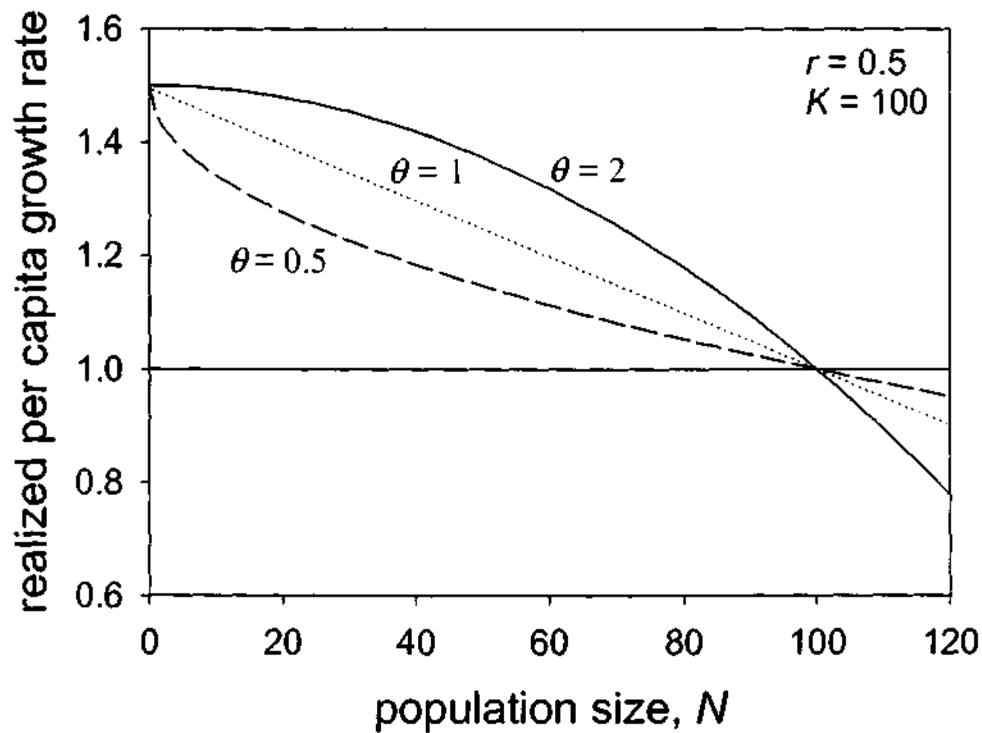

Figure. 4.3. The density-dependence of realized per capita population growth rate according to the formulation of Gilpin *et al.* (1976) shown here for some arbitrary values of r, K, and θ. Here the density-dependence can be non-linear, depending on the value of the parameter θ ($\theta = 1$ reduces to the logistic case). Note that if there were three genotypes with identical r and K values as depicted here, the one with $\theta = 2$ would be able to sustain higher realized growth rates than the others at all population densities from 0 to K.

$$w_{ij} = 1 + r_{ij}\left[1 - \left(\frac{N}{K_{ij}}\right)^{\theta}\right] \tag{4.3}$$

Their primary interest, however, is to show that under various situations traditionally considered to lie along the r-K spectrum, once one breaks out of the linearity assumption, selection can directly act upon the form of the density-dependence of realized growth rate (in this case by inducing evolutionary changes in θ). They, too, explicitly assume that intra- and inter-genotypic competition coefficients are the same.

This insistence on the equating of intra- and inter-genotypic competition coefficients is somewhat paradoxical, especially in the light of the often used interpretation of carrying capacity K reflecting in some sense the efficiency of conversion of biomass to offspring (MacArthur & Wilson, 1967). In other words, in the models considered above (Eqns. 4.1, 4.2, 4.3) even though the K_{ij} differ among genotypes, suggesting that different genotypes can make different numbers of offspring from the same quantum of food, the impact of adding one A_iA_i individual on the growth rate of A_jA_j is the same as that of adding one A_jA_j individual. Intuitively, though, addition of an individual of genotype with larger K than others should, all else being equal, have a relatively smaller impact on growth rate because these individuals consume less food. It is, thus, intuitively clear that the reductions in the maximal growth rate for different genotypes in a polymorphic population should depend not on the total population size N (as it does in Eqns. 4.1, 4.2, 4.3) but rather on the numbers of each genotype (N_{ij}). Indeed, if one ignores the issue of reproduction, then competition between genotypes is conceptually no different than competition among species and typical competition models

explicitly make the realized growth rate of each species a function of the numbers of the two species. In fact, the frame-work of the Lotka-Volterra equations for competition suggests itself as an appropriate analogue for inter-genotypic competition, embodying as it does inter- and intra-specific competition through separate competition coefficients, α_{ii} and α_{ij}.

If we recast Eqn. 4.1 in terms of the genotype-specific sensitivity of growth rate to density, we get

$$w_{ij} = 1 + r_{ij} - \sum_{k,l=1,2,\ldots,m} \alpha_{ijkl} N_{kl} \qquad (4.4),$$

where α_{ijkl} refers to the reduction in realized per capita growth rate of genotype A_iA_j due to the addition of one individual of genotype A_kA_l, and N_{kl} is the number of individuals of genotype A_kA_l present in the population. This type of formulation, often referred to as density-frequency dependent selection, is found in the models of Clarke (1972), Matessi and Jayakar (1976), Asmussen (1983), and Anderson and Arnold (1983). Once again, the focus of these analyses was on conditions permitting genetic polymorphism, and the general conclusion was that, in contrast to the pure density-dependent selection case, many interior equilibria are possible if one takes genotype-specific sensitivity to population density into account. Similarly, overdominance in carrying capacities is no longer the determining criterion for maintenance of genetic polymorphism; it is the interplay of the K_{ij} and the α_{ijkl} that determines whether or not a polymorphism will be maintained. In general, these models suggest that density-dependent fitnesses may be more likely to yield stable polymorphism than suggested by the analysis of the more restrictive formulations such as those in Eqns. 4.1, 4.2 and 4.3. A further interesting result

from numerical studies of this type of model with varying functional forms for the density-dependent genotypic fitnesses is that the total population size at a stable interior equilibrium can be greater than any of the K_{ij} (Asmussen, 1983).

Unfortunately, these more reasonable models of density-dependent selection do not seem to have achieved the representation they warrant in the literature. The formulation of Eqn. 4.1 is still what one typically encounters in textbooks (e.g. Hartl & Clark, 1997), and models such as Eqn. 4.4 are often mentioned almost as exotic extensions to Eqn. 4.1, which is typically referred to as a model of "pure" density-dependent selection. Yet, being analogues to the Lotka-Volterra competition models, one would expect models such as Eqn. 4.4 to be perfectly reasonable and acceptable abstractions of the process of inter-genotypic competition, which is what density-dependent selection is all about (essentially, these models are discrete time Lotka-Volterra models wherein the competing types are Mendelian genotypes at a single locus). The arguments as to why these models lend themselves to a more reasonable view of density-dependent selection will be presented in a later sub-section, after reviewing some empirical evidence on adaptations to crowding in *Drosophila*.

A far more complex and species specific model for density-dependent selection was developed by Mueller (1988b) to explicitly abstract the relevant ecology of *Drosophila* cultures into a formulation that could then be used to make specific predictions that could be tested using laboratory populations of *Drosophila*. The details of this model are not too pertinent to the issue at hand, but it should be noted that analysis of this model suggested that it was possible for populations to evolve increased competitive ability without increasing K or decreasing body size (Mueller, 1988b).

TRADEOFFS IN DENSITY-DEPENDENT SELECTION

Much of the interest generated by the notion of density-dependent selection is due to the suggestion that there are tradeoffs between the ability to do well under uncrowded (r-selection) and crowded (K-selection) conditions, respectively (MacArthur & Wilson, 1967; Gadgil & Bossert, 1970; Luckinbill, 1978, 1979; Mueller & Ayala, 1981; Mueller *et al.* 1991; Tanaka, 1996). Clearly, if the same genotype could do well at different densities, then there would be no difference between populations that had evolved under different densities, and the notion of density-dependent selection would have little value in explaining observed patterns of diversity. There is, thus, an expectation that the fitness of genotypes varies with density in a manner such that no one genotype has the highest fitness at all densities. The first question that we are faced with here is how one is to measure fitness. It is reasonable to argue (e.g. Mueller, 1997) that the trait that is ultimately under density-dependent selection is actually the density-specific realized growth rate and that, therefore, the appropriate fitness measure here is the realized growth rate at various densities.

Within the constraints of the logistic formulation, it is clear that if such r-K tradeoffs exist, then genotypes with higher K will sustain higher growth rates at relatively high densities (Fig. 4.4). In the model of Gilpin *et al.* (1976), it is possible for higher density-specific growth rates to evolve through changes in the parameter θ, reflecting the way in which growth rate responds to increasing density. Similarly, in the class of models incorporating genotypic interactions (Eqn. 4.4), or in the *Drosophila* model of Mueller (1988b), genotypes with high competitive ability (α) may be favoured by selection at high densities, rather than genotypes with high carrying capacity (K). Consequently, the

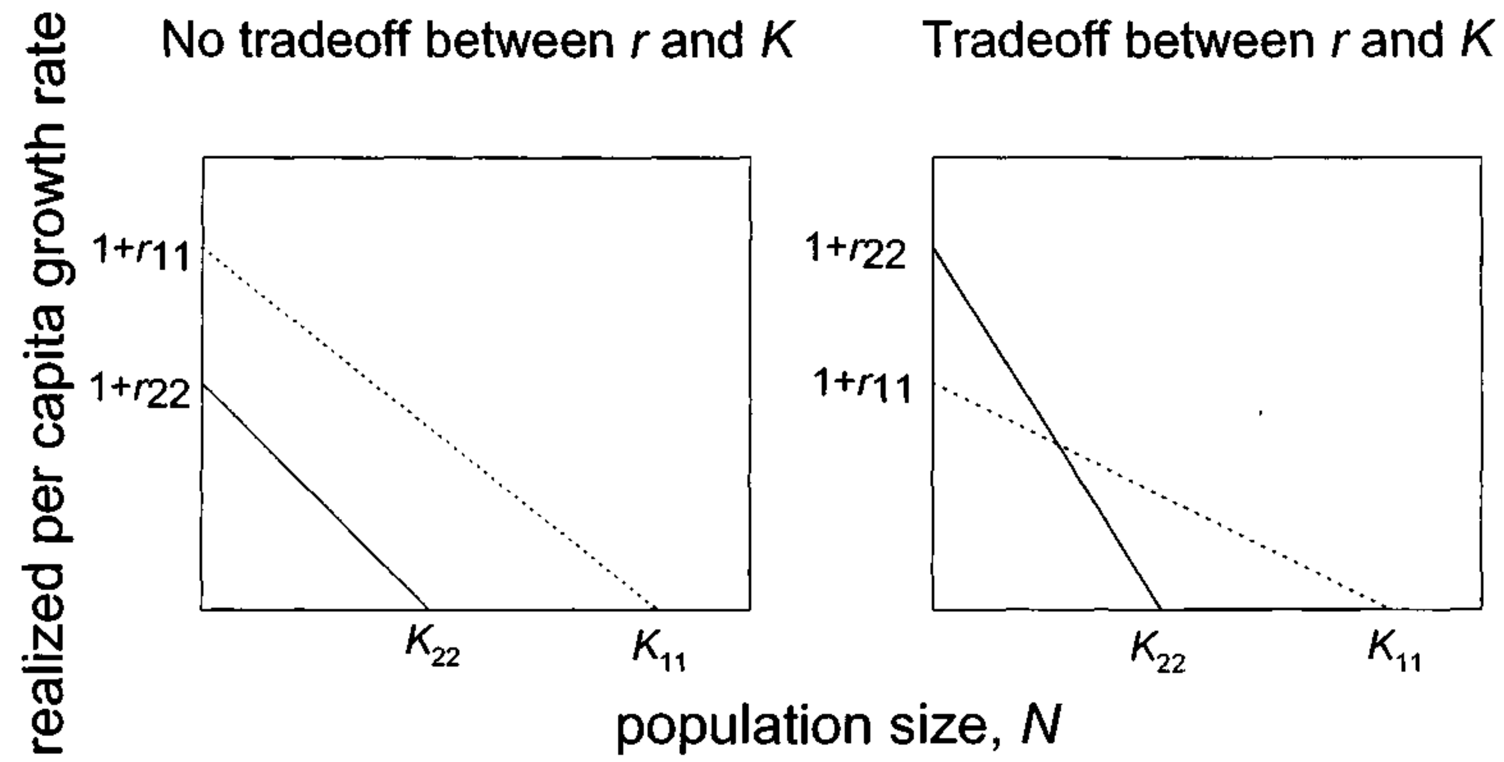

Figure. 4.4. If one considers two genotypes with different r and K values, the only mechanism within the confines of the logistic formulation whereby one genotype can have superior realized per capita growth rates (fitness) at high densities is by having greater K. In both cases shown, selection in a crowded environment will favour genotype A_1A_1.

primary tradeoff that needs to be sought is not between maximal growth rate (r or as sometimes designated, r_{max}) and carrying capacity (K), but rather between realized growth rate at low and high densities. Evidence for such tradeoffs has been seen in experiments on laboratory populations of *Drosophila* (Mueller & Ayala, 1981; Mueller *et al.* 1991), and *Paramecium* (tradeoff between competitive ability and maximal growth rate: Luckinbill, 1979), but not in populations of *E. coli* (Luckinbill, 1978; but see also Vasi *et al.* 1994: Bell, 1997, pg. 459).

As is the case with any overall measure of fitness, density-specific realized population growth rates are likely to be the culmination of a multitude of specific traits at the physiological and or behavioural level that could affect how well organisms are able to cope with a particular level of crowding. It is, therefore, only to be expected that the specific adaptations underlying the evolution of higher density-specific growth rates, and consequently the low versus high density growth rate tradeoff, will vary considerably among species with differing basic ecologies (Mueller, 1997). In the next sub-section, I will briefly review what is known about the specific adaptations underlying the evolution of higher growth rates at high density in populations of *D. melanogaster* subjected to extremely high levels of crowding for many generations in the laboratory.

ADAPTATION TO CROWDING IN *DROSOPHILA*

Two sets of selection studies on laboratory populations of *D. melanogaster* carried out over the last 20 years or so have yielded considerable insight into the mechanisms by which populations maintained at high density adapt so as to be able to sustain a higher rate of population growth at high density, relative to control ancestral populations (reviewed in Joshi, 1997a; Joshi & Mueller, 1996; Mueller, 1995, 1997). In a

Drosophila culture with very high larval density, the environment deteriorates in two ways over time: food tends to run out, and toxic nitrogenous metabolic wastes tend to accumulate. There is, thus, selection favouring the ability to develop fast under crowded conditions, and also to be able to withstand fairly toxic levels of wastes such as ammonia and urea. Adaptive evolution in response to both these selection pressures seems to occur in *Drosophila* populations.

Compared to control populations reared at low larval densities, populations subjected to many generations of crowding evolve higher population growth rates at high densities, and higher K (Mueller & Ayala, 1981; Mueller *et al.* 1991), as well as higher competitive abilities when competed against a common marked strain (Mueller, 1988a). Other traits seen to evolve in the populations maintained at high density are increased larval feeding rate (Joshi & Mueller, 1988, 1996), pupation height (Mueller & Sweet, 1986; Joshi & Mueller, 1993, 1996), larval tolerance to metabolic waste (Shiotsugu *et al.* 1997; Borash *et al.* 1998), foraging path length (Sokolowski *et al.* 1997) and minimum food required for pupation (Mueller 1990; Joshi & Mueller, 1996). Although the crowding adapted populations have shorter egg to eclosion development time, higher pre-adult survivorship, and greater weight at eclosion (Mueller *et al.* 1993; Borash & Ho, 2001) than controls when assayed at high larval density, they do not differ from controls in development time, survivorship, or size at eclosion when assayed at low larval density (Santos *et al.* 1997; Borash & Ho, 2001).

Thus, it appears that *Drosophila* populations evolve enhanced competitive ability when subjected routinely to high larval density, primarily by becoming better at acquiring food fast and by being better able to withstand relatively high levels of metabolic waste,

even though this ability comes at the cost of decreased efficiency at converting food to biomass (Mueller, 1990; Joshi & Mueller, 1996; Borash & Shimada, 2001), perhaps partly offset by greater efficiency at assimilating lipids (Borash & Ho, 2001). It is interesting to note that although evolution in crowded conditions in these populations led to an increased carrying capacity, it was not through the predicted mechanisms of greater efficiency of conversion of food to biomass or reduced body size. It is also worth noting that the evolution of increased competitive ability through a mechanism such as faster feeding, which evolved twice in separate experiments with flies from different ancestries (Joshi & Mueller, 1988, 1996), cannot be accommodated within the framework of the 'pure' density-dependent selection models (Eqns. 4.1, 4.2, 4.3).

SELECTION FOR FASTER DEVELOPMENT IN *DROSOPHILA*

One way of looking at selection at high larval density in *Drosophila* is to treat it as selection for faster development because individuals failing to eclose before a certain point in time die because food runs out, or because the medium becomes too toxic to permit survival. This view has been important in thinking about selection on wild *Drosophila* populations because larvae of many species occupy ephemeral habitats such as rotting fruits. It has, consequently, often been suggested that faster development has been under strong natural selection in *Drosophila* (Clarke *et al.* 1961; Robertson, 1963; Partridge & Fowler, 1992). Indeed, larval growth rates in wild *Drosophila* populations are thought to be an evolutionary compromise between the need to develop fast and the constraint that faster development typically reduces adult size (Santos *et al.* 1988; Partridge & Fowler, 1993). Yet, larvae in rotting fruits often have to deal with not just the need to develop fast, but also to do so under fairly crowded conditions (Atkinson, 1979;

Nunney, 1990). One consequence of this confounding of selection for faster development and for adapting to high density in ephemeral habitats has been the often implicit assumption by many workers that the two kinds of selection will have fairly similar outcomes (Tantawy & El-Helw, 1970; Wilkinson, 1987; Santos *et al.* 1988; Prout & Barker, 1989; Partridge & Fowler, 1993; Borash *et al.* 2000).

However, a comparison of results from density-dependent selection experiments and this study on FEJ and JB populations, clearly shows that the suites of traits evolving under the two types of selection regime are almost exactly opposite. The FEJ populations in which only the first 20% or so of eclosing individuals are allowed to breed each generation, evolve rapid development at low density, relative to controls, and this reduction in development time is accompanied by large reductions in adult weight at eclosion (Chapter 3). Moreover, the FEJs evolve reduced pre-adult survivorship, larval feeding rate, foraging path length, digging propensity, pupation height, larval growth rate, and minimum food requirement for successful pupation and eclosion, relative to the JB controls (Chapter 3).

EFFECTIVENESS AND TOLERANCE IN COMPETITION

One of the notions that follows from formulations of density-dependent selection that include interactions among genotypes (Eqn. 4.4) is that of selection under crowded conditions acting on competition coefficients α. The idea of α-selection was first put forward by Gill (1972, 1974) and Case and Gilpin (1974) in the context of inter-specific competition and the possible coevolution of competitors. Yet, in 12 major papers on density-dependent selection between 1972 and 1984, including a major review in 1984 and another in 1997, Gill's (1972, 1974) work is cited only 6 times, and 4 of these are

merely passing references. In his detailed review, Boyce (1984) does make the point that models like Eqn. 4.4 clearly show that selection for competitive ability and K-selection are not the same thing, but even his treatment suggests that he views these situations as somehow being a "complication" of the 'pure' density-dependent case due to the incorporation of frequency-dependent selection. Pianka (1972) suggests that the notion of α-selection should be subsumed into a broadened concept of K-selection.

I agree that the notion of density-dependent selection needs to be broadened to incorporate α-selection. In fact, I suggest that α-selection is more than a "complication"; it is one of the primary aspects of what happens when organisms are faced generation after generation with a high density environment. K-selection, in fact, is likely to be a somewhat subsidiary aspect of density-dependent selection, relative to α-selection in most cases. Viewing density-dependent selection from more of an α-selection perspective also enables us to make use of a further distinction between two types of competitive ability that is of heuristic value, and this aspect is now discussed.

Since competition is typically defined as a mutual inhibition of population growth rates by the two or more competing groups (whether genotypes or species) it is possible to think of two components of competitive ability: the ability to inhibit the other group (henceforth 'effectiveness') and the ability to withstand inhibition by the other group (henceforth 'tolerance') (Eggleston, 1985; Joshi & Thompson, 1995). To my knowledge, these concepts have not been explicitly discussed in the literature on density-dependent selection (but see Eggleston, 1985), even though there is clear empirical evidence that these two components of competitive ability are at least partially independent in situations of both inter-specific (Peart, 1989; Goldberg & Landa, 1991) and intra-specific

(Mather & Caligari, 1983; Eggleston, 1985; Hemmat & Eggleston, 1988, 1990) competition, and that they are under partly genetic control (Eggleston, 1985) and can evolve separately (Joshi & Thompson, 1995), at least in *Drosophila* species.

The main proposition that is advanced in this section is that a view of density-dependent selection that explicitly recognizes that competition coefficients will often be the primary targets of such selection (α-selection) allows us to focus on some hitherto neglected but potentially important tradeoffs that, very likely, will also clarify some of the confusion regarding evolution of developmental rates and of adaptations to crowding in *Drosophila*. To construct this view, I note that even within the confines of a logistic formulation, density-dependent selection with arbitrary intra- and inter-genotypic competition coefficients α_{ijkl}, can lead to a variety of outcomes, not all of which imply an evolutionary increase of K. Moreover, it could be argued that the α_{ijkl} in such situations are not always constrained to be simple functions of the carrying capacities K_{ij}, K_{kl} (contra Pianka, 1972).

The strict dependency of α_{ijkl} on K_{ij}, K_{kl}, in fact, need not be assumed in order to apply models such as Eqn. 4.4. Such dependency arises from a somewhat restricted view of competition involving only resource acquisition, with only the amount of resource required to survive and reproduce being considered, but not the rates at which the resource is acquired. A phenomenon like the evolution of faster feeding rate in *Drosophila* populations adapting to crowding cannot be incorporated into the classical formulation of density-dependent selection (e.g. Eqn. 4.1) at all. In such formulations, the only way to increase one's growth rate at high density, relative to other genotypes, is to have a higher K (or, for example, in Gilpin *et al.* 1976, to change θ). These formulations

do not permit a genotype to inhibit the growth rate of the other genotype, while not altering its own basic parameters like r, K or θ. Yet, this type of effect is exactly what is seen to happen in the *Drosophila* experiments: faster feeding, in itself, affects neither maximal growth rate or carrying capacity (in fact it increases the minimum food necessary for pupation, implying reduced efficiency). When in competition with relatively slower feeders, however, faster feeders have a clear competitive edge: they can greatly inhibit the population growth rate of slower feeders, and this inhibition is independent of their own sensitivity to their own density and therefore of fundamental growth rate parameters such a r, K or θ.

The notions of effectiveness and tolerance can be incorporated readily into this view of density-dependent selection. For a pair of genotypes A_iA_j, A_kA_l, the competition coefficient α_{ijkl} reflects the tolerance of A_iA_j with regard to A_kA_l, whereas the competition coefficient α_{klij} reflects the effectiveness of A_iA_j with regard to A_kA_l. This is an extension of the argument made by Joshi and Thompson (1995) in the context of inter-specific competition, and it should be noted that in this case the effectiveness of A_iA_j with regard to A_kA_l and the tolerance of A_kA_l to A_iA_j are the same quantities. Clearly, intra-genotypic competition coefficients cannot be split up in this manner: α_{ijij} reflects sensitivity of the population growth rate of genotype A_iA_j to the addition of more individuals of its own genotype, and this sensitivity subsumes both the effectiveness and tolerance of the genotype with regard to itself. There is also some empirical evidence for the evolution of these components of competitive ability; a study of inter-specific competition between *D. simulans* and *D. melanogaster* revealed that populations could

evolve higher competitive ability through changes in effectiveness or tolerance or both (Joshi & Thompson, 1995).

Interpreting the observed evolutionary responses to selection under crowding in *Drosophila* in the context of effectiveness and tolerance, it seems clear that faster feeding will result in increased competitive ability through increased inter- but not intra-genotypic effectiveness, whereas increased ability to withstand metabolic waste is likely to increase tolerance, both inter- and intra-genotypic. Moreover, the expectation of increased efficiency of food utilization now appears far less unequivocal, because a genotype that can get by on less food will also thereby leave that much more for others. Thus, increased efficiency reduces effectiveness while increasing tolerance and can, therefore, have a net negative effect on competitive ability, especially in situations where the genotype with greater efficiency of food utilization is competing against a genotype with greater efficiency of food acquisition. Exactly this sort of tradeoff has been experimentally observed in *Drosophila* populations adapted to crowding (Mueller, 1990; Joshi & Mueller, 1996). Examining the larval traits that evolved in our FEJ populations (Chapter 3), one would expect these populations to have reduced effectiveness due to slower feeding rates and lower adult weight and minimum food requirement for pupation and eclosion, perhaps partly offset by increased tolerance due to the latter. They might also be expected to have a higher K due to the reduced adult size. The following sub-section discusses some experimental results on urea tolerance, early life fecundity, and population growth rates of the FEJ populations, and the final sub-section returns to this more general theme to make an explicit prediction about competitive ability in the FEJ populations.

MATERIALS AND METHODS

Urea tolerance assay

Urea tolerance was assayed by recording egg to adult survivorship at three levels of urea – 0 g/l, 7 g/l and 14 g/l. Following Shiotsugu *et al.* (1997), these levels of urea were added to regular banana-jaggery food just before pouring it into vials. Ten such vials, with 5 ml of food each, were set up for each combination of population and urea level. Eggs from standardized flies were collected within a one-hour egg collection window, and 30 eggs were put into each of the vials. After the pupae had darkened, these vials were observed for eclosions at two hourly intervals. After four days, most of the flies eclosed. Later, the checks were relaxed to once in six hours and finally terminated after finding no eclosions in any vial over a continuous period of one week. Urea tolerance was used as a surrogate for ammonia tolerance, to which it is correlated. (Borash+Shimada, 201

Fecundity assay

Freshly eclosed progeny of standardized flies were set up in single male-female pairs in vials with ~ 3 ml of food. Forty such pairs were set up per population. Flies were transferred without anesthesia to fresh unyeasted vials every day for the first 10 days of adult life, and the eggs laid during the previous 24 hours was recorded. Any males dying were replaced by back up males of the same age. Dying females were not replaced, but there were negligible deaths during the 10 days of the assay. Total fecundity per female over the 10 days was obtained by simply summing up the daily egg count data.

Population growth assay

From each FEJ and JB standardized population, two small populations were derived by seeding two vials with 8 males and 8 females each and allowing them to lay eggs for 24 hours, after which the adults were discarded. The larvae developed and

pupated in these vials, and from day 8 through day 18 after egg-lay, any eclosing flies in these vials were collected daily into fresh vials with ~ 5 ml food in them. Eclosing flies were added daily into these adult collection vials and every other day all adults collected from a specific population till that time were shifted to a fresh vial containing ~ 5 ml food. On the 18th day after egg-lay, the egg vials were discarded and all eclosed adults of each population transferred to fresh vials. Each generation, the number of adult males and females present in each population (vial) was counted on the 21st day after egg-lay. The flies were then placed into a fresh vial with the appropriate amount of food and allowed to lay eggs for exactly 24 hours, after which the adults were discarded. This maintenance regime was continued for five generations to yield four pairs of N_t and N_{t+1} values for each population, which were used to construct a return map for the JB and FEJ derived populations.

Statistical analyses

All statistical analyses were implemented using Statistica™ for Windows release 5.0 B (StatSoft Inc., 1995). Data on survivorship of each FEJ and JB populations at the three levels of urea were subjected to an arcsine square root transformation (Freeman & Tukey, 1952) and then subjected to a mixed model ANOVA treating block as a random factor crossed with selection regime and urea level. Ten-day fecundity data of FEJ and JB populations were analyzed by comparing selection regime means by a paired *t*-test. For the data from the population growth assay, individual pairs of N_t and N_{t+1} values for each population were classified according to block, selection regime and density ($N_t < 50$: low; $50 < N_t < 100$: medium; $N_t > 100$: high). Values of N_{t+1} were treated as the dependent variable, and the data were subjected to a mixed model ANOVA with

block treated as a random factor crossed with selection regime and density. Assessment of FEJ and JB population growth rates at different densities was done by comparing mean N_{t+1} between the two selection regimes at each density through Fisher's Least Significant Difference (LSD), fixing the comparison-wise error rate at 0.01 to allow for 3 comparisons being made.

RESULTS

Urea tolerance

In general, pre-adult survivorship declined with urea level for both FEJ and JB populations, and as previously noted (Chapter 3), the survivorship of FEJ populations was significantly less than that of JB populations even in the absence of urea (Fig. 4.5). The ANOVA revealed significant effects of selection regime, urea level and the selection regime × urea level interaction (Table 4.1). The difference in survivorship between the JB and FEJ flies did not differ significantly (paired *t*-test on difference) between treatments at 0 g/L and 7 g/L urea, and then increased significantly from about 0.28 to 0.48 at 14 g/L urea (Fig. 4.5).

Fecundity

Mean fecundity over the first 10 days of adult life in the JB populations (169.3 eggs/female) was significantly greater ($p < 0.01$) than that in the FEJ populations (96.3 eggs/female) (Fig. 4.6).

Population growth

In both FEJ and JB derived populations, population numbers tended to rise over the five generations of the assay (data not shown). However, mean N_{t+1} attained when N_t fell into different density categories showed a clear pattern (Fig. 4.7), with the only

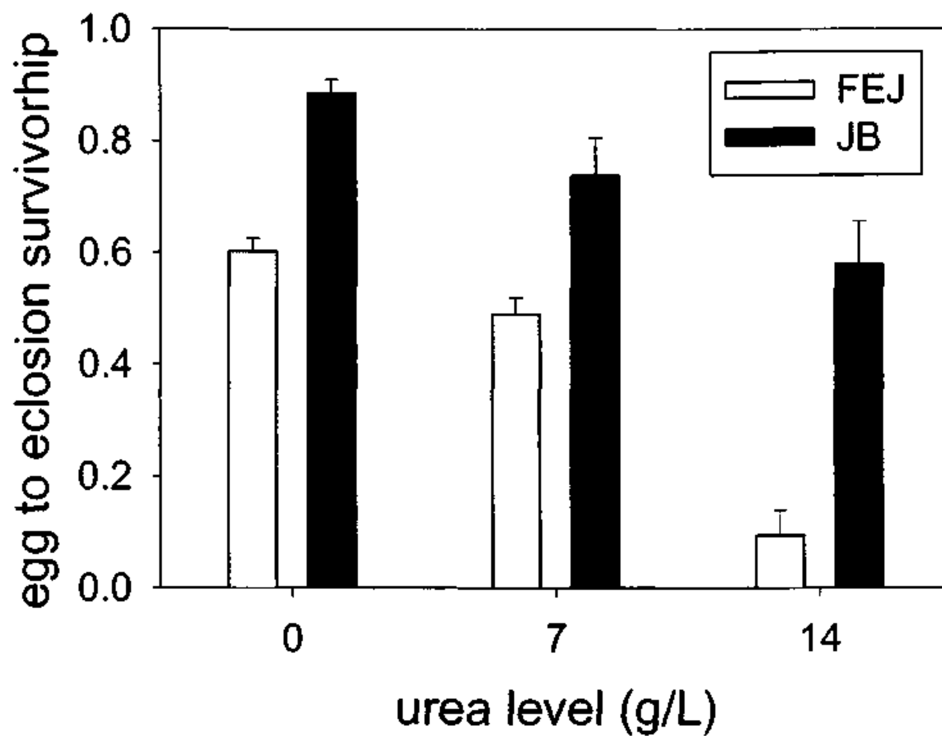

Figure. 4.5. Urea tolerance of FEJ and JB populations. The bars depict mean survivorship, averaged across the four replicate populations within each selection regime. Error bars depict 95% confidence intervals about the mean.

Table 4.1. Results of ANOVA on population mean arcsine-square root transformed survivorship data from the urea tolerance assay. In the analysis, block was treated as a random factor and, consequently, block effects and interactions involving blocks cannot be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	0.8574	339.35	0.0003
Urea level	2	0.4756	20.06	0.0022
Selection × Urea level	2	0.0748	6.73	0.0293

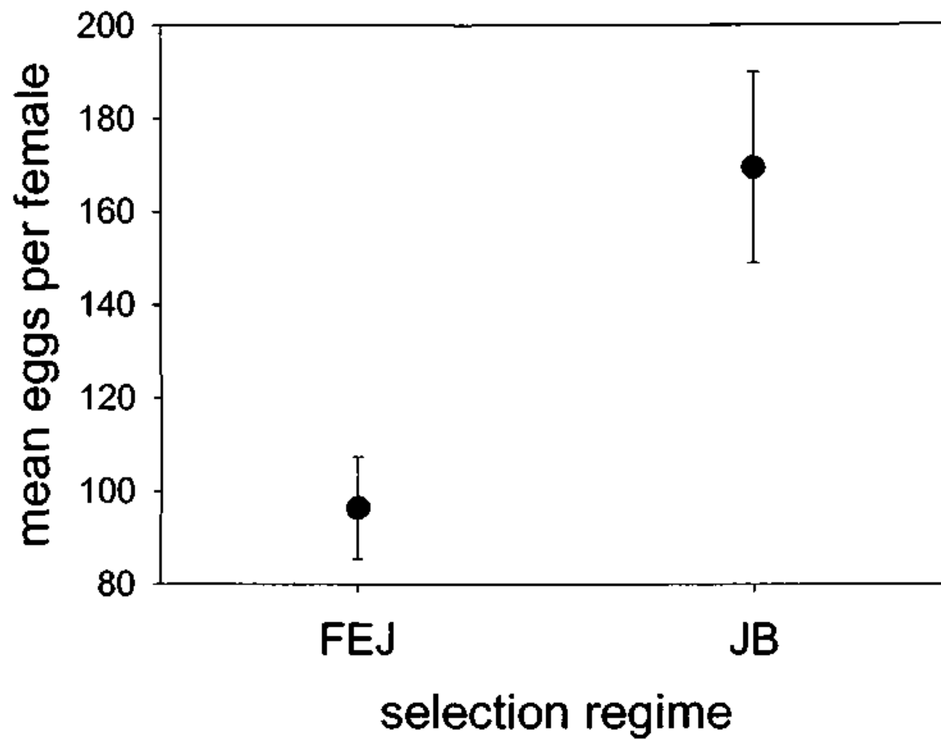

Figure. 4.6. Mean total number of eggs laid per female over the first 10 days of adult life. averaged across the four replicate populations within each selection regime. Error bars depict 95% confidence intervals about the mean.

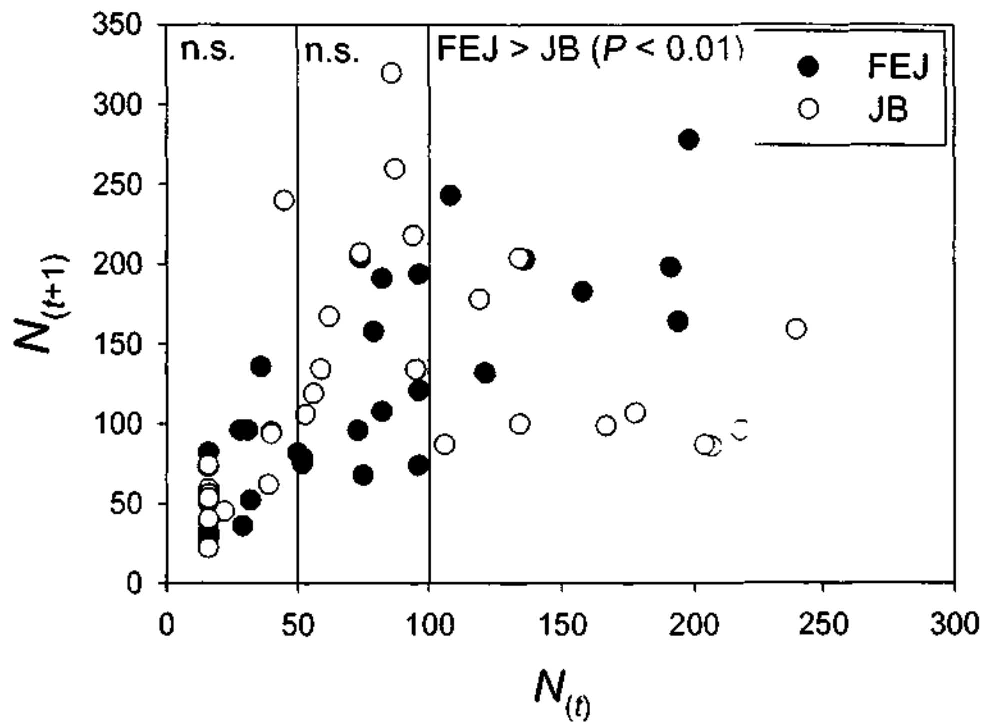

Figure. 4.7. Return map based on 5 generations of census of adult numbers in 16 small populations (2 populations derived from each FEJ and each JB population). Vertical lines divide the data into three groups based on population size N_t (N_t < 50: low; 50< N_t <100: medium; N_t >100: high). Mean N_{t+1} of FEJ and JB derived populations was not significantly different (P>0.01) for the low or medium N_t, whereas mean N_{t+1} for FEJ derived populations was significantly greater (P<0.01) than for JB derived populations for high N_t.

significant ANOVA effects being due to density and the selection regime × density interaction (Table 4.2). Multiple comparisons revealed that at low, and medium values of N_t, the mean N_{t+1} attained by FEJ and JB derived populations did not significantly differ from one another, whereas at high values of N_t, the mean N_{t+1} attained by FEJ populations was significantly greater than that of the JB derived populations (Fig. 4.7).

DISCUSSION

The experimental results clearly suggest that in monotypic cultures, FEJ populations are likely to sustain higher rates of population growth at high densities than the JB controls (Fig. 4.7). In the context of the logistic model, this would have to be interpreted as an indication that the FEJ populations have higher K than the JB controls, and this interpretation would be consistent with the reduced minimum food requirement and adult size of the FEJs relative to the JBs (Chapter 3). However, it could also be that the functional form of density-dependence in the FEJ and JB populations is different, with or without an accompanying difference in carrying capacity. The population growth assay suggests that r in the FEJ and JB populations may not be significantly different, whereas the reduced fecundity over the important early part of adult life of FEJ females at a density of 2 flies per vial (Fig. 4.6) suggests that r in the FEJ populations should be substantially smaller that in the JB controls. One reason for this apparent discrepancy may be the difference in density in the two assays. In the fecundity assay, the density was two flies/vial, whereas in the low density classification of the population growth assay data, densities ranged from 16 to 50 flies/vial, and fecundity in *Drosophila* populations closely related to the JBs and FEJs is known to decline rapidly as the density increases from 2 to 16 flies/vial (Mueller *et al.* 2000). Especially if there are any differences in the

Table 4.2. Results of ANOVA on one-step population sizes (N_{t+1}) attained by populations for whom N_t fell into the arbitrarily designated categories of low, medium or high (see Sub-section on *Statistical analysis* for details). Block was treated as a random factor, and variation among replicate small populations within Selection × Block is subsumed in the error term.

Effect	*df*	MS	*F*	*P*
Selection	1	411.67	0.182	0.6984
Block	3	3860.48	1.263	0.2999
Density	2	60839.76	73.794	0.0001
Selection × Block	3	2260.76	0.74	0.5346
Selection × Density	2	19031.57	21.544	0.0018
Block × Density	6	824.46	0.27	0.9478
Selection × Block × Density	6	882.99	0.289	0.9387
Error	40	3055.8		

functional form of the density-dependence of growth rate between FEJ and JB populations, differences in *r* may not be seen when looking at mean growth rates over a range of densities that are already in excess of 16 flies/vial.

It has been noted earlier that the FEJ populations may be expected to have evolved reduced effectiveness in competition due to slower feeding rates and lower adult weight and minimum food requirement for pupation, perhaps partly offset by increased tolerance due to the latter. To this is now added a further decrease in tolerance due to heightened sensitivity to high levels of urea (Fig. 4.5). It, thus, becomes apparent that, contrary to many earlier expectations, selection for faster development at low density is unlikely to lead to the evolution of greater competitive ability. Ironically, though, the traits classically expected to evolve in *K*-selected populations – smaller size, increased *K* or population growth rates at high density, greater efficiency of conversion of food to biomass – actually seem to evolve in the FEJ populations selected for faster development, rather than in populations selected for adaptations to high density. Yet, given the lower feeding rates, urea tolerance and minimum food requirements of the FEJ populations, I would predict that these populations should be poorer competitors than the JB controls, their greater efficiency and higher *K* notwithstanding.

I suggest that the confounding of selection for adaptations to crowding and selection for fast development in the *Drosophila* literature has been partly due to expected outcomes of density-dependent selection being based on the restrictive formulation within the constraints of the logistic equation. Although formulations incorporating genotypic interactions (arbitrary α_{ijkl}) were available in the literature, as were the notions of splitting competitive ability into components due to effectiveness and

tolerance, these ideas were not put together when viewing the issue of selection for faster development versus adaptations to crowding. It is hoped that the present arguments make the case that a broader view of density-dependent selection is useful in trying to understand these important evolutionary phenomena, and in reconciling empirical results with theoretical formulations.

Section B: Pre-adult competitive ability in the FEJ and JB populations

In the previous section of this chapter, I have shown that examining results of studies on adaptations to crowding and on selection for faster development in *Drosophila* in light of a broader conception of density-dependent selection that includes the notions of α-selection (Gill, 1974), and the effectiveness and tolerance components of competitive ability (Eggleston, 1985; Joshi & Thompson 1995), leads to the prediction that the FEJ populations should have lower competitive ability than the JB controls, even though they possess some of the attributes of a "*K*-selected" species, such as greater efficiency, carrying capacity, and population growth rates at high density.

To my knowledge, only two studies have demonstrated a positive relationship between rapid development and competitive ability. Bakker (1969) generated one slow and one fast developing line (by selecting on the larval duration) from a line carrying the *Bar* and white eye mutations, and found that the fast developing line was superior than the slow developing line when both were competed against the ancestral control population. Unfortunately, larval feeding rates were not assayed in this study. Clear interpretation of these results is, moreover, difficult because the selected fast and slow lines were not replicated, and competitive ability (and many other characteristics) of the fast line were not significantly different from the ancestral control line (Bakker, 1969). It

is, thus, possible that the slow line was a poorer competitor, not as a correlated response to being a slow developer per se, but as a result of a combination of drift and selection for poor fitness in general, the last being a common problem when selection for reduced lifespan, fecundity or development rate is done. In a more recent study, Krijger *et al.* (2001) have shown that larval development time is a good indicator of competitive ranking among seven *Drosophila* species competed against one another in pair wise trials. Similar effects are also known for competition between *D. melanogaster* and *D. simulans* (Joshi & Thompson 1995, 1997). Yet, one has to be circumspect about inferring potentially evolutionarily important within-population correlations from patterns of correlations among populations. For example, tradeoffs between early and late life fitness are well established in *Drosophila* (Service *et al.* 1988) but comparisons across species fail to detect them (Schnebel & Grossfield, 1988).

Competition assays

Two separate assays of competition were carried out after 90 and 120 generations of FEJ selection, respectively. Broadly speaking, the two assays were similar in conception: eggs collected from flies from each FEJ and JB population that had undergone one complete generation of common rearing without selection on development time were placed at specific densities either by themselves, or with an equal number of eggs from a yellow body mutant strain, in vials with 2 ml of food, and the number of eclosing adults, and their phenotypes scored. At the time of the first assay, the yellow body strain, obtained from the *Drosophila* stock centre at the University of Mysore, India, had been maintained in our laboratory for about 10 generations on a two week discrete generation cycle at the same larval densities as the FEJ and JB populations.

Generation 90 assay

Eggs collected from the four FEJ and four JB populations were used to assess the impact of density on survivorship in mono-typic cultures. Five vials per population were set up at each of three densities: 30, 300 and 600 eggs/vial. The number of adults eclosing in each vial was recorded and used to assess egg to adult survival. Five vials per FEJ and JB population were also set up to assess competitive ability against the yellow body strain. The total density of eggs was 300 eggs/vial, 150 of the test population and 150 of the yellow body strain. The number of eclosing wild type and yellow body adults in each vial was recorded.

Generation 120 assay

The purpose of the second assay was to (a) confirm the repeatability of the results of the first assay, (b) examine density effects on larval and pupal survivorship separately, and (c) assess the performance of the yellow body strain in monotypic culture so that the magnitude of competitive inhibition of its growth by the FEJ and JB populations could be compared. Since total survival at 600 eggs/vial was extremely low (see RESULTS), only densities of 30 and 300 eggs/vial were used. Five vials per population were set up at each of the densities for the mono-typic assay. Since the assays were staggered by block, a separate set of 5 vials from the yellow body strain was run with each block (FEJ_i, JB_i). The number of pupae formed and adults eclosing in each vial was recorded and used to assess larval, pupal and egg to adult survival. Five vials per FEJ and JB population were also set up to assess competitive ability against the yellow body strain. The total density of eggs was 300 eggs/vial, 150 of the test population and 150 of the yellow body strain. The number of eclosing wild type and yellow body adults in each vial was recorded.

Statistical analyses

All analyses were implemented using STATISTICA™ for Windows Release 5.0 B (StatSoft Inc., 1995). Data were subjected to separate mixed model ANOVAs in which block was treated as a random factor crossed against fixed factors such as selection regime, density or assay. Survivorship data were subjected to arcsine square root transformations (Freeman & Tukey, 1950) prior to analysis. For mono-typic cultures, egg to adult survivorship in the generation 90 assay, and life-stage specific survivorship in the generation 120 assay, were analyzed separately. Egg to adult survivorship data of FEJ and JB populations at densities of 30 and 300 eggs/vial in the two assays were analyzed together to assess the repeatability of the results. The impact of competition with FEJ and JB populations on the performance of the yellow body populations was assessed by analyzing egg to adult survivorship of yellow body individuals when in competition with themselves (mono-typic culture), and with FEJ or JB individuals (bi-typic cultures), at 30 and 300 eggs/vial in the generation 120 assay.

RESULTS

Mono-typic cultures: density-dependent survival

In all mono-typic cultures in the two assays, egg to adult survivorship declined with density. In the generation 90 assay, survivorship of the JB populations declined from a mean of 0.9 at 30 eggs/vial to 0.22 at 600 eggs/vial, whereas that of the FEJ populations declined from 0.6 at 30 eggs/vial to 0.18 at 600 eggs/vial (Fig. 4.8). The ANOVA revealed significant effects of selection regime, density and the selection regime × density interaction (Table 4.3). For both JB and FEJ populations, the decline in survivorship with density was almost perfectly linear, and the interaction was driven by

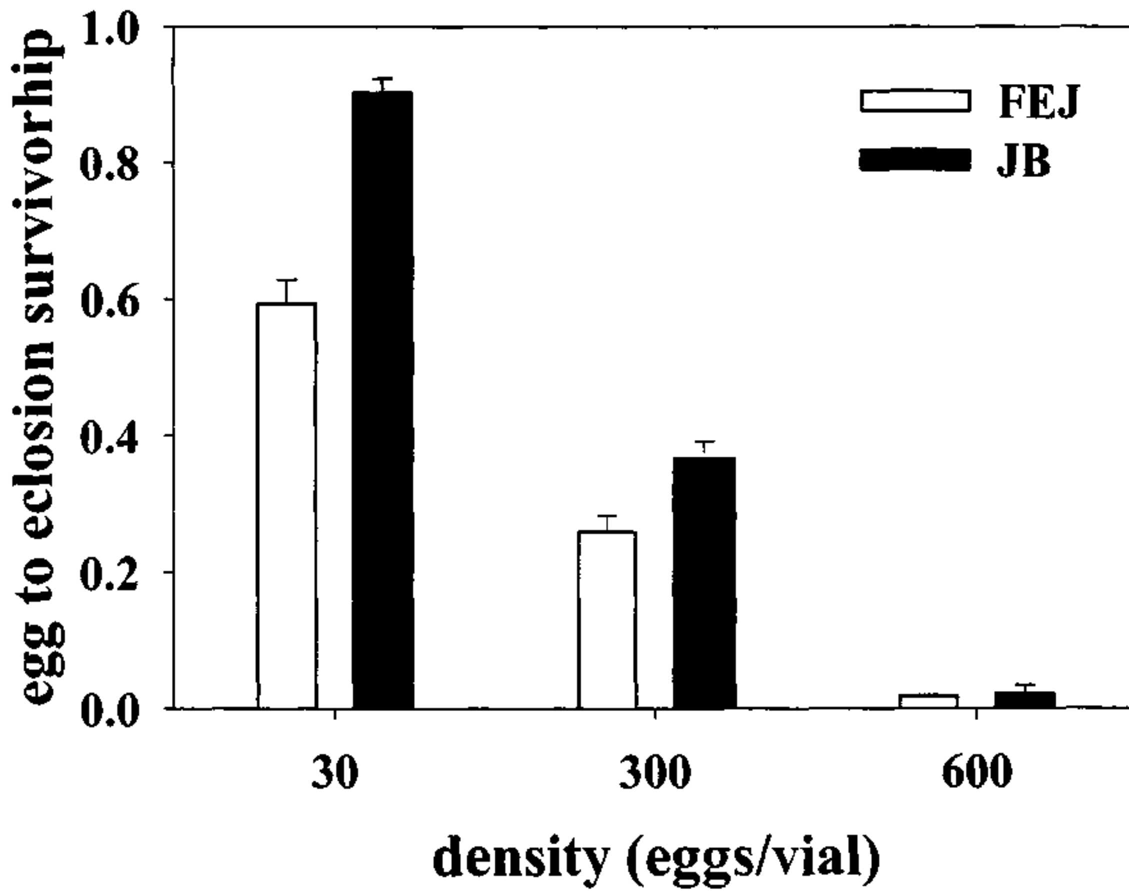

Figure 4.8. Mean (± s.e.) egg to eclosion survivorship in mono-typic cultures of FEJ and JB populations at three different densities in the generation 90 assay.

Table 4.3. Results of mixed model ANOVA on mean arcsine square root egg to adult survivorship of FEJ and JB populations in mono-typic culture at 30, 300 and 600 eggs/vial in the generation 90 assay. In this design, block and interactions involving block cannot be tested for significance, and have therefore been omitted from the table.

Effect	*df*	MS	*F*	*P*
Selection	1	0.1662	96.18	0.0023
Density	2	1.7570	857.84	< 0.0001
Selection × Density	2	0.0753	12.95	0.0067

significant differences in slope between the selection regimes (analysis not shown). In the generation 120 assay, the difference between FEJ and JB populations in egg to eclosion survivorship was seen to be entirely due to differences in larval survivorship; the only significant ANOVA effect on pupal survivorship was due to density (Table 4.4). Overall, at both 30 and 300 eggs/vial, larval and egg to adult survivorship in the FEJ populations was significantly lower than that of both JB and yellow body populations (Fisher's LSD, $p < 0.01$), which did not, however, significantly differ between themselves (Fig. 4.9).

The pattern of effects of density (30 versus 300 eggs/vial) on egg to adult survivorship of the JB and FEJ populations was consistent across the two assays (Table 4.5: non-significant ANOVA effect of selection × density × assay). In both assays, FEJ populations, regardless of density, had lower egg to adult survivorship than JB populations, with the proportionate difference becoming smaller at the higher density (Figs. 4.8, 4.9 c; Table 4.5: significant ANOVA effects of selection, density and selection × density). The principal difference between assays was that survivorship for both FEJ and JB populations at 300 eggs/vial was lower in the generation 120 compared to the generation 90 assay (Table 4.5: significant ANOVA effects of assay and density × assay). This effect is likely to be due to slight differences in food quality and/or volume between assays.

Bi-typic cultures: competitive ability

The competitive performance of the yellow body populations improved from the generation 90 to the generation 120 assay (Fig. 4.10 a), and this likely represents the effects of a further 30 generations of adaptation to the conditions in our laboratory (our food medium and temperature of rearing are different from the conditions at the stock

Table 4.4. Results of mixed model ANOVA on mean arcsine square root life-stage specific survivorship of FEJ, JB and yellow body populations in mono-typic culture at 30 and 300 eggs/vial in the generation 120 assay. Random effects and interactions that cannot be tested for significance have been omitted for brevity.

Effect	*df*	MS	*F*	*P*
Larval survivorship				
Population	2	0.0983	22.35	0.0017
Density	1	0.1376	24.53	0.0158
Population × Density	2	0.0011	0.33	0.7286
Pupal survivorship				
Population	2	0.1662	1.12	0.3858
Density	1	1.7570	62.69	0.0042
Population × Density	2	0.0753	0.91	0.4516
Egg to eclosion survivorship				
Population	2	0.1662	65.91	< 0.0001
Density	1	1.7570	35.79	0.0093
Population × Density	2	0.0753	1.40	0.3177

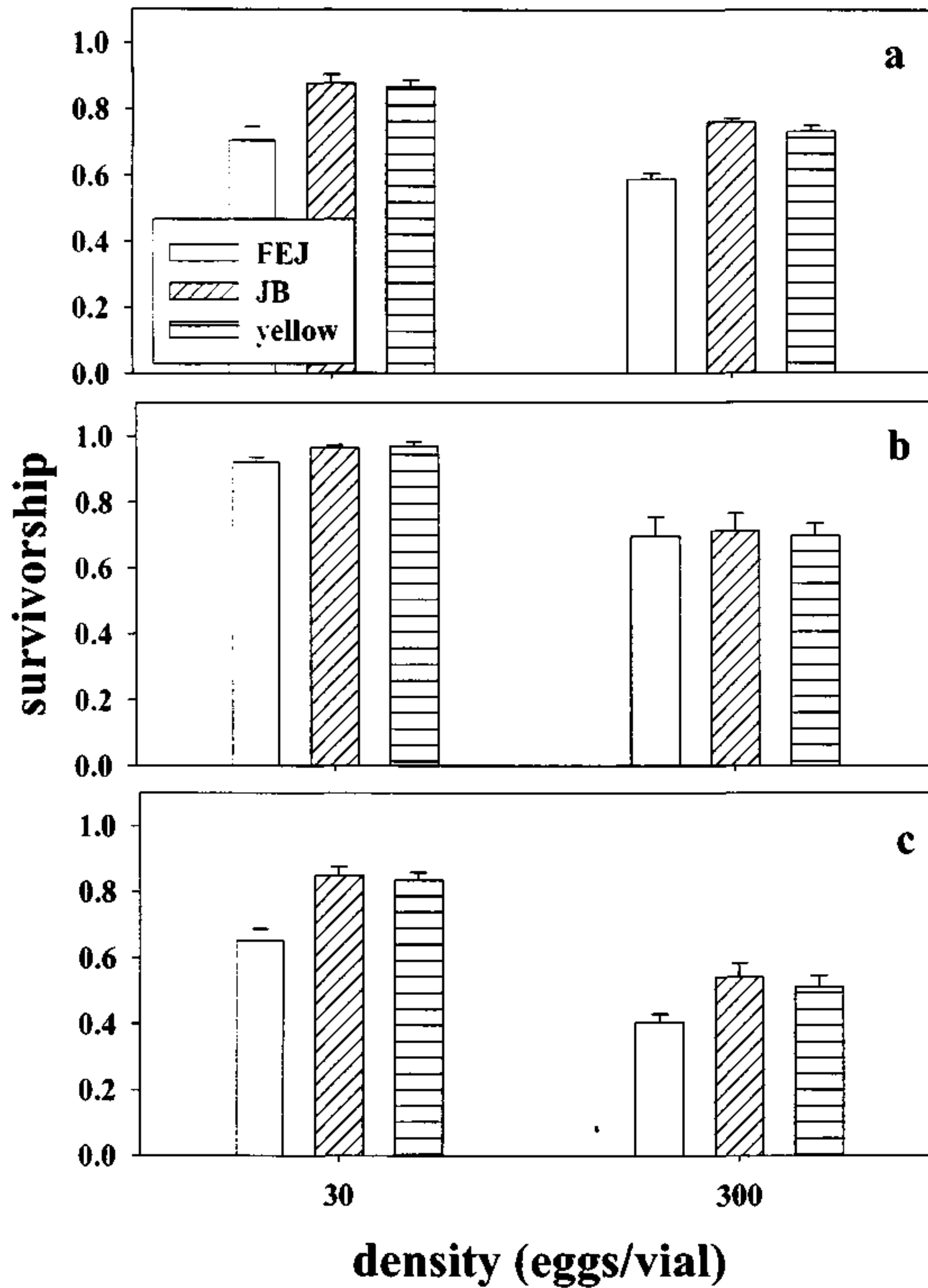

Figure 4.9. Mean (± s.e.) (a) Larval (b) Pupal and (c) Egg to adult survivorship in mono-typic cultures of FEJ, JB, and yellow body populations at two different densities in the generation 120 assay.

Table 4.5. Results of mixed model ANOVA on mean arcsine square root egg to adult survivorship of FEJ and JB populations in mono-typic culture at 30 and 300 eggs/vial in the generation 90 and 120 assays. In this design, block and interactions involving block cannot be tested for significance, and have therefore been omitted from the table.

Effect	*df*	MS	*F*	*P*
Selection	1	0.3801	125.59	0.0015
Density	1	1.2096	122.86	0.0016
Assay	1	0.0477	10.40	0.0484
Selection × Density	1	0.0644	19.40	0.0217
Selection × Assay	1	0.0076	2.15	0.2385
Density × Assay	1	0.0644	13.16	0.0360
Selection × Density × Assay	1	0.0140	2.21	0.2338

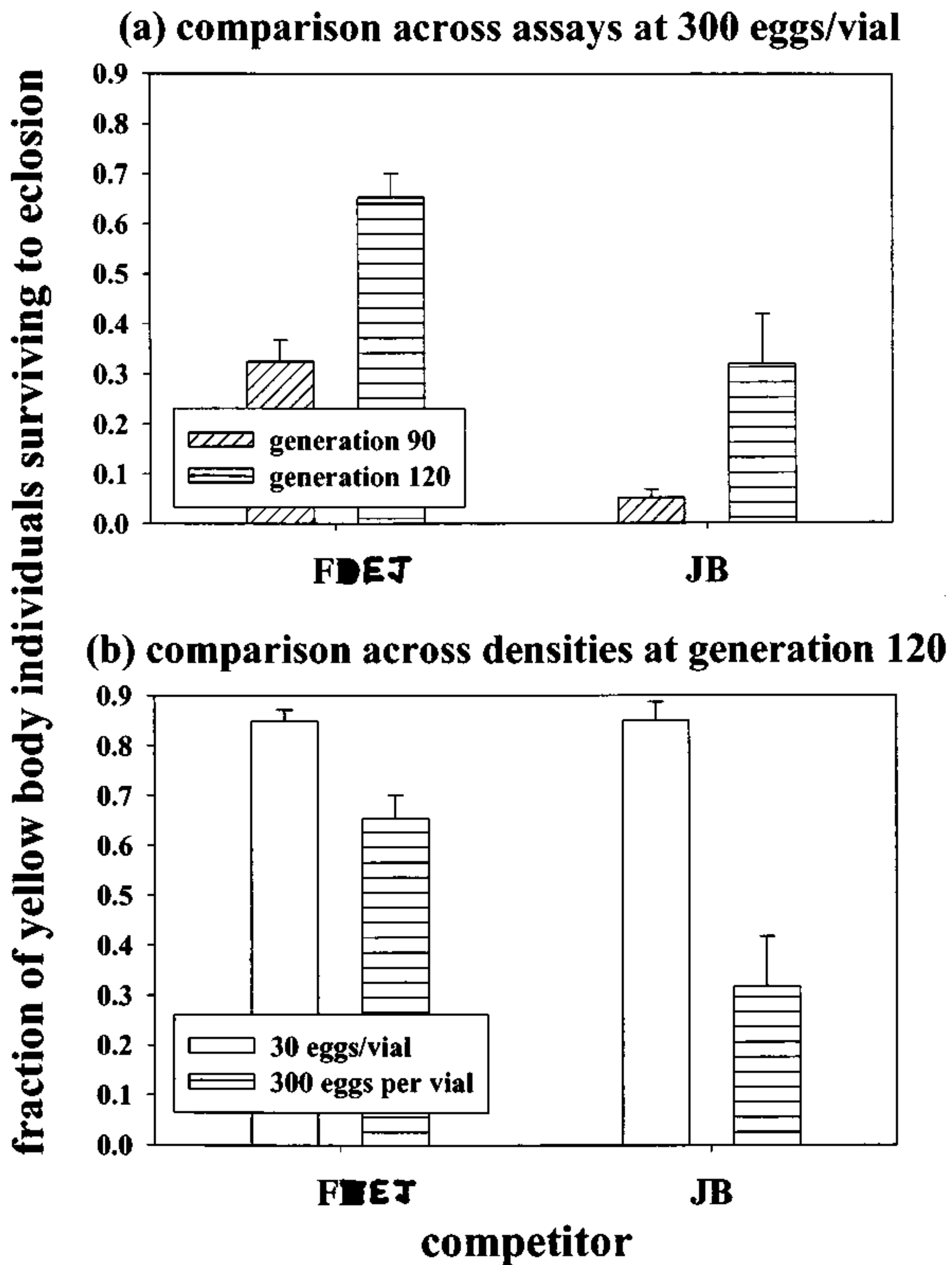

Figure 4.10. Mean (± s.e.) egg to eclosion survivorship of yellow body individuals when in competition with FEJ or JB individuals at different densities (30 eggs/vial in generation 120 assay; 300 eggs/vial in the generation 90 and 120 assays).

centre from where the yellow body flies were obtained). The pattern of relative competitive ability of the yellow body populations against the FEJ and JB populations was, however, consistent across assays (Fig. 4.10 a; no significant competitor identity × assay interaction at 300 eggs/vial: ANOVA table not shown). In the generation 120 assay, the egg to eclosion survivorship of yellow body populations was close to 0.85 at a density of 30 eggs/vial, regardless of whether the competition was with itself (mono-typic cultures) or with JB or FEJ populations (bi-typic cultures) (Figs. 4.10 b, 4.11). At a density of 300 eggs/vial, however, survivorship of yellow body populations was greatest when competing with FEJ populations, and least when competing with JB populations (Fig. 4.11, Table 4.6). However, the survivorship difference between yellow bodies in mono-typic culture and in competition against FEJ populations was not significant (Fisher's LSD, $p = 0.10$) whereas there were significant differences (Fisher's LSD, $p < 0.01$), between the survivorship of yellow body populations competing against FEJ and JB, and between yellow body populations in mono-typic culture and competing against JB populations.

DISCUSSION

The results from these two competition assays clearly show that (a) in mono-typic cultures, FEJ populations are somewhat less susceptible to the adverse effects of increasing density than the JB controls (Figs. 4.8, 4.9 c; Table 4.5), and (b) the FEJ populations are substantially poorer competitors than the JB controls (Fig. 4.11, Table 4.6). These observations are contradictory to both predictions from the restricted version of "classical" density-dependent selection theory (e.g. MacArthur & Wilson, 1967; Roughgarden, 1971) and the expectation that faster development confers a competitive

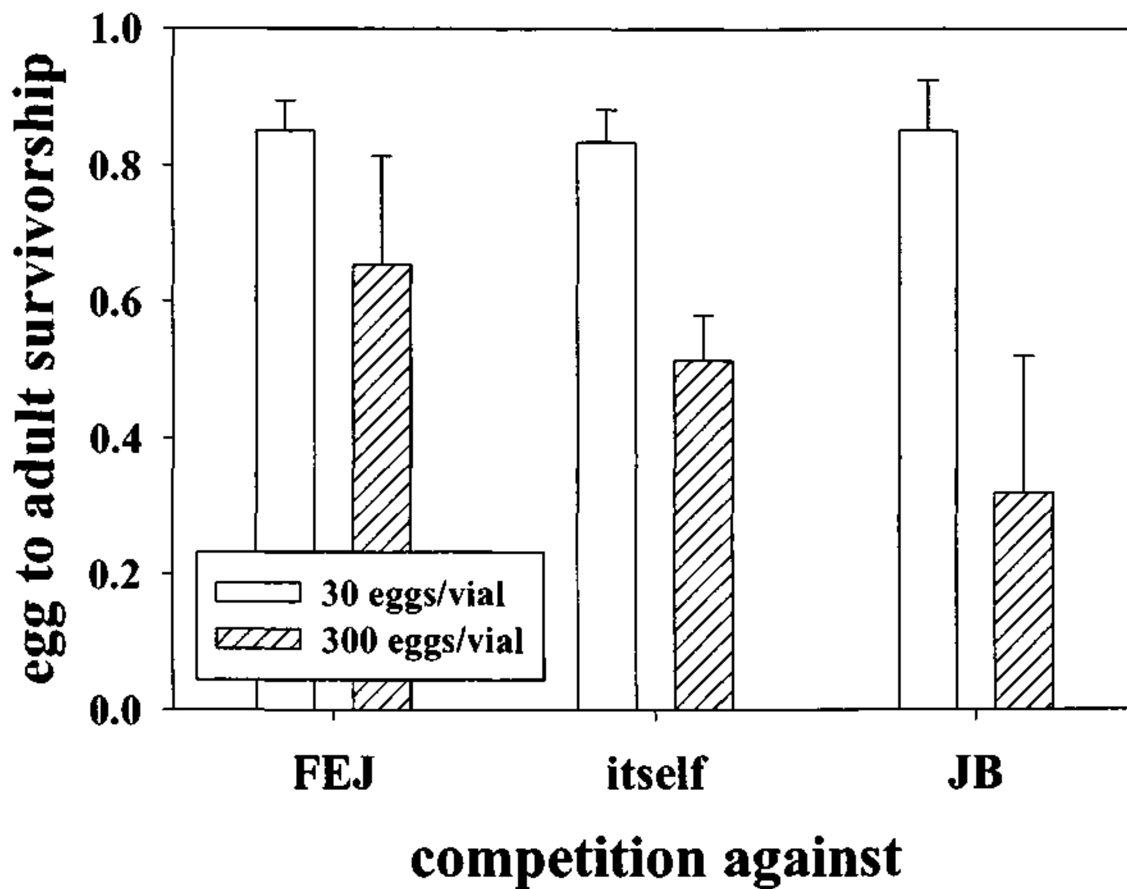

Figure 4.11. Mean (± s.e.) egg to eclosion survivorship of yellow body individuals at two different densities in mono-typic (competition against itself), and bi-typic (competition against FEJ or JB) cultures in the generation 120 assay.

Table 4.6. Results of mixed model ANOVA on mean arcsine square root egg to adult survivorship of yellow body populations in mono-typic and bi-typic cultures (competitor: itself, FEJ, JB) at 30 and 300 eggs/vial in the generation 120 assay. In this design, block and interactions involving block cannot be tested for significance, and have therefore been omitted from the table.

Effect	*df*	MS	*F*	*P*
Competitor	2	0.0630	6.13	0.0354
Density	1	0.9370	40.19	0.0079
Competitor × Density	2	0.0693	15.89	0.0040

advantage (e.g. Bakker, 1969; Prout & Barker, 1989; Borash *et al.* 2000; Krijger *et al.* 2001). A closer examination of the reasons for this apparent contradiction, in my opinion, helps underscore the subtlety of the evolutionary process and the need to be very circumspect in making broad generalizations about what kinds of trait may be expected to evolve under particular selection pressures.

Certainly, I would tend to agree with the general assertion that 'all else being equal, faster development should translate into increased competitive ability'. The point, however, that I wish to make is that is all else is clearly not equal in the case of the FEJ populations. The FEJ populations pay a heavy fitness cost, relative to the JB controls, in terms of increased pre-adult mortality (Chapter 3; Figures 4.8, 4.9 c). Moreover, the FEJ populations have evolved lower feeding rates (Chapter 3) and urea tolerance (Fig. 4.5; Table 4.1) than the JB controls, and these traits are likely to reduce the effectiveness and tolerance components, respectively, of their competitive ability. Although a discernible viability cost to faster development becomes apparent only after about 50 generations of selection (Chapter 3), reductions in larval feeding rate are apparent even after 10 generations of selection for faster development in a set of populations different from but related to the FEJs (N. G. Prasad, M. Shakarad and A. Joshi, *unpubl. data*), suggesting that even moderate reductions in development time may not yield a benefit in terms of competitive ability. I stress, however, that faster development leading to reduced competitive ability as a correlated response to evolution does not imply that fast development may not be a good indicator of competitive ability in inter-specific comparisons.

Overall, these results underscore the growing realization that correlated responses to selection in laboratory experiments are the result of myriad complex and subtle interactions between the nature of selection and the laboratory ecology of the cultures being used (Rose *et al.* 1996; Ackermann *et al.* 2001; Prasad & Joshi, 2003). For example, upon closer examination, even the widely believed and apparently well established notion of the positive association between body size and male mating success in *Drosophila* turns out to depend crucially on the factors responsible for the size variation in the population being studied and on its genetic composition (Zamudio *et al.* 1985; Santos *et al.* 1994; Santos, 1996; Joshi *et al.* 1999). Similarly, rates of larval weight gain actually decline in the FEJ populations, even though on optimality principles one might expect increased rates to be very beneficial under such a selection regime (Chapter 3). The lesson to be drawn from all these studies is that one must be very careful when formulating broad predictions about the kinds of correlated responses one expects under different selection regimes. Often the seemingly trifling details of laboratory maintenance procedures, and the specific behavioral or physiological traits underlying changes in fitness components, can give rise to unexpected patterns of correlated responses to selection.

Chapter 5: Evolution of Adult Traits

In Chapter 3, the relationship between pre-adult development time and adult size was explored in detail. In the present chapter, I enlarge the scope of the discussion by including adult traits related to the life-history, in order to better understand the evolutionary consequences of directional selection for faster development and early reproduction. As noted earlier, adult size in *Drosophila*, is determined largely by resources acquired and assimilated during the larval stage. In fact, the pre-adult duration in *Drosophila* is thought to have evolved as a compromise between the conflicting necessities of developing faster and having a larger adult soma (Santos *et al.* 1997), the latter being associated with higher adult fitness (Robertson, 1957; Partridge & Farquhar, 1983; Mueller, 1985; Partridge *et al.* 1987a; Markow & Ricker, 1992). On the other hand, the relation between development time and body size in *Drosophila* can be affected by nutritional levels (Robertson, 1963). Moreover, studies on populations of *D. melanogaster* subjected to different kinds of demographic selection selected have shown that development time and body size can evolve independently of each other (Chippindale *et al.* 1994). The generality of the positive correlation between adult size and fitness in *Drosophila* further called into question by a number of studies that find the two to be uncorrelated (Santos *et al.* 1994; Zamudio *et al.* 1995; Santos, 1996; Joshi *et al.* 1999; da Silva & Valente, 2001). Overall, it appears that body size is positively correlated with fitness in *Drosophila* populations with large size variation (e.g. wild populations or crowded laboratory populations), whereas in moderate density laboratory populations, in which size variation is relatively small, fitness and body

size are often not strongly correlated (Joshi *et al.* 1999; da Silva & Valente, 2001; Prasad & Joshi, 2003).

All studies in which decreased pre-adult development time was directly selected in *Drosophila*, yielded a correlated reduction in the adult size in the faster developing populations compared to their controls (Zwaan *et al.* 1995a; Nunney, 1996; Chippindale *et al.* 2003a; Chapter 3). A major part of the development time decline in these populations was due to reductions in larval duration rather than in pupal duration (Nunney, 1996; Chippindale *et al.* 1997a; Chapter 3) and the reduction in the third instar duration appears to constitute most of the reduction in larval duration (Chapter 3), The third instar is the stage of larval development during which most weight gain occurs. Hence, reduced adult size of fast developing *Drosophila* populations appears to be a direct consequence of reduced larval provisioning, which, in turn, is likely to affect adult traits other than just body size.

The effects of pre-adult resource acquisition and adult size on adult fitness components are likely to be mediated through traits such as adult starvation and desiccation resistance, and lipid and glycogen levels (Chippindale *et al.* 1996, 1998). Larvae from populations selected for higher starvation resistance had higher growth rate and assimilated lipids at a much higher rate than control populations (Chippindale *et al.* 1996), whereas those selected for increased desiccation resistance assimilated glycogen at a higher rate than controls (Chippindale *et al.* 1998). Adults from starvation and desiccation selected populations also had increased lipid and glycogen, respectively, than adults from control populations (Chippindale *et al.* 1996, 1998). These studies also revealed that populations

selected for increased starvation and desiccation resistance had significantly higher wet weights, dry weights and water content, along with increased development time (Chippindale *et al.* 1996, 1998), although, other studies on desiccation resistance in *D. melanogaster* found no difference in either weight, water content or development time between selected and control populations (Hoffmann & Parsons, 1993a, 1993b). The evolution of higher resistance to starvation and desiccation is also seen to exact a fitness cost in terms of lower egg to adult viability than controls in the selected populations (Chippindale *et al.* 1996, 1998). Direct selection for decreased development time decreased starvation resistance of selected populations in one study (Chippindale *et al.* 2003a), whereas it had no effect on the starvation resistance or lipid content in another study (Zwaan *et al.* 1995a).

The relationship between development time and longevity is of fundamental interest in the context of understanding the interplay between resource acquisition, partitioning and utilization in the life-history, because development time greatly affects the amount of resources acquired whereas longevity is further affected by patterns of resource allocation and utilization. The relationship between development time and longevity is also useful for testing the developmental theory of ageing (Lints, 1978, 1988) which predicts a positive correlation between these two traits. Although Chippindale *et al.* (2003a) found decreased longevity in their faster developing populations, several other studies (Chippindale *et al.* 1994; Zwaan *et al.* 1995b, Nunney, 1996; Partridge *et al.* 1999) did not find a causal relation between development time and adult longevity, suggesting that development time and longevity can evolve independently of one another.

In this chapter, I discuss the evolution of adult traits in populations of the FEJ populations selected for faster pre-adult development and early reproduction in our laboratory. The FEJ populations develop about 25% faster than their controls and have reduced larval growth rates, pre-adult survivorship and adult weights at eclosion (Chapter 3).

MATERIALS AND METHODS

Maturation time assay

Maturation time, the time duration between eclosion and first mating was assayed after 70 generations of FEJ selection. Flies were collected within one hour of eclosion and a freshly eclosed fly was transferred into a vial containing the standard banana-jaggery food and provided with two mature, three day old, virgin flies of the opposite sex. The vial was then continuously monitored until the first mating occurred. Couplings of males and females that lasted less than three minutes were not recorded as matings. For each mating, the time duration between eclosion and first mating, and the duration of copulation were recorded. Twenty vials per sex per population were set up, and the vials were arranged randomly on a table under uniform overhead lighting in a room where temperatrure was maintained at 25 ± 0.5°C.

Fecundity assay

Fecundity was measured at generations 20 and 50 of FEJ selection at two ages (day 3 post-eclosion and day 11 post-eclosion), and under two nutritional conditions (with and without yeast supplement for the adult flies for three days prior to the assay). The ages were chosen to match the age of the flies when eggs are

collected from them during the regular maintenance protocol (3 days for FEJs and 11 days for JBs). The flies were maintained as mixed sex groups at a density of about 50 flies per vial on regular banana-jaggery food with transfers to fresh food every alternate day. For nutritional conditioning, flies were sorted into vials that contained either plain banana-jaggery food (unyeasted condition) or a smear of yeast-acetic acid paste along the vial wall in addition to banana-jaggery food (yeasted condition). The flies were held in these vials for three days prior to the assay.

For measuring fecundity, flies from the conditioning vials were sorted under light carbon dioxide anesthesia and one male and one female were placed into vials containing 3 ml of banana-jaggery food. Forty such vials were set up for each nutritional status × age × population combination. After 24 hours, the flies were discarded and the number of eggs in each vial were counted.

At generation 20 of FEJ selection, I also measured the dry weight of the females on days 3 and 11 post-eclosion. Flies from the conditioning vials were freeze killed, sexed and distributed in groups of five each into clean dry vials. These were dried at 70°C for 36 hours and weighed to the nearest 0.0001g. From these data, I calculated fecundity per unit dry weight as the mean fecundity of a population divided by the mean dry weight of the females of that population.

Lifetime fecundity assay

Lifetime fecundity was measured after 70 generations of FEJ selection. Eggs were collected from the standardized flies by placing a fresh food plate in the cages for 4 hours. The eggs were then dispensed into vials containing 6 ml banana-jaggery

food at a density of 50 eggs per vial. Twenty such vials were set up per population. As there was a significant difference in the developmental time of the control and selected populations (Chapter 3), peak eclosion of flies from the selected and control populations was made to coincide by staggering the egg collection by the developmental time difference (Chapter 2). Hence, flies from FJ and JB populations were of same age when the assay was started. The early and late eclosing flies were discarded and only the flies from the peak (middle) of eclosion distribution were used in this assay. Within 6 hours of eclosion, the flies were lightly anesthetized using carbon dioxide and a male and female were put into a vial containing ~3 ml banana-jaggery food. Forty such vials were set up per population. Each pair was transferred each day at about the same time to fresh food vials. The eggs laid in each vial by the female in a 24 hour period were counted. The daily egg counts were carried out till the female died. When a male died or escaped, it was replaced with a virgin male of the same age. If a female escaped during transfer to fresh food vials, the data from such vials were not used in the analysis. For each female in this assay, I obtained daily fecundity, life time fecundity and longevity.

Female starvation resistance and lipid estimation

Starvation resistance and lipid content were assayed after 125 generations of FEJ selection on females at three ages: freshly eclosed, 3 day old and 10 day old. For 3 day old and 10 day old flies, starvation resistance was measured under both unmated and mated conditions. The flies were maintained as either single sex groups (by sorting the sexes under light carbon dioxide anesthesia, within 4 hours of

eclosion to ensure virginity) or mixed sex groups, at a density of about 50 flies per vial on regular banana-jaggery food with transfers to fresh food every alternate day.

The starvation resistance assays were carried out at 25°C, constant light and about 90% relative humidity. Eight females were placed in a vial containing 6 ml non-nutritive agar to prevent desiccation. Five such vials were set up per population at each adult age. Mortality was scored at six hour intervals until all the eight flies in the vial died. Thus, each vial yielded a mean value of starvation resistance across 8 flies. The grand mean of the five vials is taken as the mean starvation resistance of the population.

Lipid content was estimated following Zwaan *et al.* (1991). Groups of 10 flies were freeze killed, dried at 70°C for 36 hours and weighed to obtain the dry weights. These flies were then placed in excess of ether and lipid was extracted over a 24 hour period with gentle agitation. The flies were then removed from the ether, dried at 70°C for about 2 hours, and weighed to obtain lipid free dry weights. The difference between dry weight before and after ether extraction was taken as the total lipid content. Fractional lipid content was estimated as the ratio of total lipid content to the dry body weight before ether extraction. Starvation resistance per unit lipid was estimated by dividing the mean starvation resistance (in hours) by mean total lipid content for each population.

Longevity assay

Longevity of reproducing flies was assayed at generations 10, 30 and 70 of FEJ selection. One-day old flies were sorted into vials containing about 4 ml of banana-jaggery food at a density of four males and four females per vial. Ten such

vials were set up per population in the generation 10 and 30 assays, whereas 20 such vials were set up per population in the generation 70 assay. Flies were transferred to fresh food vials every alternate day, and mortality was checked every day. After 20 generations of FEJ selection, longevity of virgin flies was assayed. The assay was similar to the one described above, except for the fact that the flies were isolated as virgins and 10 vials were set up per sex per population with each vial containing eight flies of the same sex.

Statistical analyses

FEJ and JB populations bearing identical numerical sub scripts were treated as random blocks in the analyses. Population means were used as the unit of analysis, and all data were subjected to mixed model ANOVAs treating selection regime and, wherever appropriate, the age of the flies, nutritional status or mating status as fixed factors crossed amongst themselves and with the random blocks. All statistical analyses were implemented using STATISTICA™ for windows release 5.0 B (StatSoft Inc., 1995).

RESULTS

Maturation time

Mean maturation time in FEJ populations was longer than that in the JB populations, with FEJ females taking about 37% more time from eclosion to first mating compared to the JB females, whereas the difference in males was about 17% (Fig. 5.1). A three way mixed model ANOVA showed a significant effect of selection ($F_{1,3} = 26.86$, $p = 0.014$), and no significant effects of sex ($F_{1,3} = 1.95$, $p = 0.26$) or the selection × sex interaction ($F_{1,3} = 7.44$, $p = 0.072$). Multiple

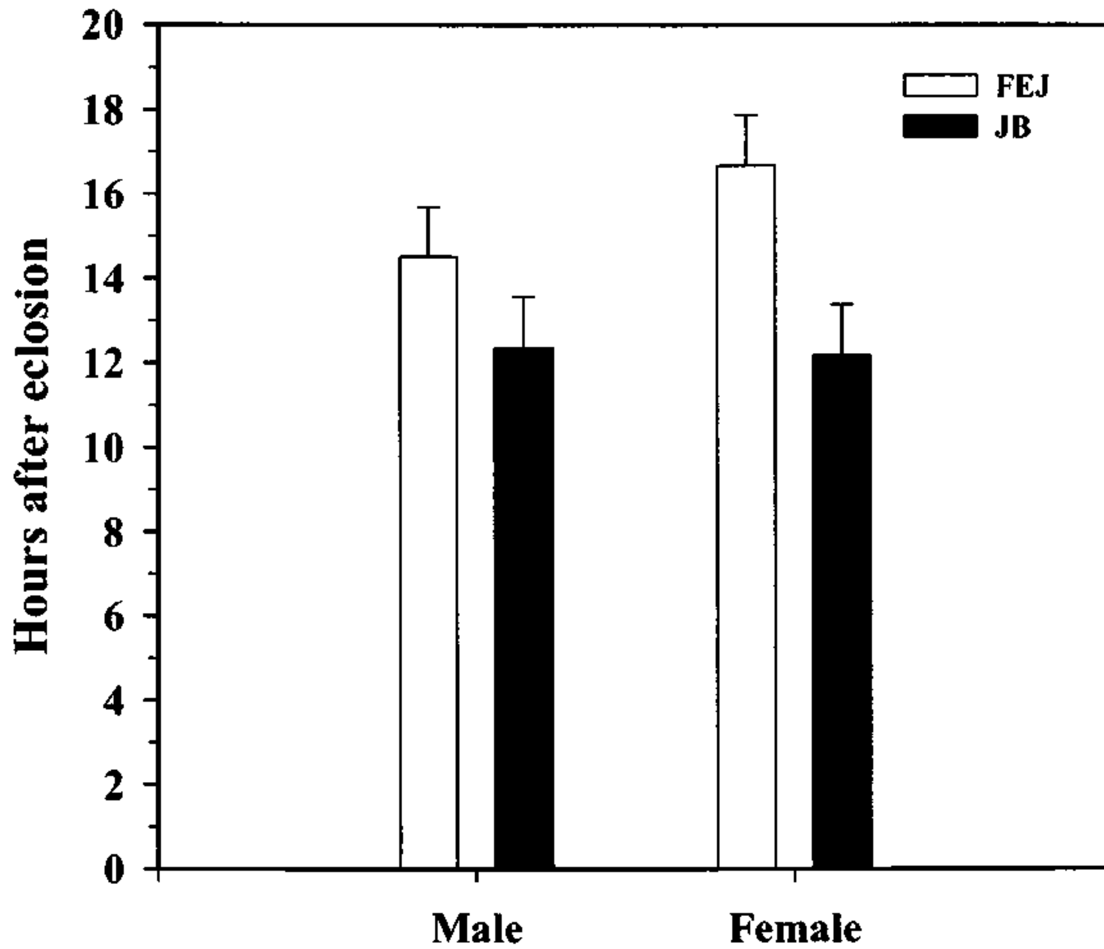

Figure 5.1. Mean maturation time (time from eclosion to first mating) in the FEJ and JB populations after 70 generations of FEJ selection. The error bars represent 95% confidence intervals based on the least squares estimate of the appropriate error mean squared term in the ANOVA and, hence, can be used for visual hypothesis testing.

comparisons revealed a significant difference in maturation time between the FEJ and JB for both females ($t = 7.32$, $p < 0.001$) and males ($t = 3.5$, $p < 0.05$).

Fecundity

The fecundity data from the two experiments (generation 20 and 50 of FEJ selection) were analysed separately using four-way mixed model ANOVAs, the results of which are summarised in Table 5.1. At generation 20, there was no significant difference in the fecundity of FEJs and JBs regardless of age and conditioning (Fig 5.2 a, Table 5.1). Fecundity earlier in life was higher than late in life, and yeasting increased the fecundity of both FEJ and JB populations. Multiple comparisons showed no significant difference between FEJ and JB females.

The results from the ANOVA on fecundity per unit weight (Fig 5.2 c, Table 5.1) were similar except for a significant selection × age × nutritional status interaction. This interaction is because, on day three post eclosion, FEJs have significantly higher fecundity per unit dry weight compared to the JBs under unyeasted conditions ($t = 5.081$, $p < 0.01$), whereas under yeasted conditions, JBs have marginally higher fecundity per unit dry weight (Fig 5.2 c).

At generation 50 of selection, the fecundity of the FEJs was significantly lower than that of the JBs (Fig 5.2 b, Table 5.1). Multiple comparisons indicated that the fecundity of FEJs was significantly ($p < 0.05$)lower than that of JBs at all ages and nutritional conditions, except on day 10 post eclosion under unyeasted condition ($t = 2.82$, $p > 0.05$).

Table 5.1. Summary of results of the separate four factor ANOVAs on age specific fecundity at generations 20 and 50 of FEJ selection. Here selection, age of the fly (3 days versus 11 days post eclosion) and condition (Nutritional condition – yeasted versus unyeasted) were treated as fixed factors crossed with random blocks. In this design, the effects of blocks and interactions involving the block cannot be tested for significance and have therefore been omitted for brevity.

Generation	Effect	*df*	MS	*F*	*P*
20 Fecundity	Selection (S)	1	98.26	0.9831	0.3945
	Age (A)	1	1295.25	219.0166	0.0007
	Condition (C)	1	14467.54	227.7584	0.0006
	Selection × Age	1	126.73	2.3065	0.2261
	Selection × C	1	159.95	3.7016	0.1501
	A×C	1	227.87	2.8772	0.1884
	S×A×C	1	20.24	0.9524	0.4011
20 fecundity per unit dry weight of female	Selection (S)	1	<0.0001	0.0528	0.8331
	Age (A)	1	0.0041	43.0976	0.0072
	Condition (C)	1	0.04	131.3971	0.0014
	Selection × Age	1	0.0003	1.7458	0.2781
	S×C	1	0.0004	1.8095	0.2712
	A×C	1	0.0003	0.6553	0.4775
	S×A×C	1	0.0002	11.707	0.0418
50 Fecundity	Selection (S)	1	2657.97	146.1635	0.0012
	Age (A)	1	1075.93	264.2306	0.0005
	Condition (C)	1	14755.38	864.2151	<0.0001
	S×A	1	45.7	1.6537	0.2887
	S×C	1	379.1	6.2374	0.0879
	A×C	1	72.16	0.8739	0.4188
	S×A×C	1	119.9	4.8972	0.1138

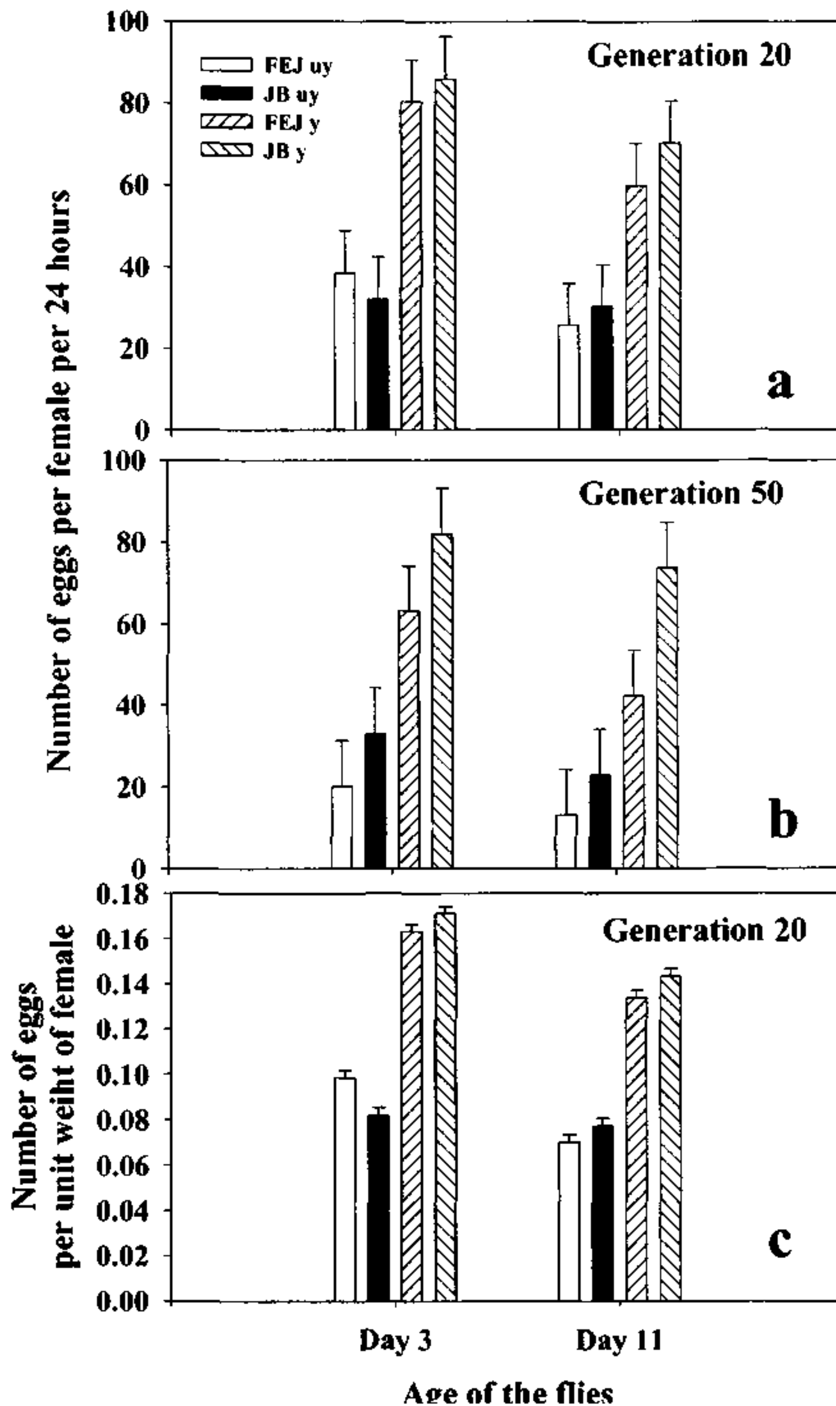

Figure 5.2. Mean fecundity of the females of the FEJ and JB populations at (a) generation 20 and (b) generation 50 of FEJ selection. Panel (c) shows the mean fecundity per unit dry weight of females in FEJ and JB populations measured after 20 generations of FEJ selection. The error bars represent 95% confidence intervals based on least squares estimate of the appropriate error mean squared term in the ANOVA and, hence, can be used for visual hypothesis testing. UY and Y in the legend refer to unyeasted and yeasted conditioning, respectively

Lifetime fecundity and longevity

After 70 generations of FEJ selection, the mean total lifetime fecundity of the FEJ females was significantly less than that of the JBs ($F_{1,3}$ = 14.31, p = 0.032) (Fig. 5.3), being reduced by about 33%. There was, however, no significant difference in the mean longevity of FEJ and JB females used in the lifetime fecundity assay ($F_{1,3}$ = 0.0177, p = 0.90). If we examine the pattern of daily fecundity over the lifetime of FEJ and JB females (Fig 5.4), it is clear that daily fecundity changes markedly over time and does so differently in FEJ and JB populations (Table 5.2). In this assay, survivorship was very high till about 15 days post eclosion, then mortality reduced the sample size over time. Hence, the daily average fecundity was calculated till at least 8 flies were left in each population.

In both JB and FEJ populations, an early peak in fecundity is followed by a dip and then a subsequent peak around day 10 after eclosion (Fig 5.4). Multiple comparisons indicated that in JBs, there was a significant difference in the average daily fecundity on days 2 and 6 (df = 102, t = 10.71, $p < 0.05$) and on days 6 and 9 (df = 102, t = 4.71, $p < 0.05$) post eclosion, whereas in the FEJs the mean daily fecundity was significantly different between days 3 and 6 (df = 102, t= 2.1, $p <$ 0.05) and on days 6 and 9 (df= 102, t = 3.05, $p < 0.05$) post eclosion. The JBs show a very strong and distinct first peak, two days post eclosion, while the second peak is less sharp and spread out between days 9 - 11 post eclosion. There is also a third peak around day 26 after eclosion. On the other hand, the first peak in FEJs is much smaller than that in the JBs, and is spread over the second and the third days. Similarly, the second peak of the FEJs is more like a plateau between days 10 - 14,

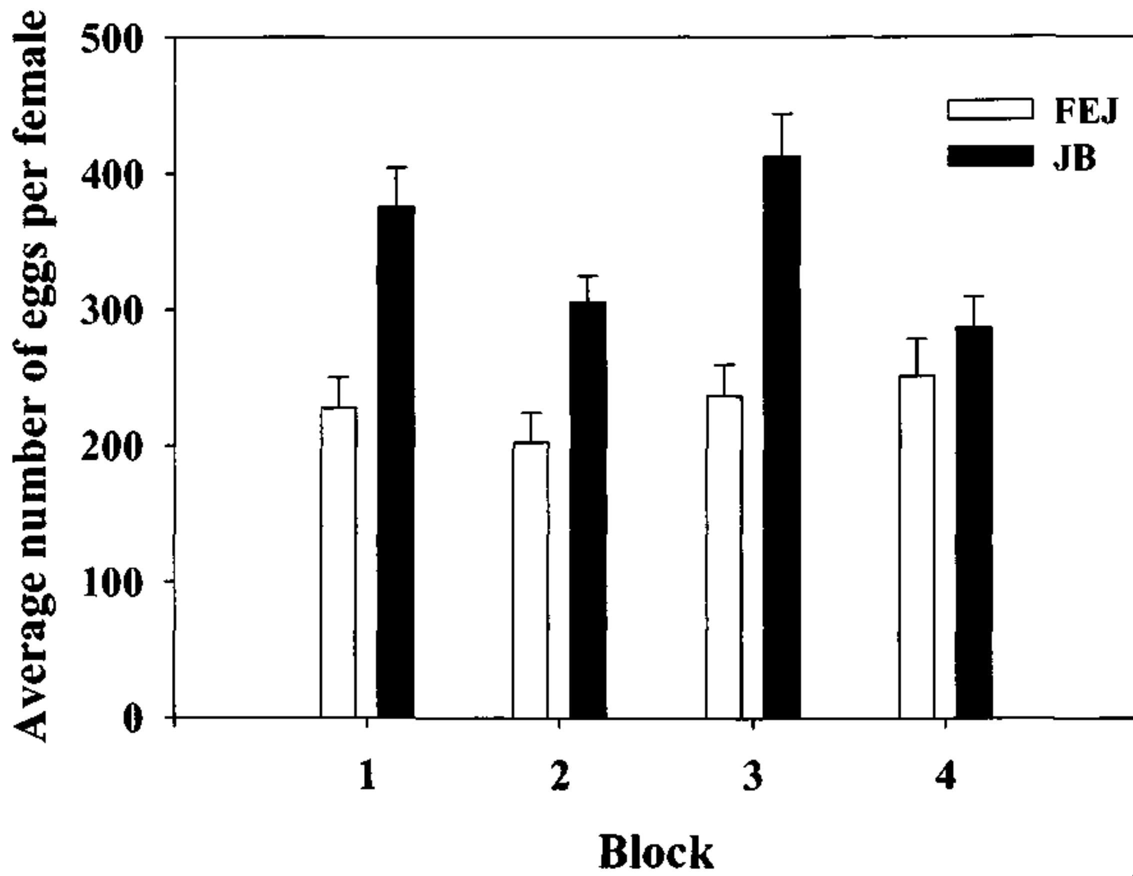

Figure 5.3. Mean total lifetime fecundity (± s.e.) of females from the four FEJ and JB populations after 70 generations of FEJ selection.

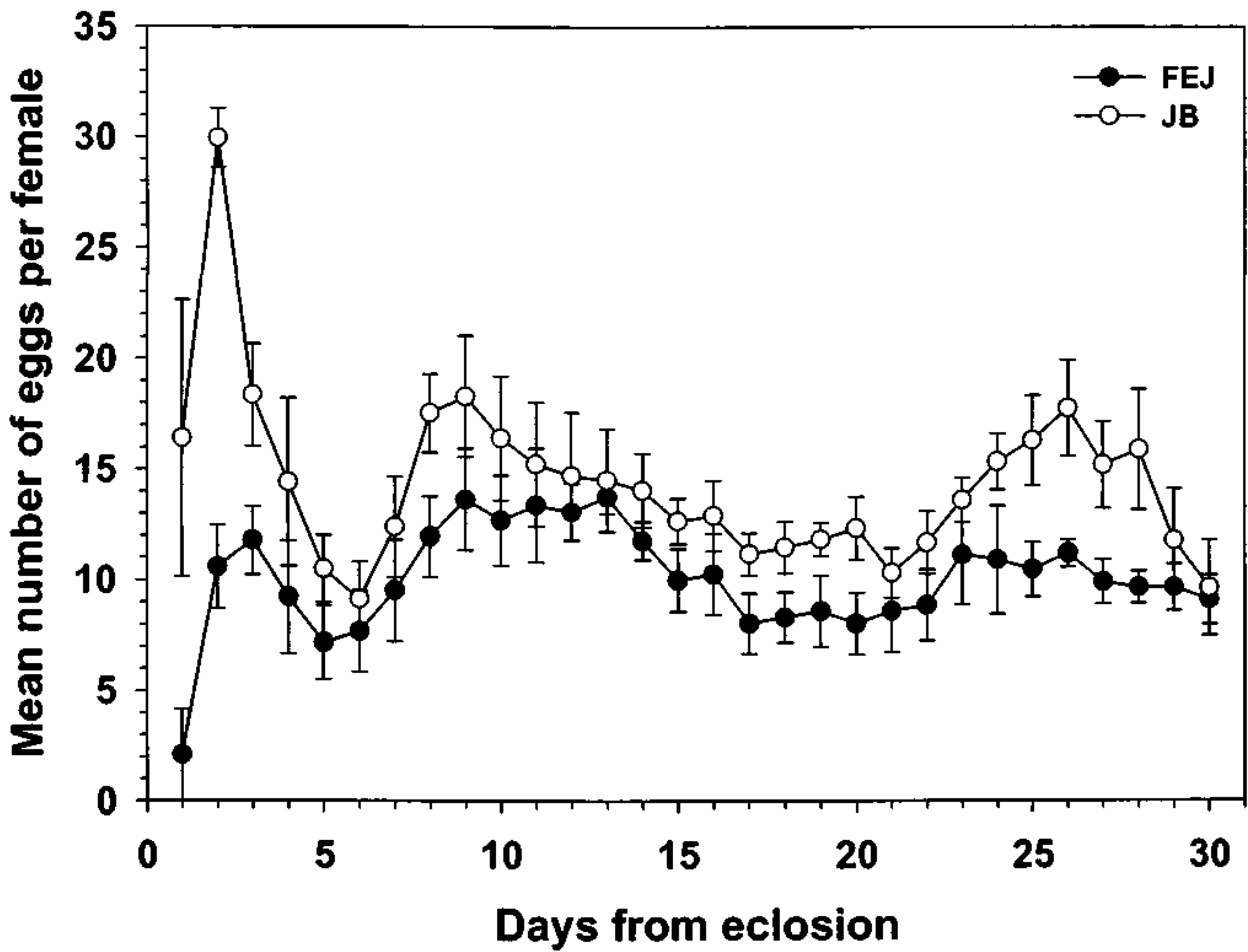

Figure 5.4. Mean daily fecundity (± s.e.) of the FEJ and JB females averaged over the four replicate populations after 70 generations of FEJ selection.

Table 5.2. Summary of the ANOVA results on the daily average fecundity of FEJ and JB populations. In this three way mixed model repeated measures ANOVA, selection regime was treated as a fixed factor crossed with random blocks with the daily fecundity being the repeated measure. In this design, the effects of blocks and interactions involving the block cannot be tested for significance and have therefore been omitted for brevity.

Effect	*df*	MS	*F*	*P*
Selection	1	1091.37	18.35	0.02
Time	34	65.69	3.33	< 0.01
Selection × Time	34	28.18	3.73	< 0.01

and there is no distinct third peak. The early peak fecundity of JBs is about 36% higher than fecundity during the second peak, and this difference is significant (t test, $p < 0.05$). By contrast, the early peak fecundity of FEJs is not significantly different from that during days 10 - 14 after eclosion (t test, $p = 0.36$). The FEJs have lower average daily fecundity than JBs all through their life. Multiple comparisons showed that the differences between FEJ and JB mean daily fecundity were significant over the first four days of eclosion, days 8 and 9 post eclosion and much later, between days 20 and 30 post eclosion.

Starvation resistance and lipid content

Starvation resistance and lipid content data were analysed in two ways. First, the starvation resistance and lipid content of flies at eclosion, and of mated flies on days 3 and 10 post eclosion, were analysed using a three way ANOVA treating selection regime and age of the flies as fixed factors crossed amongst themselves and with random blocks (Table 5.3). Second, starvation resistance and lipid content of mated and virgin flies on days 3 and 10 post eclosion were subjected to a four way ANOVA treating selection regime, age of the flies and mating status as fixed factors crossed amongst themselves and with random blocks (Table 5.4).

The FEJs had lower starvation resistance than the JBs at eclosion, as well as days 3 and 10 post eclosion (Fig. 5.5 a), with the reduction in starvation resistance of FEJs at eclosion being about 33%. Starvation resistance declined over the first three days of adult life, reducing by nearly 13% in FEJs and nearly 26% in JBs. Multiple comparisons showed that the difference in starvation resistance of FEJs and JBs was significant at eclosion ($t = 5.48$, $p < 0.001$) but not at other ages.

Table 5.3. Summary of results of the separate three factor ANOVAs on age-specific starvation resistance, starvation resistance per unit lipid, and fractional lipid content in FEJ and JB populations. Here selection and age of the fly (at eclosion, 3 days and 10 days post eclosion) were treated as fixed factors crossed with random blocks. In this design, the effects of blocks and interactions involving the block cannot be tested for significance and have therefore been omitted for brevity.

Trait	Effect	*df*	MS	*F*	*P*
Starvation resistance	Selection (S)	1	4054.96	15.8893	0.0283
	Age (A)	2	3264.96	10.3022	0.0115
	S × A	2	401.02	3.3795	0.104
Starvation resistance per unit lipid	Selection (S)	1	1579.73	56.4695	0.0049
	Age (A)	2	614.94	23.7433	0.0014
	S × A	2	398.5	15.0424	0.0046
Fractional lipid content	Selection (S)	1	0.0196	108.9007	0.0019
	Age (A)	2	0.0012	3.1309	0.1172
	S × A	2	0.0057	20.2747	0.0021

Table 5.4. Summary of results of the separate four factor ANOVAs on age-specific starvation resistance, starvation resistance per unit lipid, and fractional lipid content in FEJ and JB populations. Here selection, age of the fly (3 days versus 10 days post eclosion) and condition (mated versus virgin) were treated as fixed factors crossed with random blocks. In this design, the effects of blocks and interactions involving the block cannot be tested for significance and have therefore been omitted for brevity.

Trait	Effect	*df*	MS	*F*	*P*
Starvation resistance	Selection (S)	1	4948.88	24.28	0.016
	Condition (C)	1	2165.97	17.56	0.0248
	Age (A)	1	105.31	0.1906	0.6919
	S×C	1	390.81	3.42	0.1616
	S×A	1	8.81	0.0578	0.8255
	C×A	1	1694.64	7.6777	0.0695
	S×C×A	1	61.8	1.42	0.3197
Starvation resistance per unit lipid	Selection (S)	1	572.2	34.58	0.0098
	Condition (C)	1	13.59	0.4556	0.5481
	Age (A)	1	75.94	2.12	0.2411
	S×C	1	0.2156	0.0082	0.9337
	S×A	1	27.24	3.32	0.1661
	C×A	1	25.4764	3.41	0.162
	S×C×A	1	15.396	2.4	0.2195
Fractional lipid content	Selection (S)	1	0.0156	17.15	0.0256
	Condition (C)	1	0.0139	24.18	0.0161
	Age (A)	1	0.0064	15.11	0.0302
	S×C	1	0.0007	0.9074	0.4111
	S×A	1	0.0066	43.02	0.0072
	C×A	1	0.0009	0.998	0.3914
	S×C×A	1	0.0004	1.06	0.3799

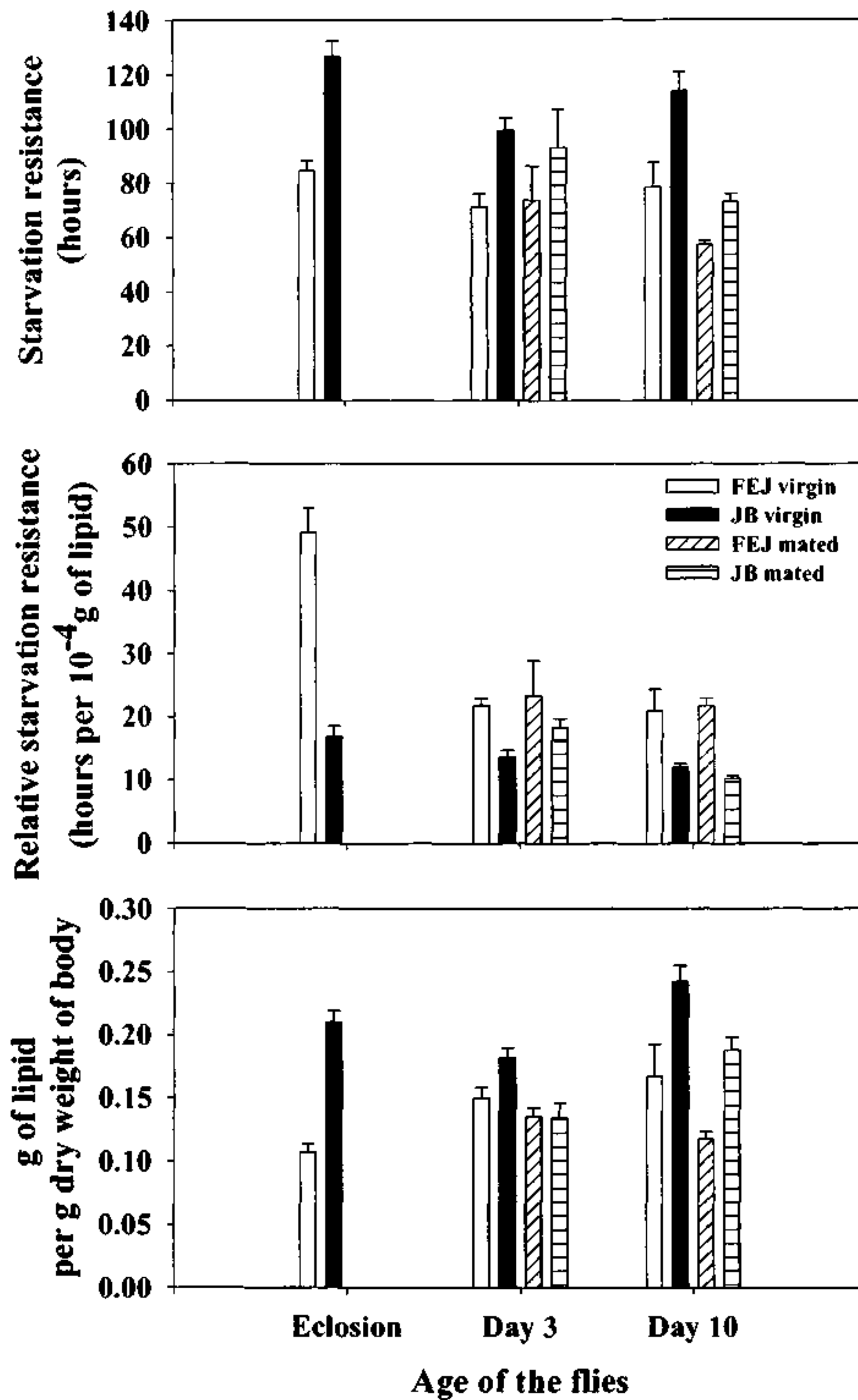

Figure 5.5. Mean (± s.e.) (a) Starvation resistance, (b) Starvation resistance per unit lipid and (c) fractional lipid content of the FEJ and JB females after 125 generations of FEJ selection.

Starvation resistance per unit lipid was significantly higher in FEJs compared to the JBs, and declined by about 53% over the first three days of adult life, whereas there was hardly any change in the relative resistance of JBs with age, resulting in a significant selection × age interaction (Fig 5.5 b, Table 5.3). From day 3 post eclosion to day 10 post eclosion, the starvation resistance per unit lipid of FEJs remained unchanged while that of JBs declined by about 44% (Fig 5.5 b). Multiple comparisons showed that the difference in relative starvation resistance of FEJs and JBs was significant at eclosion ($t = 8.82$, $p < 0.001$) but not at days 3 and 10 post eclosion.

Fractional lipid content was significantly lower in FEJs compared to the JBs (Fig 5.5 c, Table5.3). At eclosion, JBs had twice as much lipid per unit body weight than FEJs. The fractional lipid content of FEJs increased slightly with age, while the JBs showed a huge decline in fractional lipid content over the first three days of adult life, followed by an increase by day 10 post eclosion (Fig 5.5 c). The ANOVA revealed significant effects of selection and selection × age interaction (Table 5.3). Multiple comparisons showed that the difference in fractional lipid content of FEJs and JBs was significant at eclosion ($t = 7.83$, $p < 0.001$) and 10 days post eclosion ($t = 5.38$, $p < 0.001$) but not at 3 days post eclosion ($t = 0.11$, $p > 0.05$).

Examining data from mated and virgin flies at day 3 and 10 post eclosion revealed that FEJs had lower absolute starvation resistance than JBs, and mated flies had lower starvation resistance than virgin flies (Fig 5.5 a, Table 5.4). Multiple comparisons showed a significant difference between virgin and mated FEJs ($t = 4.54$, $p < 0.01$) as well as virgin and mated JBs ($t = 8.73$, $p < 0.001$) 10 days post

eclosion. Compared to the FEJs, the difference between virgin and mated JBs was significantly higher, indicating a greater cost to reproduction in JBs compared to the FEJs over the first ten days of adult life. The mated JBs were nearly 35% less resistant than virgin JBs while the mated FEJs were about 25% less resistant than virgin FEJs at 10 days post eclosion. Starvation resistance per unit lipid was significantly higher in FEJs than JBs and the only significant effect in the ANOVA was due to selection (Table 5.4). Fractional lipid content was higher in JBs than FEJs, at day 10 compared to day 3, and in virgins compared to mated flies (Fig 5.5 c, Table 5.4). Multiple comparisons show that the fractional lipid content in FEJs was significantly lower than JBs 10 days post eclosion regardless of mating status, but the difference was not significant on day 3 at either mating status.

Longevity

After 10 generations of FEJ selection, longevity of mated FEJ females had declined by about 20% relative to the JBs, whereas the longevity of FEJ and JB males remained the same, resulting in a significant effect of selection, sex and the selection × sex interaction (Fig 5.6, Table 5.5). After 30 generations of FEJ selection, longevity of both mated males and females in the FEJ populations was less than that of their JB counterparts, resulting in a significant effect of selection and sex only (Fig 5.6, Table 5.5). After 70 generations of FEJ selection, there was no difference between the longevity of mated FEJs and JBs, with the only significant effect being that of sex (Fig 5.6, Table 5.5). Longevity of virgin flies measured after 20 generations of FEJ selection showed no significant effects of selection or sex, although the difference between FEJ and JB longevity was

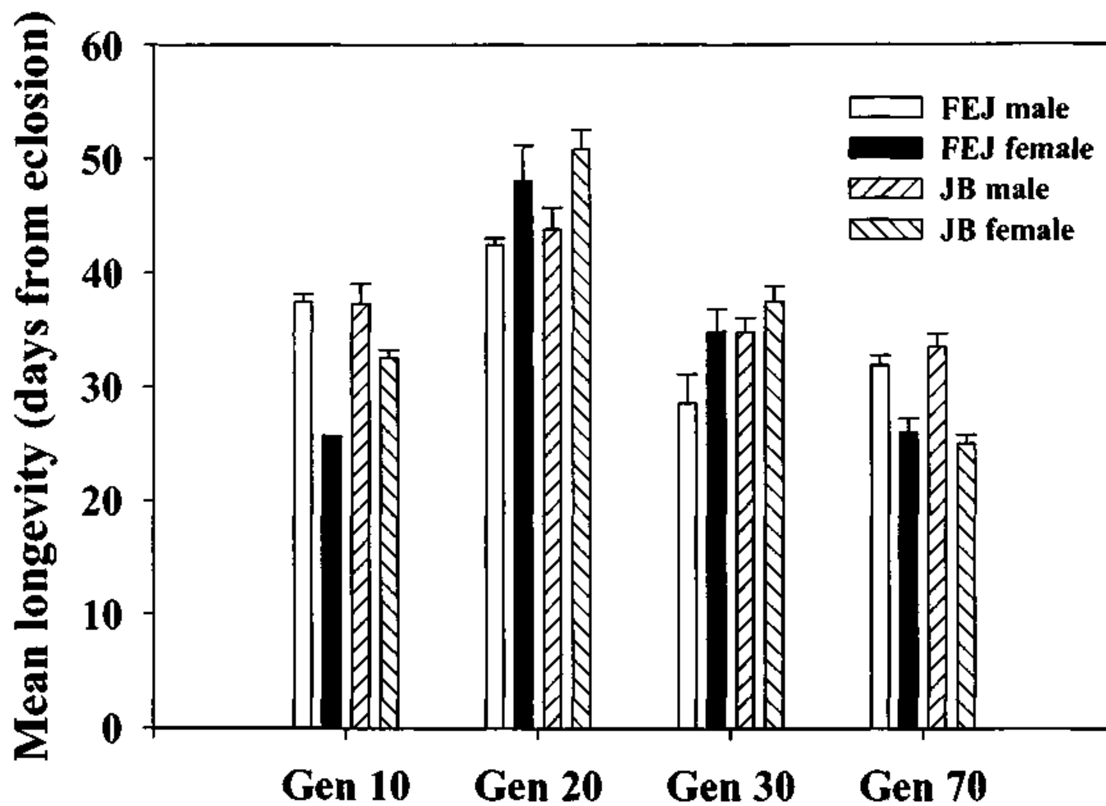

Figure 5.6. Mean longevity of the FEJ and JB populations at various generations of FEJ selection. At generation 20 of FEJ selection, longevity was assayed on virgin flies, whereas all other assays were done on reproducing flies. The error bars represent 95% confidence intervals based on least squares estimate of the appropriate error mean squared term in the ANOVA and hence can be used for visual hypothesis testing.

Table 5.5. Summary of results of the separate three factor ANOVAs on longevity of FEJ and JB populations after 10, 20, 30 and 70 generations of FEJ selection. Selection and sex were treated as fixed factors crossed with random blocks. At generation 20, longevity of virgin flies was assayed whereas at all other generations, longevity of reproducing flies was assayed. In this design, the effects of blocks and interactions involving the block cannot be tested for significance and have therefore been omitted for brevity.

Generation	Effect	*df*	MS	*F*	*P*
10 (mated)	Selection	1	45.8442	12.5433	0.0383
	Sex	1	276.5292	58.4179	0.0048
	Selection × Sex	1	50.5284	15.0397	0.0304
30 (mated)	Selection	1	78.8132	62.6619	0.0042
	Sex	1	80.2624	7.1647	0.0752
	Selection × Sex	1	12.8147	0.3038	0.6199
70 (mated)	Selection	1	0.4027	0.3785	0.5819
	Sex	1	206.1444	34.5849	0.0098
	Selection × Sex	1	7.1231	1.3933	0.3229
20 (virgin)	Selection	1	17.7140	1.6115	0.2938
	Sex	1	1.9471	0.0755	0.8014
	Selection × Sex	1	159.6947	99.0201	0.0022

somewhat greater in females compared to males, resulting in a significant selection × sex interaction. (Fig 5.6, Table 5.5).

DISCUSSION

In *Drosophila*, although the pre-adult and adult stages are sharply demarcated, they are also linked by a transfer of resources from the former to the latter stage, and this linkage can result in a selection on pre-adult traits affecting adult fitness (Chippindale *et al.* 1996, 1998, 2003a). The FEJ populations are under simultaneous selection for faster pre-adult development and early reproduction, and have evolved reduced adult size at eclosion as a correlated response to selection. The FEJs have also evolved a syndrome of reduced larval energy expenditure, presumably to conserve resources in the face of reduced larval duration and resource acquisition (Chapter 3). I now discuss the results on correlated changes in adult traits in the FEJ populations in the context of how life-histories evolve.

Maturation time and fecundity

The FEJ populations are under strong directional selection for faster pre-adult development and early reproduction compared to the JB controls. Given that longevity and fecundity beyond three days post eclosion is unimportant to the fitness of the FEJs, one would predict that the optimal life-history strategy for the FEJs would be to develop fast, mature earlier and lay more number of eggs on day three post eclosion, compared to the JBs. A careful examination of the results of assays on adult traits over the course of FEJ selection suggests that the FEJs initially approach this ideal life-history, but then move away from it. By generation 20 of FEJ selection, the pre-adult development time and adult size at eclosion was

substantially reduced in the FEJs compared to the JBs (Chapter 3). However, the fecundity of FEJs after 20 generations of selection was not significantly different from that of the JBs, despite substantial reductions in adult size at eclosion. In fact, the FEJs produced marginally more number of eggs per unit body weight compared to the JBs early in life. Thus after 20 generations of selection, FEJs had moved towards the predicted, ideal life-history. By generation 50 of FEJ selection, however, fecundity of the FEJs was significantly lower than that of the JBs at both day 3 and day 11 post eclosion (Fig 5.2 b). For FEJs, fecundity at day 3 is important to fitness, whereas for JBs it is fecundity at day 11 that is relevant to fitness in their maintenance regime. Thus, it appears that by 50 generations of selection, the FEJs were moving away from the predicted ideal life-history, and this is corroborated by the lifetime fecundity assay done after 70 generations of FEJ selection. After 70 generations of selection, the fecundity of the FEJs was much lower than the JBs throughout their life, especially early in life (Fig 5.4).

This apparently maladaptive evolution of lower early fecundity in the FEJs can be attributed to the differential fitness weighting of faster development and early reproduction in the FEJ maintenance regime. In the FEJ regime, reproduction of an individual is conditional upon being among the 20% or so fastest developing flies. Moreover, there is a two and a half day gap between eclosion and egg collection, during which the flies are supplied with rich, yeast supplemented food, and the strength of selection on fecundity is ameliorated by the vagaries of sampling eggs from a petri-plate containing many more eggs than needed to initiate the next generation. Thus, the fitness weighting of developing faster than others is likely to

be greater than that of being able to produce relatively more eggs than others early in life. The two and a half day holding period is presumably responsible for the reduced pupal duration in the FEJs, which is compensated for by an increased maturation time (see discussion in Chapter 3). In the first 20 generations of FEJ selection, though the FEJs eclosed as much smaller adults than the JBs, they were probably able to make up the difference over three days of feeding on rich yeasted food and could therefore, manage to lay as many eggs as the JBs, although their higher egg production per unit weight appears to exact a cost in terms of longevity which is, however, not strongly correlated with fitness in the FEJ maintenance regime. However, as selection progressed, the fitness advantage to being able to develop fast resulted in increasing reduction in adult size, and eclosion at a relatively more sexually immature stage. Hence, fecundity in the FEJs declined and maturation time increased over 70 generations of selection. Moreover since early fecundity is likely to be predominantly dependent upon larval resource acquisition, FEJs will have low fecundity in the first few days of adult life, compared to the JBs. Early life fecundity, moreover, cannot be increased at the expense of late life fecundity, even though late life fecundity is not correlated with fitness in the FEJ maintenance regime.

These results are in contrast to the findings of Chippindale *et al.* (2003a) who observed reduced average fecundity both early and late in life in their fast developing populations within 25 generations of selection, even though these populations were under strong selection for extremely early fecundity. This result is probably an artifact of averaging, because peak fecundity early in their life of the

fast developing populations was not different from that of the controls, and was also attained earlier than in the controls (Chippindale *et al.* 2003a). This peak was followed by a sharp decline in fecundity of the fast developing populations (at an age not relevant to the fitness of the fast developing populations in their maintenance regime), leading to a reduction in average fecundity over the early part of their life.

The reduced total lifetime fecundity of the FEJs is in agreement with the results of previous studies by Zwaan *et al.* (1995a) and Nunney (1996) who also found a reduction in fecundity of fast developing flies, although in the former case this difference was not significant. The difference in the lifetime fecundity of FEJs and JBs was, moreover, not due to a difference in longevity.

Pattern of lifetime fecundity

Contrary to the canonical unimodal, positively skewed distribution of lifetime fecundity thought to be characteristic of *Drosophila* (David *et al.* 1974), daily fecundity in both JBs and FEJs has more than one peak (Fig 5.4). The ancestors of the JB populations are the B populations (Rose, 1984), maintained on a 14 day discrete generation cycle. The lifetime fecundity pattern of the B populations is already known (Chippindale *et al.* 1993), and I have reanalyzed those data in the same way as the data from my experiments. These analyses show that the lifetime fecundity pattern of the Bs is unimodal, and has a peak at about 4-5 days post eclosion. The second peak of daily fecundity in the JBs corresponds to the day when eggs are collected from them to initiate the next generation in their regular maintenance protocol. It appears that the JB populations have evolved a second peak

in daily fecundity around day 10 post eclosion as a response to being reared on a 21 day discrete generation cycle, as opposed to a 14 day discrete generation cycle as their ancestors were. Interestingly, the first peak of daily fecundity in the JBs is still significantly greater than the second peak, even though it is the second peak that is under selection. In principle, individuals in the JB populations that could save up resources till about day 10 post eclosion and thus, increase the second peak of daily fecundity at the expense of the first, would be favoured by selection in a 21 day discrete generation maintenance regime. Clearly, this has not occurred. Similarly, in other studies where late fertility was selected for (Rose, 1984), early fecundity remained higher than late fecundity even after well over a hundred generations of selection. It may be that a long history of selection in the wild has led to erosion of additive genetic variance for investment in early fecundity such that the flies lack a mechanism to switch resources from an early life-stage to a later life-stage. This line of reasoning is supported by the observation that life time fecundity schedules in wild caught *Drosophila* or those maintained in overlapping generation mass cultures tend to be unimodal (triangular) with a pronounced peak early in life (David *et al*. 1974). Similar triangular fecundity functions are observed in a number of insects and appear to be shaped by patterns of senescence (Dixon and Agarwala, 2002). Patterns of senescence are, in turn, shaped by patterns of mortality and life expectancy. If this explanation for evolution of triangular fecundity functions holds for wild *Drosophila*, the implication would be that life expectancy of *Drosophila* in the wild tends to be low. Although some studies reveal that longevity of recently wild caught flies is quite high under lab conditions (Linnen *et al.* 2001), one major

study that assessed life expectancy of seven *Drosophila* species in the wild using mark-recapture methods concluded that life expectancy was in the range of 1 to 6 days (Rosewell & Shorrocks, 1987). The available data, thus, support the view that the retention of a large first peak in daily fecundity in the JB populations is an imprint of past history.

The point I wish to stress here is that both JB and FEJ populations have failed to evolve the fecundity schedule that would be ideal in their respective maintenance regimes, even after more than 100 generations. The JBs are saddled with an early peak in daily fecundity, presumably due to past selection history, which at best contributes nothing to fitness and at worst detracts from fitness by squandering resources that could be used to produce more eggs around day 10 post eclosion. The FEJs, constrained by the relatively greater fitness weighting of rapid development, cannot achieve a high early peak of fecundity due to paucity of resources at eclosion. These results, thus, highlight the pitfalls of predicting optimal life-histories without full knowledge of genetic and phylogenetic constraints in addition to ecological ones.

Starvation resistance

In my study, adult starvation resistance, lipid content and efficiency of the use of lipids, all evolve as correlated responses to selection on development time. The FEJ populations have evolved a reduced starvation resistance, with a corresponding decline in lipid content. The present study, thus, complements the results of previous studies which found that populations selected for increased starvation resistance had longer development time along with an increased storage

of lipids (Chippindale *et al.* 1996; Harshman *et al.* 1999). Decreased development time and growth rates in the FEJs can account for their decreased absolute lipid levels, but it is not intuitively obvious why fractional lipid levels should be affected. An examination of the development time changes in the FEJ populations provides some insight into possible reasons for the change in fractional lipid content and also a possible explanation for the observations of Chippindale *et al.* (1996) that although increase in development time of the populations selected for increased starvation resistance was only about 1-2%, lipid content in those populations increased by 2-3 fold, and starvation resistance increased by more than 80%.

In *Drosophila* the larvae have to attain a certain minimum size (critical size) in order to be able complete development and successfully eclose (Robertson, 1963). This critical size is attained in the early third instar soon after the second moult. In the period prior to attaining the critical size (pre-critical period), larval growth rates are relatively low and the increase in the weight of the larva with age can be approximated linearly (Santos *et al.* 1997). In the post-critical period, however, the larval growth rates increase rapidly and there is an exponential increase in the larval weights till a plateau is reached late in the third instar (Bakker, 1961; Robertson, 1963; Santos *et al.* 1997). In the FEJs, 67% of the decrease in development time comes from the larval stage with maximum reduction in the third instar duration (Chapter 3). Assuming that lipids are among the main metabolites that contribute to the huge increases in weight of the third instar larva, it is clear that the FEJs will have lower relative lipid levels as a result of a disproportionate reduction of the duration of the third instar. This can also explain the asymmetry in

the response of the traits observed by Chippindale *et al.* (1996). Minor increases in the third instar duration can greatly increase lipid content at eclosion due to the exponential nature of larval weight gain over age.

Starvation resistance per unit lipid can be interpreted as reflecting either increased efficiency in lipid usage, or the allocation of a greater proportion of lipid for somatic maintenance rather than for reproduction. These two possibilities are not mutually exclusive. Starvation resistance per unit lipid of the FEJs remains higher than the JBs from eclosion through day 10 of adult life (Fig 5.5 b). In fact, the starvation resistance per unit lipid of the FEJs is very high at eclosion, and is reduced sharply by the third day post eclosion whereas in the JBs, the starvation resistance per unit lipid is relatively low at eclosion and does not change much over the first 10 days of adult life (Fig 5.5 b). I believe that this is due to differing patterns of resource commitment in the FEJs and JBs. There is evidence consistent with the view that the FEJs eclose as relatively immature adults compared to JBs, due to the higher fitness weighting of developing fast, compared to that of early reproduction (Fig 5.1, see also preceding section). The present results further strengthen this view. I believe that relatively little of the available lipid is committed for reproduction at eclosion in the FEJs, thereby making more lipid available for somatic maintenance, giving rise to high starvation resistance per unit lipid. However, by the third day post eclosion a larger fraction of the available lipid is committed for reproduction, resulting in a sharp drop in starvation resistance per unit lipid. The JBs eclose as much more mature adults, with a relatively large fraction of available lipid already committed for reproduction. Hence, they have low

starvation resistance per unit lipid at eclosion compared to FEJs, and undergo a decline only between days 3 to 10 post eclosion (Fig 5.5 b) in mated individuals; this decline would correspond to increased lipid allocation to the second peak of daily fecundity.

The above argument assumes that the commitment of lipid to reproduction involves lipid acquired during both larval and adult stages, and that a definite proportion of lipid committed to reproduction becomes unavailable for somatic maintenance. Both these assumptions are in line with the Y model of resource partitioning (de Jong & van Noordwijk, 1992) and are certainly not unreasonable. my results also confirm the negative effect of mating on adult starvation resistance (Chippindale *et al.* 1997b), with mated flies having both reduced starvation resistance and fractional lipid content than virgin flies. Interestingly, the starvation resistance of 10-day old virgin JBs is significantly higher than that of 10 day old mated JBs. However, there is no significant difference between the virgin and mated flies of FEJs at any age. Overall it appears that the FEJs have evolved a lower cost of reproduction, to the extent that the cost is reflected in lipid depletion, over the first ten days of adult life, compared to the JBs.

Longevity

In the FEJ maintenance regime, continued survival beyond day 3 post eclosion is irrelevant to fitness. Given that eggs laid early in life are important to FEJ fitness, and that FEJ are smaller at eclosion and have less lipid than controls, simple optimization reasoning would lead to the prediction that longevity would be sacrificed for higher early fecundity over the course of FEJ selection. The pattern of

evolution of longevity of mated flies in the FEJs, however, does not conform to this prediction. Mean longevity of the FEJs was reduced by about 5 days compared to the JBs within 10 generations of selection, and the same difference was also observed after 30 generations of FEJ selection (Fig 5.6). By 70 generations of FEJ selection, however, there was no significant difference between the longevity of reproducing FEJs and JBs. In principle, the amelioration of the FEJ-JB difference in longevity between generations 30 and 70 of FEJ selection could be a consequence of JB longevity declining to the level of FEJs due to inbreeding, However, this is unlikely because the JBs are maintained as large populations (N ~ 1800) and, moreover, do not show any signs of inbreeding depression such as lower pre-adult survivorship or adult fecundity. Moreover, the JBs, in their regular maintenance regime, are held in vials for about a week before being transferred into population cages, while FEJs are directly transferred to cages upon eclosion and never go through a vial stage as adults. Hence, differential adaptation to vial conditions as adults, which could give rise to G × E interactions affecting assay results (e.g. see Leroi *et al.* 1994a,b), is expected in the JBs rather than FEJs. Longevity estimates obtained from lifetime fecundity experiment conducted at generation 70 of FEJ selection again confirmed that the FEJ and JB longevity did not differ significantly (data not shown). Thus the most likely conclusion is that between generation 30 and 70 of FEJ selection, the longevity of the reproducing flies in the FEJ populations increased relative to the JBs.

The rapid decline of longevity in the FEJs early in selection precludes mutation accumulation as a possible cause of accelerated senescence in these flies,

although it may contribute to reduced lifespan much later in the course of selection. The longevity of virgin FEJs and JBs assayed after 20 generations of FEJ selection did not significantly differ from one another, suggesting that the longevity reduction in FEJs early in selection was mediated through reproduction. It seems clear that, early in selection, longevity of reproducing FEJs was reduced via a tradeoff between early life fecundity and longevity. After 20 generations of FEJ selection, FEJ females, though substantially lighter than JB females at eclosion, did not differ significantly from JB females in fecundity, implying a greater production of eggs per unit resource by the FEJ females (Fig 5.2 b). Consequently, the question to be addressed is why this tradeoff seems to disappear over the course of FEJ selection, between generations 30 and 70, even though mutation accumulation would be expected to accentuate the longevity difference between FEJs and JBs as selection proceeded, especially since the fitness weighting of higher fecundity per unit dry weight would be expected to increase as the size difference between FEJ and JB females at eclosion was becoming bigger in the course of FEJ selection through generation 70.

I hypothesise that the apparently anomalous evolution of increased longevity in the FEJs between generations 30 and 70 of selection is due to the prescence of a "lipid switch" which may have evolved in the ancestors of these flies under natural selection in wild conditions, where nutritional levels presumably fluctuate over time. I envisage the lipid switch as a physiological mechanism that determines the relative allocation of lipids to reproduction versus somatic maintenance, based on the amount of lipid present in the body. Above a certain threshold level of lipid,

allocation is biased toward reproduction, while at levels of lipid below this threshold, allocation is biased toward somatic maintenance. I postulate that in the initial generations of FEJ selection, the lipid levels of the FEJs declined (as inferred from a decline in weight at eclosion) but remained above the "switch" threshold. Thus, within the reproduction versus somatic maintenance allocation range specified by the setting of the "switch", phenotypes allocating relatively more lipid to reproduction were favoured by selection, resulting in a correlated evolution of decreased longevity in reproducing flies in the FEJ populations. Between generations 30 and 70 of FEJ selection, I believe that lipid levels fell below the "switch" threshold, due to decreasing size in response to selection for reduced development time, and that the relative allocation pattern was consequently altered, leading to a decreased mean allocation to reproduction versus somatic maintenance. Thus, once the "switch" threshold was crossed, even though selection for increased reproductive output per unit lipid would still be maintained in the FEJ regime, the range of allocation phenotypes that could respond to selection would have moved down the scale of relative allocation to reproduction versus somatic maintenance, resulting in an overall greater allocation to somatic maintenance than was seen during the early generations of FEJ selection.

Such switching in relative allocation to reproduction versus somatic maintenance in response to nutritional status has been documented previously. Flies grown under crowded conditions have smaller body size and lower fecundity, but greater longevity compared to flies from uncrowded cultures (Zwaan *et al.* 1991). Similarly, flies maintained on a high yeast diet have higher fecundity but lower

starvation resistance compared to flies maintained on a low yeast diet (Chippindale *et al.* 1997b). My observations after 50 generations of selection, that fecundity and fractional and absolute lipid content, and cost of reproduction in terms of reductions in starvation resistance, was lower in FEJs than that of the JBs, whereas their starvation resistance per unit lipid was higher than that of the JBs, is consistent with the hypothesis of the "lipid switch" outlined above. If the postulated explanation for the evolution of increased longevity in FEJs between generations 30 and 70 of selection is correct, it provides a good example of how a trait evolved in the past can constrain responses to selection along maladaptive trajectories, by restricting the range of phenotypes available for selection to act upon.

My results on the evolution of longevity in faster developing populations are in contrast to those of Zwaan *et al.* (1995a) and Nunney (1996) who found no difference in the longevity of fast developing populations and their controls. However, the differences in both materials and methods between my study and those of Zwaan *et al.* (1995a) and Nunney (1996) are too numerous to permit me to identify the causes of the discrepancy in observed responses to selection. I note also that these two studies did not select for early reproduction in addition to faster development as I did, or was done by Chippindale *et al.* (1997a, 2003a,b). It is more meaningful to compare my results with those of Chippindale *et al.* (2003a) because the flies used in both these studies share a common ancestry and the general selection protocol is quite similar. Chippindale *et al.* (2003a) observed a decline in longevity of their fast developing flies by 40 generations of selection, and this difference increased, at least in some sets of flies, over 100 generations of selection.

Longevity of the fast developing populations did not revert back to the levels of the control populations in their study. I suspect that this is due to the fact that Chippindale *et al.* (1997a, 2003a) selected for extremely early reproduction (within 1 day post eclosion) compared to our protocol. In their study, eggs were collected from the flies soon after transferring them into cages, unlike my study where there was a two and a half day gap between eclosion and egg collection. This selection for extremely early fecundity probably prevented lipid levels from falling below the threshold level. Alternatively, the selection regime followed by Chippindale *et al.* (1997a, 2003a) might have selected for flies with lower levels of the lipid switch threshold.

Conclusion

Intense and continued directional selection for faster pre-adult development has major consequences for adult fitness traits. The FEJs, it appears, have sacrificed most traits normally correlated with adult fitness in the course of evolving faster pre-adult development. This is not too surprising given that selection acts with full force on the pre-adult stages (Hamilton, 1966, Chippindale *et al.* 1997a), and that the fitness weighting of faster development in the FEJ selection regime is greater than that of early reproduction. This point underscores the context specific nature of fitness. In the FEJ regime, fitness is more strongly associated with faster development than fecundity or adult survival and this is reflected in the observed patterns of life-history evolution in these populations. In this context, it will be interesting to select populations for divergent traits such as faster pre-adult development and elongated adult life-span and look at the evolution of correlated

traits. If such selection is successful. elucidation of the mechanisms underlying the response to selection would provide insights into how the genetic architecture of fitness related traits can be altered by selection as the pattern of relative fitness weighting of different traits is altered.

This study also shows that what evolves is not always what is expected based on simplistic optimization arguments. Reduced early fecundity and increased longevity of the FEJs by 70 generations of selection are just the opposite of what would be predicted by an optimality argument. It is clear that the genetic architecture of fitness related traits – itself a product of past selection history – can constrain responses to selection along unexpected or even maladaptive trajectories, a subtlety often overlooked by enthusiasts of the adaptationist programme (Chippindale *et al.* 2003b; Rose *et al.* 1996; Prasad & Joshi, 2003). Our inability to cleanly predict the evolution of life-histories in a relatively well controlled setup such as the laboratory indicates that predictions about what is optimal and, hence, what should evolve in the wild, need to be made with caution.

Chapter 6: Developmental Instability, Population Dynamics and Maternal Effects

In this chapter, I discuss three studies that were undertaken on the FEJ and JB experimental system, but that deal with issues somewhat removed from the main thrust of this thesis which is on the evolution of life-histories under strong directional selection for faster development and early reproduction. The issues discussed in this chapter are, nevertheless, relevant to life-history evolution in a broader context, and to how nutrition-related life-history tradeoffs are studied experimentally. I begin by describing results from an experiment that attempted to test the hypothesis that extreme directional selection for faster development would result in increased developmental instability. Next, I discuss a study of population dynamics in populations derived from the FEJs and JBs in which I tested the hypothesis that population stability can evolve as a by-product of life-history evolution as a result of correlated responses to selection acting on traits that are not directly demographic parameters. Finally, I describe an experiment done on one of the JB populations to ask whether the effects of nutritional environment on offspring fitness components can be modulated by the nutritional environment experienced by their mothers.

Section A: Faster development and fluctuating asymmetry

The development of a stable phenotype, buffered against environmental and developmental noise is thought to be of importance for the optimal performance of individuals (Møller, 1999a). Developmental stability typically refers to the ability of an individual to develop a stable phenotype despite adverse environmental or genetic conditions, and is thought to reflect a homeostasis that counteracts deviations from an

optimal ontogenetic trajectory (Møller & Swaddle, 1997; Møller, 1999b). In recent years, there has been increasing interest in the use of various measures of developmental stability to understand a wide range of ecological and evolutionary problems, as measures of developmental stability/instability are thought to provide reliable information about the quality (fitness) of individuals (Leung, 1999), and of the degree of environmental stress to which they were exposed (Waddington, 1960; Parsons, 1961; Hurtado *et al.* 1997). The most popular measure of developmental stability has been the departure from perfect symmetry of a bilateral character (usually measured as its fluctuating asymmetry, FA), and it has been suggested that measures of developmental instability like FA are reliable predictors of fitness (Møller & Swaddle, 1997; Møller & Thornhill, 1998; Waynforth, 1998; Møller, 1999b), although this relationship is controversial (*e.g.* Clarke, 1998; Cadée, 2000), as is the reliability of FA as an indicator of stress (Blanckenhorn *et al.* 1998; Lu & Bernatchez, 1999; Woods *et al.* 1999; Bjorksten *et al.* 2000; Bourguet, 2000).

Many factors are thought to affect developmental stability, although empirical evidence for their effects is often tentative (extensive review in Møller & Swaddle 1997). One of the major factors thought to affect developmental stability and FA is the growth rate or development time (Møller, 1997), although there is considerable disagreement about the nature of the effect of development time on developmental stability, leading to mutually contradictory predictions as exemplified by the following quotation: "In conclusion, studies of growth rates demonstrate that fast growth generally is associated with a symmetrical phenotype. Alternatively, there may be a tradeoff between developmental stability and growth rate. ... (Møller, 1997; page no. 921)". Another

suggestion is that development rate is optimised in populations, and any deviations from this optimum will tend to lower developmental stability (Clarke, 1998). Strong directional selection is another factor thought to lead to increased FA (Parsons, 1992; Møller & Pomiankowski, 1993).

If both directional selection and faster development tend to result in increased FA, one would expect populations that have been under intense directional selection for faster development for many generations to exhibit reduced developmental stability and greater FA. Previous studies addressing the relationship between development time and FA have relied either on phenotypic manipulations (Parsons, 1961) or comparisons of different breeds of animals (Møller *et al.* 1995). It is, however, known that phenotypic correlations do not necessarily reflect underlying genetic correlations (Falconer, 1981; Rose & Charlesworth, 1981; Chippindale *et al.* 1993, 1994). Moreover, comparisons of different breeds of animals with varying developmental time (Møller *et al.* 1995) are difficult to interpret because the breeds are likely to differ genetically for any number of traits, other than development time, that could have a direct effect on FA. Laboratory selection experiments in which either FA or development time were directly subjected to selection have not as yet been used to investigate the genetic relationship between development time and FA. The FEJ populations are under intense directional selection for faster development and as a consequence, have evolved a reduced development time (Chapter 3). Hence, we might expect higher levels of FA in these populations, given that they are under intense directional selection for reduced development time relative to controls. I use sternopleural bristle number (henceforth, bristle number) as the indicator trait to compute FA since this trait has been used previously as a measure of developmental

stability in *Drosophila*, and also appears to be correlated with fitness (Kearsy & Barnes, 1970).

MATERIALS AND METHODS

Measurement of sternopleural bristle number

This assay was carried out after 70 generations of FEJ selection. Eggs were collected from the standardized flies at a density of 50 eggs per vial containing 6 ml banana-jaggery food, and five such vials were set up per population. The flies used in the assay were all of the same age (see Chapter 2). Three day old flies were killed by immersing them in soap-water and the number of sternopleural bristles on the right and left sides of 30 flies of each sex from each population was counted under a stereo-zoom microscope. Thus, data from 480 flies (30 flies × 2 selection regimes × 4 replicate populations) were used for the analyses.

Checking for directional asymmetry, antisymmetry and measurement error

In studies of FA, it is important to assess the magnitude of directional asymmetry (DA), and antisymmetry (AS), if any (Palmer & Strobeck, 1986). As bristle number is a discrete variable, the methods suggested by Palmer and Strobeck (1986) for assessing directional asymmetry were not applicable to my data. Since the possibility of measurement error in bristle number is negligible, replicate measurements on the same individual were not made. I assessed directional asymmetry by constructing 95% confidence intervals around the mean of the signed (R_i - L_i) values (where L_i = number of sternopleural bristles on the left side, and R_i = number of sternopleural bristles on the right side of the ith individual) for each sex in each population separately, and tested for significant deviations of the mean from zero. I graphically checked for antisymmetry by

constructing frequency histograms of the $(R_i - L_i)$ values for each sex in each selection regime pooled across all four populations.

Size dependence of FA

As suggested by Palmer and Strobeck (1986), I regressed the absolute value of $(R_i - L_i)$ on the trait size $((R_i + L_i)/2))$ for each sex in each population separately. A three way mixed model ANOVA (with selection regime and sex as the fixed factors crossed amongst themselves and with random blocks) on the slopes of these individual regressions indicated no significant main effects or interactions of any of the factors. Hence, data from all the populations and both sexes were pooled and regressed as described earlier. The overall regression showed a significant positive slope (b = 0.16; p = 0.0002), thus making it necessary to correct for trait size while computing FA.

Computation of FA

Of the nine FA indices listed by Palmer and Strobeck (1986), I used two:

Index 1 (Index 2 in Palmer & Strobeck, 1986):

$$FA = \frac{\sum_{i=1}^{N} \frac{|R_i - L_i|}{\left(\frac{R_i + L_i}{2}\right)}}{N}$$

and

Index 2 (Index 6 in Palmer & Strobeck, 1986).

$$FA = Var\left[\frac{(R_i - L_i)}{\left(\frac{R_i + L_i}{2}\right)}\right]$$

where L_i = number of sternopleural bristles on the left side, R_i = number of sternopleural bristles on the right side of the ith individual, i = 1 to 30 for each sex in each population, implying N = 30. These two FA indices were calculated separately for each sex in each population. Index 1 is commonly used and is based on the means of the absolute right-left differences. I also calculated index 2 as it is claimed to have higher discriminatory power, being based on the variance of the right-left differences (Palmer & Strobeck, 1986).

Statistical analyses

Selected and control populations bearing identical subscripts were treated as blocks in the statistical analyses as they were closely related. The data on total bristle number and the two FA indices for each population were subjected to separate ANOVAs, treating selection regime and sex as fixed factors crossed amongst themselves and with block as a random factor. All statistical analyses were done using STATISTICA™ for Windows Release 5.0 B (StatSoft Inc., 1995).

RESULTS

There was a clear difference in the total bristle number in the flies from selected (FEJ) and control (JB) populations (Table 6.1). The ANOVA indicated significant main effects of selection regime (F= 370.970, df = 1, p = 0.0003) and sex (F=113.105, df = 1, p = 0.002), as well as a significant selection regime × sex interaction (F=35.937, df = 1, p = 0.009). The mean bristle number of FEJ males (~ 13) and females (~ 15) was significantly less than that of JB males (~ 18) and females (~ 19), respectively. In the FEJ populations, females had significantly higher number of bristles than males, whereas in the JB populations the difference between sexes was not significant (Table 6.1).

Table 6.1. Mean total bristle numbers of each sex in each population. The 95% confidence interval around the mean for each selection regime × sex combination was calculated using the least squares estimates of the appropriate error mean squared term in the ANOVA. (Pop: Population).

		Pop 1	Pop 2	Pop 3	Pop 4	Mean	95% c. i.
FEJ	Male	13.10	12.97	12.90	13.13	13.03	0.49
	Female	14.97	14.87	14.73	14.97	14.88	0.49
JB	Male	17.77	18.50	18.77	18.17	18.30	0.49
	Female	18.17	19.23	18.77	19.20	18.84	0.49

The means of the signed (R_i - L_i) values did not differ significantly from zero in either of the sexes in any of the populations, indicating the absence of directional asymmetry. The frequency distributions of the (R_i - L_i) values for each selection regime × sex combination were unimodal, with a distinct mode at zero, thus ruling out antisymmetry for the trait studied (Fig. 6.1). The results of analyses on FA in FEJ and JB populations did not differ significantly, irrespective of whether index 1 or index 2 was used for the computation of FA (Tables 6.2 and 6.3).

The fraction of perfectly symmetric individuals (R_i - L_i = 0) was higher in the FEJ populations (0.58 for males and 0.38 for females), compared to the JB populations (0.30 for males and 0.32 for females) (Table 6.4). This difference was, however, not statistically significant due to the anomalous behaviour of block 4. If the data from this block are excluded from the analysis, then there is a significant main effect of selection (F = 22.192, df = 1,2, p < 0.05) but no significant effect of sex (F = 0.309, df = 1,3, p = 0.6) or selection by sex interaction (F = 2.106, df = 1,3, p = 0.3), despite reduced degrees of freedom. Overall, it is clear that FA in bristle number is not higher in the FEJ populations that have been subjected to intense directional selection for faster development.

DISCUSSION

Studies using FA as a measure of developmental stability have typically altered FA through phenotypic manipulations, especially with respect to the effect of stress like poor nutrition and parasites on FA (Møller & de Lope, 1998; Martel *et al.* 1999; Møller, 1999a; Roy & Stanton, 1999). In *D. melanogaster*, the effects of larval stress on FA are particularly well studied: in general, larval stress decreases the trait value but increases

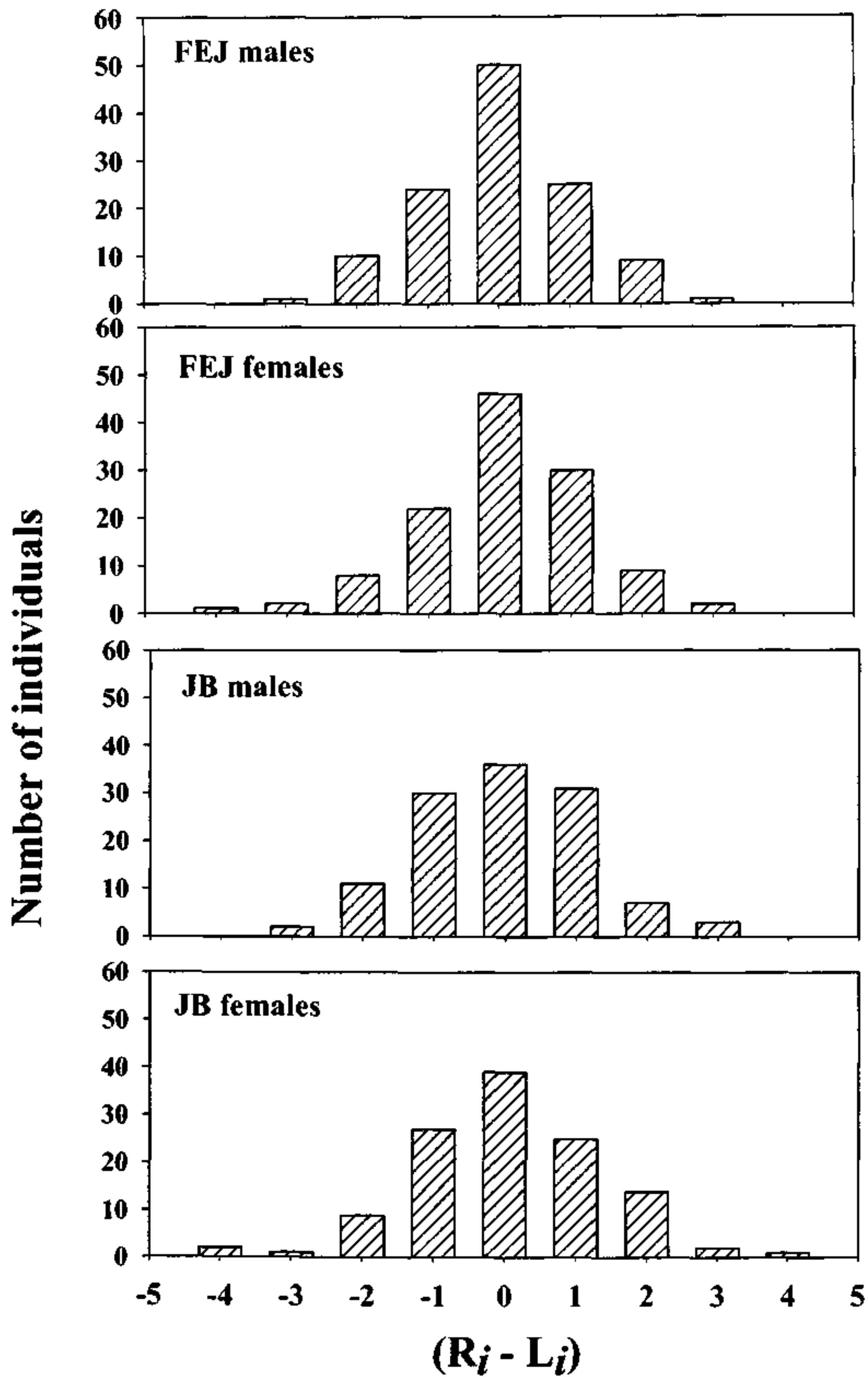

Figure 6.1. Frequency distributions of the signed $(R_i - L_i)$ values pooled over all four blocks in each selection regime × sex combination.

Table 6.2. Values of the two FA indices for each sex in each population. The 95% confidence interval around the mean for each selection regime × sex combination was calculated using the least squares estimates of the appropriate error mean squared term in the ANOVA. (Pop: Population).

	Selection	Sex	Pop 1	Pop 2	Pop 3	Pop 4	Mean	95% c. i.
Index 1	FEJ	Male	0.112	0.119	0.133	0.120	0.121	0.030
		Female	0.088	0.117	0.145	0.113	0.116	0.030
	JB	Male	0.091	0.110	0.102	0.100	0.101	0.030
		Female	0.097	0.098	0.093	0.124	0.103	0.030
Index 2	FEJ	Male	0.030	0.030	0.039	0.023	0.031	0.009
		Female	0.018	0.029	0.041	0.022	0.028	0.009
	JB	Male	0.014	0.019	0.016	0.017	0.017	0.009
		Female	0.016	0.017	0.017	0.028	0.020	0.009

Table 6.3. Summary of the two separate three-way mixed model ANOVAs on the two FA indices. Here selection regime and sex were treated as fixed factors crossed amongst themselves and with random blocks. Since the effect of blocks or their interactions cannot be tested for in this design, the table shows only the main effects and interactions of the fixed factors.

	Effect	*df*	MS	*F*	*P*
Index 1	Selection	1	0.001	3.689	0.151
	Sex	1	<0.001	0.138	0.735
	Selection × Sex	1	<0.001	0.310	0.617
Index 2	Selection	1	0.001	5.158	0.108
	Sex	1	<0.001	0.000	1.000
	Selection × Sex	1	<0.001	2.182	0.236

Table 6.4. Fraction of perfectly symmetric individuals in each sex in each population. The 95% confidence interval around the mean for each selection regime × sex combination was calculated using the least squares estimates of the appropriate error mean squared term in the ANOVA. (Pop: Population).

Selection	Sex	Pop 1	Pop 2	Pop 3	Pop 4	Mean	95% c. i.
FEJ	Male	0.500	0.433	0.433	0.300	0.417	0.142
	Female	0.500	0.366	0.333	0.333	0.383	0.142
JB	Male	0.366	0.233	0.266	0.333	0.300	0.142
	Female	0.333	0.300	0.366	0.300	0.325	0.142

the FA, and the effect of stress on FA is often trait specific (Parsons, 1961; Kearsy & Barnes, 1970; Woods *et al.* 1999; Cadée, 2000). The heritability of FA for many traits is also known to be very low (Woods *et al.* 1999). However, the evolution of developmental stability in response to directional selection is not well studied. This is a serious lacuna given that selection studies on life history traits clearly indicate that results from phenotypic manipulations are not necessarily good indicators of correlated responses to selection (Rose *et al.* 1996; Chippindale *et al.* 1993).

My study concentrates on a single trait, sternopleural bristle number. I realize that it is desirable to assess several traits for FA if one wishes to draw broad conclusions about faster development affecting the FA of an organism, but, nevertheless, I regard the present study as a useful beginning in that direction. Indeed, this study is the first attempt to assess correlated changes in FA of any trait in response to strong directional selection on development time. The point I want to stress is that my results clearly suggest that the relationship of development time, developmental stability and FA is likely to be more complex and subtle than previously thought.

In the FEJ populations, over 70 generations of selection, the total bristle number has been decreased by about 6 in males and 4 in females (Table 6.1), along with a large reduction in the adult size measured as adult dry weight at eclosion (Chapter 3) .Thus, my results are consistent with the finding of a positive correlation between fly size and bristle number reported by studies using phenotypic manipulations (Parsons, 1961; Kearsy & Barnes, 1970). A reduction in surface area of smaller adults has been suggested as a mechanistic reason for the reduction in bristle number (Parsons, 1961). In the FEJ populations, the larval resource provisioning is very low and the larvae have evolved

several mechanisms to minimize energy expenditure (Chapter 3). Therefore, it is also quite possible that the reduction in bristle number in the FEJ populations is due to low resources, or due to the available resources being utilized for faster development, a trait that has the highest fitness premium under the FEJ selection regime. The selection regime × sex interaction with respect to bristle number agrees with an earlier report that males have fewer bristles than females (Reeve & Robertson, 1954), although this difference is not significant in the JB populations. The males of the FEJ populations having lost significantly more weight than the females over 70 generations of directional selection (Chapter 3) may have also, consequently, lost more number of bristles than the females.

The nearly 20% reduction in the total bristle number and development time in the FEJ populations over 70 generations of directional selection is, however, not accompanied by a significant increase in the levels of FA. In fact, the fraction of perfectly symmetric individuals in the FEJ populations appears to be greater than in the controls. This is contrary to what would be expected on the basis of the phenotypic correlation between development time and FA in bristle number obtained by increasing or decreasing the development time by adding a tyrosine inhibitor (PTC), or increasing the rearing temperature (Parsons, 1961). As opposed to the results of such studies in which one has to contend with the potentially confounding effects of temperature and chemicals on FA directly, my results are easier to interpret since development time is under direct selection and the reduction of development time in the FEJ populations is genetic. Overall, my results clearly do not support the notion that directional selection and faster development should lead to greater developmental instability (Parsons, 1992; Møller & Pomiankowski, 1993; Clarke, 1998). I offer three possible explanations for the observed increase in the

fraction of symmetric individuals and no significant change in the levels of FA in the FEJ populations.

The evidence linking development time and developmental stability is very tentative, since it is based upon phenotypic manipulations and among-population comparisons. Selection experiments and within-population comparisons on development time and FA have never been done. Moreover, whether there is a relationship between FA and fitness of an individual is itself not clear (Møller, 1997; Clarke, 1998). Therefore, it is possible that development time and FA are not causally related, and that the expectation of greater FA under directional selection for faster development is, therefore, without foundation.

If I assume that faster development does in fact lead to increased FA in individuals, and that asymmetric individuals in the FEJ populations do have lower fitness, it is likely that selection would have favoured those individuals that can develop fast and yet have a high developmental stability. The prediction from such a scenario is, thus, opposite of what has been proposed by Parsons (1992), Møller (1997) and Clarke (1998), namely that selection for faster development should lead to greater symmetry and developmental stability.

In *D. melanogaster*, adult size critically depends on the duration of the post-critical feeding period in the middle and late third instar (Robertson, 1963). It is, therefore, likely that the total bristle number also depends on the duration of the post-critical feeding period. If one assumes that a particular minimum bristle number that can not be reduced further is associated with the minimum critical size that a larva has to attain in order to complete development, then it is possible that greater symmetry in the

FEJ populations is simply an artifact of reduced adult size. In the FEJ populations, the minimum critical size of larvae has been reduced, along with a large reduction in the third larval instar duration (Chapter 3). If the minimum bristle number is the same for both the left and right sides, then the bristle number in the FEJ populations is likely to be close to the minimum bristle number, thereby leading to a greater proportion of symmetric individuals in these populations.

Section B: Evolution of population stability

Since the demonstration that simple population growth models yield complex dynamics (May, 1974), there have been many reviews of population dynamics data, and relatively stable dynamics seem to be quite common (Turchin & Taylor, 1992; Ellner & Turchin, 1995). Why most populations show stable dynamics remains an open question, and there is no consensus on the mechanism(s) by which population stability may evolve through natural selection (Mueller & Joshi, 2000). Theoretical explanations for the evolution of population stability include group selection acting through long-term persistence (Thomas *et al.* 1980; Berryman & Millstein, 1989), individual selection acting on stability determining demographic parameters (Hansen, 1992; Ebenman *et al.* 1996), and the evolution of stability as a correlated response to life-history evolution (Mueller *et al.* 2000). As yet, however, none of these hypotheses has clear empirical support.

In the group selectionist view, unstable populations undergo more frequent extinction than relatively stable populations (Thomas *et al.* 1980; Berryman & Millstein, 1989). Consequently, the patches formerly occupied by unstable populations would likely be recolonized from nearby stable populations, and if the stability differences between

populations were primarily genetic, rather than environmental, population stability would evolve via group selection. This mechanism, however, will work only under very restrictive conditions, as pointed out by Mueller and Joshi (2000). It has also been suggested that population stability can evolve through direct selection on stability determining demographic parameters, such as population growth rate components or their sensitivity to density (Hansen, 1992; Ebenman *et al.* 1996). However, it is hard to imagine scenarios where selection favours stabilizing traits like lowered fecundity, and there is no experimental evidence for selection directly affecting the response of important demographic parameters to population density. In a rigorous test of this hypothesis, Mueller *et al.* (2000) subjected twenty populations of *D. melanogaster* to an environmental regime that leads to large and regular fluctuations in population numbers. However, even after 65 generations, stability characteristics of these populations did not evolve, nor did traits important to stability, such as the sensitivity of female fecundity to increasing adult density (Mueller & Joshi, 2000). Yet, rapid evolution of traits such as larval feeding rate did occur during the first 20 generations of this experiment, suggesting that the lack of response in stability characteristics was not due to a general absence of evolutionary change in the course of the experiment (Joshi, 1997b; Mueller *et al.* 2000).

Earlier theoretical studies suggested that tradeoffs among demographic parameters were crucial to the evolution of population stability (Turelli & Petry, 1980; Mueller & Ayala, 1981; Stokes *et al.* 1988; Gatto, 1993; Ebenman *et al.* 1996). It was also shown that a pattern of apparent stabilization over time of the dynamics of laboratory populations of blowflies (Nicholson, 1957) was consistent with an explanation involving selection for the ability of females to lay eggs even when malnourished, coupled with a

tradeoff between this ability and both survivorship and maximal fecundity (Stokes *et al.* 1988). The results from all these studies, together with the ubiquity of life-history tradeoffs, suggest that it may be most likely that population stability evolves indirectly, as a consequence of the correlated response of traits such as lower fecundity to selection on life-history traits not directly related to demography (Mueller *et al.* 2000). Here, I provide the first clear evidence supporting this hypothesis, by showing that the FEJ populations selected for rapid development in the laboratory have evolved more stable dynamics than their ancestral control JB populations, and that this result can be understood in terms of divergence in life-history traits between the FEJs and JBs as a correlated response to the imposed selection pressure.

MATERIALS AND METHODS

Assaying population dynamics

From each FEJ and JB population at generation 125 of FEJ selection, I derived eight small populations and studied their dynamics under either a stabilizing or a destabilizing food regime, following the methods of Sheeba and Joshi (1998). Each small population was maintained as a single vial culture, and was initiated by keeping 8 males and 8 females from the parent FEJ or JB population in a vial for 24 hours. The adults were then discarded, and the eggs laid during those 24 hours started generation zero of the population dynamics experiment. Once eclosion began, adults were collected into adult collection vials with 6 ml of food medium. Any new eclosing flies from the egg vials were added to these collection vials daily. Every alternate day, all adult flies eclosed in each small population till that day were transferred to a fresh vial. On the 18th day after egg collection, the egg vials were discarded, and all adult flies of each small population

were transferred to a fresh food vial with or without yeast paste (depending upon food regime) for three days of conditioning. The next generation was started by allowing the adults to oviposit for 24 hours into a new egg vial, after which they were censused and discarded. In this manner, I collected census data from all 64 small populations (2 selection regimes × 4 replicate populations within selection regime × 2 food regimes × 4 small populations for assaying population dynamics) for twenty generations. Population sizes varied between 2 - 250 adults in the various generations.

The two food regimes used were (1) a stabilizing (HL: High food levels for larvae, Low food levels for adults) regime in which egg vials contained excess (6 ml) food medium, and adults were not provided any supplementary live yeast for the three day conditioning period prior to egg collection, and (2) a destabilizing (LH: Low food levels for larvae, High food levels for adults) regime in which egg vials contained only 2 ml of food medium, and adults were provided supplementary live yeast paste during the conditioning period. The HL and LH regimes have been shown to have stabilizing and destabilizing effects, respectively, on the dynamics of *D. melanogaster* cultures, with the LH regime tending to induce large amplitude two-point cycles in population size (Mueller & Huynh, 1995; Sheeba & Joshi, 1998; Mueller *et al.* 2000). Four of the eight small populations derived from each FEJ and JB population were subjected to the LH regime, and four to the HL regime.

The coefficient of variation (CV) of population size of the FEJ and JB derived small populations was used to assess stability: a smaller CV being considered indicative of relatively stable dynamics. The CV data were subjected to ANOVA, treating selection regime (FEJ, JB), and food regime (LH, HL) as fixed factors, crossed with each other and

with block (1..4, representing ancestry of the FEJ and JB populations). CV values from the four small populations within each block × selection regime × food regime combination were treated as replicate within-cell observations.

RESULTS

As expected, the mean CV of population size in the LH food regime was significantly greater than that in the HL food regime (Table 6.5, Figure 6.2). More important, the mean CV of population size in the FEJ derived populations was significantly smaller than that in the JB derived populations (Table 6.5, Figure 6.2). There was no significant interaction between selection regime and food regime in the ANOVA (Table 6.5). The results clearly indicate that food regime had the expected effect on stability, that this effect was similar on both the FEJ and JB derived populations, and that the four FEJ populations have evolved more stable dynamics than their JB ancestors over 125 generations of selection for faster development and early reproduction.

DISCUSSION

The greater stability of the FEJs compared to the JB controls can be traced back to their respective life-histories. As a correlated response to selection for faster development, the FEJs are known to have evolved reduced fecundity (~ 35%) (Chapters 4, 5), as well as reduced body weight (~ 45%) and pre-adult survivorship at moderate larval density (~22%) (Chapter 3), compared to the JBs. These are clear correlated responses to selection for rapid development which reduces the time available for the larvae to feed and accumulate lipid reserves, and also exacts a survivorship cost (Chapter 3). Adult lifespan did not differ between FEJ and JB populations at the time of this assay

Table 6.5. Results from analysis of variance (ANOVA) on the coefficient of variation of population size in the 64 small populations.

Effect	*df*	MS	*F*	*P*
Selection regime	1	0.4493	32.77	0.0106
Block	3	0.043	6.37	0.001
Food regime	1	3.6314	489.4	0.0002
Selection regime × Block	3	0.0137	2.03	0.122
Selection regime × Food regime	3	0.0074	1.1	0.3585
Block × Food regime	1	0.0051	0.51	0.5273
Selection regime × Block × Food regime	3	0.01	1.48	0.2323
Error	48	0.0067		

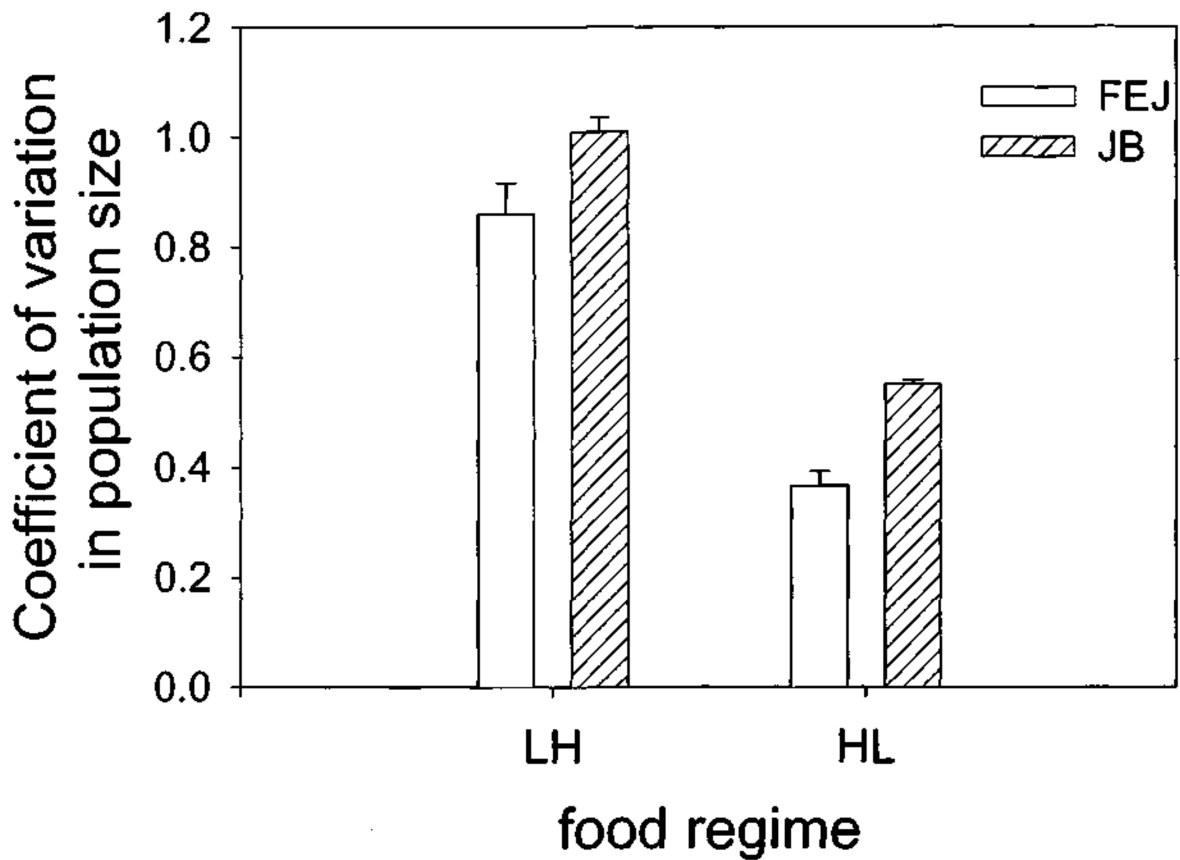

Figure 6.2. Mean (± s.e.) coefficient of variation (CV) of population size in FEJ and JB derived small populations, averaged over 16 replicates per selection regime × food regime combination.

of population dynamics (Chapter 5; N. G. Prasad *pers. obs.*). The FEJ and JB populations have been maintained on a discrete generation regime with larval and adult density regulated at a moderate level (Chapter 2). Thus, in the course of FEJ selection, there has been no differential selection on FEJs and JBs acting directly on stability determining demographic parameters, or on their sensitivity to density. I stress this point because, although development time is a demographic parameter in any population with overlapping generations, it is not directly relevant to demography in the context of the FEJ and JB maintenance regimes where adult numbers and adult and larval densities are explicitly regulated.

The higher larval density in the small populations, compared to the parent FEJs and JBs in their controlled density cultures, tends to prolong development in both FEJ and JB derived populations. Thus, all else being equal, the faster developing FEJ individuals have a greater chance of making it to adulthood before the 18 day deadline, compared to their JB counterparts. This potentially destabilizing survival advantage, however, appears to be offset by the intrinsically lower survivorship of FEJ larvae. Survivorship and fecundity in small populations could also be reduced over generations due to inbreeding, and this could have a stabilizing effect (Mueller & Joshi, 2000). However, in this assay, the JB derived populations had a lower effective (harmonic mean) population size on average (83 and 11 in HL and LH food regimes, respectively) than the FEJ derived populations (115 and 16 in HL and LH food regimes, respectively). Thus, if anything, the JB derived populations would be expected to have experienced greater inbreeding., and these results are, therefore, conservative.

I do not know at this time if the sensitivity of fecundity or survivorship to density has also changed in the FEJs, although I cannot imagine a scenario in which such changes in sensitivity might be expected given that the larval and adult densities in both JBs and FEJs are controlled at a very moderate level. However, both reduced pre-adult survivorship and fecundity are, in themselves, likely to play a stabilizing role in the dynamics of the FEJ derived small populations by contributing to a reduction in their intrinsic growth rate, a parameter observed to be the main determinant of stability/instability in most population growth models (Mueller & Joshi, 2000). The results of this study, thus, provide the first experimental evidence supporting the notion that population stability can evolve as a by-product of selection on life history traits not directly related to population dynamics.

Section C: Maternal effects and life-histories

The possible adaptive significance of non-genetic parental effects has attracted interest in recent years (Mousseau & Fox, 1998; Bateson & Martin, 1999). In particular, studies across a range of taxa suggest that the nutritional status of parents and offspring can have major effects on fitness related traits in the offspring (Shibata & Rollo, 1988; Nelson, 1991; Glazier, 1992; Gliwicz & Guisande, 1992; Rossiter, 1993; Schmid & Dolt, 1994; Lin & Dunson, 1995; Fox *et al.* 1997). Even more interesting are a growing number of examples of how parental and offspring nutritional environments can interact to affect the phenotypic expression of parental effects in the offspring (reviewed by Rossiter, 1998). In mammals, including humans, maternal nutritional status can interact with subsequent offspring nutritional status such that offspring fitness is reduced when maternal and offspring nutritional status are negatively correlated (Dahri *et al.* 1991;

Iglesias-Barreira *et al.* 1996; Ravelli *et al.* 1998). For example, the thrifty phenotype hypothesis, which has some empirical support, suggests that poor maternal nutrition predisposes the offspring towards an alternative developmental pathway which will prove beneficial if the offspring also experience a poor nutritional environment, but can be harmful if the offspring experience a nutrition rich environment (Hales & Barker, 1992; Bateson & Martin, 1999).

In addition to being of interest in their own right as evolved adaptations that may help organisms cope with varying environments (Lacey, 1998), parental effects can potentially be a major confounding factor in experiments in organismal biology, especially when the parental and offspring environments interact in their effect on the offspring phenotype (Crill *et al.* 1996). Parental effects and interactions involving nutritional levels are of particular relevance to experimental studies of life-history evolution, because much attention in such studies is focussed on tradeoffs surrounding the acquisition and allocation of nutritional resources (van Noordwijk & de Jong, 1986; Partridge & Sibly, 1991). If the phenotypic effects of offspring nutritional level can be qualitatively altered by parental nutritional levels, there is clearly reason for concern, as the experimental design for such studies typically does not include parental nutritional level as a factor (Partridge *et al.* 1987b; Leroi *et al.* 1994a,b,c; Chippindale *et al.* 1997b; Borash & Ho, 2001)

D. melanogaster has been a favoured model system for experimental studies of nutrition related life-history tradeoffs (Trevitt *et al.* 1988; Hillesheim & Stearns, 1992; Leroi *et al.* 1994a,b,c; Joshi & Mueller, 1996; Djawdan *et al.* 1998; Chippindale *et al.* 1997b, 1998; Harshman *et al.* 1999), and, in the context of laboratory selection

experiments, is increasingly being used for comparative physiology as well (Gibbs, 1999). Yet, very little is known about the possible interaction between parental and offspring environments in determining offspring phenotype in *D. melanogaster*, especially with regard to nutritional effects. Effects of parental age (Butz & Hayden, 1961), and rearing temperature (Crill *et al.* 1996; Watson & Hoffmann, 1996) on offspring fitness components have been observed in *D. melanogaster*, but nutritional level effects, to my knowledge, have not been studied. However, there is evidence from species other than *Drosophila* for interactions between the effects of parental and offspring nutritional environments on offspring phenotypes (Glazier, 1992; Parichy & Kaplan, 1992; Brett, 1993; Lin & Dunson, 1995; Ravelli *et al.* 1998). In this paper, I report results from an experiment on *D. melanogaster* in which I sought answers to the following two questions. (1) Does maternal nutritional environment affect egg weight? (2) Do maternal and offspring nutritional environments interact in their effect on offspring survivorship from egg to eclosion, and on offspring dry weight at eclosion?

MATERIALS AND METHODS

Experimental flies

For this experiment, I used the JB-1 population, one of the set of four control populations (Chapter 2). I collected eggs from the running culture and dispensed them into vials containing 6 ml of either rich or poor food (Table 6.6) at an exact density of 60 eggs per vial. I set up 120 vials each of poor and rich food in this manner. On the 18th day after egg lay, I collected all eclosed adults into cages and supplied them with the same type of food (rich or poor) that they experienced as larvae. The females in these cages are

Table 6.6. The composition of 1 litre each of regular food (used for maintenance of the running culture of the population used in the study), and the rich and poor food used for the actual assays.

Ingredient	Regular food	Rich Food	Poor Food
Banana	205 g	205 g	410 g
barley flour	25 g	25 g	50 g
jaggery (unrefined cane sugar)	35 g	35 g	none
Yeast	36 g	72 g	none
Agar	12.4 g	12.4 g	12.4 g
Ethanol	45 ml	45 ml	45 ml
Water	180 ml	180 ml	90 ml
p-hydroxymthylbenzoate	2.4 g	2.4 g	2.4 g

the 'mothers' for the assays described below, and rich or poor maternal food, thus, refers to the food on which the mother was reared, both as a larva and as an adult. I collected eggs for the assays by placing petri-plates with regular food in the cages for a 4 hour egg laying window.

Egg weight assay

I collected a total of 480 eggs laid by females reared on poor or rich food from the food plates placed in the respective cages, rinsed them in water to remove any adhering food medium, dried them on filter paper, and then weighed them in eight batches of 30 eggs each per maternal nutritional level.

Pre-adult survivorship assay

I collected eggs laid by females reared on poor or rich food from the food plates placed in the respective cages, and dispensed them into vials containing 6 ml of either rich or poor food at a density of 60 eggs per vial. I set up about 20 such vials for each maternal food × larval food combination, and recorded the proportion of eggs surviving to become pupae and adults in each vial.

Adult dry weight assay

I collected freshly eclosed flies from the vials used for the pre-adult survivorship assay, killed them by freezing, sorted them by sex, dried them for 36 hours at 70°C, and then weighed them in batches of five males or five females. I weighed eight such batches for each maternal food × larval food × sex combination.

Statistical analyses

All analyses were implemented on STATISTICA™ for Windows Release 5.0 B (StatSoft Inc., 1995). I subjected egg weight data to a one-way analysis of variance (ANOVA) with maternal food as the sole fixed factor. I transformed larval survivorship, pupal survivorship and egg to adult survivorship data by an arcsin squareroot transformation (Freeman & Tukey, 1950), and then subjected them to a two-way ANOVA with maternal food and larval food as the two fixed factors. Data on dry weights at eclosion were subjected to three-way ANOVA with maternal food, larval food and sex treated as fixed factors. I used Tukey's HSD test for all pairwise multiple comparisons.

RESULTS

The mean (± s.e.) weight per egg of eggs laid by mothers reared on poor food (0.0113 ± 0.0021 mg) was about 28 percent greater than that of eggs laid by mothers reared on rich food (0.0088 ± 0.0018), but the difference was not significant ($F_{1,14} = 0.83$, $p = 0.38$). Mean larval survivorship (fraction surviving from egg to pupation) was about 0.8 in three of the four combinations of maternal and larval food levels, and was significantly greater ($p < 0.05$) in the case of larvae growing on rich food when their mothers had been reared on poor food (Fig. 6.3). Pupal survivorship was high, and did not differ among combinations of maternal and larval food levels (Fig. 6.3). Egg to adult survivorship showed the same pattern as larval survivorship, and the patterns observed in Fig. 6.3 are supported by the ANOVA results, with both larval and egg to adult survivorship showing significant effects of larval food, and the maternal food × larval food interaction (Table 6.7).

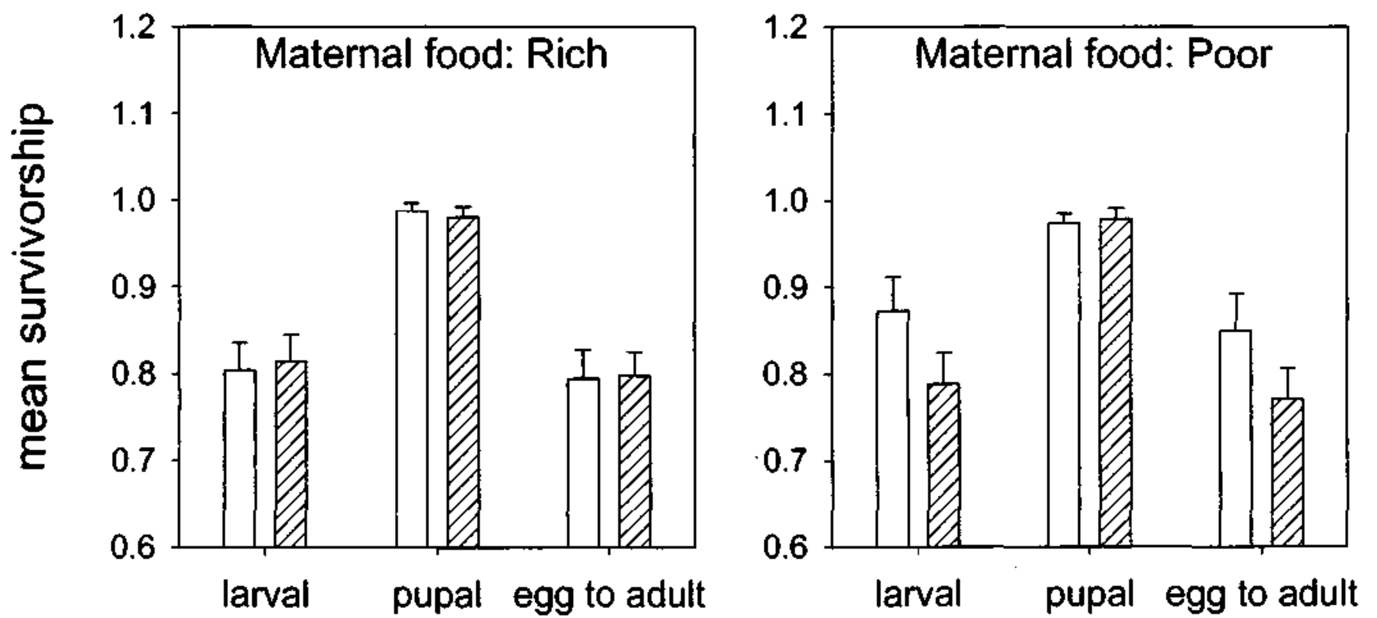

Figure 6.3. Mean survivorship of individuals in different pre-adult life stages under various combinations of maternal and larval nutritional levels. Errors represent 95% confidence intervals around the means (open bars: rich larval food, hatched bars: poor larval food).

Table 6.7. Summary of results from three separate analyses of variance (ANOVA) carried out on arcsin squareroot transformed survivorship in different pre-adult life-stages under various combinations of maternal and larval nutritional levels.

Effect	Larval		Pupal		Egg to adult	
	$F_{1,85}$		$F_{1,85}$		$F_{1,85}$	
maternal food	2.42	0.12	1.76	0.19	1.23	0.27
larval food	5.15	0.03	0.11	0.74	5.48	0.02
maternal food × larval food	7.42	0.01	2.14	0.15	5.43	0.02

The only significant effects on dry weight at eclosion were those of larval food, sex, and the larval food × sex interaction (Table 6.8). Females were significantly heavier than males, and individuals reared as larvae on rich food were significantly heavier than those reared as larvae on poor food (Fig. 6.4). The significant larval food × sex interaction was due to the fact that the dry weight of females increased proportionately more than that of males (by 72% as opposed to 66%) when reared as larvae on rich rather than poor food (Fig. 6.4).

DISCUSSION

These results provide clear evidence for an interaction between maternal and larval nutritional levels on larval, and therefore, egg to adult survivorship (Fig. 6.3, Table 6.7). Larvae whose mothers were reared on poor food and who themselves were reared on rich food had higher survivorship than larvae from all other combinations of maternal and larval food regime. I do not presently know the reason for this higher survivorship, although it is possible that some aspect(s) of egg provisioning by mothers experiencing poor food enhance the ability of the larvae hatching from those eggs to do better when they get rich food. In this experiment, mothers reared on poor food did lay heavier eggs than those reared on rich food, but the difference was not statistically significant. Lin and Dunson (1995) observed a similar interaction between parental and offspring nutritional levels on offspring fitness components in the estuarine fish *Rivulus marmoratus*. In that study, larvae from parents that experienced poor nutritional levels were able to mature earlier, and were heavier, when reared in a high nutrition environment, compared to

Table 6.8. Results from analysis of variance (ANOVA) on dry weight at eclosion (in g per 5 flies) of individuals eclosing from various combinations of maternal and larval nutritional levels.

Effect	*df*	MS	*F*	*P*
Maternal food	1	1.9×10^{-37}	1.4×10^{-29}	0.99
Larval food	1	7.4×10^{-6}	545.36	<0.001
Sex	1	2.6×10^{-6}	188.01	<0.001
Maternal food Larval food	1	1.9×10^{-37}	1.4×10^{-29}	0.99
Maternal food Sex	1	5.6×10^{-9}	0.4131	0.52
Larval food Sex	1	2.5×10^{-7}	18.361	<0.001
Maternal food Larval food Sex	1	6.3×10^{-10}	0.0459	0.83
Error	56	1.4×10^{-8}		

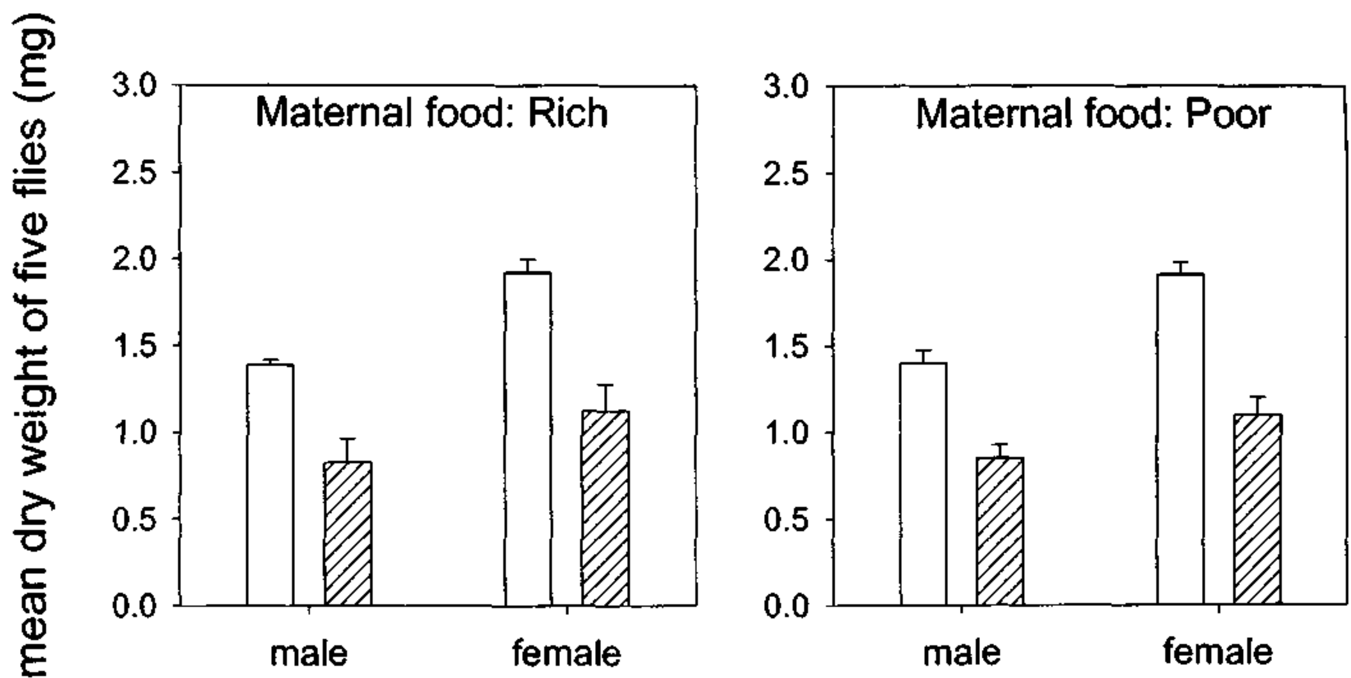

Figure 6.4. Mean dry weight of freshly eclosed males and females subjected to different combinations of maternal and larval nutritional levels. Errors represent 95% confidence intervals around the means (open bars: rich larval food, hatched bars: poor larval food).

larvae from parents that experienced high nutritional levels. Unfortunately, the physiological mechanism for this interaction was not clear (Lin & Dunson, 1995).

I observed no significant effect or interaction of maternal nutritional level on the dry weight of offspring at eclosion (Fig. 6.4, Table 6.8), suggesting that whatever effect is responsible for larvae from mothers reared on poor food surviving better on rich food does not yield a greater efficiency of assimilation. It is, however, possible that offspring of mothers reared on poor food may be superior in terms of lipid storage than individuals from other combinations of maternal and larval nutritional levels. Individuals from *D. melanogaster* populations adapted to very high larval densities are not heavier at eclosion than those from ancestral control populations, but do have a greater lipid content, which can have an effect on subsequent fecundity and stress resistance (Borash & Ho, 2001).

The results from this experiment are clearly inconsistent with the thrifty phenotype hypothesis (Hales & Barker, 1992; Bateson & Martin 1999) which would require treatments with positive correlation between maternal and offspring food levels to result in superior offspring fitness, compared to treatments where maternal and offspring food levels are negatively correlated. However, given the diversity of the various patterns of interactions between parental and offspring environments in affecting offspring phenotype (Rossiter, 1998), it would appear unlikely that generalizations about possible adaptive patterns of parental effects and interactions would have broad validity across taxa. It is far more likely that the kinds of parental effects and interactions that are seen in any taxon will depend in a complex way on the ecology, physiology and evolutionary history of the population being studied.

Overall, these results clearly show that at least one important component of fitness in *D. melanogaster* is affected by maternal and offspring nutritional levels in a complex manner. For example, the increased survivorship of larvae on rich food would not have been detected in an experiment in which mothers were raised on rich food, which is typically the case in laboratory experiments on *Drosophila*. My results, therefore, highlight the need for caution in interpreting results from nutritional manipulation experiments in *Drosophila*, and suggest that a more detailed investigation of maternal nutrition effects on fitness related traits of *Drosophila* individuals subjected to varying nutritional regimes would be very fruitful.

Chapter 7: Conclusions

In this chapter, I briefly summarize the major novel findings of fact that have emerged from the studies described in this thesis, some of which have resulted in a substantial re-orienting of our thinking about some issues in *Drosophila* life-history evolution. I also discuss these findings in the context of some broader conceptual issues in evolutionary genetics that are well illustrated by some of the results reported in this thesis.

LIFE-HISTORY EVOLUTION IN THE FEJ POPULATIONS

One major result from this study has been the identification of new tradeoffs between faster development and larval behaviours related to food acquisition (Chapter 3). In the absence of the knowledge that larval feeding rates and urea tolerance are negatively correlated with faster development, it was believed for a long time that selection for faster development and for adaptation to high larval density would lead to similar evolutionary outcomes (Bakker, 1969; Prout & Barker, 1989; Borash *et al.* 2000; Krijger *et al.* 2001). However, the identification of these tradeoffs, along with an emphasis on α-selection rather than K-selection, clearly shows that selection for faster development at moderate versus very high larval densities leads to the evolution of completely different, indeed opposite, suites of traits (Chapters 3, 4). Not only does this finding alter our view of the relationship between development time, adaptation to crowding, and competitive ability in *Drosophila* (Chapter 4), it also serves to underscore the importance of density in mediating responses to otherwise well defined selection pressures, a factor often not given enough importance while framing and testing evolutionary hypotheses.

Another major, and seemingly paradoxical, result from this study has been the strange evolution of adult life-history in the FEJ populations (Chapter 5). In the early generations of selection, the FEJs were evolving in the direction of the predicted optimal adult life-history, sacrificing longevity for relatively higher fecundity per unit weight early in life, when fecundity is correlated with fitness in the FEJ maintenance regime. Yet, after 70 generations of selection, the FEJs had moved back to a distinctly maladaptive life-history, allocating relatively more of their lipid reserves to somatic maintenance rather than reproduction early in life, in effect sacrificing early fecundity for longevity, which in the FEJ regime is irrelevant to fitness beyond day three after eclosion. If my hypothesis about the "lipid switch" (Chapter 5) is correct, this is an example of how a trait that was presumably adaptive in a fluctuating nutrition environment in the wild has become a major constraint in the selective context of the FEJ maintenance regime, and is actually moving the FEJs away from the optimal life-history.

THE GENETIC ARCHITECTURE OF FITNESS AND ITS EVOLUTION

It has long been realized that the genetic architecture of fitness components can constrain adaptive evolution by constraining or facilitating responses to selection along certain evolutionary trajectories. Moreover, the genetic architecture of fitness related traits is itself affected by selection, and can evolve, and the results in this thesis provide two examples of such an evolution of the genetic correlation between traits related to fitness. The negative genetic correlation (tradeoff) between fast development and larval growth rate seen in my study is in contrast to Chippindale *et al.* (1994) who observed a positive correlation between larval growth rate and rapid development across a set of demographically selected populations subjected to egg collection at very different adult

ages. This is somewhat surprising considering that the flies used in both my study and that of Chippindale *et al.* (1994) share a common ancestry. The reason probably lies in the intensity of selection for faster development. In the populations studied by Chippindale *et al.* (1994), development time was under mild indirect selection, whereas development time is under intense direct selection in the FEJ populations. Thus, it appears that the sign of the genetic correlation between development time and larval growth rate can evolve differently based on the intensity of selection. The other example of the evolution of genetic correlations between fitness related traits is provided by development time and longevity. The correlation between rapid development and longevity in the initial 30 generations of FEJ selection was negative, with longevity dropping as development became faster. However, between generations 30 and 70 of FEJ selection, the correlation between rapid development time and longevity had become positive, with longevity increasing as development continued to become faster. The point to note here, in the context of ongoing discussions about the rate of evolution of the network of genetic variances and covariances (the **G** matrix: reviewed in Steppan *et al.* 2002), is that these changes in the genetic architecture of fitness related traits are occurring on a relatively short time scale of tens of generations, suggesting that the **G** matrix can change quite rapidly, at least under strong selection, as also noted by Chippindale *et al.* (2003b).

TRAIT CONTRIBUTIONS TO FITNESS ARE HIGHLY CONTEXT SPECIFIC

Fitness is a multi-faceted 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. 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 Chapter 3, a difference of about 30-35 hours 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 pre-adult 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 pre-adult development.

Essentially, the life-history in an equilibrium population, which long-term laboratory adapted populations seem to be, can be viewed as being a multi-armed 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 **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 non-intuitive 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.

THE EFFECT OF HISTORY

Responses to selection can be significantly affected by past selection history. The "lipid switch" which determines the relative allocation of resources to reproduction versus somatic maintenance, probably evolved in the wild as an adaptation to a fluctuating nutritional environment. However, in the changed environmental scenario of the FEJ maintenance regime, the switch has become maladaptive, pushing the fast developing populations towards a very non-optimal adult life-history (Chapter 5). Results from reverse selection experiments reveal that often traits underlying fitness evolve differently across populations, even as fitness measures converge (reviewed in Teótonio & Rose, 2001; Teótonio *et al.* 2002). However, in some other studies, the effect of history on larval feeding rates was found to be transient and disappeared with in a few generations (Joshi, 1997b). Thus, in the process of adaptive evolution, environment, genetics and history all affect evolutionary trajectories. The case of the "lipid switch" further illustrates the point that naive ideas about selection being an inexorable force that relentlessly drives populations toward optimal adaptive peaks are misconceived.

WHAT YOU EXPECT IS NOT ALWAYS WHAT YOU GET IN EVOLUTION

Intuitive common-sense expectations of what traits should evolve under a given scenario have often proven to be wrong in experimental evolutionary studies. Needless to say, figuring out why they were wrong has led to a clearer and more detailed understanding of the subtlety of adaptive evolution. Contrary to a widely held view in the *Drosophila* literature, 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* (Chapter 4). This is just one

* which in correlated with tolerance to ammonia, The usual waste product in crowded cultures (Borash et.al. 1998; Borash & Shimada 2001).

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 (Chapter 4), 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 lower rather than greater larval growth rate compared to controls (Chapter 3), even though a higher growth rate would clearly be favored by selection on optimality arguments. Populations maintained for several hundred generations on a three week discrete generation cycle, wherein only eggs laid around day 11 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 around day 11 (Sheeba *et al.* 2000; Chapter 5). Populations directionally selected for faster development show no increase in fluctuating asymmetry, and a greater fraction of symmetrical

individuals contrary to expectations (Chapter 6 Section A). 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 & 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).

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 multi-faceted nature of fitness, the problems of G $\times$ 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 $\times$ E interactions, and cross-generational effects or 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.

LIFE-HISTORY EVOLUTION AND PARENTAL EFFECTS

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). Such interactions between parental and offspring environments can be major confounding factors in experiments in life-history evolution (Crill *et al.* 1996; Hercus & Hoffmann, 2000). This study has revealed, for the first time, evidence for interactions between maternal and larval food levels on larval survivorship (Chapter 6 Section C). While the mechanisms underlying these cross-generation and effects of nutrition are not yet known, the existence of such interactions between parental and larval environment 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 nutrition affects growth rates and 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 useful 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 and/or thermal ecologies.

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 & Bossert, 1970; Stearns, 1992; Charlesworth, 1994), and life-history traits like survivorship and fecundity, and their sensitivity to density, are the major determinants of population dynamics (Cole, 1954; Mueller & Joshi, 2000). Moreover, population size and life-history evolution are also linked through the theory of density-dependent selection (Mueller, 1997; Reznick *et al.* 2002). Yet, population dynamics and life-history evolution have remained largely separate fields and it has been argued in this context 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 & Joshi, 2000). One such question pertains to the mechanism(s) for the evolution of population stability.

My study has provided the first empirical evidence supporting the view that that population stability can indeed evolve as a correlated response to selection on life-history traits that are not themselves stability determining demographic parameters (Mueller & Joshi, 2000). The FEJ populations have evolved reduced fecundity and pre-adult survival as correlated responses to selection on development time and time of reproduction (Chapters 3, 4), both of which are not demographic parameters in the context of a discrete generation culture, although they would be in an overlapping generation culture. I have further shown that the FEJ derived populations indeed exhibit greater stability of adult census numbers than those derived from their ancestral control populations, when maintained in an uncontrolled density culture (Chapter 6, Section B).

The evolution of population stability is 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 and/or adult densities, whereas formal life-history evolution theory has been derived from models of the growth of age-structured populations with overlapping generations (Partridge & Sibly, 1991). 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 focusing debate about life-history tradeoffs (van Noordwijk & de Jong, 1986; Houle, 1991; de Jong & 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.

I 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 endeavor that poses a daunting challenge to theorist and experimentalist alike. I also see a complementary need for the development of population growth models for overlapping generation *Drosophila* cultures that include life-stage and age-class specific life-history details, as also for models predicting life-history evolution in populations with periodic rather than equilibrium dynamics.

I 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 & 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, it is hoped that a closer interaction between theory and experiment in life-history evolution, and between population dynamics and life-history evolution, will be seen in the future.

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