The Genetic Architecture of Fitness-related Traits in Populations of Three Species of *Drosophila* Subjected to Selection for Adaptation to Larval Crowding

A Thesis

Submitted for the Degree of

Doctor of Philosophy

By

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To

My Parents

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DECLARATION

I declare that the matter presented in my thesis entitled "**The genetic architecture of fitness-related traits in populations of three species of *Drosophila* subjected to selection for adaptation to larval crowding**" is the result of studies carried out by me at the Evolutionary and Organismal Biology Unit of the Jawaharlal Nehru Center 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.

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Date: September 10, 2009

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10 September, 2009

CERTIFICATE

This is to certify that the work described in the thesis entitled " **The genetic architecture of fitness-related traits in populations of three species of *Drosophila* subjected to selection for adaptation to larval crowding** " is the result of investigations carried out by Ms. Archana N in the Evolutionary and Organismal Biology Unit of the Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560 064, under my supervision, and that the results presented in the thesis have not previously formed the basis for the award of any other diploma, degree or fellowship.

Amitabh Joshi, Ph.D.

Professor

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List of Publications

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2. Sharmila Bharathi N., Archana N., Anjana B., Satish K. M., Mohan J. and Joshi A. 2007 Time to death in the presence of *E. coli*: A mass scale method for assaying pathogen resistance in *Drosophila*. *J. Genet*. **86**, 75–79.

3. Archana. N, Ghosh Modak S.*, Sharmila Bharathi N. , Mohan J., Chari S., Bose J. and Joshi A. Density-dependent selection and selection for faster development reveal novel genetic correlations in *Drosophila ananassae* . Manuscript under preparation.* Equal contribution.

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Summary

I chose to work on the genetic architecture of fitness-related traits in populations of three species of *Drosophila* subjected to selection for adaptation to larval crowding, with the primary focus being on the following questions:

1. Is the network of genetic correlations among fitness-related traits under larval crowding similar in species of *Drosophila* other than *D. melanogaster* and, if not, does it vary with evolutionary distance?

2. Do *D. ananassae* populations selected for adaptation to larval crowding exhibit a polymorphism of different genotypes for exploiting temporal variation in the environment, as seen earlier in *D. melanogaster*?

3. Can maintenance regime have an effect on the genetic architecture of fitness-related traits through promoting assortative mating for development time under crowding?

The first question is general and pertains to the degree to which the genetic architecture of fitness-related traits is conserved across congeners, whereas the other two questions arise from differences between my results and those obtained in earlier selection studies on adaptation to crowding in *D. melanogaster*. I chose to work on adaptation to crowding because previous studies in *D. melanogaster* involving selection for either adaptation to crowding or faster development, unlike studies where age at reproduction or stress tolerance was selected for, revealed a fairly consistent pattern of genetic correlations among traits like development time, pre-adult viability, larval feeding rate, larval competitive ability and weight at eclosion. Thus, adaptation to crowding seemed to be a good selection pressure

under which to examine the generality of the genetic architecture of these traits in other *Drosophila* species. I worked on three species of *Drosophila — D. ananassae, D. nasuta* and *D. melanogaster*. *D. ananassae* and *D. melanogaster* are closer evolutionarily as compared to *D. nasuta*, belong to the same sub-genus Sophophora and the Melanogaster species group, and diverged 12 MYA. *D. nasuta* belongs to a different sub-genus, *Drosophila*, and diverged from *D. melanogaster* ca. 50 MYA. Hence, to answer the first question, I inferred the signs of genetic correlations among fitness-related traits in these three species by examining correlated responses to selection for adaptation to larval crowding.

In *D. melanogaster*, an earlier selection studies on six populations, three of which were selected for adaptation to low density (*r*-populations) and three for adaptation to high density (*K*-populations), revealed a trade-off in net productivity at the two densities. *K*-populations had higher net productivity at high densities and *r*-populations had higher net productivity at low density. *K*-populations also showed evolution of higher competitive ability and larval feeding rate as compared to the *r*-populations, with no change in development time and weight at eclosion, when assayed at moderate densities. A further study in *D. melanogaster*, with selection for crowding only at the larval stage, also showed evolution of almost similar life-history and related traits. The populations selected for adaptation to larval crowding showed evolution of higher larval feeding rate, competitive ability, and tolerance to urea and ammonia, as compared to the controls. In these lines also, there was no change in development time and weight at eclosion at moderate densities, even though lipid content at eclosion was higher at high density. In both studies, efficiency of food utilization was lower in the selected populations. Thus, the two studies of density-

dependent selection in *D. melanogaster* showed evolution of similar set of traits, and this pattern was also seen in populations of *D. melanogaster* selected for rapid egg to adult development.

In my experiments, selection for larval crowding in *D. ananassae* showed evolution of traits which were different from the ones obtained in the earlier studies in *D. melanogaster*. The selected populations showed higher pre-adult survivorship and competitive ability at high density. The development time of selected populations decreased, whereas there was no change in dry weight and lipid content at eclosion, when assayed at either low and high densities. Moreover, there was no change in larval feeding rate, unlike in previous studies on *D. melanogaster*, where feeding rate was positively correlated with competitive ability. Moreover, *D. ananassae* populations selected for adaptation to larval crowding were found to be more efficient than the controls. The minimum feeding time (the minimum amount of time the larva needs to feed to reach the critical size necessary for pupation and eclosion) decreased in the selected populations and the feeding rate did not change. Thus, the flies were taking in less amount of food but were reaching the critical size early. The adult traits — life span, fecundity and starvation resistance — followed a trend similar to that seen in the earlier studies on *D. melanogaster*. There was no change in life span and fecundity in the selected populations, whereas starvation resistance was higher in flies raised at higher density in the selected populations as compared to controls. Overall, in *D. ananassae*, the network of genetic correlations among traits related to fitness under larval crowding was quite different from that seen in *D. melanogaster* in earlier studies.

Interestingly, selection for adaptation to larval crowding in my *D. nasuta* and *D. melanogaster* populations also showed the evolution of a suite of traits very similar to *D. ananassae*: selected populations evolved higher competitive ability, decreased development time and no change in larval feeding rate and weight at eclosion. Adult traits also showed similar results across all three species. Thus, even the *D. melanogaster* populations in my study showed a different pattern of genetic correlations than that seen in previous studies. Compared to the *D. ananassae* and *D. nasuta* populations that were relatively recently wild-caught, my *D. melanogaster* populations were derived from very long-term laboratory populations. The observation of a similar pattern of correlated responses to selection in all three species, therefore, suggests that differences in the duration of laboratory adaptation are not likely to be the cause for the observed differences between the traits that evolved in previous studies on *D. melanogaster* and in my *D. ananassae* and *D. nasuta* populations. A further possibility is that these differences in traits evolved in response to selection for adaptation to larval crowding could be due to a small difference in maintenance regime between my study and a previous study on *D. melanogaster*. I discuss this possibility at the end, because my next experiment further suggested that maintenance regime could have played a role in mediating the evolution of a different set of traits in my study.

An earlier study in *D. melanogaster* had revealed the existence of a polymorphism in the crowding adapted populations. Early emerging genotypes from a crowded culture showed higher larval feeding rate but lower tolerance to nitrogenous wastes than late emerging genotypes. I also wanted to check if the *D. ananassae* populations selected for adaptation to larval crowding showed such a polymorphism. However, I found no evidence for such a polymorphism in the selected populations of *D. ananassae*, with the progeny of early and

late emerging flies in a crowded culture exhibiting almost similar larval feeding rate, development time and tolerance to nitrogenous wastes. One difference in the maintenance regime I used and that used in the previous study on *D. melanogaster* that revealed this polymorphism was in the collection of adult flies from the crowded larval vials. In my experiments, emerging flies from the larval vials were collected into a cage daily, implying that flies emerging on a particular day got pooled into the same cage with all the flies that had emerged on previous days. In the previous study on *D. melanogaster*, however, flies eclosing on a particular day were housed with others eclosing the same day for several days prior to being pooled into a cage. The latter regime, in principle, could have inadvertently induced assortative mating for development time in the crowding-adapted populations and this, in turn, could have driven the maintenance of the earlier discussed polymorphism.

I examined this possibility further by doing a one-generation experiment, using *D. melanogaster* scarlet eye and red eye flies, in which I replicated the two maintenance regimes and then studied the mating system in both. In the set up replicating the earlier type of maintenance regime, I found proportionately more assortative mating for development time happening, as compared to the set up similar to my maintenance regime. This finding strengthens the view that the polymorphism seen in the earlier study might have been due to maintenance regime induced assortative mating among the early and late emerging flies. In principle, it is possible for differences in mating system to affect the pattern of genetic correlations and thus, explain the differences between my study and previous studies. However, the present data do not permit unequivocal conclusions to be drawn. It could also be the case that quantitative differences in the degree of crowding affect the fitness-weighting of different traits, thereby leading to varied patterns of correlated responses to

selection for adaptation to crowding in the different studies. Further experimental work will be required to probe this possibility.

Overall, my study shows that the different congeneric species under similar selection pressures can show a fairly similar genetic architecture of fitness-related traits, even though evolutionarily they might be far apart. Although some pair-wise correlations were species-specific, the major correlations among traits relevant to fitness under crowding were quite similar across the three species I studied. My results also exemplify how seemingly trivial aspects of maintenance regime can have important evolutionary consequences, possibly even affecting the network of genetic correlations revealed under different selection pressures.

CHAPTER 1

Introduction

Fitness can be comprehensively defined in terms of reproductive value or the expected future reproductive contribution of an individual, as a function of its age, expectation of survival and expectation of reproduction (Reznick and Travis, 1996). Thus, the study of life-history traits like reproductive age, number of offspring, life span and other traits which affect these indirectly, like resistance of an organism to various biotic and abiotic stresses or traits affecting resource acquisition and utilization, is important to both ecology and evolution. However, it was Mac Arthur and Wilson's (1967) theory of density-dependent selection which for the first time formally brought together population genetics and population ecology, as it considered the impact of population density on evolutionary trends. Since then, many theoretical models have been developed for life-history evolution over the course of time and under different environmental conditions (Gadgil and Bossert, 1970; Roughgarden, 1971; Asmussen, 1983; Boyce, 1984; Charlesworth 1994). These models predict the life-history expected to evolve under some defined selection regime. However, these models typically do not explicitly consider the genetic architecture of fitness-related traits. This lacuna has been addressed by many empirical studies on life-history evolution done in field, semi-natural or laboratory conditions (Rose 1984; Vasi et al. 1994; Hoffmann et al. 2003; van der Linde, 2005; Nussey et al. 2008).

Laboratory selection, also called experimental evolution, has been a very successful method for studying the evolution of life-history and related traits for the past 25 years or so (Rose et al. 1990, 1996). Experimental evolution studies with *D. melanogaster* in particular have helped to unravel the pleiotropic effects underlying fitness-related traits and also revealed trade-offs that constrain the evolution of such traits (reviewed by Prasad and Joshi, 2003). Laboratory selection experiments have several advantages over field and semi-natural

studies, including population level replication of control and selection treatments, thus increasing the inferential power (Rose et al. 1996), and high degree of control of the conditions in which the experiment is conducted (Gibbs, 1999; Harshman and Hoffmann, 2000). The population size in such experiments can also be kept high enough to avoid potentially confounding effects of genetic drift and inbreeding.

D. melanogaster has been one of the most popular metazoan model systems for laboratory selection experiments (reviewed in Prasad and Joshi, 2003). *Drosophila* has many advantages, such as short life-cycle, ease of breeding in large numbers, and a vast understanding of its laboratory ecology (Mueller, 1985).

Various long-term selection experiments on life-history-related traits have been conducted on *D. melanogaster*, including selection for postponed senescence (Rose, 1984; Patridge and Fowler, 1992; Roper et al.1993), for adaptations to crowding (Mueller, 1997; Joshi 1997), for starvation and desiccation resistance (Chippindale et al. 1996; Gibbs et al. 1997; Harshman et al 1999), and for rapid pre-adult development (Zwaan et al. 1995; Nunney 1996; Chippindale et al. 1997; Prasad et al. 2000, 2001). However, barring a few studies on other species of *Drosophila* (Hoffman and Cohan, 1987; Hoffmann and Parsons, 1993; Watson and Hoffman, 1996), *D. melanogaster* has remained the organism of choice for most of the laboratory selection studies. Other *Drosophila* species have been widely used to study life-history traits in the field with regard to clinal and other ecological factors (e.g. Loeschcke et al. 2000; Moreteau et al. 2003; van der Linde and Sevenster, 2006). However, the laboratory selection experiments have largely remained restricted to one species, *D. melanogaster* (Prasad and Joshi, 2003).

Given that most long-term selection experiments involving life-history traits have used *D. melanogaster*, our knowledge of the genetic architecture of fitness-related traits in *Drosophila* is largely limited to the one species. Whether the pattern of genetic correlations among fitness-related traits is largely conserved across other species of *Drosophila* or not, thus, remains an open question, the answer to which has non-trivial implications for our ability to generalize from the results obtained from experimental evolution studies on *D. melanogaster*. With an intent to address this question of the generality of patterns of genetic correlations, I chose to impose selection for adaptation to larval crowding on 3 species of *Drosophila*: *D. ananassae*, *D. nasuta* and *D. melanogaster*. The reason I chose to study adaptation to larval crowding was because, unlike selection for age at reproduction or stress tolerance, previous studies in *D. melanogaster* involving selection for either adaptation to crowding or faster development had yielded a fairly consistent pattern of genetic correlations among various fitness-related traits (Bierbaum et al. 1989; Joshi and Mueller, 1996; Santos et al. 1997; Prasad et al. 2000, 2001; Chippindale et al. 1997, 2003) and, hence, I could make direct comparisons with these studies. Choice of these 3 species was made easier by the fact that *D. ananassae* and *D. nasuta* are found abundantly in Bangalore, and our populations of *D. melanogaster* are long-term laboratory populations sharing ancestry with the UUs (Joshi and Mueller, 1996), used as controls in a previous study of density-dependent selection in *D. melanogaster*. *D. ananassae* and *D. melanogaster* are closer evolutionarily, as compared to *D. nasuta*, belonging to the same sub-genus *Sophophora* and the Melanogaster species group. They diverged 12 MYA and share 80% genes in common (*Drosophila* 12 genomes consortium, 2007). *D. nasuta* belongs to the other sub-genus, *Drosophila*, and diverged from *D. melanogaster* ca. 50 MYA.

There have been two detailed earlier studies on density-dependent selection in laboratory populations of *D. melanogaster*. One study on six populations of *D. melanogaster* maintained in laboratory for eight generations at low (*r*-selected populations) and high density (*K*-selected populations) showed that the *K*-selected populations had higher population growth rate and net productivity at high densities, whereas the *r*-selected populations were superior at lower densities (Mueller and Ayala, 1981; Mueller *et al*.1991). This finding supported the basic idea of a trade-off between adaptations to low versus high density (Mac Arthur and Wilson, 1967). Furthermore, it was seen that the egg-to-adult survivorship was higher and adults bigger when reared at high density in *K*-selected populations compared to *r*-selected populations, although these differences were not significant at low densities. It was also observed that the adults from *K*-selected populations eclosed faster at low densities but took longer than the *r*-selected populations to develop at high density (Bierbaum et al. 1989; Borash et al. 2000). Competitive ability and larval feeding rate were positively correlated, as the *K*-selected populations had higher competitive ability (Mueller, 1988) and larval feeding rates (Joshi and Mueller, 1988). However, there was no change in adult size at eclosion (Bierbaum et al. 1989)

One of the drawbacks of this selection experiment was that the *K*-populations were kept on an overlapping generation cycle, whereas the *r*-populations were kept on a 14-day discrete generation cycle, thereby confounding potential selective effects on age at reproduction with those of larval/adult density. The *K*-populations also experienced higher densities than the *r*-selected populations, both as larvae and adults, and also had a larger population size. Hence, a different set of *D. melanogaster* populations, the UUs (Uncrowded as larvae and adults) and the CUs (Crowded as larvae and Uncrowded as adults), were studied (Mueller et al.

1993). In this system, both types of populations were maintained on 21-day discrete generation cycles and differed only in the larval density experienced. Larval feeding rates in CU populations evolved to be higher than that of UUs (Joshi and Mueller, 1996) as also earlier seen with *K*-populations (Joshi and Mueller, 1988). There was, however, no difference between CUs and UUs in dry weights at eclosion or in the development time assayed at moderate densities (Santos et al. 1997). Pupation height evolution in the CUs also followed a course somewhat different from the *K*-populations (Joshi et al. 2003). Santos et al. (1997) also showed that the growth rates of the larvae did not differ between the control and selected populations. Interestingly, both the CUs and the *K*-populations evolved reduced efficiency of conversion of food to biomass (Mueller, 1990; Joshi and Mueller, 1996), at odds with the predictions of earlier verbal theory of density-dependent selection. The reduced efficiency was believed to be due to a trade-off between larval feeding rate and efficiency (Joshi and Mueller, 1996).

The pre-adult life stages of *Drosophila* in nature inhabit ephemeral and often crowded habitat patches (like rotting fruits), wherein strong competition for the limited resources is caused by not only individuals of the same species, but also by individuals of same genus and other insect species. It has therefore been believed that in nature *Drosophila* are under strong selection for faster development and competitive ability. Hence, it was typically thought that selection for faster development and larval crowding would lead to similar evolutionary outcomes (Tantawy and El-Helw 1970; Wilkinson 1987; Santos et al. 1988; Partridge and Fowler 1993; Borash et al. 2000). However, subsequent laboratory selection experiments on *D. melanogaster* contradicted this widespread belief.

When *D. melanogaster* populations were selected for rapid egg-to-adult development, they evolved a very different suite of traits than that seen with *K*-populations or the CUs (Prasad *et al.* 2000, 2001). The faster developing populations had reduced development time at low and high densities and weighed less than their controls. Moreover, the duration of the first and third larval instars and pupa was shorter, and the larval feeding rates, foraging path lengths and pupation heights were significantly lower in the faster developing populations than the controls by about 50 generations of selection (Prasad *et al.* 2001). Hence, selection for adaptation to larval crowding and faster development in *D. melanogaster* led to the evolution of very different, indeed opposite, sets of traits. Moreover, a picture emerged of genetic correlations between traits related to food acquisition and competition that were consistent across various studies. In the experiments on selection for rapid development or adaptations to larval crowding, larval feeding rate was positively correlated with both larval competitive ability and development time, and body weight and development time were also positively correlated. However, the correlation between competitive ability and development time was not consistently seen across these studies.

In a crowded culture vial, there is a progressive deterioration of food resources and build up of nitrogenous wastes. This raises the possibility of certain genotypes to specialize in early part of the environment when the food is more and the environment is less toxic, and other genotypes to specialize in the late part of the environment when the food is scarce and there is build up of nitrogenous wastes. This possibility was tested on the CUs by Borash et al. (1998) and it was found that, indeed, there are two distinct genotypes: one specializing in the early part and having higher larval feeding rates and lower tolerance to urea and ammonia, and another specializing in the late part with lower larval feeding rates and higher tolerance

to urea and ammonia. Hence, I wanted to see if there is a similar polymorphism of genotypes shown by selected populations of *D. ananassae* as well.

Since, there was a consistent pattern of genetic correlations between these competition-related traits in *D. melanogaster*, I wanted to see if these correlations are similar or different in other *Drosophila* spp. Therefore, I imposed selection for adaptation to larval crowding on three *Drosophila* species – *D. ananassae*, *D. melanogaster* and *D. nasuta*, with the main focus on addressing the following questions:

1. Is the network of genetic correlations among fitness-related traits under larval crowding similar in species of *Drosophila* other than *D. melanogaster* and, if not, does it differ more in more distantly related species?

2. Do *D. ananassae* populations selected for adaptation to larval crowding exhibit a polymorphism of different genotypes for exploiting temporal variation in the environment, as seen earlier in *D. melanogaster*?

3. Can maintenance regime have an effect on the genetic architecture of fitness-related traits through promoting assortative mating for development time under crowding?

Chapter 2 gives the description of populations used, their maintenance in the laboratory and the methods used for the various assays.

Chapters 3, 5 and 7 detail the evolution of larval life-history traits and trace the network of genetic correlations among these traits in these three *Drosophila* species. Here, I also compare these results with the earlier studies of density-dependent selection in *D.*

melanogaster. Chapters 4, 6 and 8 discuss the evolution of adult fitness-related traits when the three *Drosophila* species were selected for adaptations to larval crowding. Chapters 1-8 give a detailed account of studies on the three species of *Drosophila* which I have used to address the first question I have put forward.

The second question that I have raised in my thesis is addressed in chapter 9. It describes the result from one-time study on *D. ananassae* control and selected populations to see if there is polymorphism of genotypes to exploit the temporal variation in the environment which occurs as a result of crowding causing depletion of resources and built up of nitrogenous wastes.

In chapter 10, I address the third question. I describe here the results of an experiment which was designed to test if there was any difference in the mating system in my type of selection regime and that of CUs (Joshi and Mueller, 1996).

Thus, this thesis reports on detailed studies on different congeneric species to know more about the generality of life-history evolution using laboratory selection experiments. Moreover, my study also points to the necessity of caution while imposing various selection regimes since, small differences may alter the signs of genetic correlations.

CHAPTER 2

Materials and methods

The experiments reported in this thesis were done on populations of three species of *Drosophila – D. ananassae, D. melanogaster* and *D. nasuta*. Our *D. melanogaster* are from a set of long standing laboratory populations, while the *D. ananassae* and *D. nasut*a populations are derived from local caught populations from Bangalore which had been in laboratory conditions for 6-7 years (caught in 2001) when the experiments were done. The populations of *D. ananassae* and *D. nasuta* were initiated from 300 and 70 inseminated females, respectively. Initially, they were kept as a single population each for 35 generations and later split into four replicate populations. After two generations of rearing them as four replicates, the selection lines were started from these populations (Sharmila Bharathi et al. 2003).

2.1. EXPERIMENTAL POPULATIONS

The study was done on eight populations each of *D. ananassae, D nasuta* and *D. melanogaster*. Four of these populations, in each case, served as controls (AB_{1-4}- *Ananassae Baseline*, NB_{1-4}- *Nasuta Baseline*, MB_{1-4}- *Melanogaster Baseline*). These control populations are maintained on a 21-day discrete generation cycle at 25 ± 1ºC, about 90% relative humidity, constant light (LL) and on cornmeal medium (table 2.1). The larval density is regulated at about 60-80 larvae per 8-dram vial with 6 ml food. The number of breeding adults is about 1500-1800 per population and the adults are maintained in Plexiglas cages with abundant food. Eggs are collected from these flies by placing Petri dishes with food into the cages for 18 h. The eggs collected off these food plates are then dispensed into 40 vials (52 vials in case of the NBs) at a density of 60-80 eggs per vial. On the 12^{th} day

after egg collection, the eclosed flies are transferred into Plexiglas cages containing a Petri dish of food and moist cotton to keep the humidity high. A generous smear of yeast and acetic acid paste is given 4 days (3 days in case of the MBs) prior to egg collection.

The selected populations, ACUs (*Ananassae* Crowded as larvae and Uncrowded as adults), NCUs (*Nasuta* Crowded as larvae and Uncrowded as adults) and MCUs (*Melanogaster* Crowded as larvae and Uncrowded as adults) were derived from their respective controls with ACU-1 being derived from AB-1, and so on. The selected populations are maintained on a 21-day discrete generation cycle at 25 ± 1ºC, about 90% relative humidity, constant light (LL) and on cornmeal media. The number of breeding adults is about 1500-1800 per population and the adults are maintained in Plexiglas cages with abundant food. Eggs are collected from these flies by placing Petri dishes with food into these cages for 18 h. The eggs collected off these food plates are then dispersed into 20 vials (40 for NCUs) at different high egg densities. For ACUs, 550-600 egg/vial are collected in 1.5 ml food. In NCUs, the egg density is maintained at 350-400 eggs/vial in 2 ml of food. For MCUs, the egg density is 750-800 eggs/vial in 1.5 ml food. Once the flies start eclosing, they are transferred daily into the cages; this process is continued till no more flies eclose in the vials (generally, all flies eclose by day 15 after egg collection). For the ACUs and NCUs, the selection was started two generations after the control populations were split into four; however, for MCUs, the selection was started one generation after the control populations were shifted to cornmeal media from banana-jaggery medium in which they were already maintained as four replicates. MBs were initially created by mixing all the JB populations (as they might have started experiencing effects of inbreeding) and then, splitting them into five populations.

Table 2.1. Composition of cornmeal medium (1 litre)

Cornmeal	100 g
Yeast	40 g
Sugar	40 g
Agar	12 g
Activated charcoal	0.5 g
Water	1 L

Since each ACU, NCU and MCU population was derived from one AB, NB and MCU population, respectively, selected and control populations bearing identical numerical subscripts are more related to each other than to other populations with which they share the selection regime (AB_i and ACU_i are more closely related than AB_i and AB_j or ACU_i and ACU_j and so on; i,j = 1-4). Therefore, control and selected populations with identical subscripts were treated as blocks in the statistical analyses.

Collection of flies for the assays

Non-genetic parental effects could be induced in the populations due to differences in maintenance regimes between the selected and control populations. Hence, all selected and control populations were maintained under common rearing conditions for one complete generation prior to assaying, in order to eliminate all non-genetic parental effects. Eggs were collected from different population cages and dispensed into vials containing 6 ml cornmeal media at a density of 60-80 eggs per vial, and 40 vials were maintained per population. The egg collection window for both control and selected populations was 18 hours. On the 12^{th} day after egg collection, the flies were transferred into Plexiglas cages with abundant food. The adult numbers were around 1200-1500 per population. They were supplied with yeast-acetic acid paste for three or four days (depending on the assay) before egg collection for assays. The progeny of these flies, hereafter referred to as standardized flies, were used for the various assays.

2.2. ASSAYS DONE ON THE THREE SPECIES SELECTED FOR ADAPTATIONS TO LARVAL CROWDING

Most of the assays described below were performed on all the three species. However, few assays were done on only one or two of the three species. The populations on which the assays were done and the generation of selection at which these were done are mentioned in the description of each assay.

2.2.1. Assays of larval traits

Egg-to-adult survivorship

At various points in the ongoing selection (the number of generations was different for the three species), standardized flies were yeasted for three days before egg collection. A fresh food plate was kept in the cage and the flies were allowed to lay eggs for about 14 h, after which the eggs were removed from the food plate with a moistened brush and placed on agar pieces. Eggs were collected at two different densities – moderate and high– with the vial containing 1.5 or 2 ml of food, depending on the species. Eight such vials were set up for each density and each replicate population of both selected and control populations. The number of flies eclosing from each vial was recorded and used to assess egg to adult survival at the two densities.

NB and NCU - Generation 76 of selection and egg densities of 70 and 350 eggs/vial in 2 ml of food.

MB and MCU - Generations 10 and 30 of selection and egg densities of 70 and 800 eggs/vial in 1.5 ml of food. At generation 10, the egg density was 600 eggs/vial.

This assay was done on the ABs and ACUs by Sharmila Bharathi (2006).

Pre-adult competitive ability

The pre-adult stages of the control and the selected populations were competed against a common competitor population carrying a morphological marker for the convenience of identification *i. e.* a white eye mutant (WE) of *D. melanogaster* obtained as a spontaneous mutation in one of our existing wild type stocks. The WE population was being maintained

in the laboratory for about 90 generations on a 21-day discrete generation cycle on banana-jaggery medium at constant light conditions, 25 ± 1ºC and 90 % relative humidity. Eight vials were set up at two different densities: moderate and high with 1.5/2 ml per vial of cornmeal medium to assess competitive ability against white eye mutants. The moderate density (70 eggs per vial) bitypic cultures contained 35 eggs of the test population and 35 WE eggs whereas the high density assay vials comprised half from the test population and half from the white eye mutant population. The number of eclosing flies of the test populations and the white eye strains were recorded.

NB and NCU - Generation 76 of selection and egg densities of 70 and 350 eggs/vial in 2 ml of food.

MB and MCU - Generation 10 and 30 of selection and egg densities of 70 and 800 eggs/vial in 1.5 ml of food.

This assay done on the ABs and ACUs is reported in Sharmila Bharathi (2006).

Instar duration and pre-adult development time

Egg hatching time, larval instar duration, pupation time and egg-to-adult development time of the control and selected populations were assayed.

For hatching time, 30 eggs per vial were arranged as 6×5 rows and columns and 10 such vials were set up per population. From 15 h after egg-laying, each vial was checked for the number of hatched eggs every one hour. The checks were continued till no eggs hatched for 3 continuous hours.

The contribution of different pre-adult life stages to the overall egg-to-adult development time was assessed. Eggs of approximately identical age were collected from the standardized flies by placing a fresh food plate in the cage for 3 h. The eggs were then dispensed into vials with 6 ml food at a density of 30 eggs per vial and incubated at 25 ± 1°C. Sixty such vials were setup per population. Forty three hours after the midpoint of the 3 h egg collection time window, four vials from each population were removed from the incubator and the larvae killed by immersion in hot water. The larvae were then preserved in 70% alcohol. Every two hours, four vials from each population were removed and the larvae killed and preserved.

Identification of different larval instars

Two characteristics, the spiracles and the chitinous mouthparts can distinguish the three larval instars. In our experiments, the larvae were distinguished by the increase in number of teeth of the larval mouth hooks (chitinous mouthparts). Identification was done as follows:

INSTAR	MEAN TEETH NUMBER
1st	0-1
2nd	4-5
3rd	>5

In the transition states, the larvae had two pairs of hooks. In this case the hooks which were prominent were considered. This was repeated till all larvae were in the next instar. From the

data obtained, the fraction of larvae in 2nd instar was calculated and a graph of fraction of 2nd instar larvae vs. time was plotted. The median times of each molt were obtained by extrapolation. The difference between the mean hatching time and time of 1st molt was taken as the duration of 1st instar. The time difference between the 1st and 2nd molts gave the duration of 2nd instar. Similarly, the 3rd instar duration was taken as the difference between the mean pupation time and the time of 2nd molt.

Pupation time and egg-to-adult development time were assayed at the density of 30 eggs per vial containing 6 ml food. A fresh food plate was introduced into the cages and the adults were allowed to lay eggs for 3 h. At the end of this period, the eggs were removed from the food plate with a moistened brush and placed on agar pieces. Ten vials were set up per replicate population. After the first pupa was observed, the vials were checked regularly at 2 h intervals. Any new pupae that had formed were scored and marked using a color pen. The observations were continued until no new pupae were formed in any of the vials for 3 consecutive checks. The vials were then monitored for the first eclosion. Thereafter, the vials were checked regularly at 2 h intervals and the number of eclosing males and females recorded. These observations were continued until no flies eclosed for 1 day in any of the vials. From these data, we obtained mean egg-to-pupation development time and mean egg-to-adult development time.

Development time was also assayed at the higher egg density (350 eggs per vial for NB and NCU, and 800 eggs per vial for MB and MCU). Eggs were collected off the food plates on which the flies were allowed to lay eggs for 12 hours. Exact number of eggs was dispensed into vials with either 1.5 ml (MB and MCU) or 2 ml (NB and NCU) of cornmeal medium.

Eight such vials were set up per population. The vials were incubated at 25±1°C and were observed for first eclosion. Thereafter, the vials were checked every 6 hrs for eclosion. Checks were continued till no flies eclosed in three continuous checks.

AB and ACU - Generations 71 of selection (instar duration assay). Egg-to-adult development time at high egg density of 600 eggs/vial was done by Sharmila Bharathi (2006).

NB and NCU - Generation 62 and 84 of selection for instar duration assay and development time at 350 eggs/vial, respectively.

MB and MCU - Instar duration was not checked for the MCU populations. Egg-to-adult development time at 800 eggs/vial was assayed at generation 37 of selection. The pre-adult development time was assayed at 30 eggs/vial after generations 10, 30 and 43 of MCU selection.

Larval feeding rate

The feeding rates of larvae from control and selected populations were measured at physiologically equalized ages, based on the difference in their development times. This was performed by collecting eggs from the standardized flies of selected populations 5 h later than the control flies. Thus, at the time of assay, the larvae of both selected population and controls were in the same relative stage of their larval development. Eggs were collected from both control and selected flies in a 4 h time window. At the end of this time period, strips of food were cut and placed into four agar plates, two of the control populations and two of the selected populations. Twenty-four hours later, twenty-five newly hatched larvae

were transferred into Petri dishes containing a thin layer of non-nutritive agar overlaid with 1.5 ml of 37.5% yeast suspension. Four such Petri dishes were set up per population. The larvae were allowed to feed till they all entered the early third instar. At this point, 20 larvae from each population were assayed for feeding rates, following the procedure of Joshi and Mueller (1996), by placing them individually in a Petri dish containing a thin layer of agar overlaid with a thin layer of 10 % yeast suspension. After allowing for a 15 s acclimation period, feeding rates were measured as the number of cephalopharyngeal sclerite retractions in a 1 min interval. 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.

For *D. ananassae* (generation 71 of selection), I checked the feeding rates at late 2^{nd}, early 3^{rd} and middle 3^{rd} instars to confirm whether early 3^{rd} instar is the fastest feeding stage before doing the feeding rate assay.

NB and NCU - Generations 77 of selection.

MB and MCU - Generations 15, 30, 37, and 43 of selection.

AB and ACU - Generation 71 of selection.

Larval foraging path length

Foraging path length was measured in their early 3^{rd} instar stage for all the three species at different generations.

The eggs were collected from the standardized flies from the selected populations 5 hrs later than from the controls to approximately match their physiological age at the time of the assay. Eggs were collected from both control and selected flies in 4 h time window. At the end of this time period, strips of food were cut and placed into four agar plates, two of the control populations and two of the selected populations. Twenty-four hours later, twenty-five newly hatched larvae were transferred into Petri dishes containing a thin layer of non-nutritive agar overlaid with 1.5 ml of 37.5% yeast suspension. Four such Petri dishes were set up per population. The larvae were allowed to feed till they all entered the early third instar. At this stage, 20 larvae from each population were assayed for their foraging path lengths, by placing them individually in a Petri dish containing a thin layer of agar overlaid with a thin layer of 10 % yeast suspension. After allowing for a 15 s acclimation period, the larvae were allowed to move on the Petri plate surface for one minute. The paths that the larvae travelled in one minute interval were traced onto a transparency sheet and later measured with thread and ruler. Selected and control populations, matched by subscripted indices, were assayed together.

AB and ACU - Generations 40 of selection.

NB and NCU - Generations 81 of selection.

MB and MCU - Generations 37 of selection.

Pupation height

Pupation height was measured at 30 eggs/vial for all the three sets of selected lines and their respective controls. Fresh plates were put in the standardized cages for four hours after three

days of yeasting. Eggs were collected off the plates with moistened brush and exactly 30 eggs were placed in a vial with 6 ml of cornmeal medium. 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 touching or on the surface of the food were given a pupation height of zero.

NB and NCU - Generations 34 of selection.

MB and MCU - Generations 34 of selection.

Pupation height assayed for the AB and ACUs is reported in Sharmila Bharathi (2006).

Ammonia and urea tolerance

The egg-to-adult survivorship in different concentrations of ammonia in food was assayed for the NB, NCU and MB, MCU populations. These two assays were done on the ABs and ACUs by Sharmila Bharathi (2006).

For the NB and NCU populations, the ammonia tolerance was measured at three concentrations of ammonia- 0, 15, 20 g/l and for MB and MCU populations, it was done at 0, 15, 25 g/l

Urea tolerance was measured at 0, 9, 11 g/l for NB and NCU populations and at 0, 14, 18 g/l for MB and MCU populations.

For urea and ammonia tolerance, the fresh plates were placed in cages with standardized flies for approx. 12 hrs, after yeasting them for 3 days. The eggs were collected off these

plates with moistened brushes, and exactly 30 eggs were placed in each vial with different concentrations of urea and ammonia. Ten such vials were set up for each of these three concentrations per population. The number of flies eclosing in each vial was recorded. The tolerance was calculated as the egg to adult survivorship in the different concentrations.

NB and NCU - Generation 76 of selection.

MB and MCU - Generation 30 of selection.

Dry weight after minimum feeding for pupation

This assay was done only for AB and ACU populations. After 68 generations of selection, eggs from standardized flies were collected off agar plates and transferred on to a Petri dish containing non-nutritive agar. Twenty-five freshly hatched larvae from the plates were transferred to a Petri dish containing a thin layer of nonnutritive agar overlaid with 1.5 ml of 37.5 % yeast suspension. Sixty such Petri dishes were set up per population and were randomly distributed within the incubator. Later, at intervals of 3 h, all the plates of each population were pulled out, 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 nonnutritive agar at a density of 15 larvae per vial. These transfers were done at different percentage of survivorship of the flies (known from the critical minimum feeding time assay, Sharmila Bharathi, 2006) and, hence, AB and ACU transfers were staggered accordingly. Each vial was then monitored for pupation and eclosion. Once the flies started eclosing, they were collected within 3-4 hrs of eclosion and frozen. For the dry weight, the flies were sexed and weighed in batches of five flies per vial with 8 vials per sex per

population per percentage survivorship. The flies were dried 70° C for 36 hrs before weighing.

2.2.2. Assays of adult traits

Dry weight at eclosion

The dry weight of the flies was assayed with flies raised at two different densities- moderate (70 eggs/vial for AB, ACU, MB, MCU and NB,NCU) and high (600 eggs/vial for AB and ACU, 350 eggs/vial for NB and NCU and 800 eggs/vial for MB and MCU). Freshly eclosed flies were sexed and divided into batches of 5 each and placed in clean dry glass vials. Eight such vials were set up per density, per replicate population of both control and selected populations. After drying at 70°C for 36 h, these were cooled and weighed immediately.

AB and ACU - Generation 64 of selection.

NB and NCU - Generation 84 of selection.

MB and MCU - Generation 37 of selection.

Lipid content

The same flies which were used for the dry weight assay were also used for lipid estimation. The lipid content was estimated following the method given in Zwaan *et al.* (1991). The flies eclosing from moderate and high egg densities were dried at 70ºC for 36 h in batches of 5 flies/sex/vial/population. Eight such vials were set up per population. The lipid content of the flies from the control and selected populations was estimated by taking the difference in

the dry weights of the flies before and after extraction of lipids. After taking the initial dry weight, the flies from each vial were put in approximately 1.5 ml of ether in eppendorf tubes and kept on a rocker for 24 h. After 24 h, the ether was decanted and fresh ether was put in the eppendorf tubes. This was kept on rocker for another 12 h. Ultimately, the ether was decanted and the flies were again kept in an oven at 70^{o}C for 12 h. The dry weight of the flies was taken again as before and the difference between the two readings was noted.

AB and ACU - Generation 64 of selection

NB and NCU - Generation 84 of selection.

MB and MCU - Generation 37 of selection.

Adult life span

Adult life span of flies eclosing from moderate and high egg densities was measured from the time of eclosion till death. Four males and four females were introduced into each vial with 3 ml of food. Twenty such vials were set up per density per population. Every alternate day the flies were transferred to fresh food vials and checks were done every 24 h and any death was noted.

AB and ACU – Generation 64 of selection. Egg densities of 70 in 6 ml food and 600 in 1.5 ml food.

NB and NCU - Generation 84 of selection. Egg densities of 70 in 6 ml food and 350 in 2 ml food.

MB and MCU - Generation 37 of selection. Egg densities of 70 in 6 ml food and 800 in 1.5 ml food.

Female fecundity

Female fecundity was assayed at day 3 after eclosion and day 21 from egg collection after raising the flies at two different densities – moderate and high. The fecundity was checked at these two stages because the day 3 fecundity measures the early fecundity of the flies and day 21 fecundity measures fecundity on the day the egg collection is normally done in these populations.

Once the flies started eclosing, we transferred the flies into cages. They were transferred till no more flies eclosed. For day three fecundity, fecundity vials were set up with one male and female in each vial on day 2. Twenty such vials were setup per population. The flies in the vials were transferred to new vials every 24 h. In case of any male deaths, the male was replaced from the population. This was done till day 4. On day 5, the flies were discarded. The number of eggs laid in each vial was counted. The fecundity on day three was calculated as average of fecundity on days two, three and four. Before assaying fecundity on day 21, the flies were yeasted for 3 days (i.e., on day 18 after egg collection) and the set up was done similar to that of day 3. The day 21 fecundity was calculated as average of fecundity on days 20, 21 and 22.

AB and ACU - Generation 64 of selection. Egg densities of 70 in 6 ml food and 600 in 1.5 ml food.

NB and NCU - Generation 84 of selection. Egg densities of 70 in 6 ml food and 350 in 2 ml food.

MB and MCU - Generation 37 of selection. Egg densities of 70 in 6 ml food and 800 in 1.5 ml food.

Virgin starvation resistance

Starvation resistance of freshly eclosed virgin flies of control and selected populations raised at moderate and high egg densities was assayed. Five males or females were introduced into each vial containing 6 ml of non-nutritive agar to prevent desiccation. Eight such vials were set up per sex per density per population. The vials were checked every two hours for deaths. Starvation resistance was measured as the mean time to death from eclosion under starvation conditions.

AB and ACU - Generation 64 of selection. Egg densities of 70 in 6 ml food and 600 in 1.5 ml food.

NB and NCU - Generation 84 of selection. Egg densities of 70 in 6 ml food and 350 in 2 ml food.

MB and MCU - Generation 37 of selection. Egg densities of 70 in 6 ml food and 800 in 1.5 ml food.

Statistical analyses

Data obtained from experiments involving ACU, NCU and MCU populations were subjected to separate analyses of variance (ANOVA) with corresponding AB, NB and MB

values serving as controls. Data from all the assays were analyzed by separate mixed-model ANOVA, treating block as a random factor and selection regime as a fixed factor crossed with block. Wherever applicable, density and sex were treated as fixed factors crossed with block and selection regime, for the analyses. For the minimum feeding time dry weight assay, percentage survivorship was used as a fixed factor. For all the traits, the means of the replicate populations were used as the units of analysis, and therefore, only fixed factor effects and interactions could be tested for significance (Chippindale et al. 1994). All analyses were implemented using Statistica for Windows (rel.5.0 B, Stat Soft 1995). For multiple comparisons, wherever required Tukey's HSD test was performed.

CHAPTER 3

Evolution of larval traits in

D. ananassae

In this chapter, I present the results of assays on the larval traits of *D. ananassae* populations selected for adaptation to larval crowding and their ancestral controls. The methodology and the generations at which the assays were done have already been described in chapter 2 of this thesis.

Instar duration and development time

ANOVA results showed that there was no difference in the egg hatching time between AB and ACU populations ($F_{1,3}$ = 0.083, P = 0.79). The mean hatching time of AB and ACU populations was 18.39 hrs and 18.32 hrs, respectively (fig. 3.1(a)).

Durations of the three larval instar were significantly different between AB and ACU populations as revealed by the ANOVA ($F_{1,3}$ = 46.63, P = 0.006). There was a difference of two hrs in 1st instar and 4 hrs at 2nd and 3rd instar between AB and ACU populations (fig. 3.1(b)). Moreover, the duration of instars was also significantly different from each other (table 3.1).

Pupation time of AB and ACU was significantly different from each other ($F_{1,3}$ = 37.15, P = 0.008). Average pupation time of AB and ACU populations was 119 hrs and 109 hrs, respectively (fig. 3.2(a)).

ANOVA revealed significant effect of selection regime with ACU taking almost 11 hrs less than AB to develop from egg to adult ($F_{1,3}$ = 109.22, P = 0.002, fig. 3.2 (b) and table 3.2). There was also significant effect of sex ($F_{1,3}$ = 513.93, P < 0.001) with males taking longer time to develop than the females (fig. 3.2 (b)).

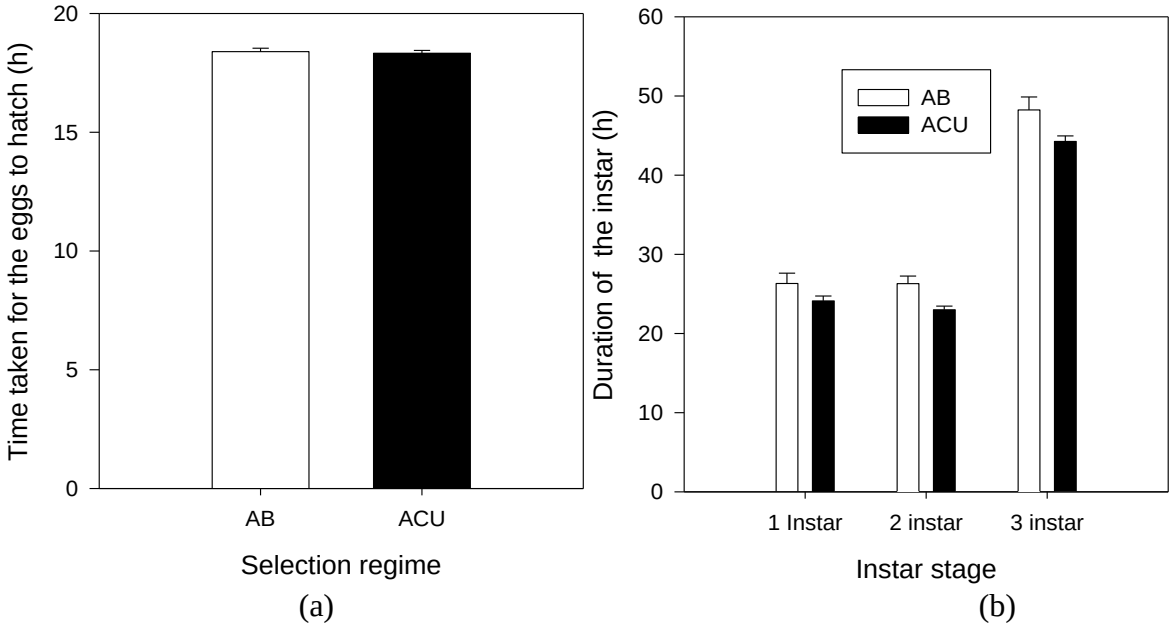


Figure 3.1. (a) Mean hatching time of AB and ACU eggs, and (b) mean duration of the three instars in the larval stage of AB and ACU populations at the density of 30 eggs/vial. Error bars represent the standard error around the mean of four replicate populations.

Table 3.1. Summary of three-way ANOVA on instar duration AB and ACU populations. The effects of block and interactions involving block could not be tested for significance in this design.

Effect	*df*	MS	*F*	*P*
Selection	1	59.831	46.625	0.006
Instar	2	1212.99	179.472	< 0.001
Selection × Instar	2	1.62749	0.3083	0.745

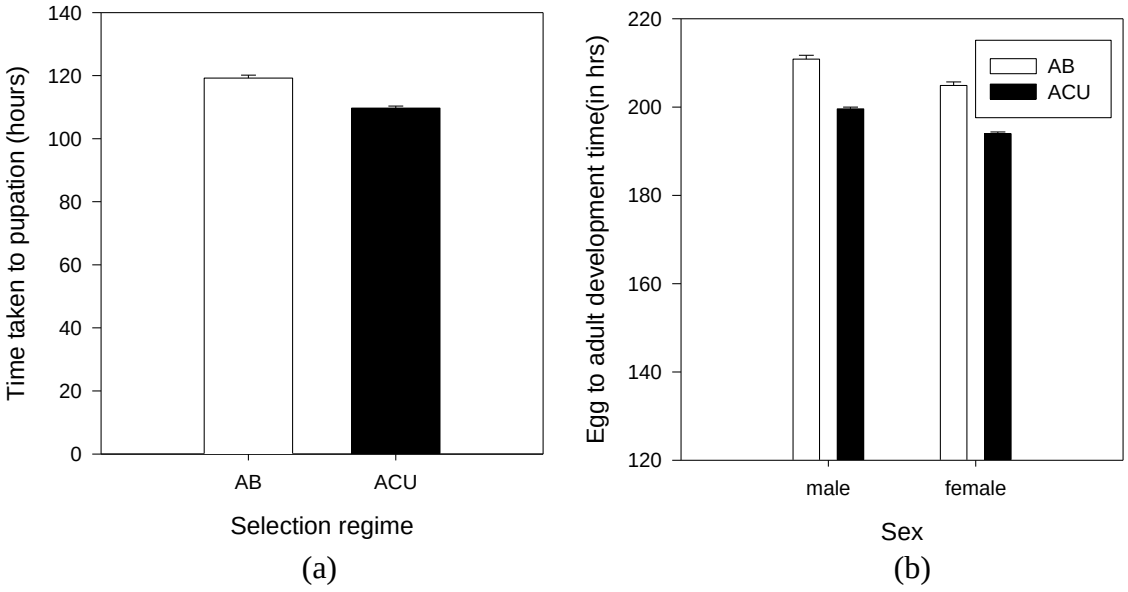


Figure 3.2. (a) Mean egg-to-pupation development time and (b) Mean egg-to-adult development time at the density of 30 eggs/vial in AB and ACU populations. Error bars represent standard errors around the mean of four replicate populations.

Table 3.2. Results of ANOVA on mean egg- to-adult development time at 30 eggs/vial in the AB and ACU populations. The effects of block and interactions involving block cannot be tested for significance in this design and have, therefore, been omitted from the table.

Effect	*df*	MS	*F*	*P*
Selection	1	344.664	225.008	< 0.001
Sex	1	104.805	81.495	0.002
Selection × Sex	1	1.495	1.915	0.26

Larval feeding rate at different stages

At generation 72 of selection, the larval feeding rate of ABs was measured at three different stages to check at which stage the feeding rate was the highest. It was measured at late 2nd, early and middle 3rd instars. There was a significant effect of instar stage ($F_{2,6}$ = 35.63, P = 0.0005, table 3.3). Tukey's HSD revealed that the difference in feeding rates was different between late 2nd and early 3rd instars and also between late 2nd and middle 3rd instars. But, there was no difference between the early and middle 3rd instars (figure 3.3).

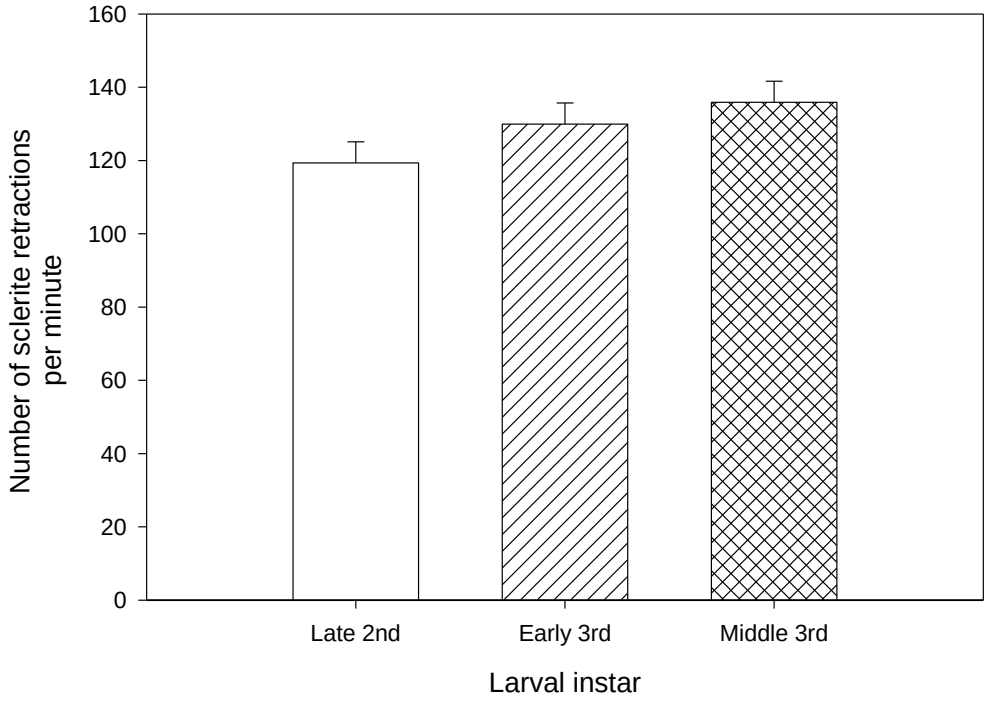


Figure 3.3. Mean (± 95% c.i.) number of sclerite retractions per minute for three different larval stages of ABs. Error bars are Tukey's HSD based confidence intervals derived from the appropriate mean squared error term in the ANOVA.

Table 3.3. Summary of three-way mixed-model ANOVA on feeding rate of different larval stage of AB and ACU populations, with selection regime and instar stage treated as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	10.14	0.645	0.48
Larval stage	2	561.988	35.63	< 0.001
Selection × Larval stage	2	5.59	0.29	0.759

Larval feeding rate

ANOVA revealed no significant difference between the feeding rates of AB and ACU populations after 71 generations of selection ($F_{1,3}$ = 0.541 , P = 0.515). The feeding rates of AB and ACU populations were 128.32 and 131.6 sclerite retractions per minute, respectively (fig. 3.4).

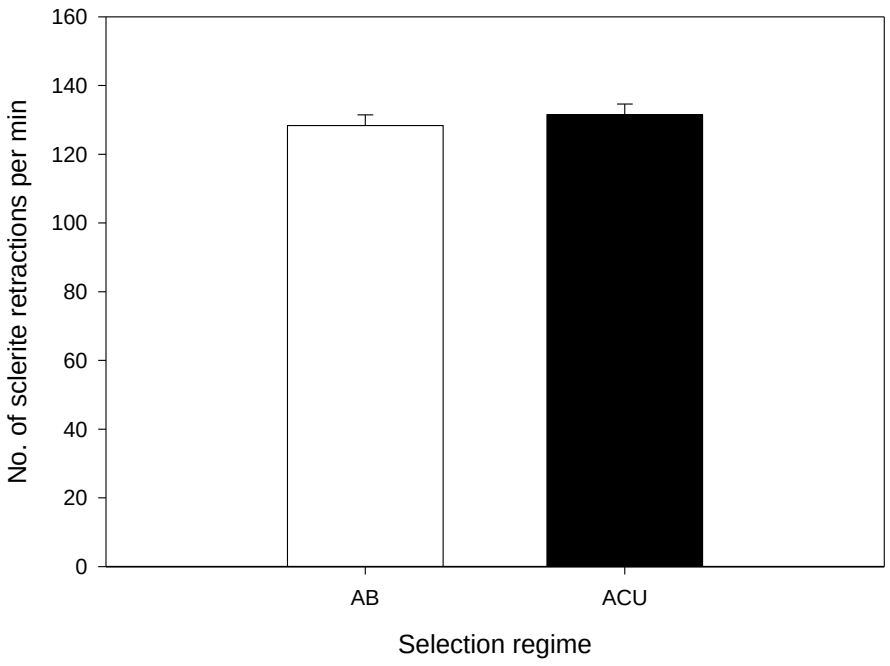


Figure 3.4. Mean larval feeding rate (± s.e.) of AB and ACU populations after 71 generations of selection.

Larval foraging path length

The ANOVA results revealed significant effect of selection regime ($F_{1,3}$ = 94.51 , P = 0.002), with ACUs having higher foraging path length as compared to ABs (fig. 3.5).

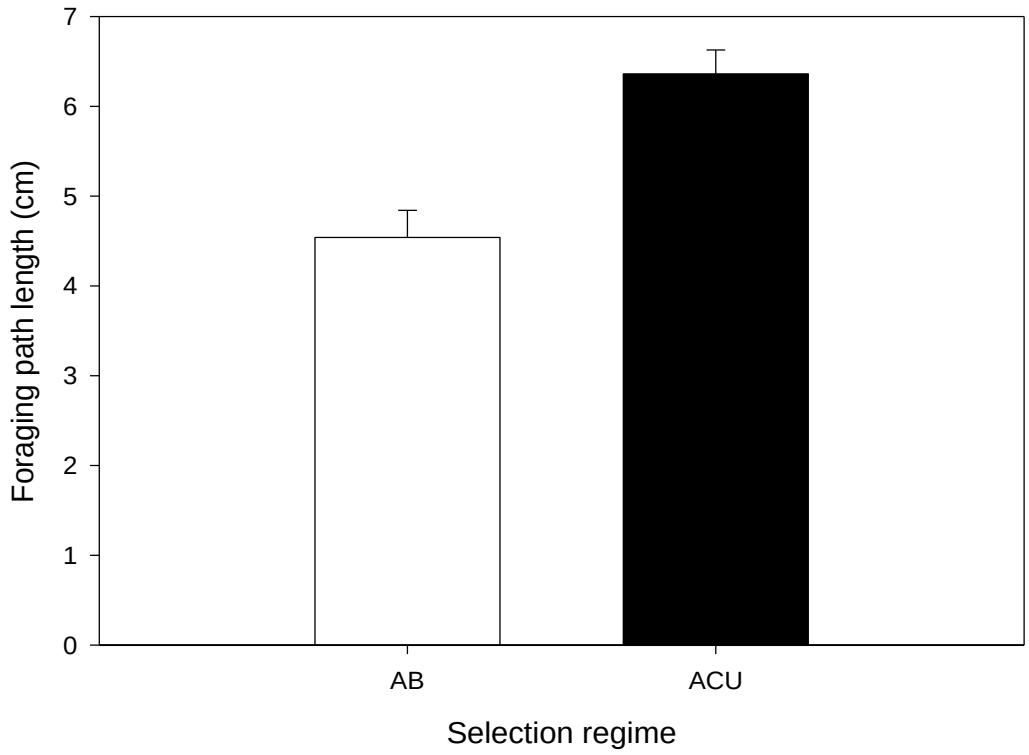


Figure 3.5. Mean foraging path length (in cm) of AB and ACU populations. The error bars represent standard errors around the mean of four replicate populations.

Minimum feeding time dry weight

The ANOVA revealed no significant effect of selection regime ($F_{1,3}$ = 0.008 and P = 0.94) on dry weights of ABs and ACUs at eclosion when larvae were allowed to feed for different time points (the comparison was done at different percentages of survivorship of flies). There were significant effects of percentage survivorship ($F_{3,9}$ = 16.39, P < 0.001) and also sex ($F_{1,3}$= 227.7, P < 0.001), with dry weights increasing with increased survivorship and females weighing more than males, respectively (table 3.4 and fig. 3.6).

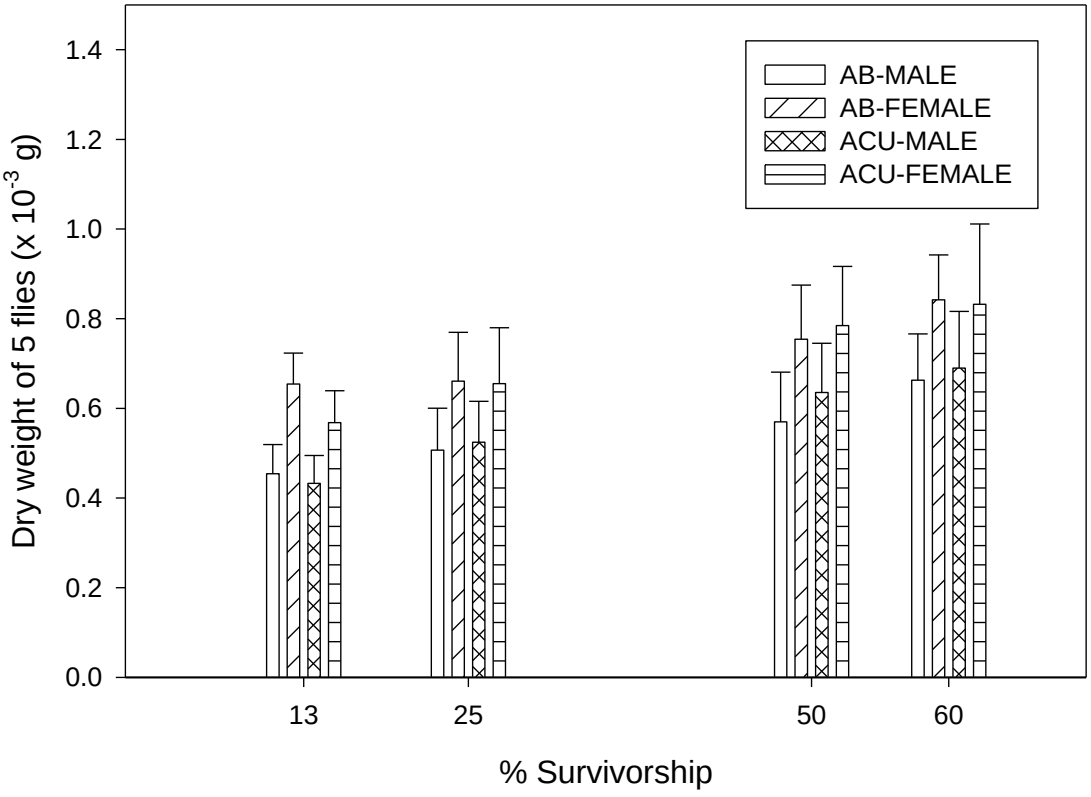


Figure 3.6. Mean dry weight (± s.e.) of the flies at eclosion of AB and ACU populations at different percentages of survivorship when larvae were allowed to feed for different time periods.

Table 3.4. Summary of four-way ANOVA on dry weight at eclosion of flies from different survivorship percentage in the minimum feeding time assay. Selection, survivorship and sex were treated as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	0.00006	0.008	0.935
Survivorship	3	0.16661	16.387	<0.001
Sex	1	0.40616	223.274	0.001
Selection × Survivorship	3	0.00704	2.762	0.103
Selection × Sex	1	0.0063	3.25	0.169
Survivorship × Sex	3	0.0005	1.402	0.304
Sel × Surv × Sex	3	0.0003	0.479	0.705

Discussion

Selection for adaptation to larval crowding in *D.ananassae* showed that the correlations between various larval traits are quite different than that seen earlier in *D. melanogaster* populations selected for adaptation to larval crowding.

Earlier work on the ACUs (Sharmila Bharathi, 2006) had shown that there was a decrease in the development time when assayed at both moderate and high densities. This was quite

contrary to the results from the earlier density-dependent selection studies in *D. melanogaster*, where there was no change in development time at moderate densities and increase in the development time assayed at high densities in the selected lines (Bierbaum et al. 1989; Santos et al. 1997). In ACUs, with increase in competitive ability there was no change in feeding rate and there was increase in ammonia tolerance but, no change in urea tolerance (Sharmila Bharathi, 2006). In the previous studies in *D. melanogaster*, competitive ability was positively correlated with larval feeding rate (Joshi and Mueller, 1988, 1996; Prasad et al. 2001; Rajamani et al. 2006). Moreover, ammonia and urea tolerance were found to be positively genetically correlated in *D. melanogaster* (Borash and Shimada, 2001).

Further experiments done on these populations of *D.ananassae* selected for adaptations to larval crowding showed that the decrease in development time at moderate densities was mainly due to reduction in the larval duration (assayed at generation 71) and not pupal duration. Pupal duration is relatively fixed in *Drosophila* species as it is tightly controlled by hormones and genetic cascades, even though it was shown to decrease in *D. melanogaster* selected for faster development for many generations (Prasad et al. 2001). The larval duration, however, can change with the quality and quantity of food available (Robertson, 1963). In the ACUs, there was significant decrease only in the third instar duration. This can be explained by the fact that the critical minimum size is achieved at late second or early third instar. Once the minimum critical size is reached, the larvae can successfully pupate and eclose as adults, but the larvae keep feeding for some time before pupating and thus, the duration of 3rd instar can be reduced without compromising on the egg to adult survivorship. The difference in egg-to-adult development time between ABs and ACUs was still

approximately 11 hrs after 71 generations of selection. There was no further reduction even after another 30 generations of selection.

The feeding rate is measured in early 3rd instar in *D. melanogaster* as the rate is highest at this stage (Joshi and Mueller, 1996). Hence, I wanted to see if the same is true for *D.ananassae* as well. Thus, I assayed the feeding rate at late 2nd, early 3rd and middle 3rd instars and found that the middle 3rd instar is the fastest feeding stage in *D.ananassae*, however, it was not significantly different from the early third instar. Larval feeding rate was assayed at generation 42 (Sharmila Bharathi, 2006) and no difference was seen between AB and ACU populations. I again assayed the feeding rate and found no difference between the ABs and ACUs after another 30 generations of selection.

In a crowded culture vial, the amount of food decreases over time and thus, the larvae may need to move about more in order to find food. Thus, the foraging path length (the distance travelled by the early 3rd instar larva in a minute interval) was found to increase in the crowding selected populations in *D. melanogaster* (Soklowski et al. 1997). This held true for ACUs as well, which showed a higher foraging path length as compared to the controls. Contrary to expectations, in the earlier studies in *D. melanogaster* populations, the efficiency got reduced over the course of selection (Mueller 1990; Joshi and Mueller, 1996). The selected populations required more food to reach the same critical size for pupation. However, when we measured the minimum feeding time (the minimum time required by the larvae to feed so as to successfully pupate and eclose as adults) in ACUs, it was found to be reduced as compared to that in the controls (Sharmila Bharathi, 2006). This could be attributed to the fact that now ACUs were faster developing than the ABs and thus, required

less time for reaching critical size, which was similar to the trend seen in *D. melanogaster* populations selected for faster development (Prasad et al. 2000). We also measured the dry weights of flies eclosing from larvae allowed to feed for different time points based on percentage survivorship, i.e., both AB and ACU larvae were allowed to feed for different time points at which their egg to adult survivorships were 13, 25, 50 and 60 %, respectively. However, there was no difference in dry weights between ABs and ACUs. The ABs and ACUs were reaching the same critical size but, the ACUs were taking less time to reach the size and there was no difference in feeding rates of ABs and ACUs. Thus, ACUs appear tp have evolved higher efficiency as compared to ABs as they consumed less food but eclosed at the same size.

Thus, overall *D.ananassae* evolved a very different set of larval traits as compared to the earlier studied *D. melanogaster* populations when under density-dependent selection. As mentioned before, *D.ananassae* and *D. melanogaster* belong to the same genus and species group and, moreover, occupy similar ecological niches in nature. Thus, the difference between the results from the two species could be because the correlations could be species specific.

CHAPTER 4

Evolution of adult traits in

D. ananassae

In this chapter, I have put together results of 5 adult traits: dry weight at eclosion, lipid content at eclosion, adult life span, female fecundity and starvation resistance. Dry weight and lipid content at eclosion lie at the juncture of larval and adult traits, being determined by larval resource acquisition and utilization and in turn strongly affecting adult fitness traits like life span and fecundity.

Dry weights at eclosion

The ANOVA results showed that there was no effect of selection regime ($F_{1,3}$ = 1.68, P = 0.29), with AB and ACU dry weights being almost the same at eclosion. There were, however, significant effects of density ($F_{1,3}$ = 111.47, P = 0.002) and sex ($F_{1,3}$ = 247.63, P < 0.001) (table 4.1), with flies from 600 eggs/vial weighing less than that from 70 eggs/vial and males weighing less than the females (fig.4.1).

Lipid content at eclosion

There was no difference in lipid content at eclosion between the AB and the ACU populations as revealed by ANOVA ($F_{1,3}$ = 0.701 , P = 0.46). However, there were significant effects of density ($F_{1,3}$ = 16.29, P = 0.027) and sex ($F_{1,3}$ = 13.8, P = 0.03) (table 4.2). The flies raised at 600 eggs/vial had more lipid at eclosion as compared to the flies raised at 70 eggs/vial and the females had higher lipid content than the males (fig.4.2).

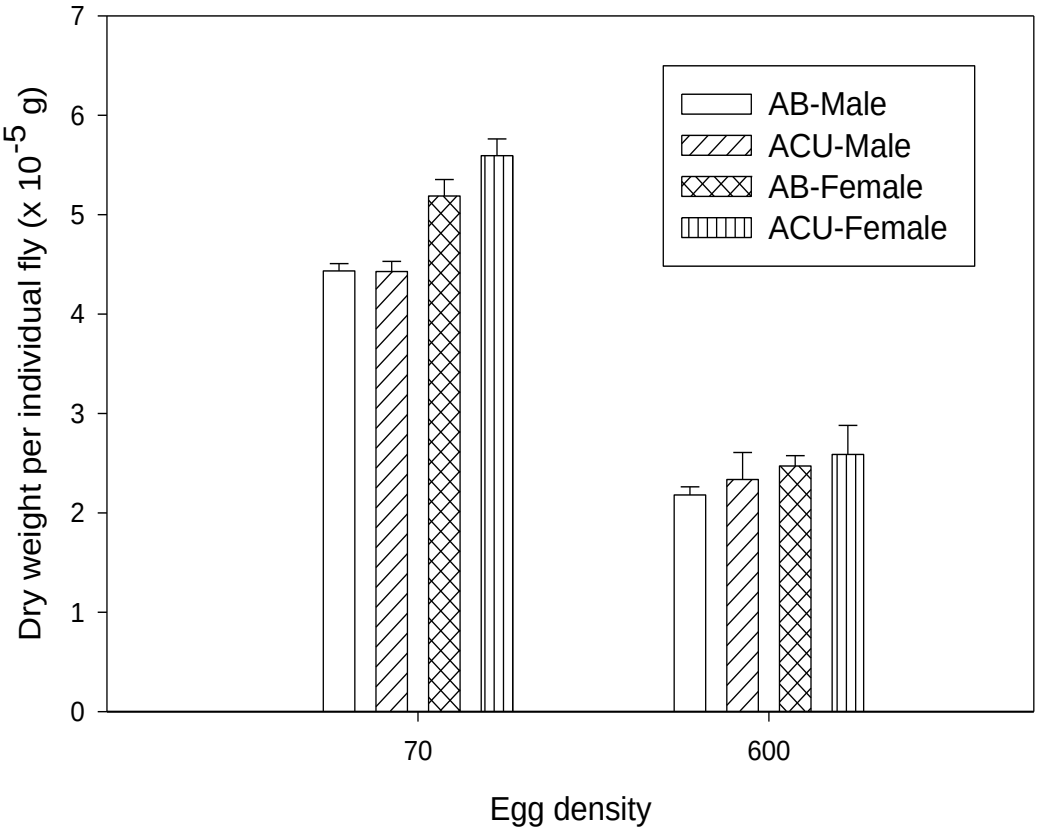


Figure 4.1. Mean dry weight per fly (± s.e.) at eclosion of AB and ACU populations when raised at 70 and 600 eggs/vial.

Table 4.1. Results of ANOVA on dry weights at eclosion of flies raised at 70 and 600 eggs/vial. The effects of block and interactions involving block could not be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	0.001	1.681	0.285
Density	1	0.127	111.47	0.001
Sex	1	0.008	247.62	< 0.001
Selection × Density	1	0.00002	0.038	0.857
Density × Sex	1	0.0023	33.94	0.01
Sel × Den × Sex	1	0.0002	73.5	0.003

Table 4.2. Summary of ANOVA on lipid content at eclosion of flies from AB and ACU populations raised at 70 and 600 eggs/vial.

Effect	*df*	MS	*F*	*P*
Selection	1	0.00003	0.008	0.46
Sex	1	0.0001	13.8	0.033
Density	1	0.0004	16.29	0.27
Selection × Density	1	8.32×10^{-7}	0.027	0.88
Selection × Sex	1	2.03×10^{-6}	2.423	0.217
Density × Sex	1	5.73×10^{-7}	0.08	0.79
Sel × Sex × Den	1	1.97×10^{-7}	0.457	0.547

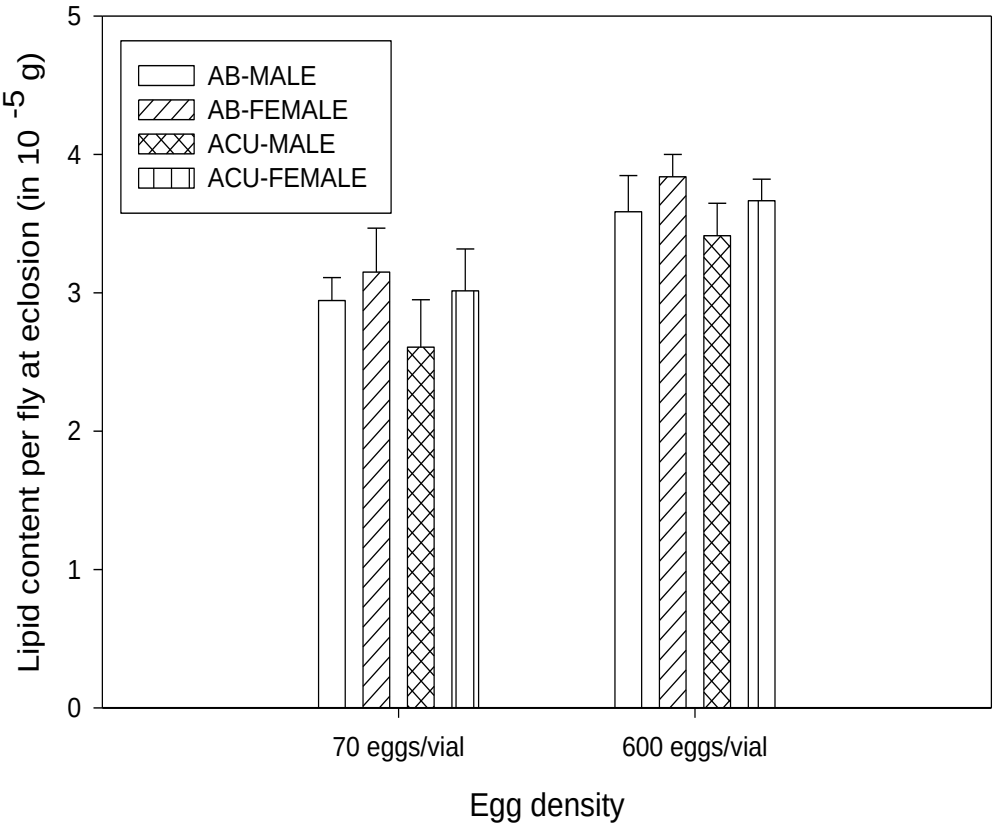


Figure 4.2. Mean lipid content per fly (± s.e.) at eclosion of AB and ACU populations when raised at 70 and 600 eggs/vial.

Adult life span

There was no change in the adult life span of the selected populations after 65 generations of selection, as revealed by ANOVA ($F_{1,3} = 0.38$, $P = 0.58$). The average life span of ABs and ACUs were 30.52 and 30.15 days, respectively. The effects of density ($F_{1,3} = 0.71$, $P = 0.46$) and sex ($F_{1,3} = 3.58$, $P = 0.15$) (Table 4.3) were also not significant. There was no difference between the flies from 70 and 600 eggs/vial and also between males and females in their life span (fig.4.3).

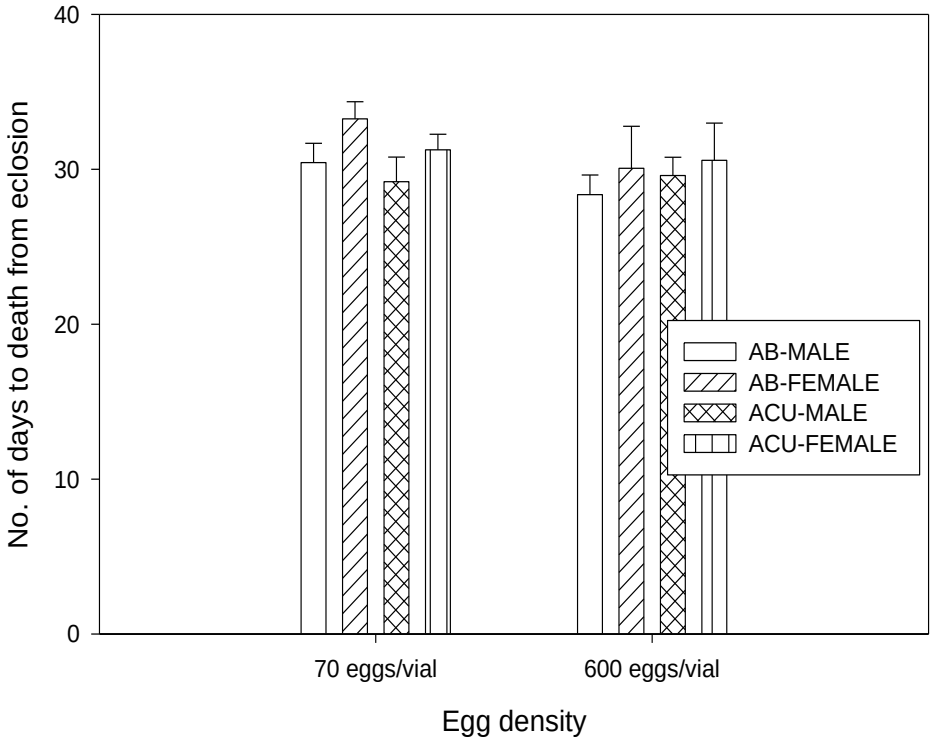


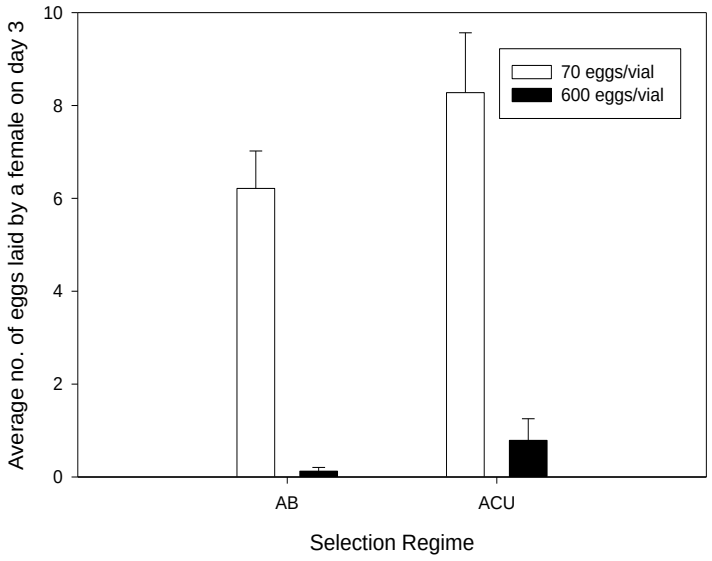
Figure 4.3. Mean lifespan (± s.e.) of AB and ACU populations when raised at two different densities of 70 and 600 eggs/vial.

Table 4.3. Summary of four-way mixed model ANOVA on mean life span of AB and ACU population raised at two different densities of 70 and 600 eggs/vial.

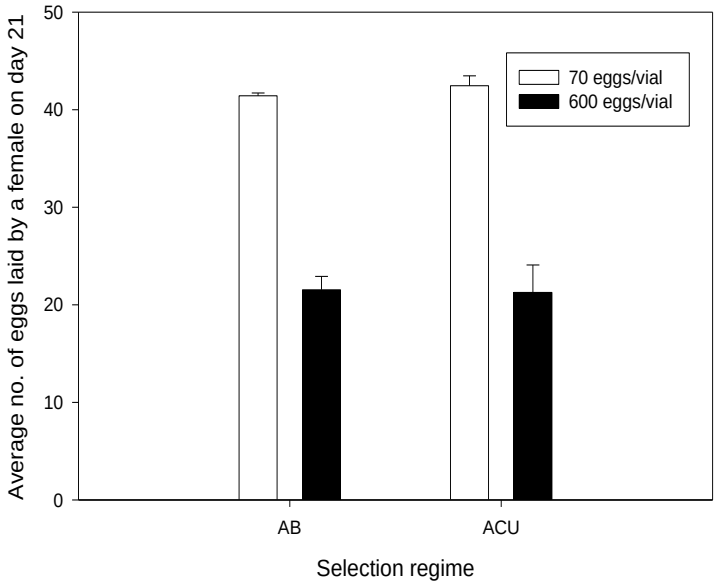
Effect	*df*	MS	*F*	*P*
Selection	1	1.103	0.384	0.579
Density	1	15.322	0.706	0.462
Sex	1	28.677	3.584	0.154
Selection × Density	1	12.395	3.23	0.17
Selection × Sex	1	1.081	0.49	0.53
Density × Sex	1	2.375	1.64	0.290
Sel × Den × Sex	1	0.001	0.002	0.968

Fecundity on day 3 after eclosion and day 21 after egg collection

ANOVA revealed no significant difference in fecundity between the AB and ACU populations on day 3 after eclosion and day 21 after egg collection, raised at 70 and 600 eggs/vial ($F_{1,3}$ = 1.04, P = 0.38). There were significant effects of density ($F_{1,3}$ = 137.15, P = 0.001) and day ($F_{1,3}$ = 358.73, P < 0.001), with flies raised at 600 eggs/vial and flies after 3 days of eclosion having less fecundity than the flies raised at 70 eggs/vial and flies from day 21 after egg collection, respectively (fig 4.4(a) and (b), table 4.4).



(a)



(b)

Figure 4.4. Mean fecundity after eclosion of flies raised at 70 and 600 eggs/vial of AB and ACU populations on (a) day 3 after eclosion and (b) day 21 from egg collection. The error bars represent standard errors around the mean of 4 replicate populations.

Table 4.4. Summary of four-way ANOVA on fecundity of AB and ACU populations raised at 70 and 600 eggs/vial on day 3 after eclosion and day 21 from egg collection with selection, density and day as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	6.097	1.0361	0.383
Density	1	1493.34	137.151	0.001
Day	1	6190.22	358.73	<0.001
Selection × Density	1	3.65	3.469	0.159
Selection × Day	1	1.92	1.85	0.267
Density × Day	1	378.14	151.56	0.002
Sel × Den × Day	1	0.005	0.011	0.922

Starvation resistance

The ANOVA revealed that the starvation resistance was greater in ACU populations as compared to the AB flies from populations raised at both 70 and 600 eggs/vial ($F_{1,3}$ = 16.09, P = 0.03). There was no significant effects of either density ($F_{1,3}$ = 3.67, P = 0.15) or sex ($F_{1,3}$ = 7.65, P = 0.07) (table 4.5). The mean time to death under starvation conditions were 71.4 and 80.6 h for ABs and ACUs raised at 70 eggs/vial and 73.7 and 86.5 h, respectively at 600 eggs/vial (fig 4.5).

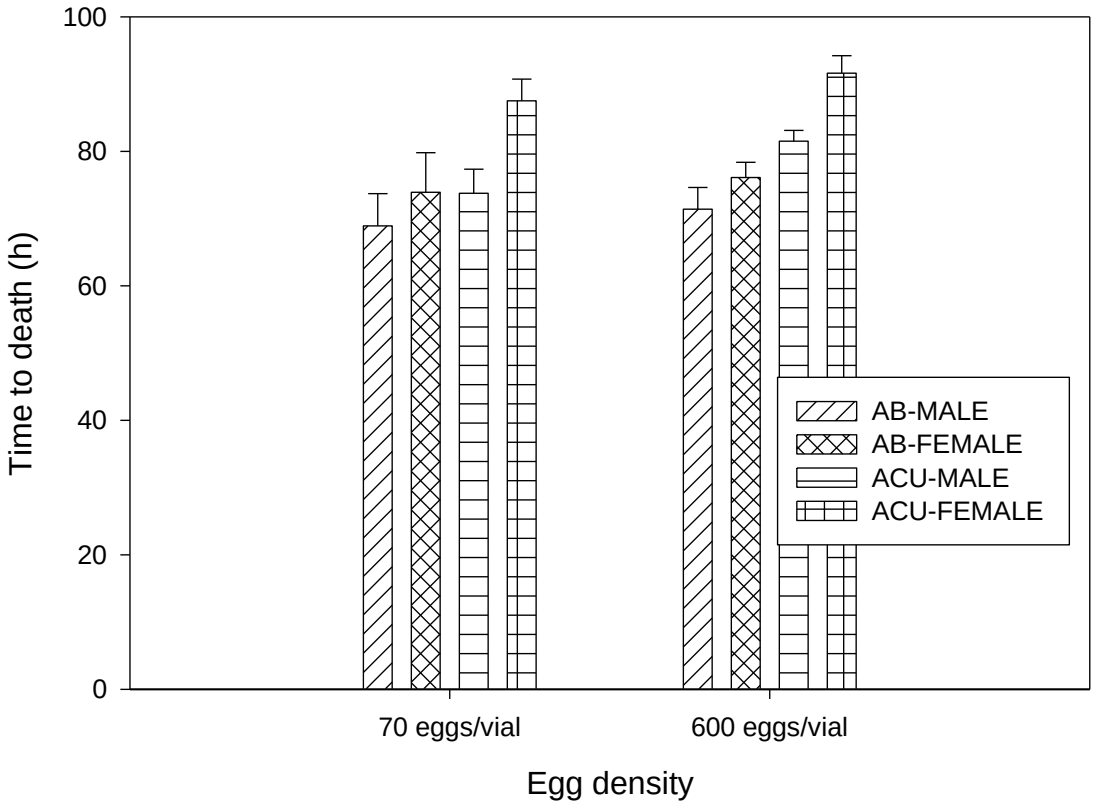


Figure 4.5. The starvation resistance (measured as the mean time to death) (± s.e.) of flies of AB and ACU populations raised at 70 and 600 eggs/vial.

Table 4.5. Results of four-way mixed-model ANOVA on mean time to death of AB and ACU populations, raised at 70 and 600 eggs/vial, under starvation conditions. Selection, density and sex were treated as fixed factors and block as random factors.

Effect	*df*	MS	*F*	*P*
Selection	1	971.85	16.09	0.027
Density	1	136.33	3.67	0.151
Sex	1	564.48	7.65	0.069
Selection × Density	1	25.83	0.21	0.681
Selection × Sex	1	100.1	10.62	0.05
Density × Sex	1	7.61	1.59	0.296
Sel × Den × Sex	1	5.45	0.22	0.67

Discussion

Adaptations to larval crowding can have effect on the adult fitness traits, mainly through changes in the resource acquisition and utilization during the larval stages.

Previous studies of adult traits in *D. melanogaster* selected for larval crowding (CUs) showed that even though the flies did not differ in their weight at eclosion (Santos et al. 1997) but, they had higher lipid content at eclosion when reared at both low and high larval densities (Borash and Ho, 2001). Higher body weight and lipid content largely determine the

adult fitness characters, lifespan and fecundity in *Drosophila* (Partridge and Fowler, 1992). Moreover, higher lipid content is also associated with higher starvation resistance (Chippindale et al. 1996, Djawdan et al. 1998) and increase in longevity was associated with correlated increase in lipid content and starvation resistance (Chippindale et al. 1996). However, *D. melanogaster* populations selected for adaptation to larval crowding did not show any increase in lifespan as compared to the controls, but these populations had higher resistance to starvation when reared at both low and high larval densities. Fecundity seemed to be slightly higher in populations adapted to larval crowding relative to the controls (Borash and Ho, 2001).

Adult fitness traits in *D. ananassae* showed a more or less similar pattern of evolution as seen in *D. melanogaster* when under selection for larval crowding in contrast to differences seen for the larval traits in the two species. In *D. ananassae*, there was no change in either the weight or lipid content at eclosion of ACUs when reared at both low and high densities. Even though there was no change in lipid content, the starvation resistance was higher in ACU populations. This suggests an increase in the efficiency of utilization of lipids in ACUs, which is again in contrast to the study of Chippindale et al. (1996) where they showed that there was no increase in efficiency of utilization of lipid reserves for starvation resistance. However, there was no difference in life span and fecundity on day three after eclosion and day 21 from egg collection between the ABs and ACUs when flies were raised at both low and high densities.

Evolution of similar adult traits in earlier studied *D. melanogaster* and *D. ananassae*, in spite of differences in larval trait correlations is interesting and shows how the adaptation to

larval crowding can occur through different evolutionary routes but, ultimately leading to similar outcomes in terms of adult fitness.

CHAPTER 5

Evolution of larval traits in

D. nasuta

This chapter summarizes the results from assays of larval traits in *D. nasuta* populations selected for adaptations to larval crowding and their controls.

Hatching time

ANOVA revealed no difference between egg hatching time of NB and NCU populations ($F_{1,3}$ = 0.407, P = 0.57). The mean hatching time of NB and NCU was 23.27 h and 23.10 h, respectively. (fig. 5.1).

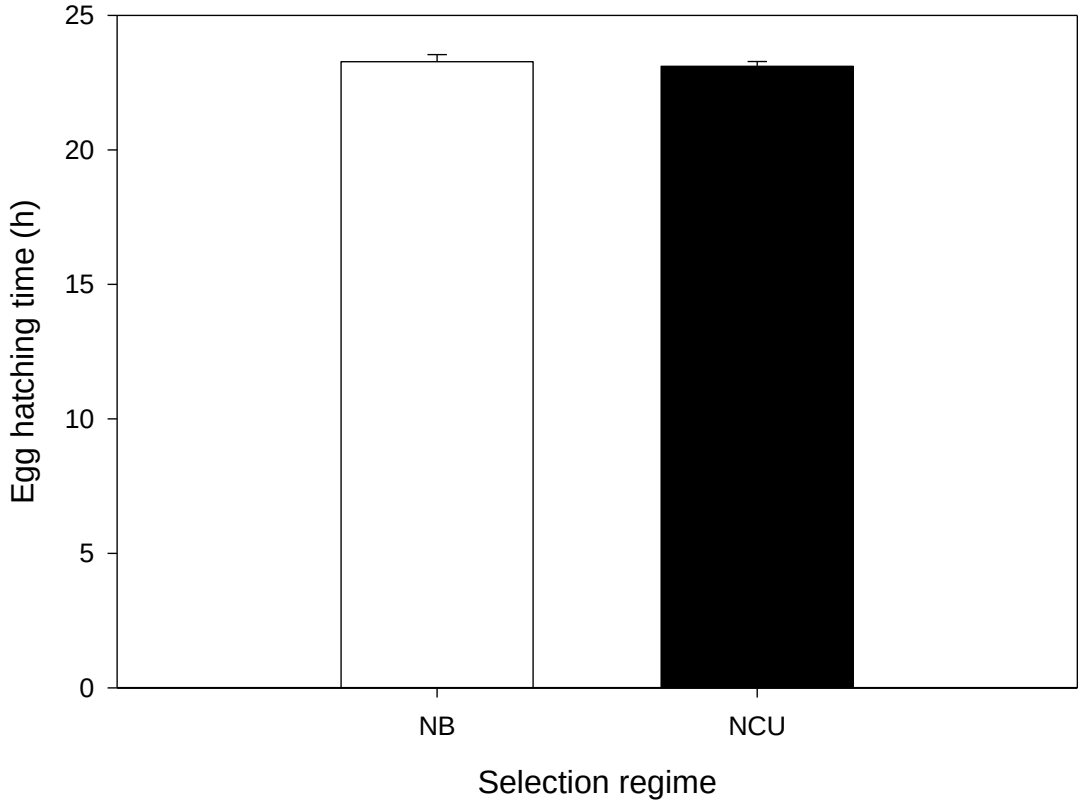


Figure 5.1. Mean hatching time (± s.e.) of NB and NCU populations at 30 eggs/vial.

Instar duration

There were significant effects of selection regime ($F_{1,3}$ = 285.7, $P <$ 0.001), instar stage ($F_{2,6}$ = 5052.1, P < 0.001) and selection × instar stage ($F_{2,6}$ = 23.04, P = 0.001, table 5.1) as revealed by ANOVA. NCUs had significantly shorter instar duration as compared to the NBs. Pair-wise comparisons using Tukey's test revealed that there difference between NB and NCU 1st and 2nd instars was not significant. However, duration of 3rd instar was significantly different between the NBs and NCUs (fig. 5.2).

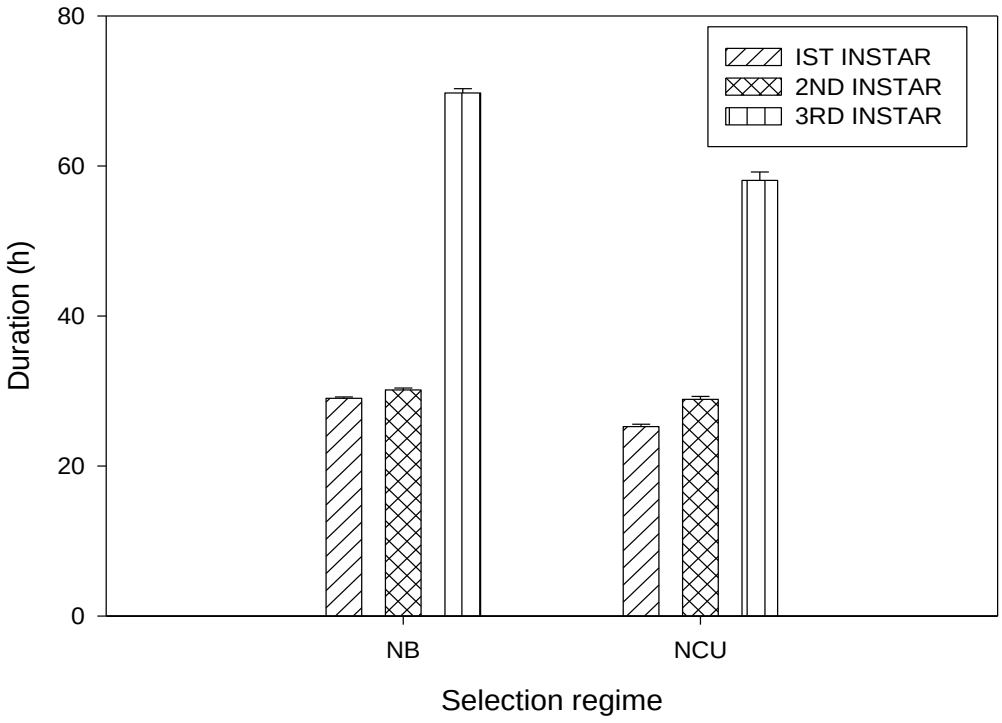


Figure 5.2. Duration (± s.e.) of three instars of NB and NCU populations at 30 eggs/vial.

Table 5.1. Summary of three-way ANOVA on instar duration of AB and ACU populations at 30 eggs/vial. Selection and instar stage were fixed factors and block was a random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	185.78	285.73	<0.001
Instar	2	3388.11	5052.12	<0.001
Selection × Instar	2	59.15	23.04	0.001

Pupation time

There was significant effect of selection on the pupation time ($F_{1,3}$ = 196.8, P <0.001) with NCUs pupating around 17 h before NBs, as revealed by ANOVA. The mean pupation time of NB and NCU was 152.15 h and 135.29 h, respectively (figure 5.3).

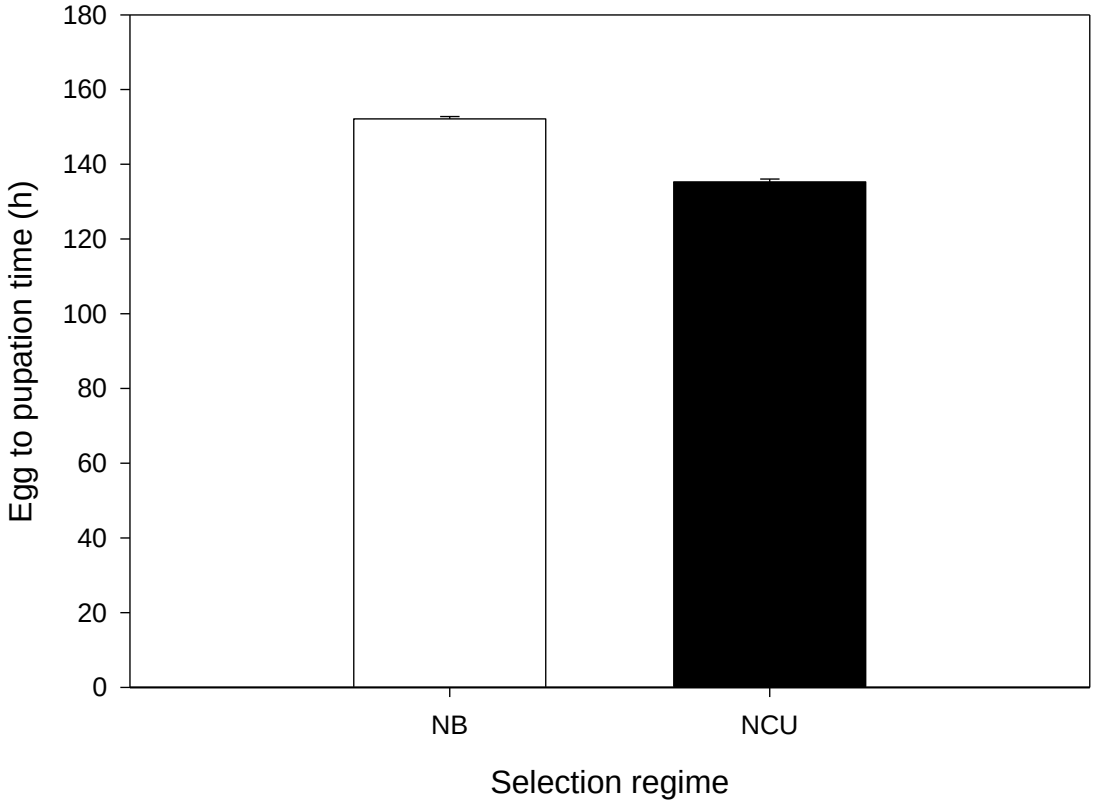


Figure 5.3. Mean (±) pupation time of NB and NCU populations.

Egg-to-adult development time

ANOVA revealed significant effect of selection on the egg-to-adult development time of NB and NCU populations ($F_{1,3}$ = 115.5, P = 0.002) (table 5.2) at 30 eggs/vial. There was no significant effect of sex ($F_{1,3}$ = 3.75 , P = 0.15), with males and females taking almost the same time to develop. The mean development time of NB and NCU populations were 239.27 h and 222.68 h, respectively (fig.5.4(a)).

At 350 eggs/vial, ANOVA showed significant difference between NB and NCU egg to adult development time ($F_{1,3}$ = 364.65, P <0.001) (table 5.3). The difference between NB and NCU development time was approx. 29 h (fig.5.4(b)). There was no significant effect of sex ($F_{1,3}$ = 13.09, P = 0.04), with males and females taking almost the same time to develop from egg to adult.

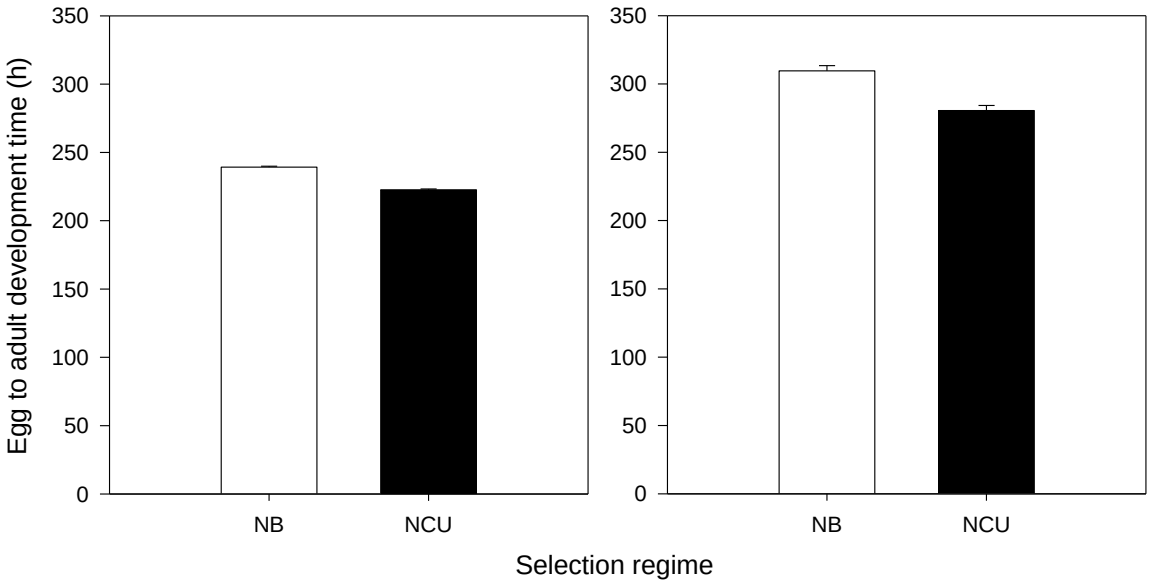


Figure 5.4. Mean egg-to-adult development time (± s.e) of NB and NCU populations at (a) 30 eggs/vial and (b) 350 eggs/vial.

Table 5.2. Summary of three-way mixed model ANOVA on mean egg-to-adult development time of NB and NCU populations at 30 eggs/vial, with selection and sex as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	1101.42	115.50	0.001
Sex	1	2.210	3.754	0.148
Selection × Sex	1	1.472	18.61	0.022

Table 5.3. Result of ANOVA performed on the egg-to-adult development time of NB and NCU populations at 350 eggs/vial with selection and sex as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	3388.81	364.65	<0.001
Sex	1	74.550	13.088	0.036
Selection × Sex	1	1.929	5.491	0.101

Egg-to-adult survivorship

ANOVA revealed that there was no significant effect of selection ($F_{1,3}$ = 3.6, P = 0.15). However, there was a significant selection × density interaction ($F_{1,3}$ = 85.42 , P = 0.002, table 5.4). Pair-wise comparisons using Tukey's HSD test revealed that NB and NCU did not differ significantly from each other at 70 eggs/vial, but at 350 eggs/vial, NB and NCU survivorships were significantly different. At 350 eggs/vial, NCUs had higher egg to adult survivorship as compared to NBs (fig.5.5).

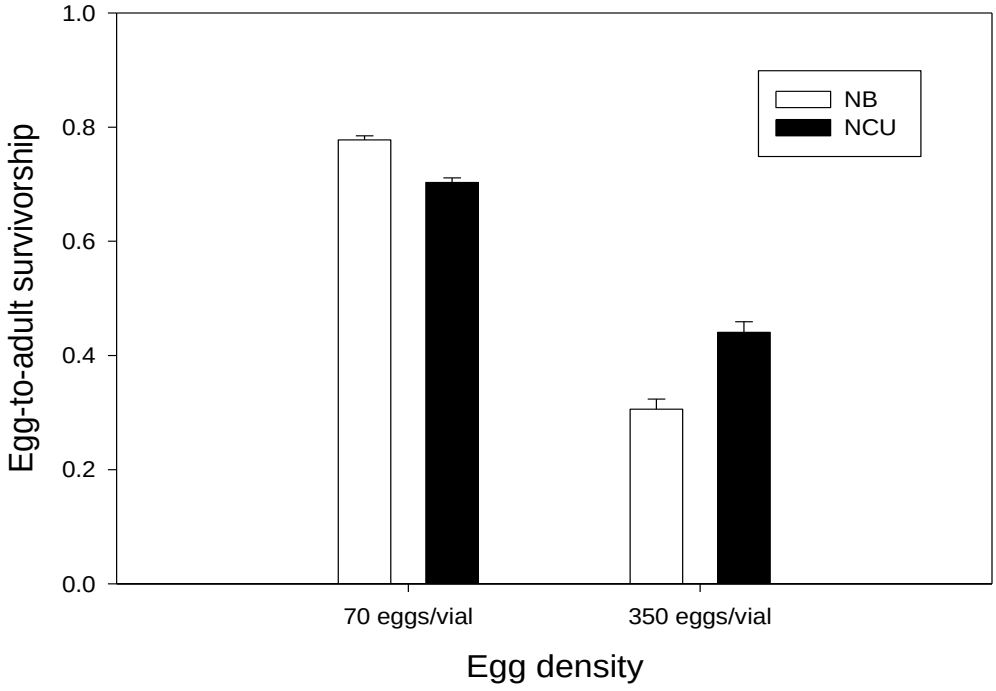


Figure 5.6. Mean egg-to-adult survivorship (± c.i.) at 70 and 350 eggs/vial.

Table 5.4. Result of three-way ANOVA on mean egg-to-adult survivorship of NB and NCU populations at 70 and 350 eggs/vial. The survivorship data were arcsine square-root transformed before performing ANOVA. Selection and density were used as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	0.002	3.602	0.153
Density	1	0.582	693.837	<0.001
Selection × Density	1	0.050	85.417	0.002

Larval competitive ability

There was significant effect of selection ($F_{1,3}$ = 18.81, P = 0.02) with NCUs having better larval competitive ability than the NBs at both 70 and 350 eggs/vial (table 5.5) when competed against a common competitor, white-eyed mutant of *D. melanogaster*. There was no significant effect of density ($F_{1,3}$ = 4.51, P = 0.124). At 70 eggs/vial, the NB survivorship was 0.56 and that of NCU was 0.61, and at 350 eggs/vial, the NB and NCU survivorships were 0.36 and 0.54, respectively (fig.5.6).

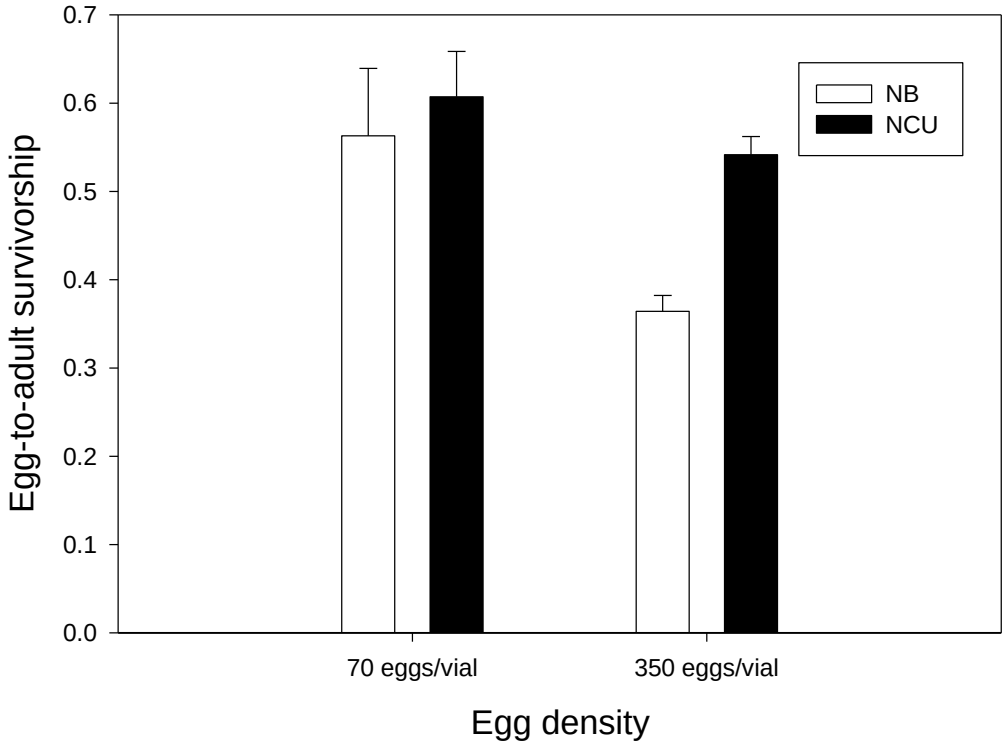


Figure 5.6. Mean larval competitive ability of NB and NCU populations against white-eyed mutant of *D. melanogaster* at 70 and 350 eggs/vial.

Table 5.5. Summary of three-way mixed model ANOVA on egg to adult survivorship of NB and NCU populations at 70 and 350 eggs/vial when competed against white-eyed mutant of *D. melanogaster*. Selection and density were fixed factors and block was a random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	0.049	18.814	0.022
Density	1	0.074	4.511	0.123
Selection × Density	1	0.018	7.741	0.068

Larval feeding rate

ANOVA showed no difference in larval feeding rate between NB and NCU populations as there was no significant effect of selection ($F_{1,3}$ = 0.623, P = 0.818). The mean larval feeding rate of NB and NCU populations was 112.76 and 113.03 sclerite retractions per minute, respectively (fig 5.6).

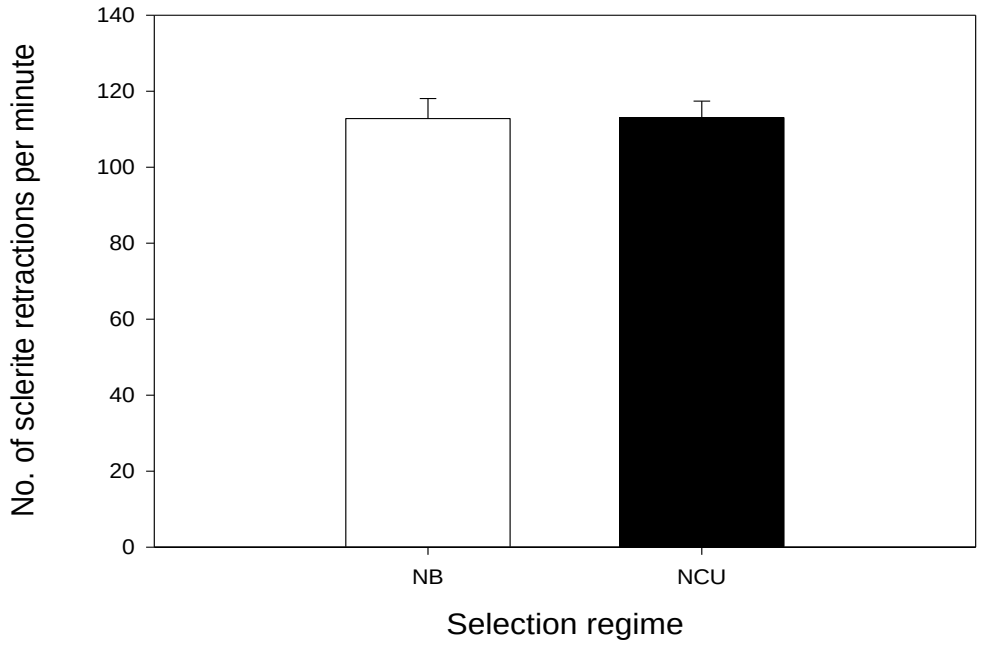


Figure 5.6. Mean larval feeding rate (± s.e.) of NB and NCU populations.

Larval foraging path length

ANOVA revealed no significant effect of selection regime ($F_{1,3}$ = 0.48, P = 0.54) with no significant difference between foraging path length of NB and NCU populations. The mean foraging path length of NBs and NCUs were 4.24 and 4.08 cms, respectively (fig.5.7).

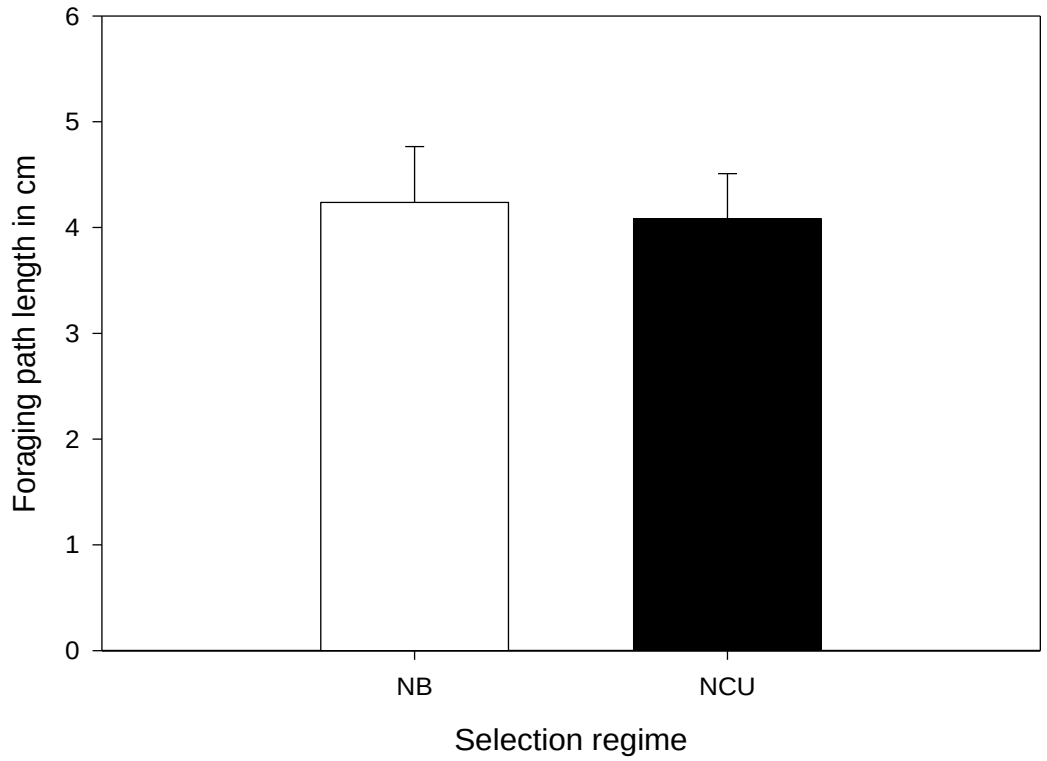


Figure 5.7. The mean larval foraging path length of NB and NCU populations. The error bars are standard errors around the mean of four replicate populations.

Ammonia and urea tolerance

Results of ANOVA performed on the egg-to-adult survivorship on food containing different concentrations of ammonia showed significant effects of selection regime ($F_{1,3}$ = 45.29, P = 0.006) and concentration ($F_{2,6}$ = 417.76, P < 0.001) (table 5.6). The egg-to-adult survivorship of NCUs was less than that of NBs at 0 g/vial and therefore, I scaled the survivorship from all the concentrations with that at 0 g/vial. The NBs had higher survivorship than the NCUs at the three different concentrations of ammonia and with increasing concentration, the survivorship of both NBs and NCUs decreased (fig 5.8(a)).

There was no significant selection × concentration interaction ($F_{2,6}$ = 0.878, P = 0.463), indicating no difference between NBs and NCUs in ammonia tolerance.

A separate ANOVA performed on urea tolerance of NBs and NCUs showed no significant effect of selection regime ($F_{1,3}$ = 0.142, P = 0.731) (table 5.7). There was no difference in egg-to-adult survivorship of NBs and NCUs on food containing different concentrations of urea (fig 5.8(b)). The effect of concentration was significant ($F_{2,6}$ = 24.88, P = 0.001), with survivorship of both NBs and NCUs decreasing with increasing concentrations of urea.

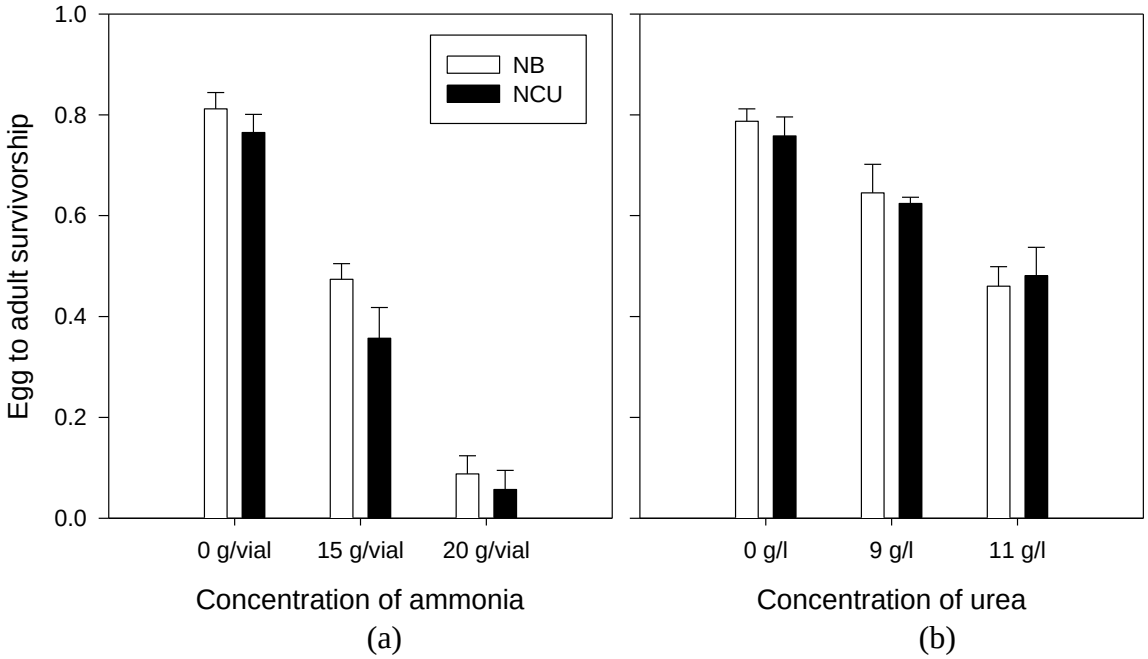


Figure 5.8. Mean egg to adult survivorship of NB and NCU populations (± s.e.) in food containing different concentrations of (a) ammonia and (b) urea.

Table 5.6. Summary of three-way mixed-model ANOVA on egg-to-adult survivorship at 30 eggs/vial of NB and NCU populations in food containing different concentrations of ammonia. Selection and concentration were used as fixed factors and block was a random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	.035	45.285	0.006
Concentration	2	.417	417.758	< 0.001
Selection × Concentration	2	.003	0.878	0.463

Table 5.7. Result of ANOVA on egg-to-adult survivorship of NB and NCU populations at 30 eggs/vial in food containing different concentrations of urea. Selection and concentration were fixed factors and block was a random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	0.001	0.142	0.731
Concentration	2	0.207	24.882	0.001
Selection × Concentration	2	0.002	0.32	0.738

Discussion

The evolution of larval traits in *D. nasuta* selected for adaptation to larval crowding showed patterns similar to that in *D. ananassae* (chapter 3 of this thesis), but quite unlike the ones seen in previous studies of *D. melanogaster* populations selected for adaptations to larval crowding.

After 76 generations of selection, both pre-adult survivorship and larval competitive ability increased at high densities as an adaptation to larval crowding. This result is similar to that seen in the ACUs and also *K*- and CU populations of earlier studies on *D. melanogaster* (Mueller, 1988; A. Joshi, pers. obs.). However, there was again no change in larval feeding rate (measured at middle 3rd instar, as in *D. nasuta* feeding rate is maximum at early as well as middle 3rd instar, data not shown) with increase in larval competitive ability. Both the *K*-populations (Joshi and Mueller, 1988) and CUs (Joshi and Mueller, 1996) evolved higher feeding rates than their respective controls. In fact, feeding rate is always highly correlated with competitive ability in *D. melanogaster* (Burnet et al. 1977; Shakarad et al. 2005).

NCUs also showed shorter egg-to-adult development time when assayed at both low and high densities. Again this was similar to that seen in ACUs (chapter 3 of this thesis), but unlike the *K*-populations (Bierbaum et al. 1989) and CU populations (Santos et al. 1997), where there was no change in development time at moderate density. However, *K*-populations had longer development time at higher densities compared to their controls. Decrease in development time in NCUs was of the order of ~ 16 h at moderate density and ~ 30 h at higher density. Thus, *D. nasuta* (like *D. ananassae*), when selected for larval crowding, also showed a negative correlation between larval competitive ability and egg-to-adult development time. This is opposite to that seen in *D. melanogaster* populations

selected for shorter development time, where decrease in development time was accompanied by correlated decrease in larval competitive ability (Shakarad et al. 2005). In NCUs, this decrease in development time at 30 eggs/vial can be mainly attributed to the larval stage and not the pupal stage. Pupal duration did not change in NCUs. This reduction in instar duration and not pupal duration in the NCUs is similar to that seen in ACUs (chapter 3 of this thesis). By contrast, again, pupal duration got reduced in *D. melanogaster* populations selected for faster development (Prasad et al. 2001), accounting for almost 33 % of total reduction in egg-to-adult development time. However, a similar reduction in pupal duration was not seen by Chippindale et al. (1997) in their faster developing populations of *D. melanogaster*. The likely reasons for this discrepancy in the results of the two experiments are discussed in detail in Prasad et al. (2001). Briefly, in the ACOs (faster developing lines in Chippindale et al. (1997)), the eggs were collected within 24 h of eclosion, whereas in FEJs (faster developing lines in Prasad et al. (2001)), the egg collection was after three days of eclosion. This might have permitted the postponement of some aspect of reproduction (like sperm maturation) to happen after eclosion in FEJs rather than in the pupal stage and, therefore, pupal duration reduction was possible in FEJs, unlike in the ACOs.

There was no evolutionary change in either larval foraging path length or urea tolerance in NCUs after more than 70 generations of selection. Surprisingly, ammonia tolerance decreased in the NCUs as compared to the NBs. The *D. melanogaster* populations under density-dependent selection (CUs) evolved both higher urea and ammonia tolerance (Shiotsugu et al. 1997; Borash et al. 1998). In a crowded culture, nitrogenous wastes build

up over time (Borash et al. 1998) and, hence, the larvae which are able to survive the toxic effects of ammonia eclose successfully as adults.

The results of my study on two species other than *D. melanogaster* have revealed that the adaptation to larval crowding could be achieved by following different evolutionary trajectories, especially with regard to pre-adult traits. Thus, in *D. melanogaster* selected for larval crowding, adaptation occurs largely through increased pre-adult survivorship and competitive ability due to increased feeding rate and higher tolerance to ammonia and urea. However, *D. nasuta* and *D. ananassae* (Chapter 3) populations subjected to high larval density for many generations have evolved a different route of adaptation to occur through increased pre-adult survivorship and competitive ability, probably primarily by decreased egg-to-adult development time and, in *D. ananassae*, by increased efficiency of food conversion to biomass (efficiency was not assayed in NCUs and NBs).

These differences between my study on *D. ananassae* and *D. nasuta*, and previous studies on *D. melanogaster* are quite surprising. *D. ananassae* and *D. melanogaster* are more closely related and belong to the same sub-genus *Sophophora* whereas *D. nasuta* belongs to a different sub-genus *Drosophila*. Thus, the differences in trait correlations among these 3 species do not seem to be directly correlated to the evolutionary distance between the groups. In the next few chapters, I show that the differences are indeed not among the species, but rather likely to be due to subtle differences in the maintenance regime of my study and the earlier studies.

CHAPTER 6

Evolution of adult traits in

D. nasuta

This chapter summarizes the results of assays of adult traits in *D. nasuta* populations selected for adaptations to larval crowding and their controls.

Dry weight at eclosion

The dry weight at eclosion of flies of NB and NCU populations was not significantly different as revealed by ANOVA ($F_{1,3}$ = 0.21 , P = 0.68) (table 6.1 and fig.6.1). There were significant effects of density ($F_{1,3}$ = 1139.02 , P < 0.001), with flies raised at 350 eggs/vial being lighter than the flies raised at 70 eggs/vial, and sex ($F_{1,3}$ = 245.05, P < 0.001), with females being heavier than the males at both the densities.

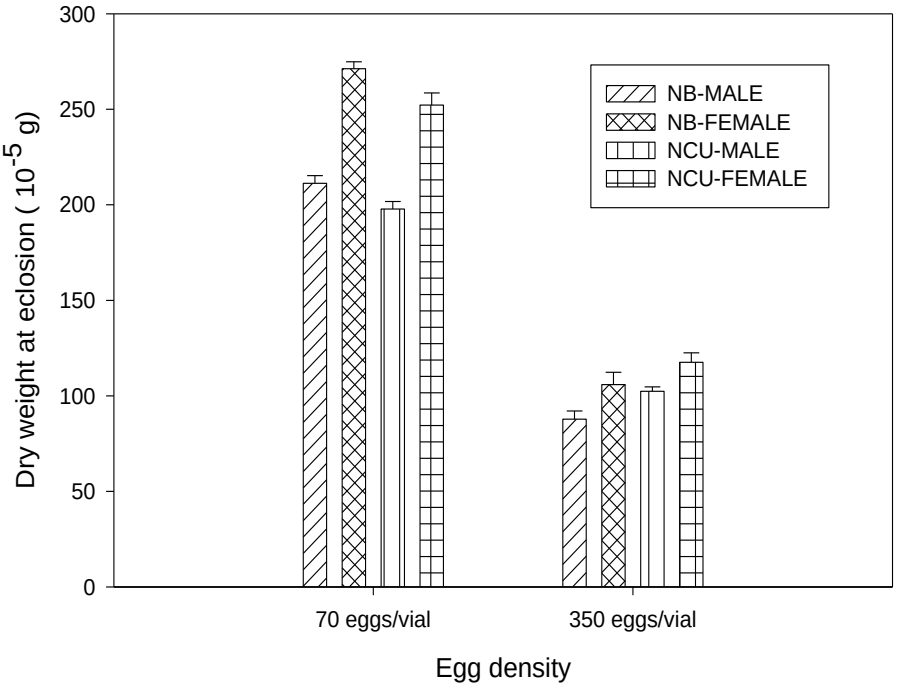


Figure 6.1 Mean dry weight at eclosion (± s.e.) of NB and NCU populations raised at 70 and 350 eggs/vial.

Table 6.1. Results of ANOVA on mean dry weight at eclosion of flies of NB and NCU populations raised at 70 and 350 eggs/vial.

Effect	*df*	MS	*F*	*P*
Selection	1	0.789	0.207	0.679
Density	1	5385.27	1139.02	0.001
Sex	1	437.53	245.05	0.001
Selection × Density	1	68.96	5.816	0.094
Selection × Sex	1	1.434	2.344	0.223
Density × Sex	1	131.93	247.84	<0.001
Sel × Den × Sex	1	0.141	0.059	0.824

Lipid content at eclosion

ANOVA revealed that there was no significant effect of selection ($F_{1,3}$ = 4.895, P = 0.11, table 6.2): flies from both NB and NCU populations had similar amount of lipid (fig.6.2). There was a significant effect of density ($F_{1,3}$ = 146.73, P = 0.001), with flies raised at 350 eggs/vial having significantly less lipid content at eclosion as compared to flies raised at 70 eggs/vial. There was also a significant selection × density interaction ($F_{1,3}$ = 60.90 , P = 0.004) with no difference in lipid content between NB and NCU populations at 70 eggs/vial and NCUs showing higher lipid content than NBs at 350 eggs/vial (fig. 6.2). There was also

significant effect of sex ($F_{1,3}$ = 76.78 , P = 0.003) with males showing less lipid content as compared to females (fig.6.2).

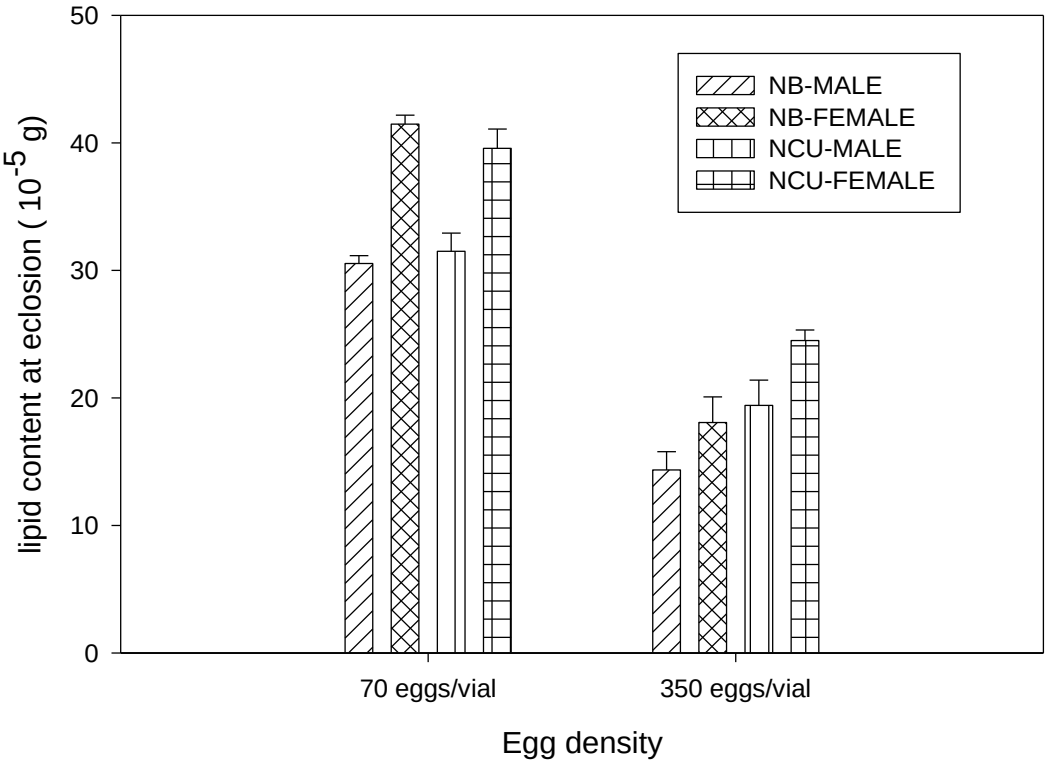


Figure 6.2. Mean lipid content at eclosion of NB and NCU flies raised at 70 eggs/vial and 350 eggs/vial. The error bars represent standard errors around the mean of four replicate populations.

Table 6.2. Summary of three-way ANOVA on lipid content at eclosion of NB and NCU populations raised at 70 and 350 eggs/vial with selection, density and sex as fixed factors and block as random factor. The effects of block and interactions involving block could not be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	2.232	4.895	0.113
Density	1	89.06	146.73	0.001
Sex	1	15.45	76.778	0.003
Selection × Density	1	3.084	60.904	0.004
Selection × Sex	1	0.043	0.712	0.461
Density × Sex	1	2.075	14.310	0.032
Sel × Den × Sex	1	0.361	0.822	0.431

Fecundity on day three after eclosion and day twenty one from egg collection

The ANOVA was performed only on day 21 fecundity as flies from both the NB and NCU populations did not lay any eggs on day 3 after eclosion.

ANOVA revealed that there were no significant effects of selection regime ($F_{1,3}$ = 5.96 , P = 0.96) or density ($F_{1,3}$ = 7.98 , P =0.07) on the fecundity of NB and NCU populations (table

6.3). The mean fecundity (eggs laid/female) of NB and NCU flies raised at 70 eggs/vial was 37.48 and 29.28, respectively, and 23.68 and 24.08, respectively, at 350 eggs/vial (fig. 6.3).

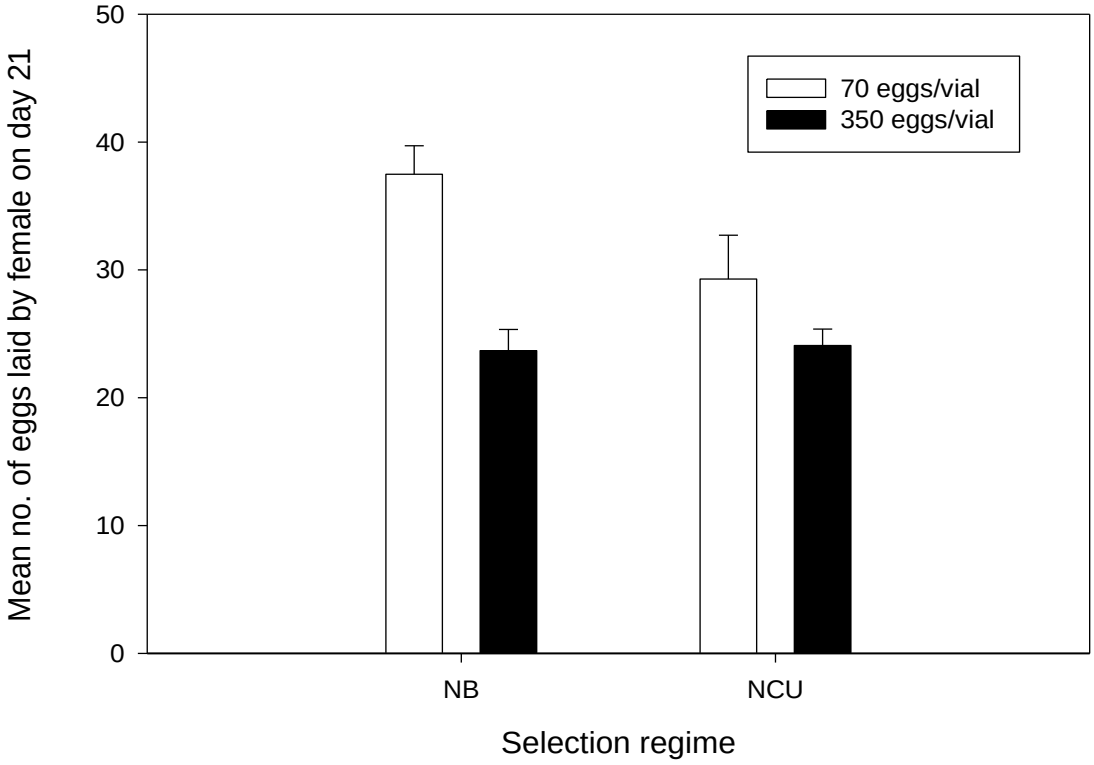


Figure 6.3. Mean fecundity on day 21 after egg collection of NB and NCU flies raised at 70 and 350 eggs/vial.

Table 6.3. Summary of ANOVA on mean fecundity on day 21 after egg collection of NB and NCU flies raised at 70 and 350 eggs/vial.

Effect	*df*	MS	*F*	*P*
Selection	1	60.79	5.962	0.092
Density	1	360.71	7.982	0.066
Selection × Density	1	73.97	5.409	0.102

Adult life span

Results of ANOVA showed significant effects of selection regime ($F_{1,3}$ = 312.05, P < 0.001) and sex ($F_{1,3}$ = 81.42, P = 0.002) (table 6.4). The NCUs had longer life span as compared to the NBs and males had shorter life span than the females. The NB and NCU populations had life span of 14.8 and 17.1 days, respectively, when raised at 70 eggs/vial, and 12.5 and 17.09 days, respectively, when raised at 350 eggs/vial (fig.6.4). There was significant density × sex interaction ($F_{1,3}$ = 19.52, P = 0.021), with males getting more affected by density than the females. The mean adult male life span decreased to half at 350 eggs/vial from the life span at 70 eggs/vial (fig.6.4).

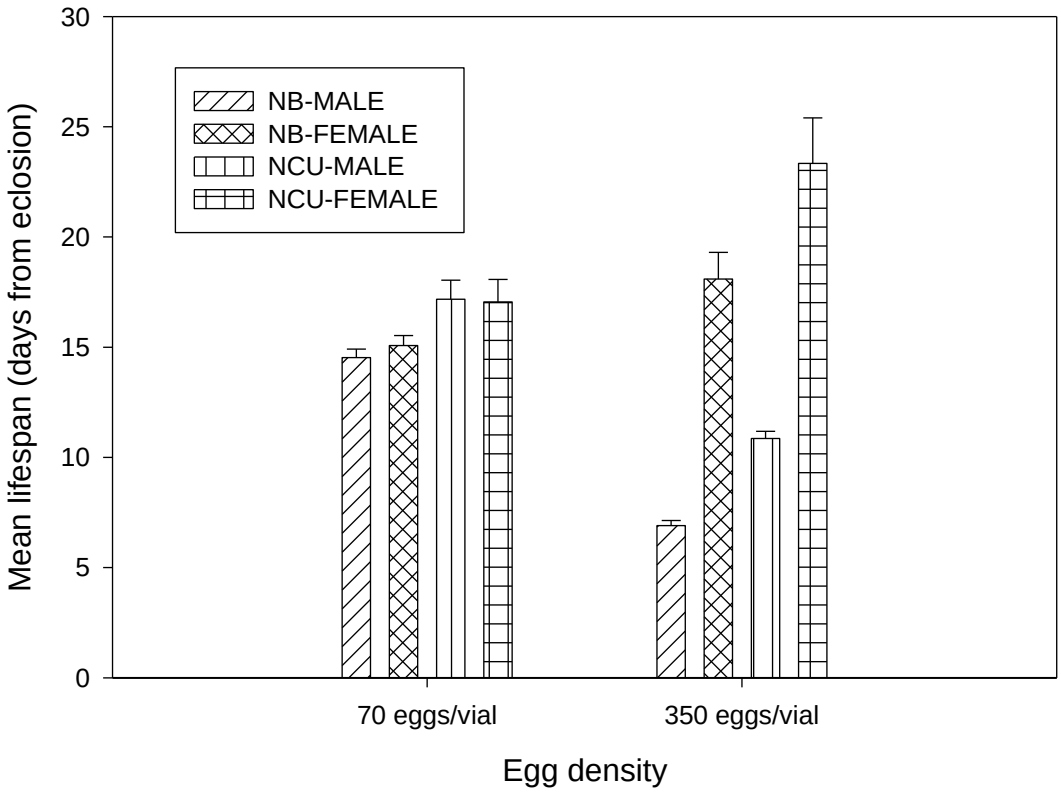


Figure 6.4. Mean adult life span (± s.e.) of NB and NCU populations raised at 70 and 350 egg/vial.

Table 6.4. Result of four-way ANOVA on mean life span of NB and NCU flies raised at 70 and 350 eggs/vial. Selection, density and sex were used as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	95.387	312.05	<0.001
Density	1	10.708	1.551	0.301
Sex	1	290.276	81.415	0.002
Selection × Density	1	10.378	9.382	0.054
Selection × Sex	1	0.194	0.073	0.805
Density × Sex	1	270.01	19.52	0.022
Sel × Den × Sex	1	1.933	2.112	0.242

Starvation resistance

ANOVA revealed that there was no significant effect of either selection regime ($F_{1,3}$ = 6.91, P = 0.078) or density ($F_{1,3}$ = 8.17, P = 0.064) (table 6.5) on the starvation resistance of NB and NCU populations raised at 70 and 350 eggs/vials. There was a significant effect of sex ($F_{1,3}$ = 88.15, P = 0.002) with females living longer than males under starvation. However, there was significant selection × density interaction ($F_{1,3}$ = 15.64 , P = 0.028). Pair-wise comparisons using Tukey's HSD test revealed that the starvation resistance of NBs and

NCUs did not differ at 70 eggs/vial but at 350 eggs/vial, NCUs had higher starvation resistance than the NBs (figure 6.5).

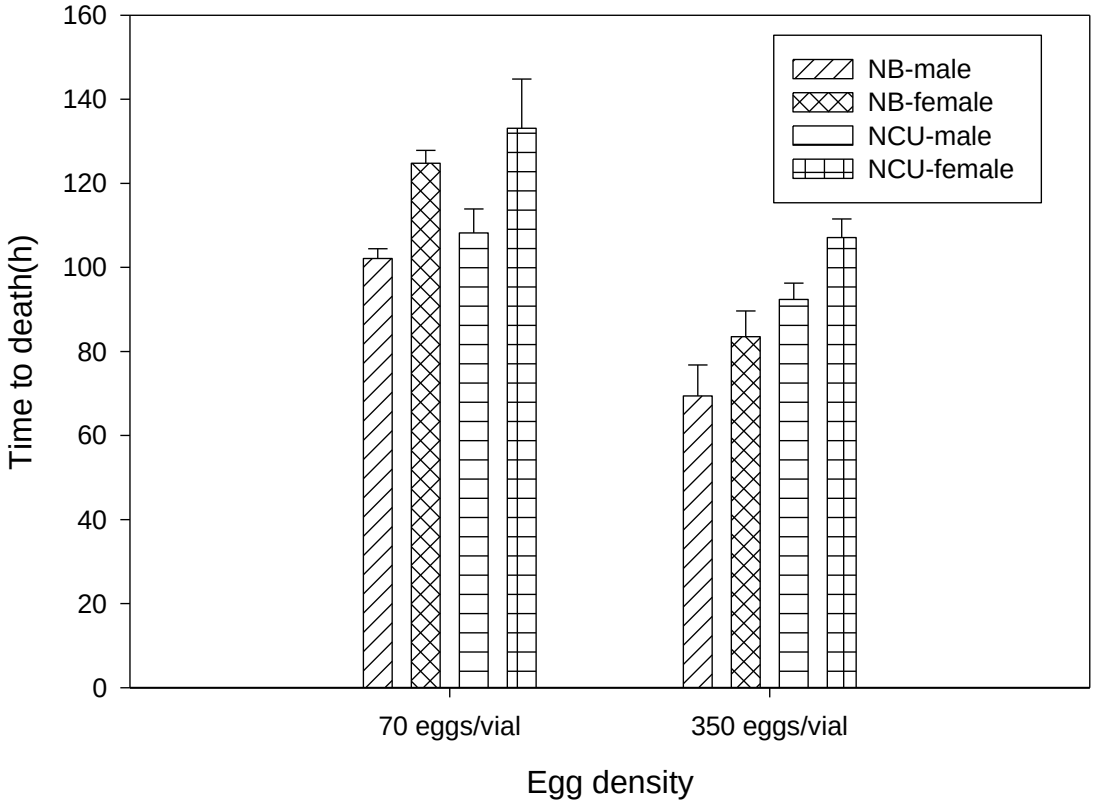


Figure 6.5. Mean starvation resistance of NB and NCU populations raised at 70 and 350 eggs/vial. The error bars represent standard errors around the mean of four replicate populations.

Table 6.5. Summary of four-way ANOVA on mean starvation resistance of NB and NCU flies raised at 70 and 350 eggs/vial. Selection, sex and density were fixed factors. Block was a random factor and could not be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	1857.88	6.906	0.078
Density	1	6704.18	8.171	0.064
Sex	1	2911.74	88.146	0.002
Selection × Density	1	517.44	15.639	0.028
Selection × Sex	1	0.194	0.725	0.805
Density × Sex	1	270.01	19.52	0.022
Sel × Den × Sex	1	1.933	2.112	0.242

Discussion

D. nasuta populations selected for adaptation to larval crowding showed evolution of adult traits similar to that seen in *D. ananassae* selected for larval crowding (chapter 4 of this thesis) and also previous studies on *D. melanogaster*, even though certain correlations were restricted to *D. nasuta* alone.

The NB and NCU populations showed no difference in dry weight at eclosion at both low and high densities. However, NCUs had higher lipid content at eclosion than the NBs when

raised at 350 eggs/vial. This was also seen in *D. melanogaster* selected for larval crowding (Santos et al. 1997; Borash and Ho, 2001) but, not in the ACUs (Chapter 4 of the thesis)

There was also a correlated increase in starvation resistance of the NCU flies raised at 350 eggs/vial. They had higher starvation resistance than the NBs at higher density, but no there was no difference at the low density. Starvation resistance has been shown to be directly proportional to the lipid content of the flies in *D. melanogaster* (Service et al. 1985; Rose et al. 1992; Chippindale et al. 1996; Borash and Ho, 2001). When starved, flies primarily use up lipid reserves. Selected populations of *D. ananassae*, however, showed higher starvation resistance without any increase in lipid content (chapter 4 of the thesis), suggesting that they may have evolved an increase in efficiency in lipid breakdown and utilization. In the NCUs, moreover, there was an increase in life span as compared to the NBs. This was unlike the trend seen in either ACUs (chapter 4 of the thesis) or earlier studies in *D. melanogaster* (Borash and Ho, 2001), where there was no change in life span. There is one interesting observation to note here, though. I found that the ACUs had higher absolute lipid content when raised at 600 eggs/vial as compared to that at 70 eggs/vial; however, NCUs showed lower absolute lipid content at 350 eggs/vial as compared to that at 70 eggs/vial. At this point I do not have any idea why this is so.

There was no change in fecundity in NB and NCU populations, which was similar to that seen in *D. melanogaster* (Borash and Ho, 2001) and *D. ananassae* (chapter 4 of the thesis).

Overall, the pattern of genetic correlations between larval and adult traits in *D. nasuta* populations selected for larval crowding is similar to that seen in *D. ananassae* under the same selection pressure, but different from that seen in *D. melanogaster* from the previous

studies. As discussed in the previous chapter, even though certain correlations are species-specific, but network of correlations between most traits I studied, remained constant in *D. ananassae* and *D. nasuta*. This study, thus, shows that though the two species, *D. ananassae* and *D. nasuta* were evolutionarily separated by approx. 35 million years, still the correlations seen between the two species is quite similar when under long term selection for adaptation to larval crowding.

CHAPTER 7

Evolution of larval traits in

D. melanogaster

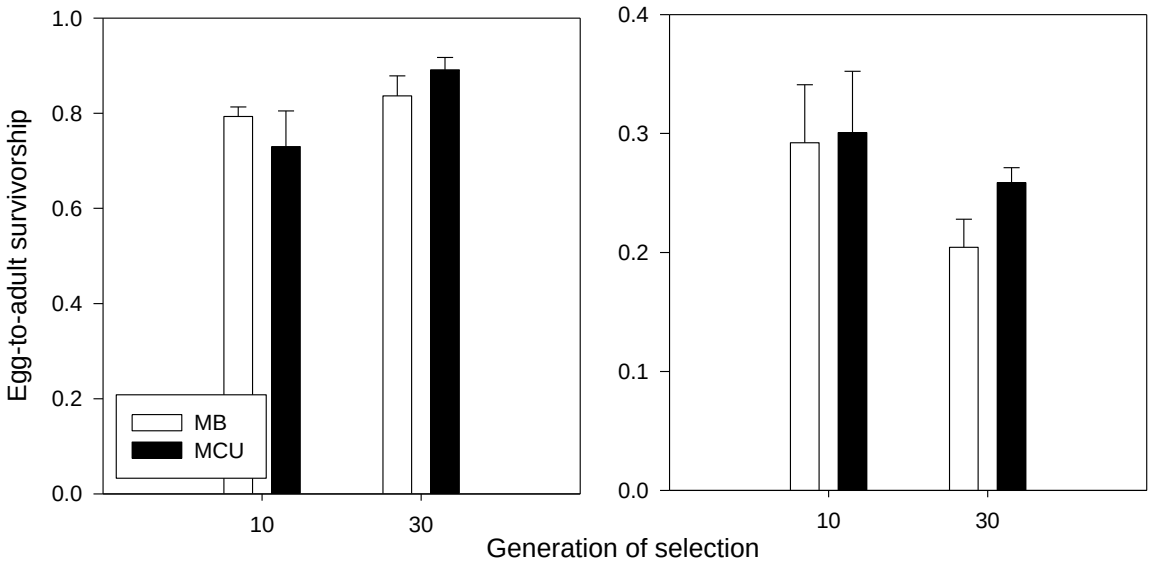
In this chapter, I have summarized the results of assays done on the larval traits of *D. melanogaster* selected for adaptations to larval crowding.

Egg-to-adult survivorship assay

Egg-to-adult survivorship was assayed at generations 10 and 30 of MCU selection. Separate ANOVAs were performed on the data from each generation.

At generation 10, there was no effect of selection regime ($F_{1,3} = 0.035$, $P = 0.864$), with MB and MCU having similar survivorships at both the densities (fig.7.1). However, there was significant effect of density ($F_{1,3} = 662.75$, $P < 0.001$) (table 7.1), with survivorship of both MB and MCU populations lower at higher density as compared to that at lower density (fig.7.1 (a) and (b)). There was no significant selection × density interaction (table 7.1).

At generation 30, again, there was no effect of selection regime ($F_{1,3} = 2.88$, $P = 0.188$), even though, the MCUs had higher survivorship than the MBs at both densities. ANOVA revealed significant effects of density ($F_{1,3} = 875.35$, $P < 0.001$) (table 7.1) on the egg-to-adult survivorship of MB and MCU populations. The egg-to-adult survivorship was lower for both MB and MCU populations at higher density (fig.7.1(b)) as compared to that at low density (fig.7.1(a)). There was no significant selection × density interaction (table 7.1).



(a) (b)

Figure 7.1. Egg-to-adult survivorship of MB and MCU populations in generations 10 and 30 of selection at (a) low egg density (70 eggs/vial) and (b) high egg density (600 eggs/vial at generation 10 and 800 eggs/vial at generation 30).

Table 7.1. Summary of three-way ANOVA on mean egg-to-adult survivorship of MB and MCU populations at 70 and 800 eggs/vial. The survivorship data were arcsine square-root transformed before performing ANOVA. Selection and density were used as fixed factors and block as random factor.

	Generation			
	10		30	
Effect	*F*	*P*	*F*	*P*
Selection	0.035	0.86	2.88	0.188
Density	662.74	<0.001	875.35	<0.001
Selection × Density	0.15	0.728	0.0704	0.807

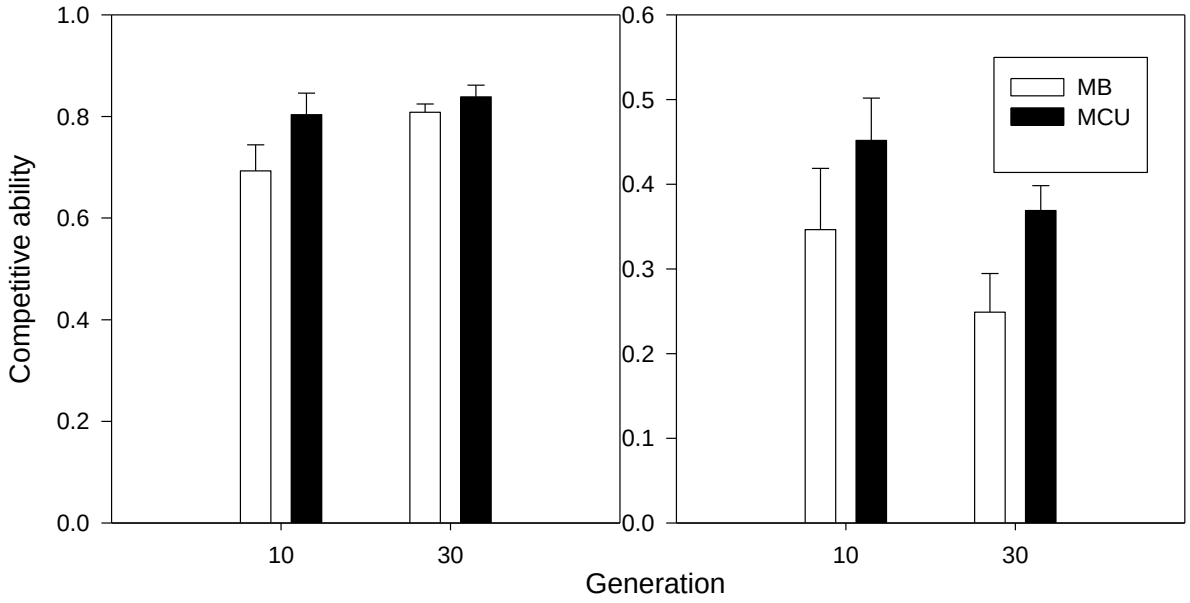
Larval competitive ability

Larval competitive ability was also assayed at generations 10 and 30. Separate ANOVAs were performed for each generation.

ANOVA revealed that there was no significant effect of selection regime ($F_{1,3}$ = 2.16, P = 0.24) and density ($F_{1,3}$ = 6.5, P = 0.08) (table 7.2) on the larval competitive ability of MB and MCU populations at generation 10, the trend was for MCU competitive ability to be higher than the MBs (fig.7.2). The mean egg-to-adult survivorship when

competed against the white-eyed mutant of *D. melanogaster* of MB and MCU populations raised at 70 egg/vial and 600 eggs/vial were 0.69 and 0.80,and 0.35 and 0.45, respectively (fig.7.2 (a) and (b)).

At generation 30, ANOVA showed that there was a significant effect of selection regime ($F_{1,3}$ = 35.82, P = 0.009) and density ($F_{1,3}$ = 169.46, P < 001) on the larval competitive ability of MB and MCU populations (table 7.2). The MCUs had higher survivorship than the MBs at 800 eggs/vial and the survivorship was lower at higher densities (fig.7.2 (a) and (b)).



(a) (b)

Figure 7.2.The mean egg-to-adult survivorship of MB and MCU populations in generations 10 and 30 of selection at (a) low egg density (70 eggs/vial) and (b) high egg density (600 eggs/vial at generation 10 and 800 eggs/vial at generation 30), when competed against a common competitor, white-eyed *D. melanogaster*.

Table 7.2. Summary of three-way mixed model ANOVA on egg to adult survivorship of MB and MCU populations at 70 and 600 or 800 eggs/vial when competed against white-eyed mutant of *D. melanogaster*. Selection and density were fixed factors and block was a random factor.

	Generation			
	10		30	
Effect	*F*	*P*	*F*	*P*
Selection	2.16	0.238	35.82	0.009
Density	6.50	0.083	169.46	<0.001
Selection × Density	0.64	0.482	3.25	0.169

Egg-to-adult development time

Egg-to-pupation time

Egg-to-pupation time was assayed at generations 10 and 30 of selection. Separate ANOVAs were performed on data from the two generations.

At generation 10, ANOVA revealed significant effect of selection regime ($F_{1,3}$ = 24.14, P = 0.015). The mean pupation time of MB and MCU populations was 120.17 h and 117.19 h, respectively (fig.7.3).

ANOVA showed that at generation 30 also there was significant effect of selection regime ($F_{1,3}$ = 47.96, P = 0.006) (table 7.3). The average pupation time of MBs and MCUs was 120.37 h and 114.06 h, respectively, at generation 30.

Egg-to-adult development time at 30 eggs/vial

Egg-to-adult development time was assayed at generations 10, 30 and 43. Separate ANOVAs were performed at each generation.

At generation 10, ANOVA revealed significant effects of selection regime ($F_{1,3}$ = 47.13, P = 0.006) and sex ($F_{1,3}$ = 104.69, P = 0.002) (table 7.4) with MCUs developing faster than the MBs, and females developing faster than the males. The mean development time of males and females of MB and MCU populations was 214.08 h and 210.37 h and 210.64 h and 207.97 h, respectively.

At generation 30 also, there was significant effect of selection regime ($F_{1,3}$ = 45.86, P = 0.007) and sex ($F_{1,3}$ = 165.8, P = 0.001). The difference between MBs and MCUs was 8 hrs.

By generation 43, the difference between MB and MCU populations was still 8 hrs (fig.7.4). ANOVA on generation 43 data revealed significant effect of selection regime ($F_{1,3}$ = 198.53, P < 0.001) and sex ($F_{1,3}$ = 108.21, P = 0.002) (table 7.5).

Egg-to-adult development time at 800 eggs/vial

There was no significant effect of either selection regime ($F_{1,3}$ = 0.64, P = 0.29) or sex ($F_{1,3}$ = 1.38, P = 0.32) on the egg to adult development time of MB and MCU populations when assayed at 800 eggs/vial (table 7.4). Though the MCUs had shorter development time than the MCUs, the difference was not significant at generation 37 of selection (fig.7.5).

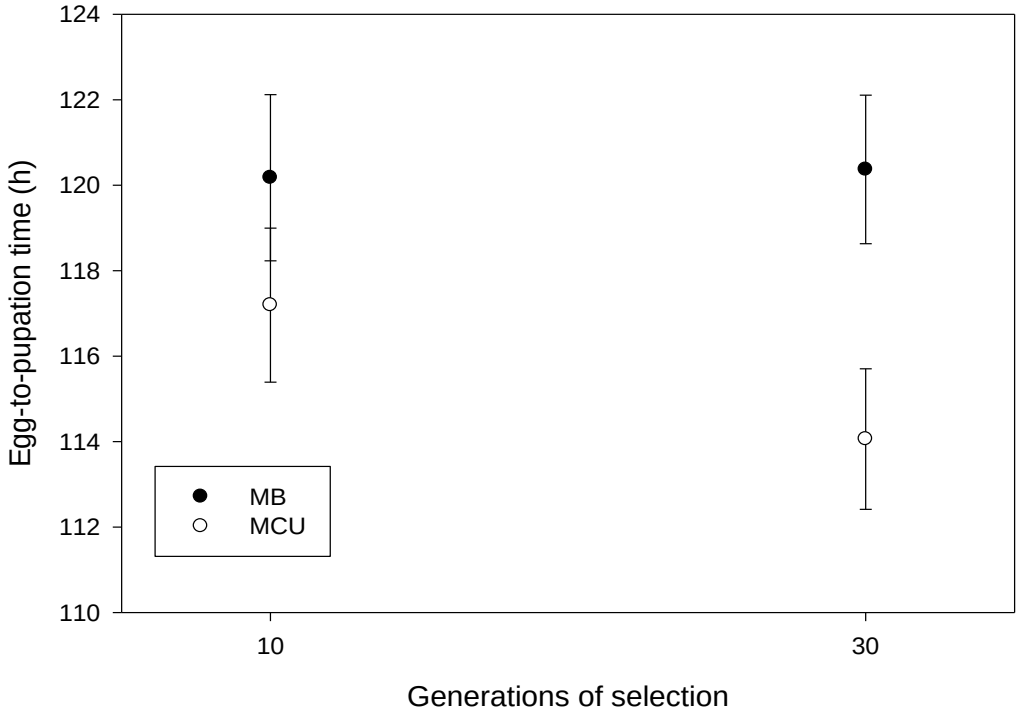


Figure 7.3. Egg-to-pupa development time in MB and MCU populations at 30 eggs/vial. Assay was done at generations 10 and 30 of selection. The error bars represent standard errors around the mean of four replicate populations.

Table 7.3. Summary of ANOVA on egg-to-pupation time of MB and MCU populations at generations 10 and 30 of selection at 30 eggs/vial. Selection regime was a fixed factor and block was a random factor.

	Generation			
	10		30	
Effect	*F*	*P*	*F*	*P*
Selection	24.84	0.015	47.96	0.006

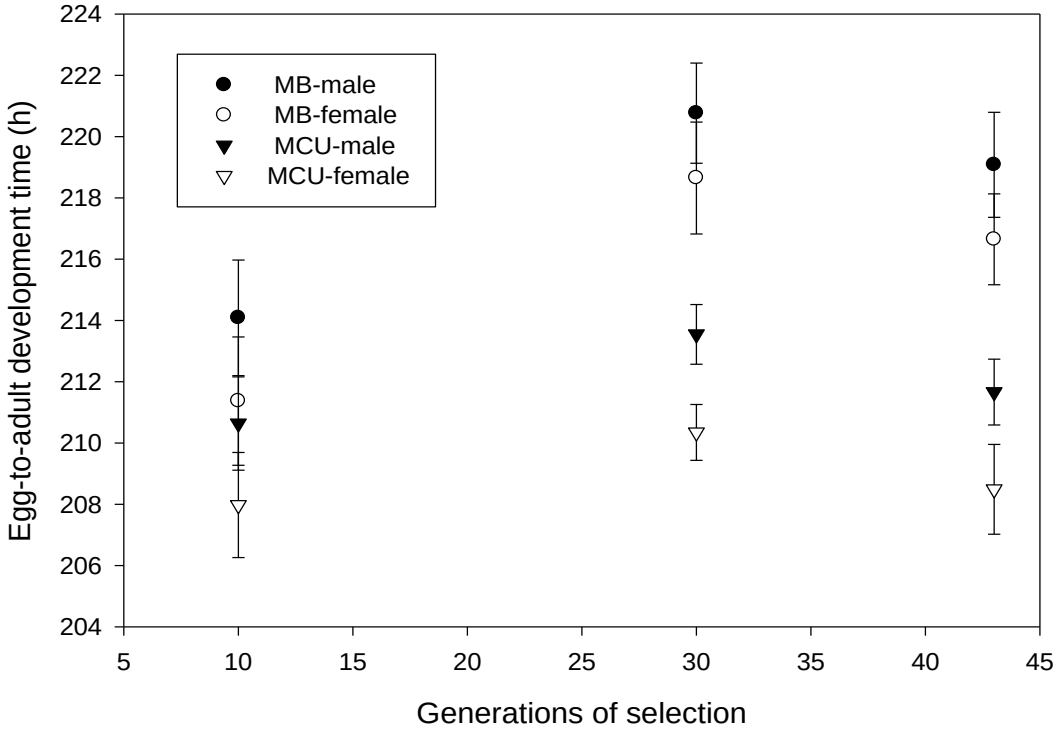


Figure 7.4. Egg-to-adult development time of MB and MCU populations at 30 eggs/vial over 43 generations of selection. Error bars represent standard errors around the mean of four replicate populations.

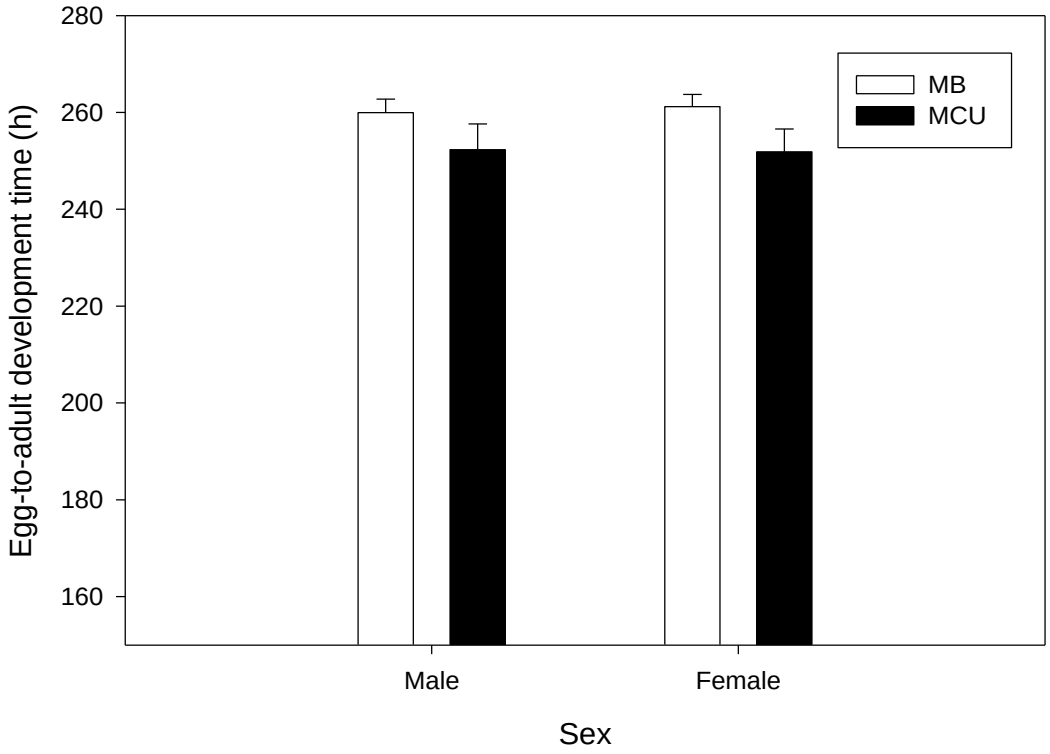


Figure 7.5. Egg-to-adult development time of MB and MCU populations at 800 eggs/vial. Error bars represent standard errors around the mean of four replicate populations.

Table 7.4. Summary of ANOVA on egg-to-adult development time of MB and MCU populations at 800 eggs/vial. Selection and sex were used as fixed factors and block as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	287.58	1.64	0.289
Sex	1	0.617	0.20	0.684
Selection × Sex	1	2.73	1.38	0.324

Table 7.5. Result of separate three-way ANOVA on egg-to-adult development time of MB and MCU populations at 30 eggs/vial assayed at generations 10, 30 and 43 of selection. Selection regime and sex were used as fixed factors and block was used as random factor.

	Generation					
	10		30		43	
Effect	*F*	*P*	*F*	*P*	*F*	*P*
Selection	47.13	0.006	45.86	0.006	198.53	<0.001
Sex	104.698	0.002	165.81	0.001	108.22	0.001
Selection × Sex	0.027	0.88	3.53	0.156	0.78	0.443

Larval feeding rate

The larval feeding rate was assayed at generations 15, 30, 37 and 43. Separate ANOVAs were performed at each generation.

ANOVA revealed that there was no significant effect of selection regime (table 7.6) at any generation assayed. There was no difference in larval feeding rate of MB and MCU populations even after 43 generations (fig.7.6).

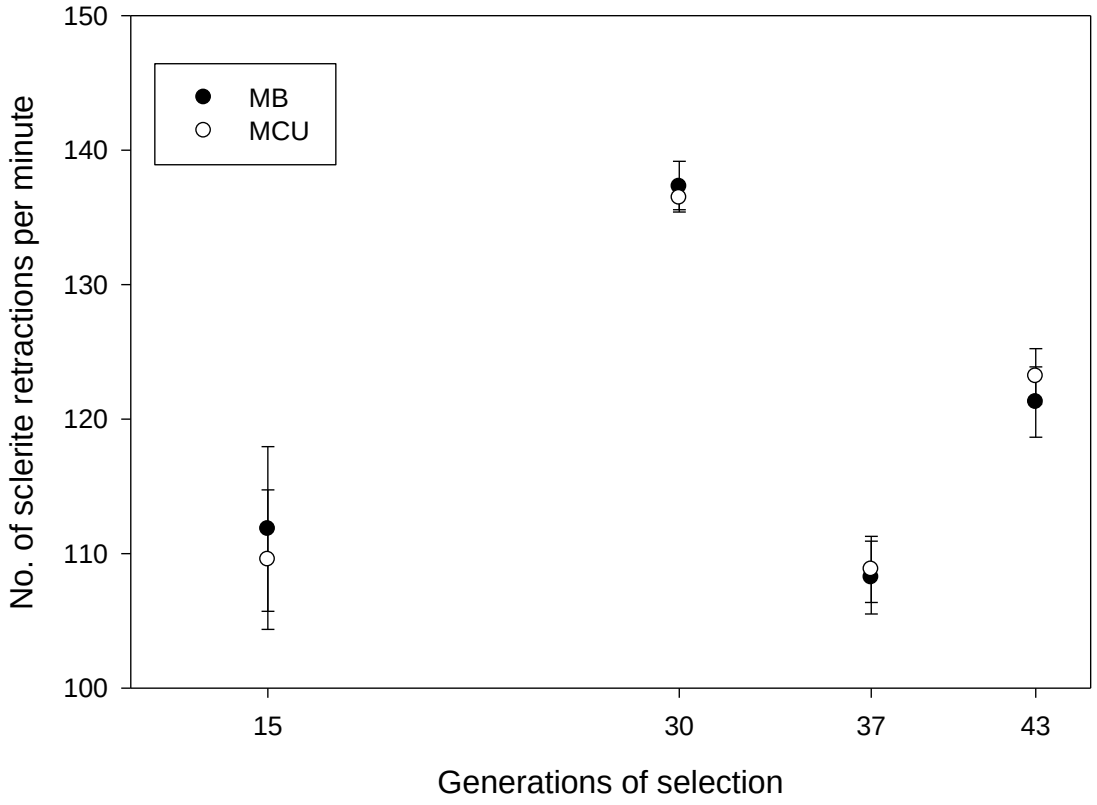


Figure 7.4. Mean feeding rate of MB and MCU populations of 43 generations of selection. The error bars represent the standard errors around the mean of four replicate populations.

Table 7.6. Summary of results of ANOVAs on larval feeding rate of MB and MCU populations at generations 15, 30, 37 and 43. Selection regime was used as fixed factor and block was a random factor.

	Generation							
	15		30		37		43	
Effect	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Selection	0.61	0.491	0.17	0.706	0.51	0.525	2.15	0.239

Larval foraging path length

After 37 generations of selection, there was no significant effect of selection regime ($F_{1,3}$ = 1.92, P = 0.26) on the foraging path length of MB and MCU populations. The mean foraging path length of MBs and MCUs was 3.83 and 3.65 cm, respectively (fig.7.5).

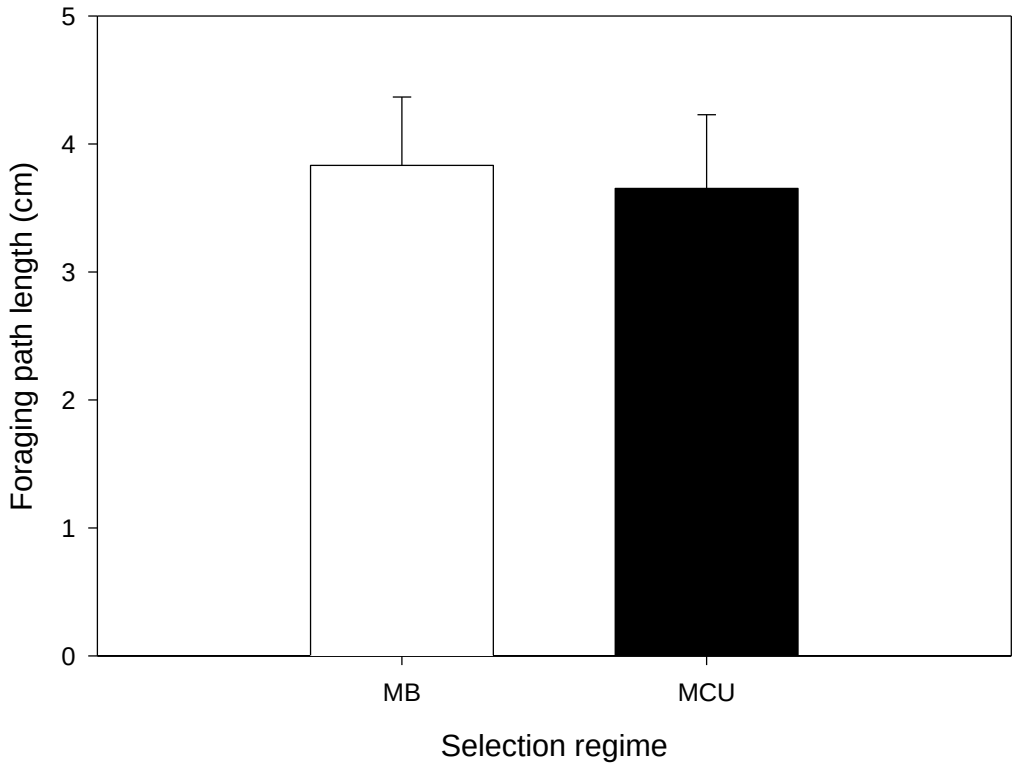


Figure 7.7. Mean foraging path length of MB and MCU populations. Error bars represent standard errors around the mean of four replicate populations.

Pupation height

There was no difference in the pupation height of MB and MCU populations as revealed by ANOVA ($F_{1,3}$ = 1.44, P = 0.316). The mean pupation height of MB and MCU populations was 1.36 and 1.18 cm, respectively (fig.7.8).

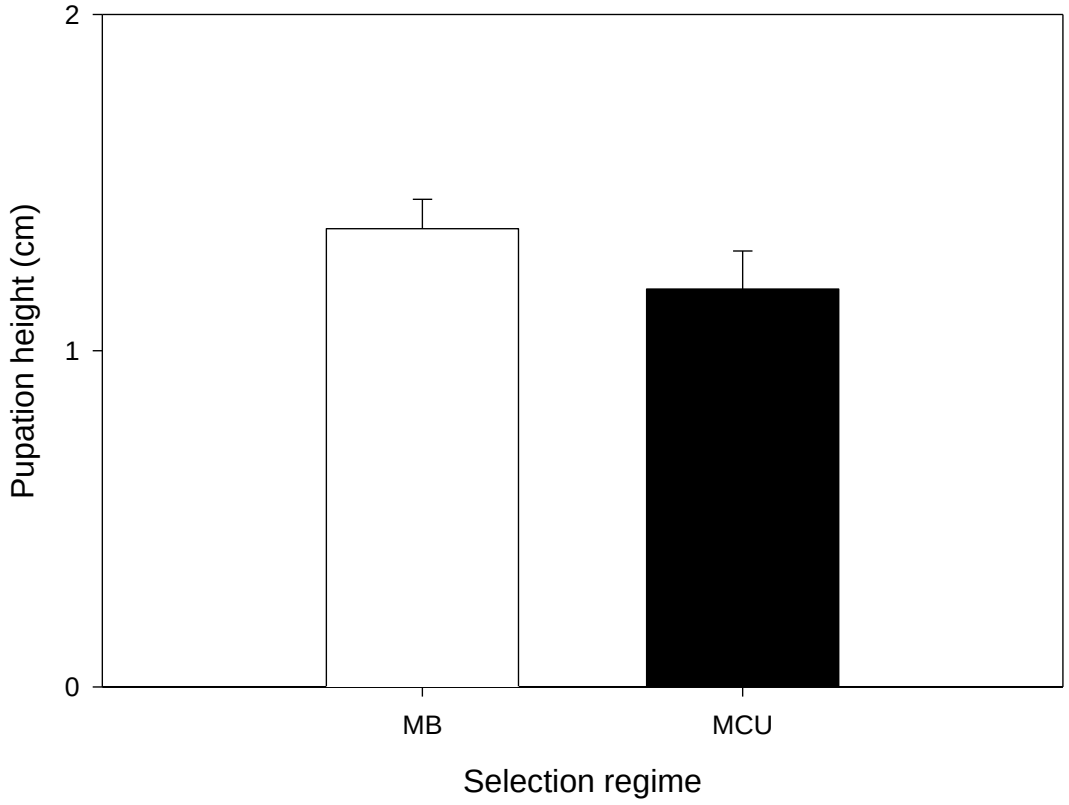


Figure 7.8. Mean pupation height (± s.e.) of MB and MCU populations at 30 eggs/vial.

Ammonia tolerance

ANOVA showed that there was no significant effect of selection regime ($F_{1,3}$ = 3.82, P = 0.146) and selection regime × concentration interaction ($F_{2,6}$ = 627.7, P < 0.001). The MB and MCU populations did not differ in their tolerance to ammonia (fig.7.9). There was,

however, significant effect of concentration ($F_{2,6}$ = 1.64 , P = 0.27). The egg-to-adult survivorship of both MBs and MCUs decreased with increasing concentrations of ammonia (table 7.7).

Urea tolerance

There was no difference in tolerance to urea between MBs and MCUs as revealed by ANOVA ($F_{1,3}$ = 4.83, P = 0.115). However, there was an effect of concentration ($F_{2,6}$ = 89.45, P < 0.001) with tolerance of both MB and MCU populations decreasing with increasing concentrations of urea (fig.7.10 and table 7.8).

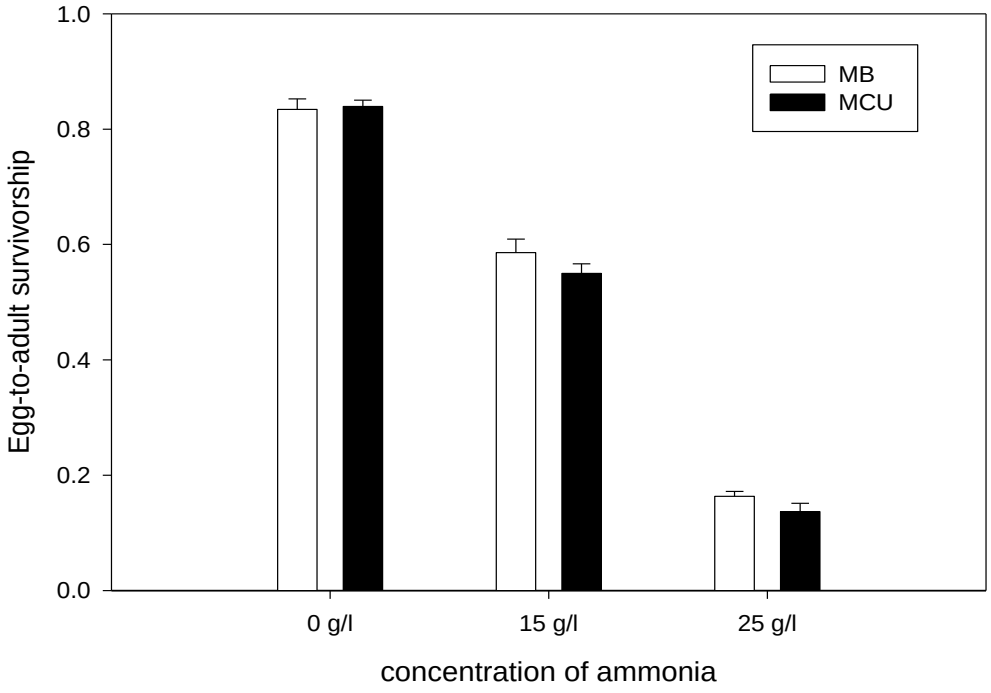


Figure 7.9. Mean egg-to-adult survivorship of MB and MCU populations in three different concentrations of ammonia. The error bars represent standard errors around the mean of four replicate populations

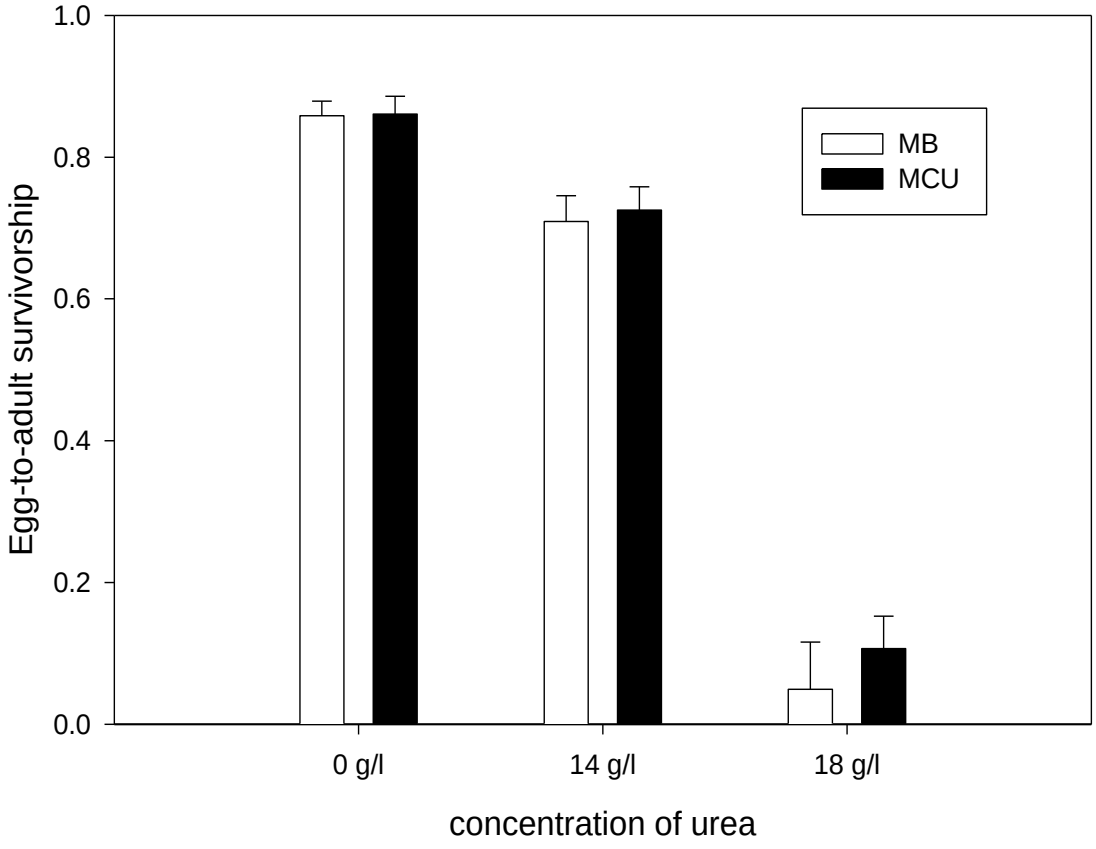


Figure 7.10. Mean egg-to-adult survivorship (± s.e.) of MB and MCU populations in different concentrations of urea.

Table 7.7. Results of three-way ANOVA on egg to adult survivorship of MB and MCU populations in food containing different concentrations of ammonia. Selection and concentrations were fixed factors and block was a random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	0.003	3.82	0.145
Concentration	2	1.17	627.73	<0.001
Selection × Concentration	2	0.0013	1.64	0.27

Table 7.8. Results of three-way ANOVA on egg to adult survivorship of MB and MCU populations in food containing different concentrations of urea. Selection and concentrations were fixed factors and block was a random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	0.012	4.83	0.115
Concentration	2	1.99	89.45	<0.001
Selection × Concentration	2	0.007	2.69	0.146

Discussion

I again imposed larval crowding selection on *D. melanogaster* to confirm whether the genetic correlations seen in *D. ananassae* and *D. nasuta*, which were different from those seen in earlier studies of *D. melanogaster*, were truly species differences, or could have been due to specific details of maintenance.

Selection on *D. melanogaster* was started when the selection on *D. ananassae* and *D. nasuta* had crossed almost 45 generations. Hence, most of the results reported here for *D. melanogaster* have been assayed at earlier generations of selection as compared to the other two species.

The egg-to-adult survivorship at low as well as high densities did not differ significantly between the MB and MCU populations after 30 generations of selection. However, MCU survivorship was higher than that of MBs at both the densities. Perhaps, by another 10 generations of selection, they could have diverged further. The ACUs (chapter 3 of this thesis; Sharmila Bharathi, 2006) evolved higher egg-to-adult survivorship only after ~ 40 generations of selection and NCUs (chapter 5 of this thesis) took more than 60 generations of selection to evolve significantly higher egg-to- adult survivorship than the control NBs.

Unlike survivorship, larval competitive ability in MCUs evolved to be greater than MBs by generation 30. This is similar to that seen in NCUs, which had evolved higher competitive ability by generation 40 but, took longer to evolve higher egg-to-adult survivorship (chapter 5 of the thesis). These two traits were also seen to evolve in earlier studies on *D. melanogaster* subjected to crowding (Mueller, 1988; Bierbaum et al. 1989; A. Joshi pers. obs.).

One of the traits highly correlated with competitive ability in *D. melanogaster* is the larval feeding rate (Burnet et al. 1977; Joshi and Mueller, 1988, 1996). However, MCUs did not show a correlated increase in larval feeding rate with increase in competitive ability. This is similar to that seen in ACUs (Sharmila Bharathi, 2006; chapter 3 of the thesis) and NCUs (chapter 5 of this thesis). It is not clear at this point why the MCUs did not undergo any increase in larval feeding rate.

Moreover, like ACUs and NCUs, MCUs showed reduced egg-to-adult development time at low density, and this reduction was mainly in the egg-to-pupation time, thereby implying reduction in the larval instar durations. However, unlike in the other two species, I did not assay the durations of instars in MCUs. These *D. melanogaster* selected lines also showed reduction in egg-to-adult development time when reared at 800 eggs/vial but this difference was not significant till 37 generations of selection. This reduction is similar to that seen in ACUs (Sharmila Bharathi, 2006) and NCUs (chapter 5 of this thesis). On the contrary, Bierbaum et al. (1989) reported an increase in development time of *K*-selected populations (populations maintained at high density).

Various previous studies either have shown that there is no correlation between competitive ability and development time (Bierbaum et al. 1989; Santos et al. 1997) or that they are positively correlated (Shakarad et al. 2005). However, I found that the two traits were negatively correlated in all the three species studied.

I also assayed foraging path length, pupation height, and ammonia and urea tolerance. However, these traits did not evolve in the MCUs and were not significantly different than in MBs. This is unlike the results seen in the earlier studies where urea and ammonia

tolerance evolved in response to selection for crowding. There is a buildup of nitrogenous wastes when the vials are crowded with larvae and, thus, the larvae, over generations, are expected to evolve tolerance to these wastes (mainly ammonia). Urea and ammonia tolerance in *D. melanogaster* were found to be positively genetically correlated (Borash et al. 2000a). However, in all the three species I studied, I could not find such a correlation. Foraging path length is a trait well studied in *D. melanogaster* in relation to larval crowding and it was longer in lines selected for larval crowding (Soklowski et al. 1997), but MCUs did not evolve longer foraging path length. However, these traits are correlated responses to selection and are not directly under strong selection, and since I did these assays very early in the course of selection, it would be better to be cautious here in drawing conclusions about these traits. It would be interesting to see how these traits have evolved after some 50 generations of selection.

In my study, *D. melanogaster* populations selected for larval crowding showed evolution of larval traits similar to that shown by *D. ananassae* and *D. nasuta* but, unlike that seen in earlier studies of *D. melanogaster*. To summarize briefly, the positive correlations seen between larval feeding rate and competitive ability and between development time and feeding rate were not seen in my study. Moreover, the correlations between development time and survivorship, and between development time and competitive ability, were negative in my study although they were positive in the earlier studies.

Competitive ability can be conceptualized to consist of two components: effectiveness (the ability to inhibit the other group) and tolerance (ability to withstand inhibition by the other group) (Joshi et al. 2001), and it has been shown that these two components can evolve

independently in *Drosophila* spp. (Joshi and Thomson, 1995). In the earlier studies on crowding selection, *D. melanogaster* populations evolved both the components: effectiveness through higher feeding rate and tolerance through higher ammonia and urea tolerance. In my study, as these traits have not evolved and development time has reduced, I suggest that reduced development time by few hours, which evolved as a correlated response, probably gives these populations enough time to avoid the toxic buildup of ammonia. This is further supported by the fact that the maximum reduction of development time was in the larval stages (chapter 3 and 5 of this thesis) when they would have been subjected to the vagaries of their immediate environment. Thus, they increased their effectiveness by reducing the development time by few hours and thereby, reaching the third instar (major resource acquisition stage in *Drosophila*) early and thus, deprive the other larvae of food. However, there is no change in tolerance component of competitive ability.

Thus, *D. ananassae*, *D. melanogaster* and *D. nasuta* showed evolution of similar larval traits, barring few traits which were species-specific, suggesting that different species can respond similarly to the same selection pressure. However, the difference between my results on *D. melanogaster* and results from the early studies could be due to.

1. Food medium used to conduct the studies.
2. The difference in the maintenance regime of the two studies.

Some preliminary studies done in my lab (N. Sharmila Bharathi, unpublished data) showed that the food medium did not affect the life-history and related traits. Hence, we can ignore the 2nd point as well. Thus, the most plausible reason could be the difference in the

maintenance regime of the two studies. To test and accept/reject this hypothesis, I did a one generation experiment, details of which are described in chapter 9 of this thesis.

CHAPTER 8

Evolution of adult traits in

D. melanogaster

In this chapter, I have put together the results of assays on the adult traits of D. melanogaster populations selected for adaptations to larval crowding and their ancestral controls.

Dry weight at eclosion

There was no difference between MB and MCU dry weights as ANOVA did not show any significant effect of selection regime ($F_{1,3}$ = 1.799, P = 0.272). There was, however, a significant effect of density ($F_{1,3}$ = 1531.3, P < 0.001) and sex ($F_{1,3}$ = 349.9, P < 0.001) (table 8.1), with flies from 70 eggs/vial weighing more than that from 800 eggs/vial and females weighing more than the males at both the densities (fig.8.1).

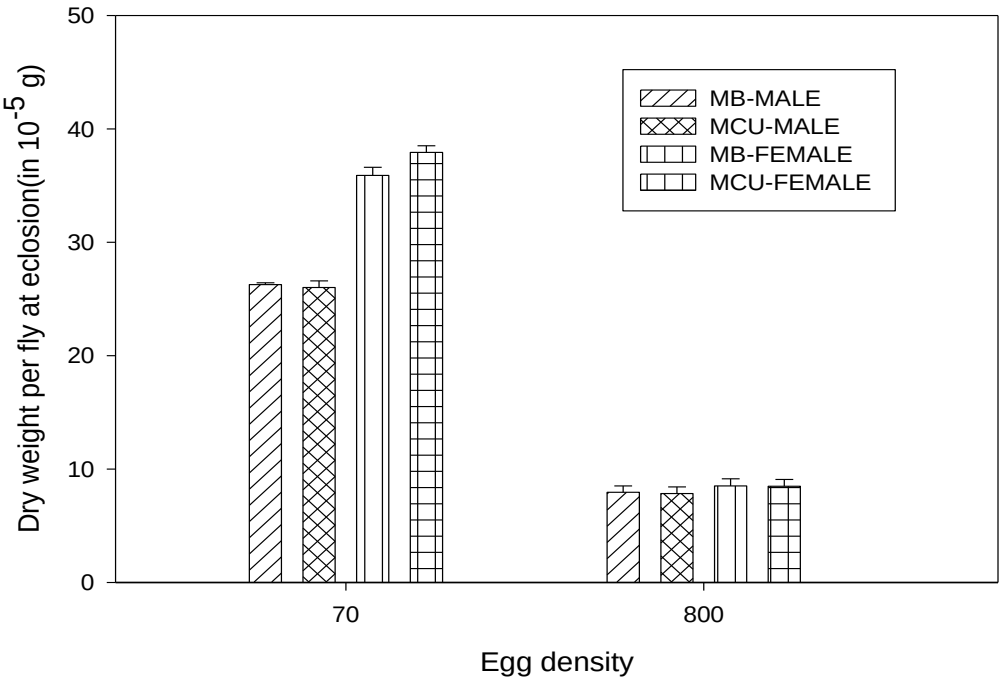


Figure 8.1. Mean dry weight at eclosion (± s.e.) of flies of MB and MCU populations raised at 70 and 800 eggs/vial.

Table 8.1. Results of three-way ANOVA on mean dry weight at eclosion of MB and MCU populations raised at 70 and 800 eggs/vial. Selection, sex and density were used as fixed factors and block was a random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	$1.32E\times 10^{-10}$	1.799	0.272
Density	1	4.35×10^{-7}	1551.3	<0.001
Sex	1	2.58×10^{-8}	349.9	<0.001
Selection × Density	1	1.81×10^{-10}	2.42	0.217
Selection × Sex	1	2.79×10^{-10}	6.75	0.081
Density × Sex	1	2.07×10^{-8}	235.17	<0.001
Sel × Den × Sex	1	2.39×10^{-10}	11.24	0.04

Lipid content at eclosion

ANOVA revealed no significant effect of selection regime ($F_{1,3} = 0.92$, $P = 0.41$),with MBs and MCUs having similar lipid contents at eclosion. However, there was a significant effect of density ($F_{1,3} = 16.82$, $P = 0.026$). The flies raised at 800 egg/vial had almost half the lipid content as the flies raised at 70 eggs/vial (fig.8.2 and table 8.2).

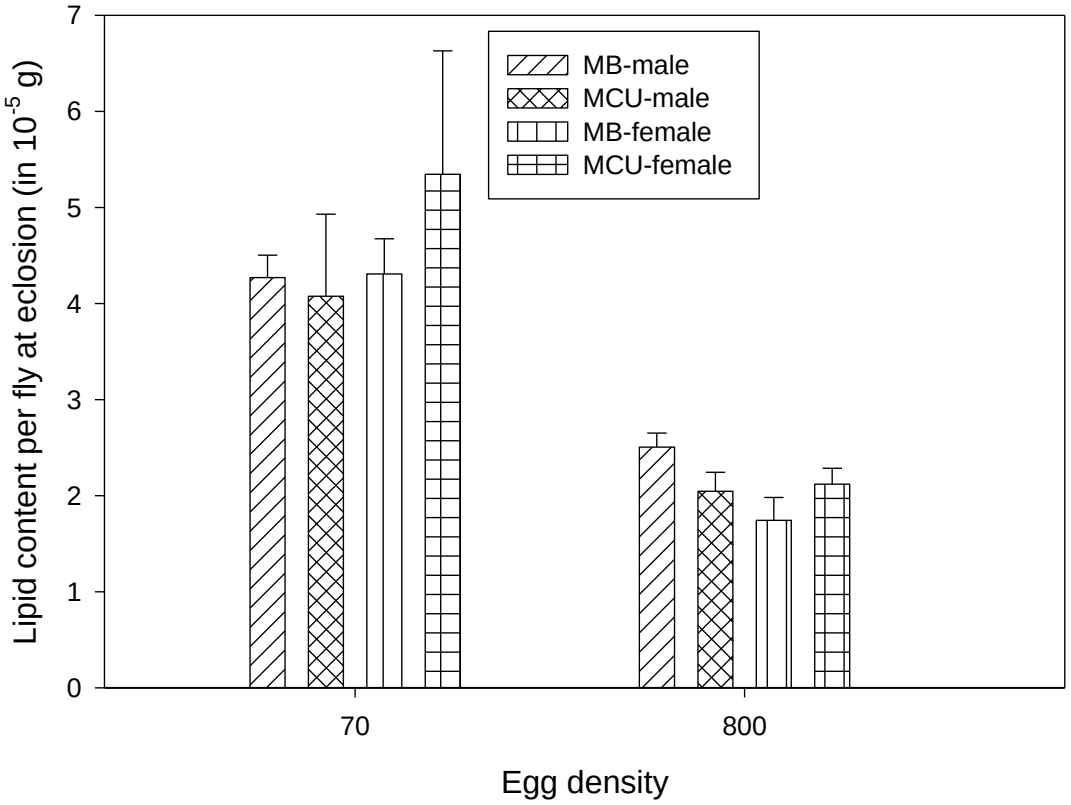


Figure 8.2. Mean lipid content (± s.e.) at eclosion of MB and MCU populations at 70 and 800 eggs/vial.

Table 8.2. Results of three-way ANOVA on mean lipid content at eclosion of MB and MCU populations raised at 70 and 800 eggs/vial. Selection, sex and density were used as fixed factors and block was a random factor. Hence, effects of block and interactions involving block could not be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	1.36×10^{-10}	0.916	0.409
Density	1	1.49×10^{-9}	16.82	0.026
Sex	1	1.14×10^{-10}	5.10	0.109
Selection × Density	1	6.58×10^{-14}	4.3×10^{-4}	0.985
Selection × Sex	1	6.92×10^{-14}	9.58	0.054
Density × Sex	1	6.12×10^{-11}	3.55	0.155
Sel × Den × Sex	1	8.30×10^{-11}	10.55	0.047

Life span

The ANOVA showed that there was no significant effect of selection regime ($F_{1,3}$ = 3.5, P = 0.16), density ($F_{1,3}$ = 1.03, P = 0.38) or the selection × density interaction ($F_{1,3}$ = 0.81, P = 0.43) (table 8.3). However, there was significant effect of sex ($F_{1,3}$ = 32.62, P = 0.01). There was no difference in the life span of MBs and MCUs at both low and high densities. The mean life span of MB females was 32.67 and 36.36 days and that of MB males was 37.34

and 39.76 days when raised at 70 and 800 eggs/vial, respectively. The MCU females had life span of 31.18 and 32.59 days and MCU males had life span of 37.71 and 36.55 days at 70 and 800 eggs/vial (fig 8.3).

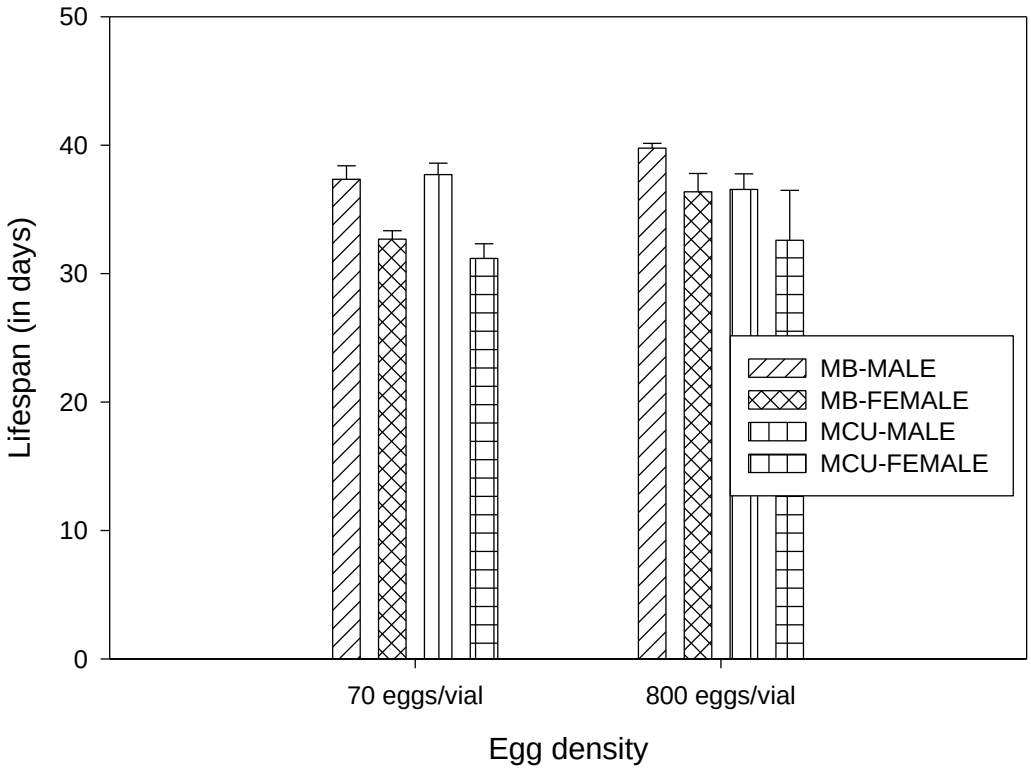


Figure 8.3. Mean life span of flies from MB and MCU populations raised at 70 and 800 eggs/vial. Error bars represent standard errors around the mean of four replicate populations.

Table 8.3. Results of mixed-model ANOVA performed on mean life span of MB and MCU populations raised at 70 and 800 eggs/vial. Selection, sex and density were considered as fixed factors and block was taken as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	32.881	3.503	0.158
Density	1	20.296	1.034	0.384
Sex	1	172.21	32.613	0.01
Selection × Density	1	17.219	0.812	0.433
Selection × Sex	1	2.99	0.211	0.677
Density × Sex	1	7.39	1.727	0.280
Sel × Den × Sex	1	0.855	0.239	0.658

Fecundity on day 3 after eclosion and day 21 from egg collection

ANOVA revealed that there was no significant effect of selection regime ($F_{1,3}$ = 3.798, P = 0.146) on the fecundity of MB and MCU populations on day 3 after eclosion and day 21 from the egg collection (table 8.4). However, there was a significant effect of density ($F_{1,3}$ = 1064.52, P < 0.001), with flies raised at 70 eggs/vial having higher fecundity than the flies raised at 800 eggs/vial.

ANOVA also showed significant effect of day on which fecundity was counted ($F_{1,3}$ = 213.38, $P < 0.001$), with flies of both MB and MCU populations having higher fecundity on day 21 after egg collection (fig.8.4).

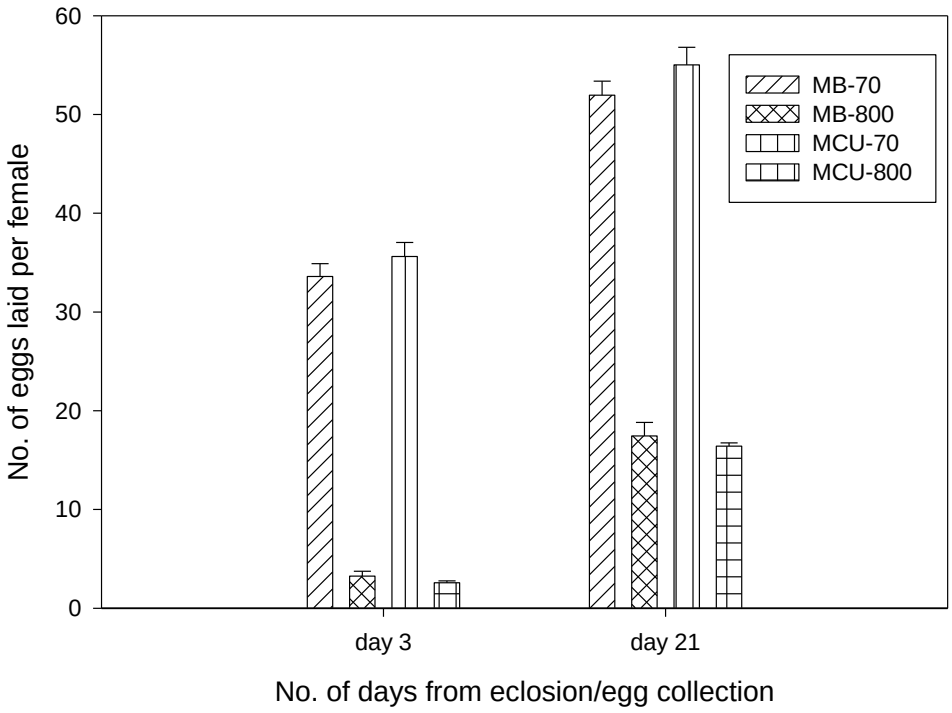


Figure 8.4. Mean fecundity (± s.e.) of MB and MCU populations on day 3 after eclosion and day 21 from egg collection.

Table 8.4. Results of four-way ANOVA on fecundity of MB and MCU populations raised at 70 and 800 eggs/vial on day 3 after eclosion and day 21 from egg collection with selection, density and day as fixed factors and block as random factor. Hence, effects involving block and interactions involving block could not be tested for significance.

Effect	*df*	MS	*F*	*P*
Selection	1	5.698	3.797	0.146
Density	1	9313.83	1064.52	<0.001
Day	1	2165.08	213.38	<0.001
Selection x Density	1	23.06	16.89	0.026
Selection × Day	1	0.246	9.198	0.056
Density × Day	1	47.39	3.021	0.181
Sel × Density × Day	1	0.996	0.486	0.536

Starvation resistance

ANOVA showed that there was no significant effect of selection regime ($F_{1,3}$ = 6.67, P = 0.08) on starvation resistance of MB and MCU populations after 37 generations of selection. However, a trend (albeit in the opposite direction to that seen in ACUs and NCUs) was noticed, with MCUs having less starvation resistance than the MBs (fig.8.5). There were significant effects of density ($F_{1,3}$ = 152.05, P = 0.001) and sex ($F_{1,3}$ = 101.14, P = 0.002)

(table 8.5), with flies raised at 70 eggs/vial and females showing higher starvation resistance than flies raised at 800 eggs/vial and males, respectively.

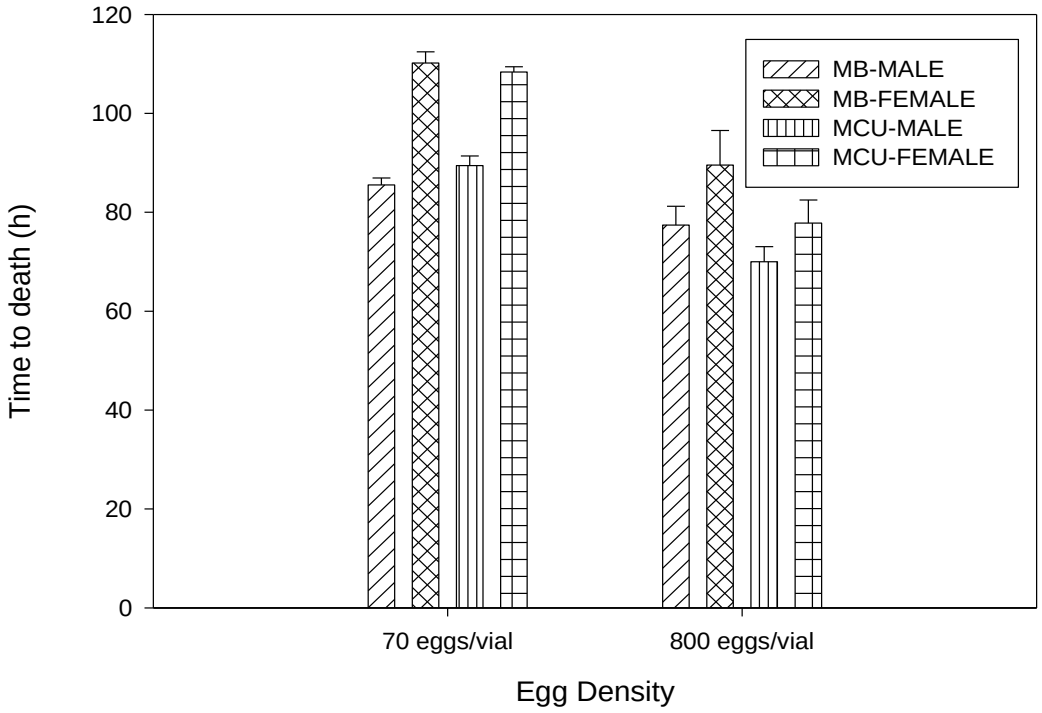


Figure 8.5. Mean resistance to starvation of MB and MCU flies raised at 70 and 800 eggs/vial. The error bars represent standard errors around the mean of four replicate populations.

Table 8.5. Results of mixed-model ANOVA performed on mean starvation resistance of MB and MCU populations raised at 70 and 800 eggs/vial. Selection, sex and density were considered as fixed factors and block was taken as random factor.

Effect	*df*	MS	*F*	*P*
Selection	1	146.47	6.67	0.081
Density	1	3101.03	152.03	0.001
Sex	1	2015.93	101.14	0.002
Selection × Density	1	225.32	5.068	0.109
Selection × Sex	1	50.16	8.19	0.064
Density × Sex	1	279.14	27.28	0.014
Sel × Den × Sex	1	0.993	0.565	0.506

Discussion

Evolution of adult traits in my study on *D. melanogaster* selected for larval crowding, showed trends similar to that seen in *D. ananassae* and *D. nasuta* selected for larval crowding (chapter 4 and 6 of this thesis) and also to the previous studies on *D. melanogaster* barring few traits (Santos et al. 1997, Borash and Ho, 2001).

There was no difference in the dry weight and lipid content at eclosion between MB and MCU populations raised at both low and high egg densities. This trend was similar to the ACUs (Chapter 4). However, in an earlier D. melanogaster study, the CUs were not heavier than controls but showed higher lipid content at higher densities (Santos et al. 1997; Borash and Ho, 2001). Corresponding to the increase in lipid content, CU flies also showed higher starvation resistance (Borash and Ho, 2001); the MCUs, however, did not show any such increase in starvation resistance, although the NCUs and ACUs (Chapters 4 and 6 of the thesis) did. This discrepancy could be due to the fact that I assayed this trait in MCUs quite early in the course of selection, i.e. by generation 37. However, the CUs had undergone > 90 generations and ACUs and NCUs had undergone > 60 generations of selection before the starvation resistance assays were done. Again, it would be interesting to look at these traits after another 30-40 generations of selection in the MCUs. Moreover, in the ACUs I could not see a genetic correlation between the lipid content and starvation resistance. There was an increase in starvation resistance without any increase in the lipid amount. This could have been due to better efficiency of utilization of lipids in ACUs as compared to ABs. It would be interesting to see if the same holds true for *D. melanogaster* as well.

MCUs showed no evolutionary increase of life span or fecundity after 37 generations of selection, which is similar to the case of ACUs (chapter 4) and CUs (Mueller et al. 1993; Borash and Ho, 2001), although the CUs did show higher (but not statistically significant) fecundity than their controls.

My *D. melanogaster* populations (MCUs) also showed genetic correlations between larval traits and adult traits similar to *D. ananassae* and *D. nasuta* but different from the earlier

studies in *D. melanogaster*. This difference in correlations could have been due to the difference in the maintenance regime of the two studies (our study and the CU populations described in Joshi and Mueller, 1996). I explored this possibility further and the results of those experiments are presented in the next chapter.

CHAPTER 9

Study on the temporal polymorphism of genotypes in D. ananassae

Introduction

In a crowded *Drosophila* culture, there is a progressive deterioration of the environment, availability of food going down and nitrogenous wastes (generated by the larvae) going up with time. This temporal variation in the environment can potentially be exploited by different genotypes in a population, as seemed to be the case with the CU populations of *D. melanogaster* in an earlier study (Borash et al. 1998). Natural selection in the crowded CU cultures led to the maintenance of a polymorphism that could be discerned along an axis of development time, with different genotypes specialized on the early and late parts of the environment in the culture vials (Borash et al. 1998). In the crowded culture vials, fast developing larvae of the selected populations had high feeding rates but lower absolute viability than slowly developing larvae, which showed a significantly higher egg-to-adult viability than the early larvae, and also increased tolerance to ammonia (Borash et al. 1998). In this experiment, I examined populations of *D.ananassae* selected for adaptation to larval crowding to see if they also showed polymorphism of genotypes to exploit the temporal variation in the environment, similar to that seen with CU populations of *D. melanogaster*.

Materials and methods

Collection of flies for assay

The flies collected for the assay were from the generation 51 in case of ABs, and generation 49 of ACUs, and the flies for the assay were generated in a manner similar to that described

in Borash et al. (1998). Both ABs and ACUs were reared at high larval densities of 600 eggs/vial, with 24 such vials being collected for each replicate population of both ABs and ACUs. Flies emerging during the first 36 hrs of eclosion were transferred into cages (approx. 200-250 flies) and classified as early flies. Flies emerging for the next 36-40 hrs were discarded. Then, the adults emerging for the next 36 hrs were taken and classified as late flies (figure 9.1).

Standardization of the flies for assay

Early and late eclosing flies from both control and selected populations were passed through one generation of common rearing conditions to eliminate non-genetic parental effects. 60-80 eggs/vial were collected from both early and late flies of control and selected populations. The emerging flies were transferred into Plexiglas cages on the 12^{th} day after egg collection. All assays were performed using these flies, i.e. the standardized progeny of early and late eclosing flies.

Egg-to-adult development time assay

Eggs for the assay were collected from the standardized flies by putting a cut plate in the cage for 2 h. 30 eggs were dispensed into each vial. 10 such vials were set up for each combination of population and eclosion phenotype. Once the first pupa was observed, vials were checked every 2 h and the number of pupae formed noted. Two hourly checks were continued till no new pupae formed. The vials were then monitored for eclosion. Once the first fly eclosed, the vials were checked every two hours for new eclosions and the number of eclosing males and females recorded. Checks were continued till no flies eclosed.

From these data, egg-to-pupa development time and egg-to-adult development time were calculated.

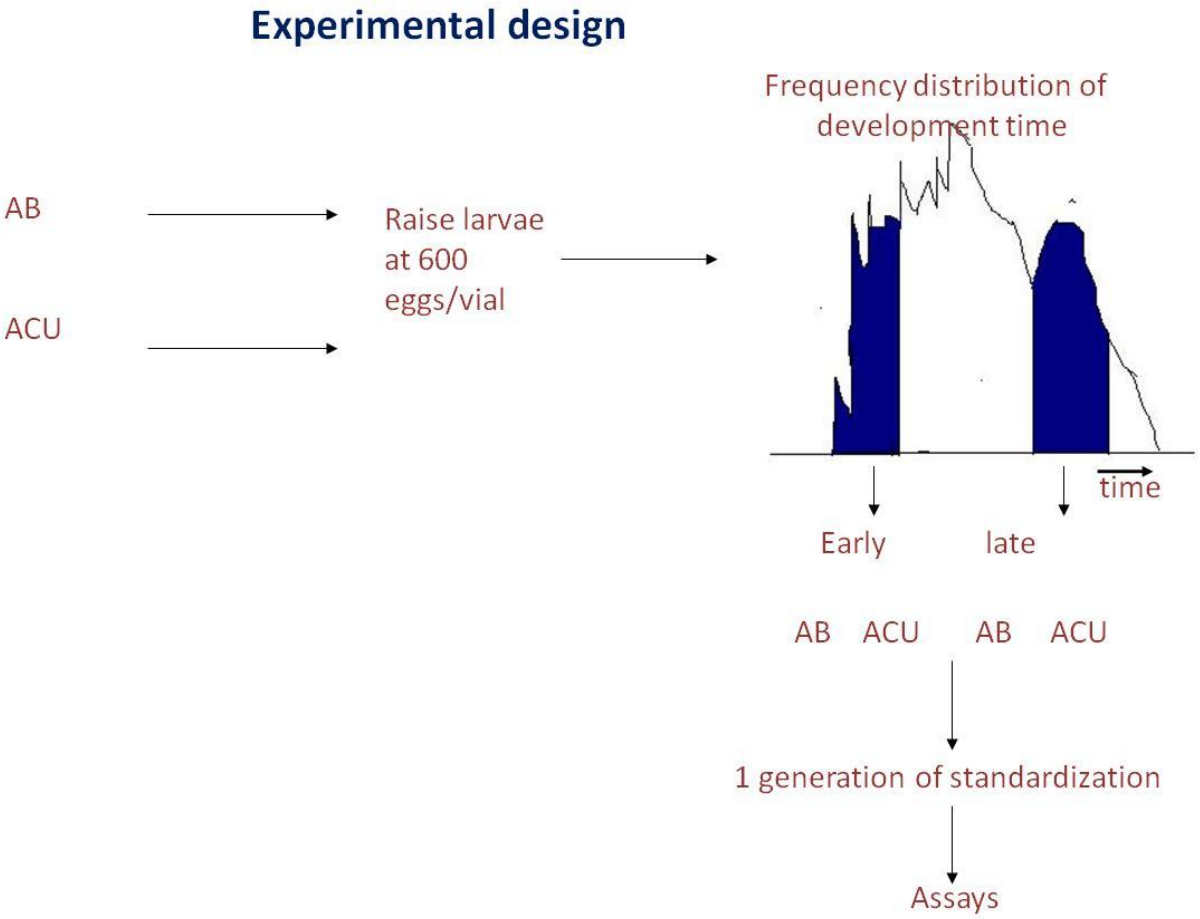


Figure 9.1. Schematic depicting the experimental design for testing the polymorphism of genotypes.

Survivorship assay

The standardized flies were yeasted for four days before egg collection, and a fresh food plate was kept in the cages on which the flies were allowed to lay eggs for about 14 h. The eggs were then removed from the food plate with a moistened brush and placed on agar pieces. Eggs were collected at densities of 70 and 600 per vial containing 1.5 ml of food. Eight such vials were set up for each density and each replicate of both early and late populations derived from the selected and control lines. The number of flies eclosing from each vial was recorded and used to assess egg-to-adult survival.

Larval competition assay

Larval competition assay was performed on the early and late populations of ABs and ACUs. The pre-adult stages of the AB and ACU populations were competed against a common competitor with a morphological marker for convenience of identification *i. e.* a white eye mutant (WE) of *D. melanogaster*. Eight vials were set up at two different densities: 70 and 600 eggs per vial with 1.5 ml of cornmeal medium to assess competitive ability against white eye mutants. The low density (70 eggs per vial) cultures contained 35 eggs of the test population and 35 WE eggs whereas the high density assay vials (600 eggs) comprised of 300 eggs from the test population and 300 eggs from the white eye mutant population. The vials were then monitored for eclosion and the number of eclosing wild type (*D. ananassae*) and white-eyed (*D. melanogaster*) adults in each vial were recorded and used to assess egg-to-adult survival.

Urea tolerance and ammonia tolerance assay

Urea tolerance was measured at three different concentrations of urea, 0 g/l, 14 g/l and 18 g/l, and ammonia tolerance was measured at three different concentrations of ammonia, 0

g/l, 15 g/l, 30 g/l. Thirty eggs were dispensed into each vial. Ten vials were set up per concentration level of urea and ammonia. Tolerance to urea and ammonia was measured as egg-to-adult survivorship and, hence, number of adults eclosing from the vials were recorded.

Larval feeding rate assay

The feeding rates of AB and ACU larvae were measured at physiologically equalized ages by collecting eggs from ACUs 5 h later than ABs. The assay was carried out on eggs collected from adults after 1 generation standardization procedure. The assay was done according to the method mentioned in chapter two of the thesis.

Statistical analyses

Data from all the assays were subjected to separate mixed-model ANOVA, treating block as a random factor and selection as a fixed factor crossed with block. Eclosion phenotype (early or late) was treated as a fixed factor in all the analyses. For the competition, starvation, life span, lipid content and fecundity assays, density was treated as a fixed factor. For urea and ammonia tolerance assays, concentration was treated as a fixed factor. All the fractional data (survivorship) were arcsine-square root transformed before analysis. In all cases, the population means were used as the units of analysis and, therefore, only fixed-factor effects and interactions can be tested for significance. All analyses were implemented using STATISTICA for Windows Release 5.0 B (StatSoft Inc. 1995).

Results

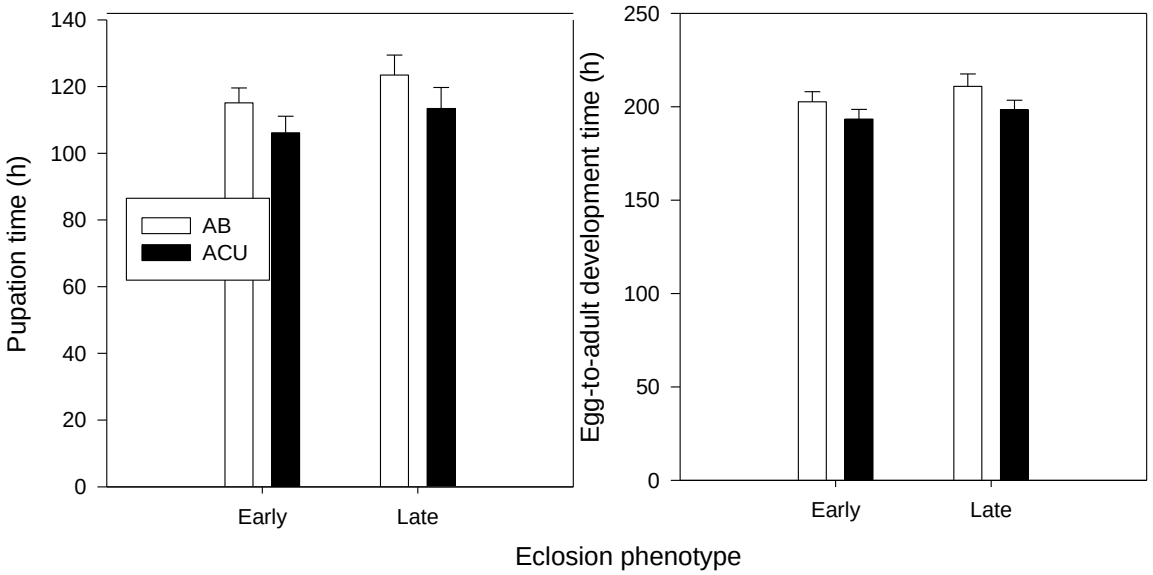
Egg-to-adult development time

Egg-to-pupation time

ANOVA revealed a significant effect of selection regime ($F_{1,3}$ = 90.21, P = 0.002). ACUs had shorter egg-to-pupation time as compared to the ABs (fig.9.2 (a)). The difference was approx. 10 hrs. However, there was no difference between the early and late populations ($F_{1,3}$ = 1.76, P = 0.28) (table 9.1)

Egg-to-adult development time

There was a significant effect of only the selection regime ($F_{1,3}$ = 338.26, P < 0.001) for egg-to-adult development time, with ACUs having significantly lesser duration than ABs (table 9.2 and fig.9.2(b)). Early and late eclosing populations did not differ ($F_{1,3}$ = 1.75, P = 0.28) from each other in egg-to-adult development time.



(a) (b)

Figure 9.2 The mean (± s.e.) (a) egg-to-pupation time, and (b) egg-to-adult development time of progeny of early and late flies of ABs and ACUs at 30 eggs/vial.

Table 9.1. Results of ANOVA on pupation time of progeny early and late flies from the AB and ACU populations.

Effect	*df*	MS	*F*	*P*
Selection	1	361.50	90.21	0.002
Eclosion phenotype	1	244.76	1.76	0.276
Selection × Ecl. ph	1	1.28	0.88	0.417

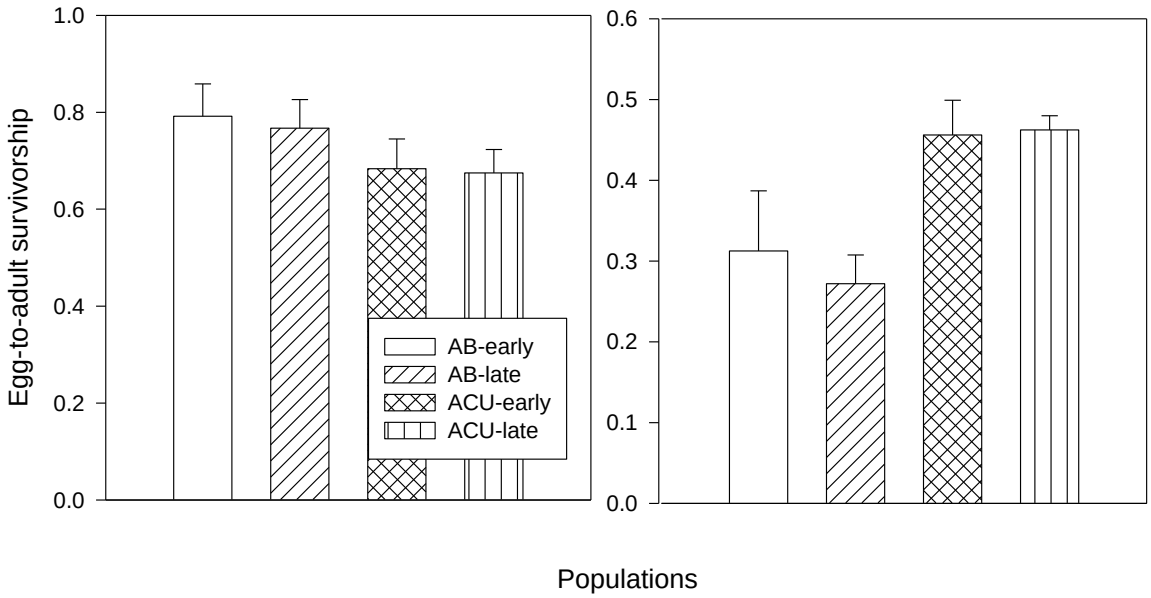
Table 9.2. Result of ANOVA on egg-to-adult development time of progeny of early and late flies of AB and ACU populations at 30 eggs/vial.

Effect	*df*	MS	*F*	*P*
Selection	1	476.75	338.26	<0.001

Eclosion phenotype	1	175.18	1.747	0.278
Selection × Ecl. ph	1	11.29	0.5697	0.505

Survivorship assay

ANOVA revealed that there were no significant effects of eclosion phenotype ($F_{1,3}$ = 0.17, P = 0.70) or eclosion phenotype × selection regime ($F_{1,3}$ = 2.44, P = 0.21). However, there was a significant effect of density ($F_{1,3}$ = 29.32, P = 0.012), with survivorship being lower at density of 600 eggs/vial than at 70 eggs/vial. There was also a significant effect of selection regime × density interaction ($F_{1,3}$ = 12.05, P = 0.04). ABs had a higher survivorship than ACUs at 70 eggs/vial, but ACUs had higher survivorship than ABs at 600 eggs/vial (table 9.3, fig.9.3(a) and (b)).



(a) (b)

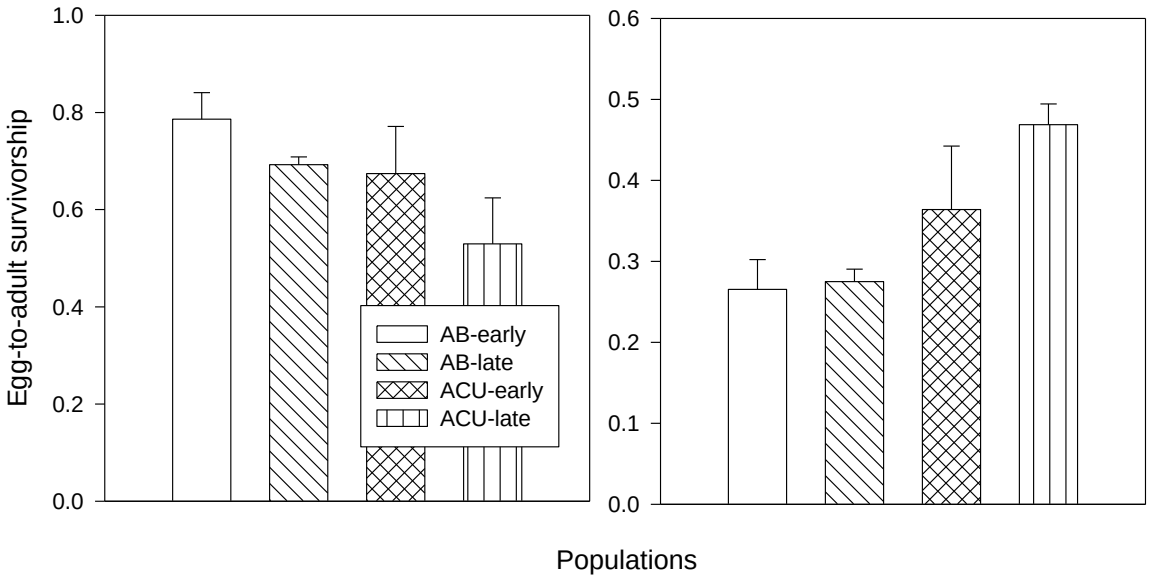
Figure 9.3. Egg-to-adult survivorship of progeny of early and late AB and ACU flies at (a) 70 eggs/vial and (b) 600 eggs/vial.

Table 9.3. Results of ANOVA on egg-to-adult survivorship of progeny of early and late flies of AB and ACU populations at 70 and 600 eggs/vial.

Effect	*df*	MS	*F*	*P*
Selection	1	0.009	2.10	0.242
Eclosion phenotype	1	0.0029	0.17	0.704
Density	1	1.00	29.32	0.012
Selection × Ecl. ph	1	0.002	2.55	0.208
Selection × Density	1	0.14	12.05	0.04
Ecl. ph × Density	1	7.65×10^{-7}	0.011	0.922
Sel × Ecl. Ph × Den	1	0.0005	0.782	0.442

Pre-adult competitive ability

ANOVA revealed no significant effect of selection regime ($F_{1,3}$ = 0.006, P = 0.95). There was a significant effect of density ($F_{1,3}$ = 36.49, P = 0.009) and density × selection regime interaction ($F_{1,3}$ = 25.10, P = 0.015). ACUs survived better in the presence of competitors as compared to the ABs at the density of 600eggs/vial (table 9.4, fig.9.4(a) and (b)). However, neither early nor late flies differed in both ABs and ACUs ($F_{1,3}$ = 1.04, P = 0.38).



(a) (b)

Figure 9.4. Mean egg-to-adult survivorship of progeny of early and late flies of AB and ACU populations at (a) 70 eggs/vial and (b) 600 eggs/vial, when competed against white-eyed mutant of *D. melanogaster*.

Table 9.4. Results of ANOVA on egg-to-adult survivorship of progeny of early and late flies of AB and ACU populations at 70 and 600 eggs/vial when competed against a common competitor, white-eyed *D. melanogaster*.

Effect	*df*	MS	*F*	*P*
Selection	1	0.0002	0.006	0.945
Eclosion phenotype	1	0.008	1.04	0.382
Density	1	0.86	36.49	0.009
Selection × Ecl. ph	1	0.001	0.198	0.686
Selection × Density	1	0.16	25.10	0.015
Ecl. ph × Density	1	0.062	5.768	0.096

Sel × Ecl. ph × Den	1	0.011	3.127	0.175

Larval feeding rate

Feeding rate did not differ between ABs and ACUs, as there was no significant effect of selection regime ($F_{1,3}$ = 0.44, P = 0.58). There was no significant effect of eclosion phenotype either ($F_{1,3}$ = 10.05, P = 0.09) (table 9.5.). Thus, the early and late populations of ABs as well as ACUs did not differ from each other (fig.9.5).

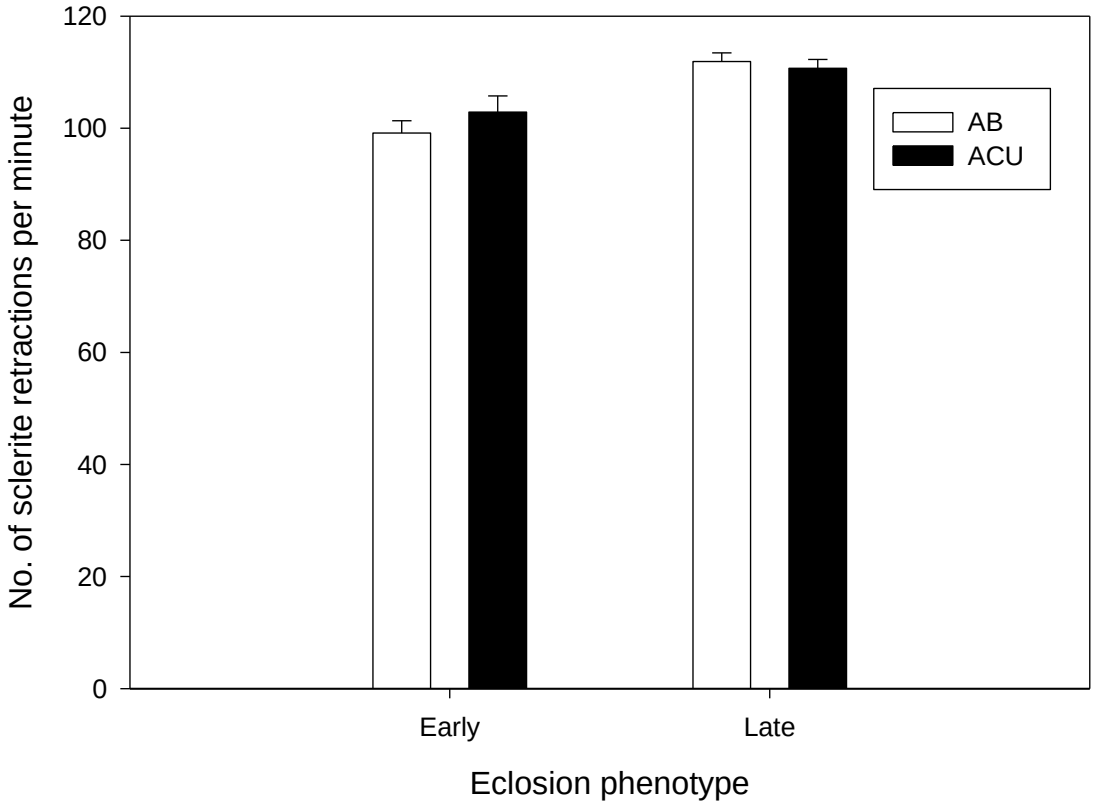


Figure 9.5. Mean (± s.e.) larval feeding rate of progeny of early and late populations of ABs and ACUs.

Table 9.5. Results of three-way ANOVA on mean larval feeding rate of progeny of early and late AB and ACU flies.

Effect	*df*	MS	*F*	*P*

Selection	1	4.75	0.44	0.575
Eclosion phenotype	1	317.76	10.05	0.086
Selection × Ecl. ph	1	18.13	2.26	0.271

Ammonia tolerance

In general, pre-adult survivorship declined with increasing ammonia level for both ABs and ACUs as revealed by significant effects of concentration ($F_{2,6}$ = 36.39, P < 0.001) in the ANOVA. The selection regime × ammonia level interaction was also significant ($F_{2,6}$ = 8.27, P = 0.019) (table. 9.6, fig.9.6). However, there was no significant effect of eclosion phenotype (early or late) ($F_{1,3}$ = 8.9, P = 0.06) on ammonia tolerance. Early and late flies of the ABs and ACUs did not differ significantly

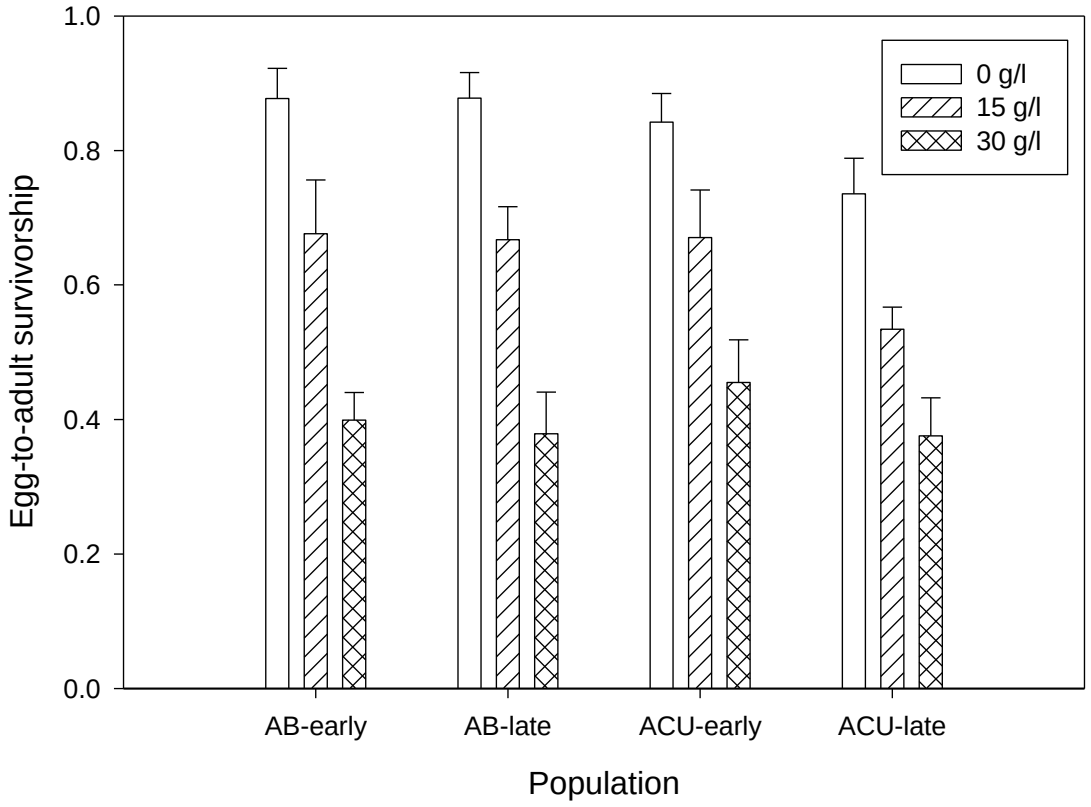


Figure 9.6. Mean egg-to-adult survivorship of progeny of early and late flies of AB and ACU populations in food containing different concentrations of ammonia. Error bars represent standard errors around the means of four replicate populations.

Table 9.6. Results of ANOVA performed on egg-to-adult survivorship at 30 eggs/vial of progeny of early and late flies of ABs and ACUs in food containing different concentrations of ammonia.

Effect	*df*	MS	*F*	*P*
Selection	1	0.023	1.34	0.331
Eclosion phenotype	1	0.04	8.91	0.058
Concentration	2	0.75	36.39	<0.001
Selection × Ecl. ph	1	0.03	6.13	0.089
Selection × Conc	2	0.02	8.27	0.018
Ecl. ph × Conc	2	0.001	0.137	0.875
Sel × Ecl. ph × Conc	2	0.001	1.16	0.375

Urea tolerance

In general, pre-adult survivorship declined with increasing concentrations of urea for both ABs and ACUs. The ANOVA revealed significant effects of concentrations of urea ($F_{2,6}$ = 42.82, $P < 0.001$) and the selection regime × urea level ($F_{2,6}$= 15.44, $P = 0.004$) interaction (table.9.7). However, there was no significant effect of selection regime ($F_{1,3}$ = 4.85, P = 0.115) or eclosion phenotype (early or late) ($F_{1,3}$ = 0.001, P = 0.97) on urea tolerance. Thus,

the early and late larvae from the ACUs or ABs did not show any difference in their tolerance to urea (figure 9.7).

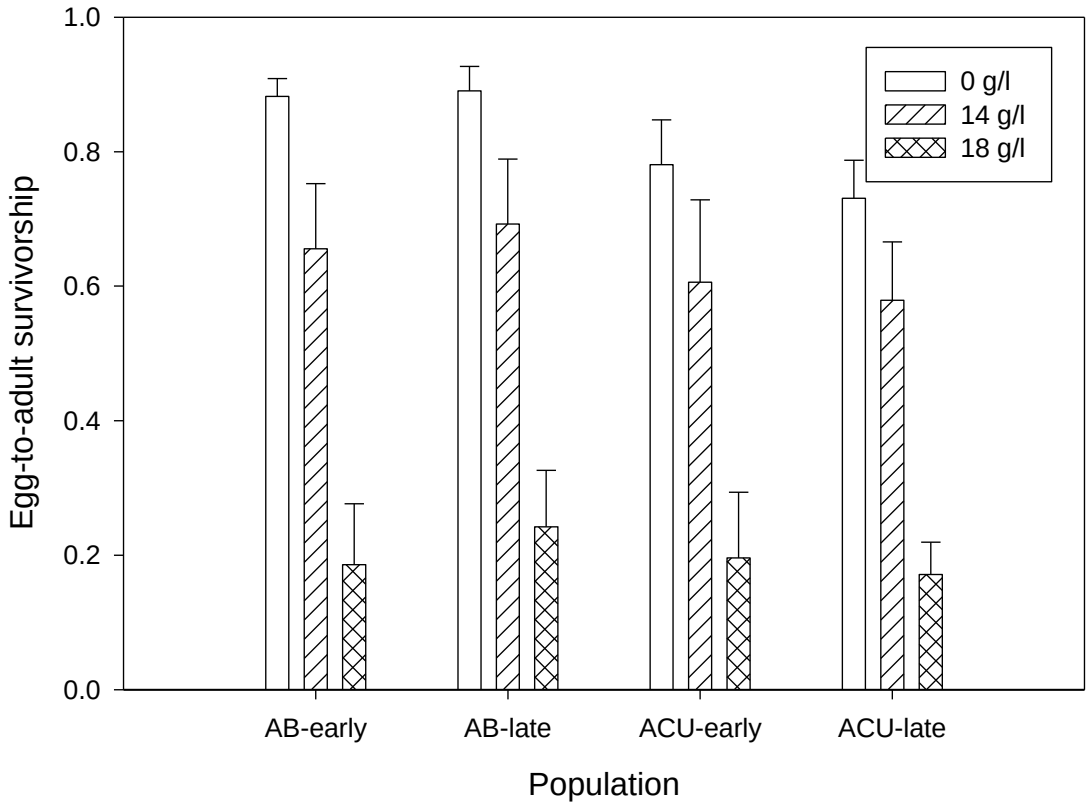


Figure 9.7. Mean egg-to-adult survivorship of progeny of early and late flies of AB and ACU populations in food containing different concentrations of ammonia. Error bars represent standard error around the mean of four replicate populations.

Table 9.7. Results of ANOVA performed on egg-to-adult survivorship at 30 eggs/vial of progeny of early and late flies of ABs and ACUs in food containing different concentrations of urea.

Effect	*df*	MS	*F*	*P*
Selection	1	0.18	4.85	0.115
Eclosion phenotype	1	4×10^{-5}	0.001	0.971
Concentration	2	2.58	42.82	<0.001
Selection × Ecl. ph	1	0.035	2.08	0.244
Selection × Conc	2	0.04	15.44	0.004
Ecl. ph × Conc	2	0.001	0.032	0.969
Sel × Ecl. ph × Conc	2	0.0005	0.038	0.963

Discussion

This study on *D. ananassae* populations selected for adaptation to larval crowding showed no evidence for polymorphism of genotypes along an eclosion time axis to exploit the progressive temporal deterioration in a crowded culture.

This result was in contrast to that seen in CUs (Borash et al. 1998), where progeny larvae of the flies eclosing early were fast feeders and less tolerant to ammonia and urea as compared to the progeny larvae of the flies that eclosed late which were more tolerant to urea and

ammonia but were slow feeders. This polymorphism was seen in the CU populations selected for adaptation to larval crowding, but not in the control UUs. However, in *D. ananassae*, neither ACUs nor the controls, ABs showed any such polymorphism. The differences observed between the ABs and ACUs in this study were the same as that presented in Sharmila Bharathi (2006) and chapter three of the thesis.

The results described in this chapter and the results of my selection experiment on *D. melanogaster* (chapter 7 of the thesis), where the genetic correlations seen between various larval traits were similar to that in *D. ananassae* and *D. nasuta* (chapter 3 and 5 of the thesis) but were different from the CUs and the *K*-populations (reviewed in Joshi, 1997), suggested that the differences between my study and previous studies of *D. melanogaster* could possibly be due to a small but potentially important difference in the maintenance regime of my study and that used for the CUs.

In the CUs, as soon as the flies started eclosing in the egg vials, they were transferred to new food vials, with additional daily transfers to a new set of vials till no flies eclosed. Thus, by day 14-15 from egg collection, there was a set of vials for each day of adult eclosion. These flies were transferred to a cage only on day 18 after egg collection, following which they were yeasted and egg collection for the next generation was done on day 21 (Joshi and Mueller, 1996). This kind of maintenance regime might have inadvertently caused assortative mating to occur in the holding vials before the adults were transferred to cages. In this vial transfer system, for example, flies eclosing on 1st day of eclosion would get to mate only with other flies that eclosed on day 1 till almost day 10 after eclosion when they would be transferred to cages and be exposed to flies that eclosed subsequently.

However, in the ACUs, as soon as the flies started eclosing, they got transferred into the cages once a day till no more flies eclosed. Thus, flies from day 1 of eclosion could potentially mate with flies from day 2 of eclosion on the very next day and so on (Sharmila Bharathi, 2006). This is likely to reduce the probability of assortative mating for development time.

I tested this hypothesis that the CU maintenance regime can lead to greater assortative mating for development time than the ACU regime, by a one generation experiment which is described in the next chapter.

Chapter 10

Study on the maintenance regimes in D. melanogaster

Introduction

In this chapter, I address the possible role of differences in maintenance regime in giving rise to some of the differences seen in my study and previous studies on *D. melanogaster*. In the earlier studies, the *D. melanogaster* populations when selected for crowding showed evolution of increased feeding rate and competitive ability. However, there was no change in development time and body weight at eclosion at moderate densities (reviewed in Joshi, 1997). However, in all the three species I studied, including *D. melanogaster*, there was evolution of higher competitive ability without any change in feeding rate and weight at eclosion, but accompanied by reduced development time in the selected populations.

Moreover, *D. ananassae* populations selected for adaptation to larval crowding, when assayed for polymorphism of genotypes, did not show any difference between the life-history traits between the progeny of the early and late eclosing flies in crowded cultures (chapter 9 of the thesis) as was seen in the *D. melanogaster* CUs (Borash et al. 1998).

Hence, I hypothesized that some minor differences in the selection regimes of my study and previous study (CUs and UUs) could have led to the evolution of different suite of traits under similar selection pressures, through differences in the mating system.

In both the selection regimes, populations were on 21-day discrete generation cycle. The main difference was in the maintenance of adult flies till three days before egg collection. In our selection regime (henceforth, called ACU-type), the flies were transferred to cages daily once they started eclosing, till no more flies eclosed (Sharmila Bharathi, 2006). However, in the CUs (henceforth called CU-type regime), the flies were transferred daily to new vials till no more flies eclosed. Thus, there was a set of vials for each day of adult eclosion, which

were transferred to new vials with fresh food every alternate day till day 18, when they all were transferred to the cages and provided with generous smear of yeast paste. Thus, while in ACU-type selection regime, the flies eclosing on a particular day could potentially mate in the cages with flies eclosing from the other days, immediately or as and when they eclosed, in the CU-type regime the flies could mate only with flies which eclosed on the same day till day 18 after egg collection. This could have led to positive assortative mating based on development time to happen, which in turn, could help maintain such polymorphism (Borash et al. 1998). Although *Drosophila* mate many times during their life, the mating propensity decreases after each mating (Markow, 1996).

Thus, I postulated that assortative mating based on development time in the CU-type regime might have been a reason why the CU results were so different from my studies as the assortative mating might have led to changes in genotype frequencies, different from what is expected under random mating. Thus, to test the hypothesis that CU-type regime leads to assortative mating for development time, compared to ACU-type regime, I did a one-generation experiment using the control populations of *D. melanogaster* (MB_{1-4}).

Materials and Methods

This study was a one-generation experiment done using the MBs (*D. melanogaster* control populations described in detail in chapter 2) and scarlet-eyed mutants (autosomal recessive) of *D. melanogaster* (kindly supplied by Dr. N. G. Prasad, Indian Institute of Science Education and Research, Mohali, India). These scarlet-eyed populations were introgressed with MB-1 for three generations to obtain a similar genetic background to the MBs before

starting the experiment. The objective here was to use the scarlet-eye flies as a phenotypic marker for this study.

Standardization of the flies

Both MBs and scarlet-eyed *D. melanogaster* populations were passed through one generation of common rearing conditions before starting the experiment.

The assortative mating assay

The MBs (1-4 replicates) and the scarlet-eye mutant populations were reared for 1 generation in 2 sets: one set maintained similar to the ACU-type regime and another to the CU-type regime (fig.10.1). The aim was to see if the ratio of the progeny (scarlet-eye to red eye) of the flies from different stages of the eclosion distribution based on development time is the same from both type of selection regimes.

ACU-type maintenance regime

20 vials of MB (1-4) and 10 vials of scarlet-eye were reared under high egg densities (400-450 eggs/vial) on 2 ml of cornmeal media. The egg density was not taken as 600 eggs/vial (the number at which ACUs are maintained in the lab) because MBs and scarlet-eye populations were not adapted to this high density and would not have survived. Thus, to obtain enough flies for the experiment, as well as to get an eclosion distribution similar to high density selection regime, 400-450 eggs/vial was chosen.

The flow chart in the fig.10.2 further describes the maintenance. The eclosing flies were further divided into 3 categories: early, middle and late, based on their egg-to-adult development time. Flies eclosing on days 1 and 2 were taken as early, days 3 and 4 as

middle and days 5 and 6 as late. Once the flies started eclosing in these vials, they were transferred to 3 cages. In cage 1, the scarlet-eyed early flies were transferred along with middle and late eclosing MB flies. Cage 2 had middle scarlet-eye flies and early and late MB flies while in cage 3, I transferred late scarlet-eye and early and middle MB flies. The transfer to cages was done daily as is done in the ACU-type maintenance regime (chapter 2 of the thesis). On day 18, the cages were provided with fresh food plates containing a generous amount of yeast paste. On day 20, 100 scarlet-eyed females were taken from each of these cages and introduced individually into separate vials containing 4 ml of cornmeal media. On day 21 (after 18-19 hrs of introduction of flies into vials), the flies were discarded and the progeny were allowed to eclose. Once, they started eclosing, the proportion of scarlet-eyed to red eyed progeny was noted for each vial.

CU-type maintenance regime

20 vials of MB (1-4) and 10 vials of scarlet-eyed mutants were raised at 400-450eggs/vial. The major differences between CU-type and ACU-type maintenance regimes are as follows:

1. When the flies started eclosing from the egg vials of both MB and scarlet-eyed flies, instead of being transferred into cages as in ACU-type, here the flies were transferred to fresh food vials (with 4 ml food) daily. A separate set of fresh vials were used daily for the transfer till no more flies eclosed. Thus, if flies eclosed for 6 continuous days, there were 6 sets of vials for both MB and scarlet-eyed populations.

2. The flies were transferred into cages only on day 18 after egg collection (approx. day 10 after eclosion started) and provided with food plates containing yeast paste (fig.10.3).

The flies were transferred into 3 cages based again on distribution of eclosing flies – early, middle and late. From here on, the process was similar to that followed in the ACU-type maintenance regime, including the set up of experimental vials.

If the ratio of scarlet-eyed to red-eyed progeny was more in the CU-type regime as compared to the ACU-type regime, it would indicate greater assortative mating for development time in the CU-type regime.

Statistical analyses

The proportion of scarlet-eyed progeny from scarlet-eyed females was the dependent variable in the statistical analyses. Three-way mixed model ANOVA was performed with type of maintenance regime and eclosion phenotype (early, middle or late) as fixed factors and block as a random factor. To see if the mating in both the maintenance regimes is random or assortative (if random, the proportion of scarlet-eyed progeny should be 0.33, since the ratio of scarlet-eyed to red-eyed flies was 1:2 in the cages), I plotted the confidence interval derived from the appropriate mean square term as the error bar on the graph and checked whether 0.33 fell within this confidence interval.

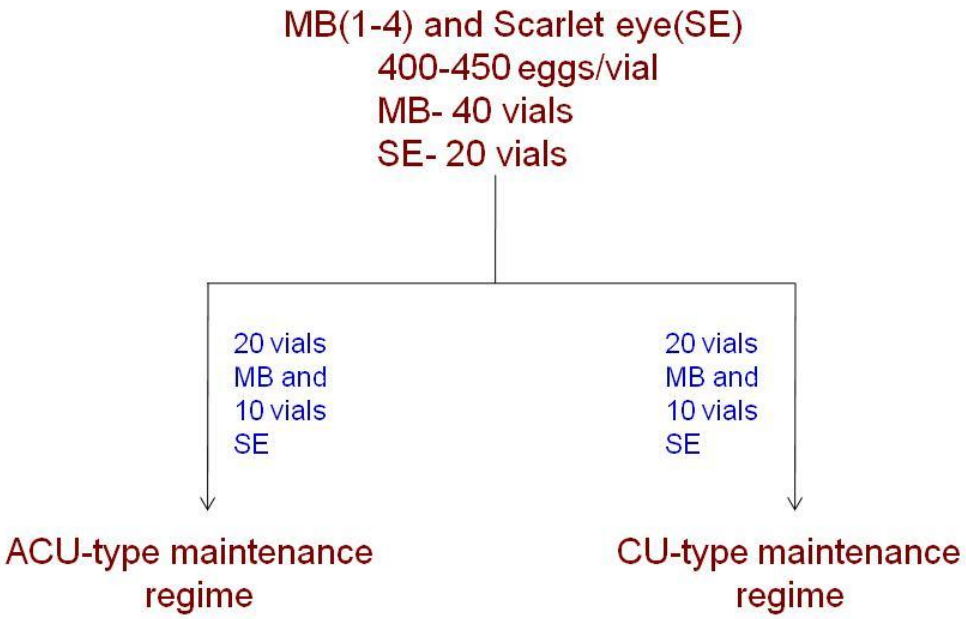


Figure 10.1. Overall schematic of the experiment. The control populations of *D. melanogaster* (MBs) and scarlet-eyed mutants of *D. melanogaster* were used for the experiments.

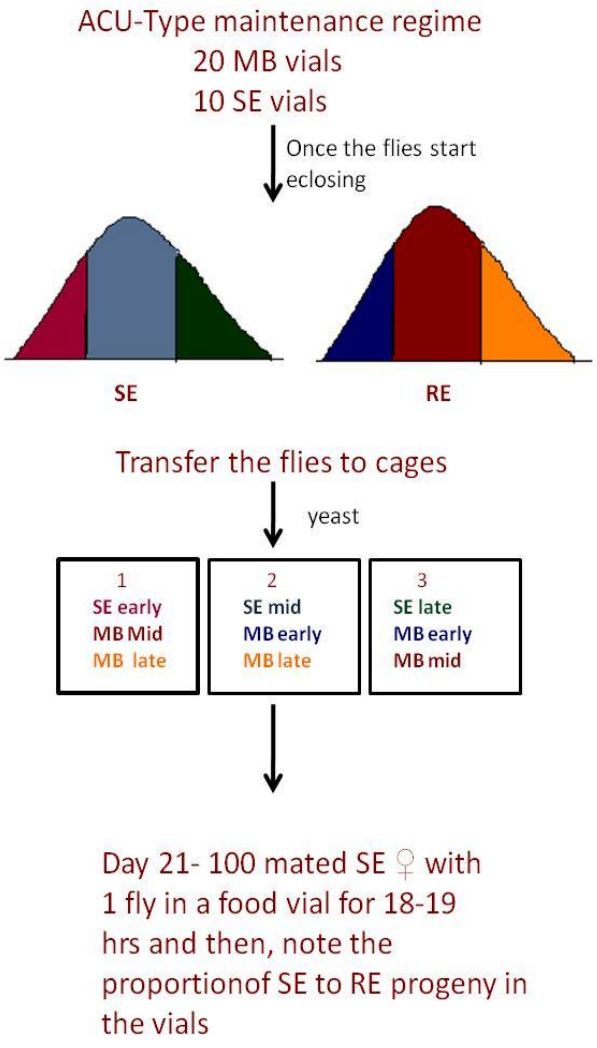


Figure 10.2. Schematic of the ACU-type maintenance regime.

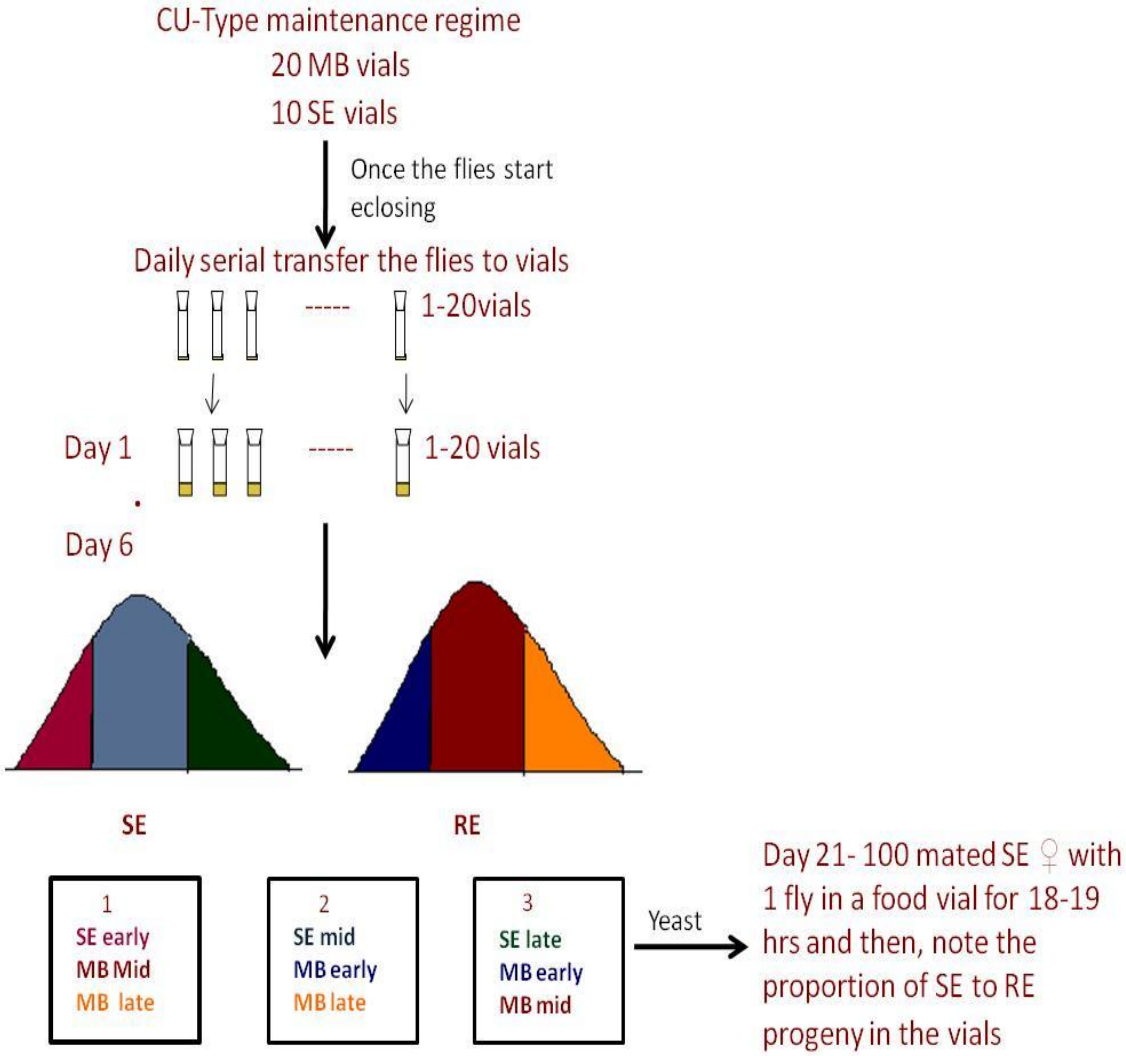


Figure 10.3. Schematic of the CU-type maintenance regime.

Results

ANOVA revealed significant effect of maintenance regime ($F_{1,3}$ = 37.43, P = 0.009), with CU-type regime showing significantly higher proportion of scarlet-eyed progeny as compared to the ACU-type regime. There were no significant effects of either eclosion phenotype ($F_{2,6}$ = 1.24, P = 0.353) or maintenance regime × eclosion phenotype ($F_{2,6}$ = 1.688, P = 0.262) (table 10.1), with CU-type regime having higher proportion of scarlet-eyed progeny in eclosion phenotypes, early, middle and late (fig.10.4). Thus, the CU-type regime showed more assortative mating based on development time compared to the ACU-type regime. When the confidence interval was plotted for maintenance regimes, in ACU-type regime, 0.33 was within the confidence interval unlike in the CU-type regime. Thus the ACU-type regime showed random mating whereas CU-type regime showed positive assortative mating for development time.

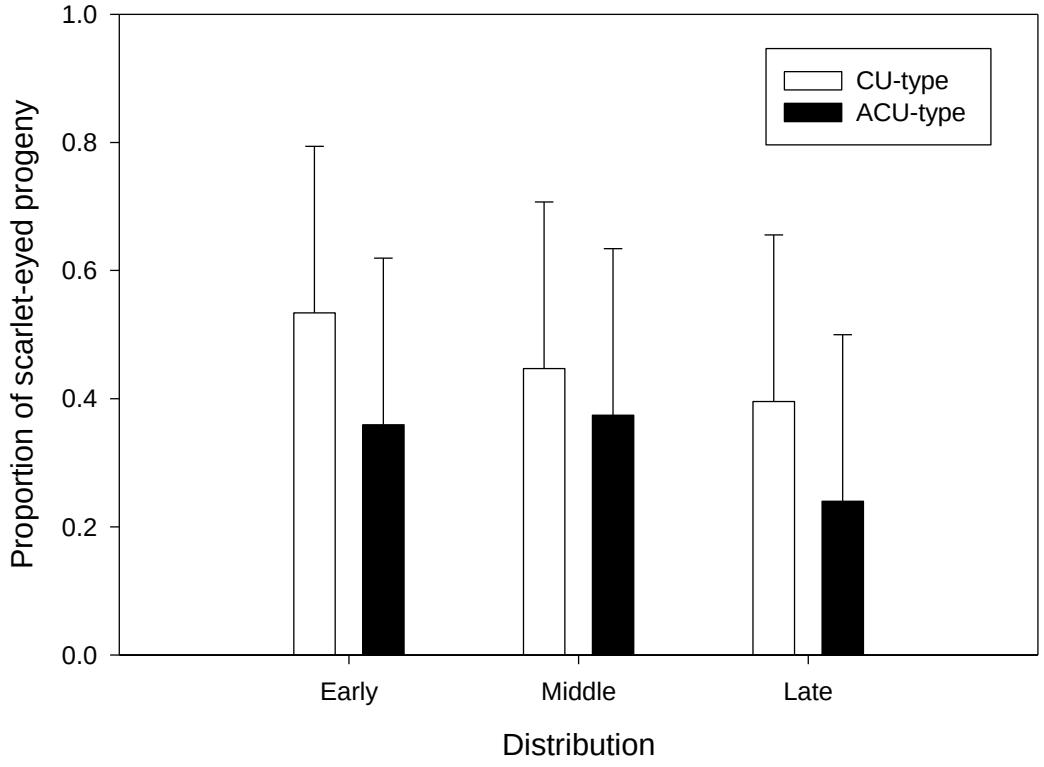


Figure 10.4. Proportion of scarlet-eyed progeny of CU-type and ACU-type selection regime at early, middle and late parts of distribution of eclosion. The error bars are confidence intervals calculated from the appropriate mean square terms.

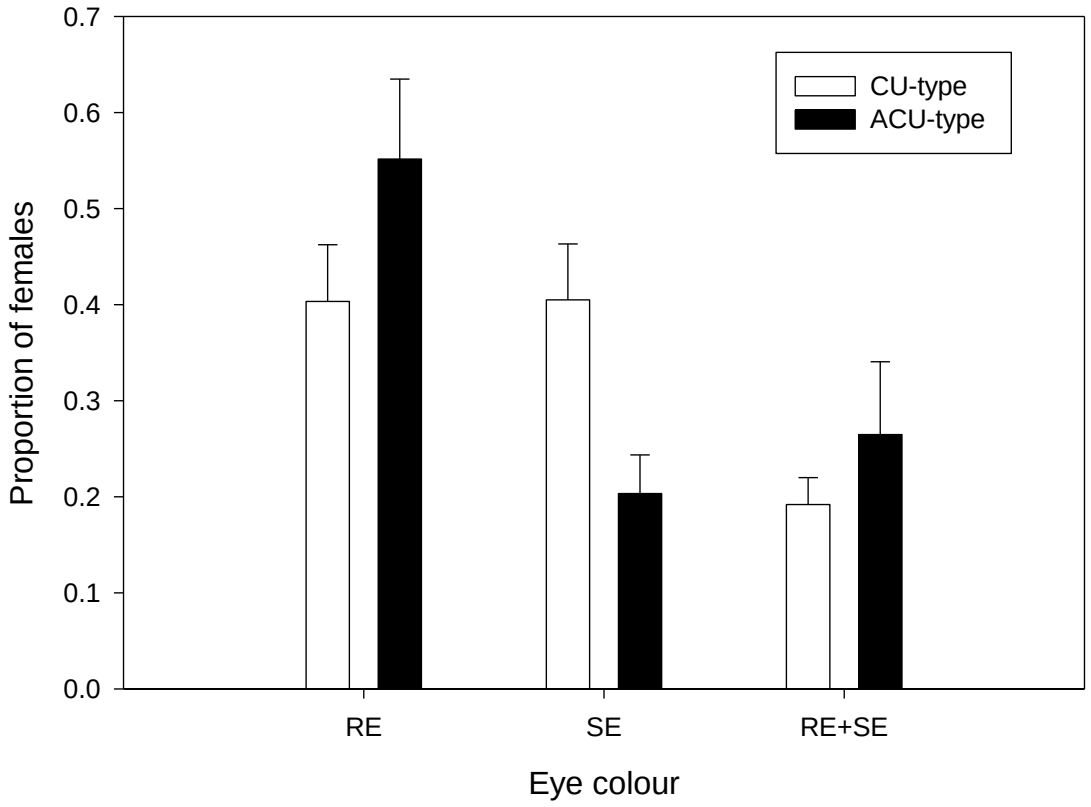


Figure 10.5. The proportions of red-eyed, scarlet-eyed and red-eyed + scarlet-eyed progeny in CU-type and ACU-type selection regimes. The error bars are standard error around the mean.

Table 10.1. Summary of three-way ANOVA on asin transformed proportion of scarlet-eyed progeny from the scarlet-eyed mothers. Selection-type and eclosion phenotype were taken as fixed factors and block as random factor. Thus, effects of block could not be tested for significance and have been omitted from the table.

Effect	*df*	MS	*F*	*P*
Population	1	0.31	37.43	0.008
Eclosion phenotype	2	0.09	1.24	0.353
Population × Ecl. ph.	2	0.01	1.69	0.262

Discussion

This experiment shows that the CU-type maintenance regime leads to assortative mating as compared to the ACU-type regime.

Since the genetic correlations observed by me, when different *Drosophila* species were selected for adaptation to larval crowding (Chapters 3, 5 and 7), were very different from the earlier studies on the CUs (reviewed in Joshi, 1997), I tried to examine if the maintenance regimes of the two studies, in some way, might have caused these differences. The results of this experiment clearly show that in the CU-type regime, there is assortative mating happening based on development time. However, in the ACU-type regime, the flies can

potentially mate with flies of different eclosion phenotype as and when they eclose and there was no detectable assortative mating for development time.

The study by Borash et al. (1998), had also discussed the role of assortative mating in maintaining the temporal polymorphism of genotypes in a crowded Drosophila culture.

This one-generation study showed that the CU-type regime led to greater assortative mating for development time as compared to the ACU-type regime. This difference in the selection regimes could be one of the reasons for differences in genetic correlations between various traits in the two studies, since the mating system can affect genetic correlations by changing the genotype frequencies and therefore, also breeding values and heritabilites. However, the other possible reason could be that the specific levels of crowding used may differently weigh different traits with respect to fitness and thus, affect outcomes of similar selection pressures.

CHAPTER 11

Conclusions

In this chapter, I summarize the major findings that have come out of my Ph D work. I briefly discuss how these findings have affected our understanding of life-history evolution in *Drosophila* and I reiterate the need for caution while extrapolating results from experiments on one species to a whole spectrum of organisms, even congeners.

In this thesis, I have addressed the lack of detailed systematic studies on life-history evolution in *Drosophila* species other than *D. melanogaster*.

Genetic correlations between various fitness-related traits have been studied in great detail in *D. melanogaster* (reviewed in Joshi and Prasad, 2003). It is known that the genetic correlations are not fixed entities but, are transient (reviewed in Prasad and Shakarad, 2004). They can change over a few tens of generations of selections and some correlations breakdown in the course of selection (Phelan et al. 2003; Archer et al. 2003), and, sometimes, they depend upon inbreeding, G × E interaction, genetic background, etc. (Rose et al. 2005). However, correlations among certain life-history related traits have tended to remain constant across various studies and various selection pressures (reviewed in Prasad and Joshi, 2003). The positive correlation between competitive ability and larval feeding rate was seen across studies from different labs (Mueller, 1988; Joshi and Mueller, 1988, 1996; Prasad et al. 2001, Shakarad et al. 2005). Similarly, the correlations between development time and larval feeding rate (Prasad et al. 2001; Rajamani et al. 2006) and development time and weight at eclosion were also consistent (Prasad et al. 2001). Adult traits like longevity and starvation resistance were also positively correlated (Chippindale et al. 1996; Prasad, 2003). Thus, the evolution of life-history and related traits under different selection pressures is very well studied in *D. melanogaster*. However, there have been very

few such studies on other species of *Drosophila*. One such study showed that when *D. melanogaster* and *D. simulans* were selected for increased resistance to desiccation, both the species showed similar correlated responses; however, the realized heritabilities were more in *D. melanogaster* (Hoffmann and Parsons, 1993). However, more detailed studies are needed to address the issue of generality of life-history and related trade-offs and correlations. The work detailed in this thesis is one such attempt. My study on three species of *Drosophila* selected for adaptation to larval crowding is first of its kind where long-term selection has been done and a detailed comparative study has been executed.

I have worked here on three species: *D. ananassae*, *D. nasuta* and *D. melanogaster* of which *D. ananassae* and *D. nasuta* were selected for over 50 generations before most of the assays done on them. Selection on *D. melanogaster* was started later and, thus, we could follow the early trajectory of trait evolution in this species. I have shown here that the three species exhibited an almost similar network of genetic correlations. The correlations are consistent across the three species, especially the ones between the larval traits. However, these results are quite contradictory to that seen in earlier the studies on *D. melanogaster* populations selected for larval crowding (reviewed in Joshi, 1997). I have summarized the network of correlations between five fitness-related traits- larval competitive ability, egg to adult survivorship, egg to adult development time, larval feeding rate and weight at eclosion in fig.11.1 (a) and (b). Fig.11.1(a) shows the correlations as seen in the earlier studies and (b) represents results from my study.

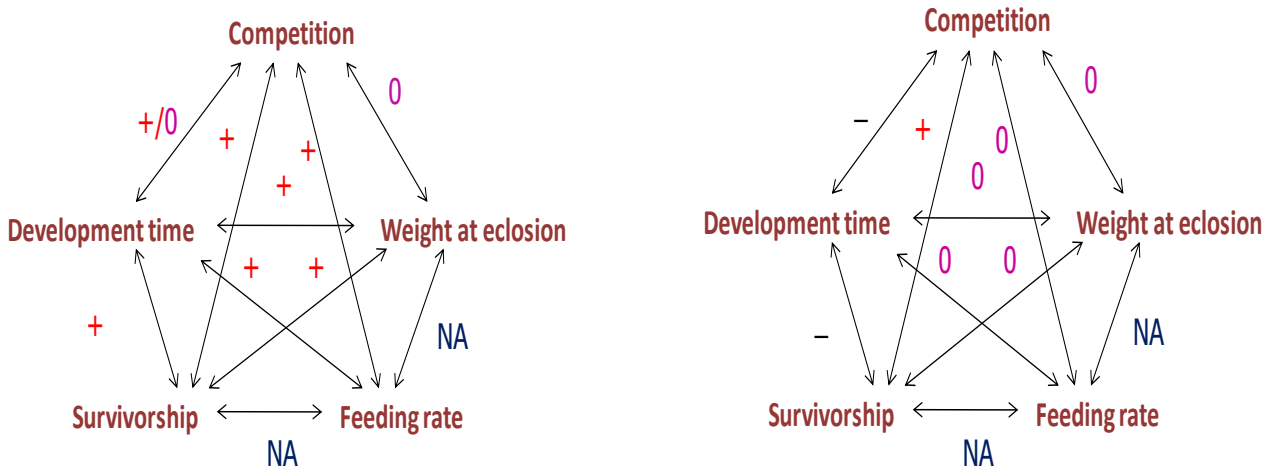


Figure 10. The network of genetic correlations between five fitness-related traits as seen in (a) earlier studies and (b) my study. NA- Not assayed. + shows positive correlation, – shows negative correlation and 0 shows no correlation.

In my study, there was no correlation between the larval feeding rate and competitive ability and also between development time and feeding rate. Even, the correlation between survivorship and development time was not seen here and a negative correlation was observed between development time and competitive ability (chapters 3, 5 and 7). Moreover, apart from these traits, I assayed tolerance to urea and ammonia. Except for *D. ananassae*, which showed evolution of increased ammonia tolerance, selected populations of other species did not evolve either increased urea or ammonia tolerance. The decrease in development time, though significant, was not very large (approx. 10 h); however, this is much less as compared to that seen by Prasad et al. (2000, 2001) when they directly selected

for rapid development in flies. I think, however, this modest decrease in development time of a few hours gives a competitive edge to selected populations. Krijger et al. (2001) have reported that development time is a good predictor of competitive ability in species under inter-specific competition. They showed that *Drosophila* species with shorter development time have higher competitive ability. This could be possible in my study as well, as the larvae from selected populations would have been able to reach the third instar in comparatively less time and thus, acquire more resources before they run out. Moreover, these flies were probably able to avoid the toxic environment caused due to the buildup of nitrogenous wastes, by pupating early.

As for no change in feeding rate, its possible that due to opposing forces of development time pulling the feeding rate down and competitive ability pushing it up, there was no change. Moreover, the changes associated with the faster developing flies (Prasad et al. 2000, 2001) were direct and correlated response to direct selection on development time. In my study, development time evolved as a correlated response and thus, the suite of traits evolved here could be different.

In chapters 4, 6 and 8, I have shown the results of assays on the adult traits. The evolution of adult traits in my study was similar to that seen in the earlier studies on selection for adaptation to larval crowding in *D. melanogaster* (Bierbaum et al. 1989; Mueller et al. 1993; Borash and Ho, 2001).

In all the three species, the selected lines showed no changes in fecundity and dry weight. *D. ananassae* and *D. nasuta* showed increased starvation resistance when reared at high densities but, not *D. melanogaster* populations, probably because they were assayed much

earlier in their course of selection. Lipid content and starvation resistance are known to be correlated, but there was increase in starvation resistance without any change in lipid content in *D. ananassae*, whereas in *D. nasuta* there was increase in lipid content at high densities. It could be possible that the ACU flies have evolved to become efficient at converting biomass to energy, or possibly starvation resistance in these populations depends on some other physiological mechanisms which doesn't involve lipid content. Baldal et al. (2005) showed, in *D. ananassae* grown in high larval density condition for one generation, the starvation resistance of adult flies is not solely dependent on lipid. Thus, this may reflect differences in resource acquisition and allocation in the flies in my study.

In chapter 9, I have discussed the results from one-generation experiment on *D. ananassae* selected and control populations which was done with the aim of seeing if there is a polymorphism of genotypes in the selected populations for exploiting the temporal variation in a crowded *Drosophila* culture, as was seen in earlier study on *D. melanogaster* populations (Borash et al. 1998). However, I could not find any polymorphism of genotypes in the ACUs.

The differences in the network of correlations seen in all the three species I studied and also, non-existence of polymorphism of genotypes in *D. ananassae*, led me to hypothesize that the small differences in my maintenance regime and the earlier study (CUs and UUs, reviewed in Joshi 1997) could be causing positive assortative mating based on development time to happen in the CUs unlike the ACUs. We tested this hypothesis by a one-generation experiment with the MBs and scarlet-eyed mutants of *D. melanogaster* and found that,

indeed, the CU-type maintenance regime led to greater assortative mating as compared to that in ACUs.

Positive assortative mating based on development time seen in the CUs could be one of the reasons for differences in genetic correlations between various traits in the two studies, since the mating system can affect genetic correlations by changing the genotype frequencies and therefore, also breeding values and heritabilities. However, the other possible reason could be that the specific levels of crowding used may differently weigh different traits with respect to fitness and thus, affect outcomes of similar selection pressures.

Overall, the thesis underlines the need for detailed studies on more congeneric species in life-history evolution before generalizing the results and also the need for caution while imposing various selection regimes since, as small differences may alter the signs of genetic correlations.

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