Dynamics of Crowded Populations of *D. melanogaster*

A Thesis

Submitted for the Degree of

Master of Science

By

Geetanjali P. Vaidya

Evolutionary and Organismal Biology Unit

Jawaharlal Nehru Centre for Advanced Scientific Research

Jakkur, Bangalore - 560064, India

April 2013

Table of Contents

DECLARATION

I declare that the matter presented in my thesis entitled "**Dynamics of Crowded Populations of *D. melanogaster*"** is the result of studies carried out by me at the Evolutionary and Organismal Biology Unit of the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India, under the supervision of Prof. Amitabh Joshi and that this work has not been submitted elsewhere for any other degree.

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

Geetanjali P. Vaidya

Place: Bangalore

Date: April 1, 2013

CERTIFICATE

This is to certify that the work described in the thesis "**Dynamics of Crowded Populations of *D. melanogaster*"** is the result of investigations carried out by Ms. Geetanjali P. Vaidya in the Evolutionary and Organismal Biology Unit of the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India, under my supervision, and that the results presented in this thesis have not previously formed the basis for the award of any other diploma, degree or fellowship.

Amitabh Joshi, Ph.D.

Professor

Acknowledgements

I would like to thank Prof. Amitabh Joshi, for introducing me to evolutionary biology and population dynamics and letting me play with these lovely problems. I don't know where else I would have found someone who could teach me so much about math, biology, history and poetry all in one, and hope for many more discussions.

Dr. Snigdhadip Dey has been a wonderful teacher, role model, helper and friend. This project would not have been possible without his practical help, his moral support and many long discussions, and his seemingly endless supplies of patience and good humour.

Thank you especially to Sajith and to Abhilash Lakshman for huge amounts of help with experiments and data analysis. Thanks to Manaswini Sarangi for many useful insights and discussions about her own findings which I have included here. Thanks also to Avani Mital and Dr. B.M. Prakash for their help and input with these experiments. I would also like to thank members of the Chronobiology and Behavioural Neurogenetics labs, who cheerfully allowed me to use equipment and computers in their labs whenever that need arose.

I would also especially like to acknowledge Rajanna and Muniraju for their help in the kitchen, their tireless efforts to keep vials supplied regardless of how large the need, and their unfailing good humour and friendliness.

Several people gave very helpful input for this project, including Sudipta Tung, Dr. Sutirth Dey and Prof. Laurence Mueller. I wish I had had more time to discuss ideas with each of them.

Thanks to my father, P.G. Vaidya, for his enthusiastic help with the mathematical side of this project, not all of which was at a stage where it could be included here. Much of my understanding of the logistic model comes from his helpful drawings in the sand.

Thanks to Gaurav Mendiratta, for being interested in anything and everything in the universe, for help with mathematics in this project, for giving me a physicist's view of biology, and for in general maintaining my sanity.

Chapter 1: Introduction

The question of how environmental and genetic factors interact to place limits on the dynamics of biological populations has been a matter of debate throughout the 20th century (for review, see Turchin 1995; Mueller & Joshi 2000). History is replete with examples of uncontrollable population expansions and crashes, and often unsuccessful interventions to impose artificial regulation. However, a range of studies examining the stability of laboratory and natural populations have found relatively stable dynamics in terms of constancy stability, more often than not (Hassell et al. 1976; Thomas et al. 1980; Mueller & Ayala 1981b; Turchin & Taylor 1992; Ellner & Turchin 1995). A theory which has gained acceptance over the past century is that the dynamics of biological populations are regulated via density-dependent mechanisms (Turchin 1995). This idea in its basic form arose with the logistic model of population growth, proposed in 1838 by the French mathematician Pierre-François Verhulst. (Verhulst 1838)

Verhulst proposed the "logistique" model of population growth in response to the logarithmic model suggested by Malthus (1798). In Malthus' model, population regulation could only occur by density-independent factors, loosely categorized under "natural causes," "misery" and "vice." Here, an exponentially growing population would swiftly outstrip its resources and crash. Verhulst's logistic model, on the other hand, introduced an alternative, very elegant theory with far-reaching implications: he suggested that populations might demonstrate self-regulation, through an inverse relationship between growth rates and population density. These ideas were picked up by mainstream ecology 80 years later, when Verhulst's logistic model was rediscovered and expanded upon by Pearl & Reed (1920). It was later applied to multi-species models of predator-prey conflict and competition by Lotka (1925) and Volterra (1926).

Debates ranged back and forth over the middle of the 20th century regarding the exact nature of population regulation in the natural world, and the relative contribution of density-dependent

and –independent factors. Nicholson (1933) argued that density-dependent regulation was key for the production of balance, claiming that whichever factors controlled population dynamics must themselves vary with population size. Others argued that population expansion could be controlled through the action of density-independent factors alone (Andrewartha & Birch 1954; Den Boer 1968). Further arguments have suggested a fine-tuning of theories of density dependent regulation, proposing that such regulation may only be strongly felt at high densities. (Milne 1958; Dempster 1983; Strong 1986). The general consensus at present, however, is that population regulation is chiefly brought about via density dependence (for review, see Turchin 1995; Mueller & Joshi 2000). The questions that remain, then, are the very non-trivial ones that lie in the details: how is density-dependence achieved? And how do the specifics of density-dependent factors influence dynamics?

Stability and Regulation

To begin with, it may be useful to pin down semantics: what is meant by stability, and what does it mean for a population to be regulated? Biological populations are subject to both stochastic and deterministic processes which will inevitably cause population numbers to fluctuate. One cannot therefore define a regulated population as one which reaches an equilibrium value and then stays there. At best, a regulated population might maintain an equilibrium cloud (Turchin 1995). A regulated biological population might therefore be described as one whose fluctuations in population numbers follow a stationary probability distribution (Dennis & Taper 1994), with consistent upper and lower limits in population size, and a relatively stable frequency distribution of population sizes.

In terms of *stability*, we can refine the above description of a stationary probability distribution to include the requirement that a population must behave in such a way that it has a relatively low probability of going extinct. For these purposes, both the lower limits of the stationary probability distribution and its absolute span and shape begin to make a difference

(Hildenbrandt et al. 2006). All else being equal, a population whose span of fluctuation is very high is more likely to hit zero, as is one with a lower floor, as is one with a bottom-heavy probability distribution. Extinction probability can be measured directly in terms of ***persistence***, which is calculated for the purposes of this project as the number of extinctions faced by a population divided by the total number of generations. Extinction probability will also be influenced by the magnitude of population fluctuations relative to typical population sizes (Turelli 1978), which will here be called ***constancy*** (Grimm & Wissel 1997). A population's size may be regulated, therefore, through density-dependent mechanisms, but the relative stability of its dynamics will depend on the details of those mechanisms. Theories of density-dependent regulation and how it might actually be achieved will be expanded upon below.

The Paradox of the Self-Limiting System

Density-dependent population regulation would imply that growth rates are themselves a decreasing function of density, and that through that inverse relationship the system is able to limit itself. Presented as pure theory such a model looks beautiful, and we can see evidence from the natural world that density-dependent regulation must exist. However, if one steps beyond a simple description of density-dependent regulation and seeks mechanisms for such regulation, a paradox emerges. The parameter which must be limited within the bounds of the system is growth rate, directly or indirectly, which can be seen as a function of age-specific fecundity and survivorship. If one speaks of placing limits *within the system itself* upon fecundity and / or survivorship, one runs across the problem that these are traits which, all else being equal, it would be in the interest of any individual to maximise. Indeed, direct selection for stability has no impact on either trait, and does not appear to influence stability either (Mueller et al. 2000). Stripped to its essentials, the question becomes that of the tragedy of the commons. If the maximization of each individual's gain means the downfall of all, how is it that civilization does not crash and burn? In the free-market scenario that people imagine imposed upon the natural

world, with no external hand to guide the system's progress, how is balance maintained?

In 1974, a paper was published by ecologist/mathematician Robert May which pointed out that simple discrete-time models of density-dependent population growth were able to show instability followed by chaotic behaviour, given sufficiently high values of the intrinsic growth rate. In other words, if density-dependence operates with a delay in feedback – a property of any discrete time model of population growth – then instability can easily result if the system is sufficiently sensitive to changes in density. This information was not in itself new, although the terminology used to describe it was. As mentioned earlier, the logistic equation had been developed 150 years earlier and had been in common use in ecology for fifty years. Its use had, by the 1970s, spread far and wide beyond the ecological implications which Verhulst had first described, finding its place in fields from physics to economics. The behaviour of the logistic equation was therefore well explored, as was its more recent descendent, the Ricker (1954) model. The concept that feedback delays can lead to unstable dynamics in a self-regulated system was an old one by the early 1970s. Robert May also asserted that similar behaviour to what he described as "chaos" had been described in meteorology ten years earlier (May, 1974).

However, Robert May's paper caused a large stir, and it may be interesting to speculate why this was the case. He included a question in his conclusion which may not have gained notice fifty years before, but that by the 1970s was growing to be a pressing issue. If a simple model of population growth can show instability arising from deterministic processes (as opposed to stochastic factors), then might such instability be possible in the natural world as well? At the time, this was just beginning to be an issue worthy of comment. By the 1970s, an awareness was growing that although nature might exemplify balance in the public imagination, it would not necessarily be able to bounce back from damage inflicted by the intrusion of civilization. The publication of "Silent Spring" by Rachel Carson in 1962 helped spur a growing consciousness of the environmental impacts of human activities. In 1970 the US passed its first environmental protection laws in response to rising levels of industrial pollution, coincident with the first Earth

Day. In 1971, Greenpeace was founded in Canada, and in 1972, Indira Gandhi spoke at the UN Conference on the Human Environment on the link between environmental protection and poverty alleviation. The 1970s saw a growing consensus in the scientific community regarding the possibility of global warming. The time was right, perhaps, for the suggestion that instability could be instigated more easily in the natural world than had been previously thought.

Several studies followed the publication of Robert May's 1974 paper which examined the stability of both natural and laboratory populations. Hassell et al. (1976) found that, across a range of insect populations, stability tended to be the norm, and measured parameter values were below those that would cause instability in the logistic or the Ricker models. Thomas et al. (1980) found stable dynamics across multiple species of *Drosophila*. Further studies by Mueller & Ayala (1981b) found that lab populations of *Drosophila* were asymptotically stable even in the face of initial perturbations. Turchin & Taylor (1992) studied long-term time series data of insect, mammal and bird populations in the wild, however, and found a range of dynamics in the populations examined, spanning from exponential stability to quasiperiodic oscillations and chaos. Ellner & Turchin (1995) analysed time-series data from a range of lab populations and found, as with natural populations, that dynamics ranged from stability to chaos. Guckenheimer et al. (1977) further pointed out that the earlier work by Hassell et al. (1976), which had found near-universal stability, used models that lacked overlapping generations and were therefore perhaps biased towards less complex dynamics. Regarding the tendency towards stability found by Mueller & Ayala (1981b), Mueller & Huynh (1994) hypothesized that this result may have been partly due to characteristics of the rearing conditions used which were in themselves stabilizing.

Based on the above, it is not possible to make broadly applicable generalizations regarding the natural stability or instability of populations, although chaotic dynamics (if one accepts that we have the tools to accurately distinguish chaos from biological noise) do appear to have been identified infrequently in those studies so far carried out. However, there are still many

questions that arise out of the previous sets of results. If it is possible for stability to evolve, through what avenues is it likely to do so? What biological traits of a population with the potential for additive genetic variation would lend themselves to stability? And given that dynamics are a function of both genes and the environment, what insights can be gained by decoupling these two factors?

These questions are pursued on a small-scale in the present study by examining the dynamics of crowded populations. Crowding has the potential to provide insight into population dynamics in several ways. In a fundamental sense, crowding lies at the root of theories of population regulation. From Verhulst (1838) to Nicholson (1933), early models of population dynamics have suggested that non-linear responses to density are key to the maintenance of stability. In terms of more recent theory, a trade off between fecundity and the ability to survive at high densities has been hypothesized as one route through which stability might evolve (Mueller & Ayala 1981b; Prasad et al. 2003). Two avenues have been taken here, looking at both immediate effects of larval crowding on dynamics and its ultimate effects, in populations which have evolved in response to larval crowding.

Chapter 2 will describe the dynamics of *D. melanogaster* populations which have been selected for adaptation to larval crowding for over 100 generations. These are the **M**elanogaster **C**rowded **U**ncrowded (MCU) populations, which were subjected to a 10 generation time series experiment alongside their controls, the **M**elanogaster **B**aseline (MB) populations. In the time series experiment, both sets of populations were subjected to the LH regime, a food regime previously found to be destabilizing in *Drosophila* populations, in which low levels of food are provided from the egg to pupal stages and high levels of food are provided to adults (Mueller & Huynh 1994; Sheeba & Joshi 1998).

Chapter 3 will present the results of further work that was done on the MCU and MB populations, looking at the possible evolution of age- and density-specific fecundity and survivorship, both of which have been theorized to be important for determining the dynamics of a population (Mueller 1988; Mueller et al 2000; Tung 2012). Two density-dependent relationships were examined: the effect of larval crowding on 21st day post-egg collection fecundity, and the effect of adult crowding on 11-21st day post-egg collection adult survivorship.

Chapter 4 will describe the dynamics of *D. melanogaster* populations which are adapted to standard lab conditions. These are the **J**oshi **B**aseline (JB) populations, which were here subjected to three regimes over the course of a 10 generation time series experiment, each an LH type regime, but with a different low level of larval food (1 mL, 2 mL and 3mL).

Experimental Populations and Maintenance Regimes

MB and MCU populations

The MCU (**M**elanogaster **C**rowded at the larval stage, **U**ncrowded at the adult stage) populations of *D. melanogaster* have been maintained for over 110 generations on a selection regime which subjects them to high levels of larval crowding and normal levels of adult crowding. These populations are maintained in four distinct blocks, each block having its corresponding MB (**M**elanogaster **B**aseline) control (maintained for over 120 generations). Background details for these populations are provided in Archana (2010).

Both the MB and MCU populations are maintained on 21-day discrete generation cycles, reared on standard corn-sugar-yeast-agar food. Flies are kept in 2.4 cm diameter x 9 cm height vials for the entirety of the egg, larval and pupal stages, and then collected as adults into 25 x 20

x 15 cm^3 Plexiglas cages where food plates are changed every alternate day. Food plates are provided on the 18th day post-egg collection that are covered with a thick layer of yeast paste in order to stimulate egg laying. Eggs are collected from a given generation on the 21st day post-egg collection, after flies are allowed to lay eggs on fresh food for 16 hours. Adults are then kept as back ups until the subsequent generation's egg collection 21 days later, and then discarded.

The MCU populations are maintained at densities of 550-600 eggs/1.5 mL food at the larval stage. From the 9th to the 16th day post-egg collection, freshly eclosed adults are dumped from vials into corresponding Plexiglas cages, which are maintained at densities of 1000-2000 adults in a cage. The MB populations are maintained at 60-80 eggs/6 mL food at the larval stage, and eclosed adults are collected into cages on the 11th day post-egg collection. For both the MB's and the MCU's, adult density in cages is manually brought down to 1000-2000 adults in order to prevent crowding at that stage, if the density has gone above 2000 in a cage. If the MCU survivorship is low, the fact is noted at the pupal stage and eggs collected once more from backup populations in order to avoid population size bottlenecks.

JB populations

Background details for the JB (**J**oshi **B**aseline) populations of *D. melanogaster* are provided in Sheeba et al. (1998). The JB populations have been maintained under standard lab rearing conditions as four replicates for over ten years. Each replicate is maintained on a 21-day discrete generation cycle, on banana-barley-jaggery-yeast-agar food. As above, flies are kept in 2.4 cm diameter x 9 cm height vials for the entirety of the egg, larval and pupal stages, with 60-80 eggs/6 mL of food collected into each vial. On the 12th day post-egg collection, eclosed adults are transferred to fresh vials with ~4 mL banana food. Food change is given every alternate day until the 18th day, when adults are dumped into 25 x 20 x 15 cm^3 Plexiglas cages. Adult density is manually brought down to 1000 – 2000 flies per cage if the density exceeds that number.

Food plates are provided on the 18th day post-egg collection that are covered with a thick layer of yeast paste in order to stimulate egg laying. Eggs are collected from a given generation on the 21st day post-egg collection, after flies are allowed to lay eggs on fresh food for 16 hours. Adults are then kept as back ups until the subsequent generation's egg collection 21 days later, and then discarded.

Chapter 2: Evolution of Stability

Introduction

Several theories have been developed in the past thirty years regarding possible mechanisms for the evolution of stability. Those which apply to a single species model will be reviewed below.

1. Thomas et al. (1980) suggested that stability might evolve through group selection. The argument here is that patches with unstable dynamics in a population would have a higher chance of extinction than patches with stable dynamics, and that a patch which has gone extinct would be more likely to be successfully recolonized by a patch that demonstrates relatively higher stability. The basic assumption, of course, is that genes must play a uniformly larger role than environment in the determination of stability, which places fairly specific requirements on both the genetic and environmental structuring of the global population across all patches. Consequently, the applications of this theory are slightly limited.

2. Theories have also been put forward that stability might evolve through selection acting at the level of the individual, which would imply that direct selection for stability would cause (a) the evolution of a relative increase in stability, and (b) a corresponding shift in traits associated with population stability (Hansen 1992; Ebenman et al. 1996). The problem with this kind of theory was discussed in chapter 1: direct selection for a lower intrinsic growth rate may not be possible, since all else being equal the individuals that are favoured by selection will be those with higher survivorship and/or fecundity. Indeed, attempts to directly select for stability have thus far failed (Mueller et al. 2000).

3. Finally, a third class of theories have suggested that stability might evolve through trade offs among individual life-history traits such that traits enhancing population stability can evolve due

to trade offs with other traits favoured by direct selection (Turelli & Petry 1980; Mueller & Ayala 1981b, Stokes et al. 1988; Gatto 1993; Ebenman et al. 1996). This is the theory with the most empirical support thus far, and the basis for the current set of experiments.

The evolution of stability via trade offs between life-history traits

May (1974) highlighted the fact that in the logistic and Ricker models of population growth, manipulations of the parameter *r*, or the intrinsic growth rate, could influence the stability of a population. As discussed in chapter 1, however, direct selection for a reduction in *r* may prove problematic. Rather, one could theorize that stability might be enhanced via direct selection for traits which trade off with *r*, indirectly resulting in a reduction in the intrinsic growth rate and therefore enhanced stability.

Several experiments in the past ten years have given empirical support for this theory. Prasad et al. (2003) and Dey et al. (2008) found that populations of *D. melanogaster* selected for faster development demonstrated greater stability than their controls, which was presumed to be partly a consequence of trade offs between faster development on the one hand and fecundity and pre-adult survivorship on the other. Dey et al. (2012) found that populations of *D. ananassae* selected for adaptation to larval crowding also showed higher stability than their controls. A possible trade off between *r* and *K* was implicated in that study, with a significantly higher *K* in the selected populations than controls and a large, though non-significant difference in *r*, with lower values of *r* in the selected populations.

Density-dependent selection and population dynamics

Theories of density-dependent selection were first described verbally by MacArthur & Wilson (1967), and expanded upon mathematically soon after by several others (Gadgil & Bossert 1970; Roughgarden 1971; Clarke 1972; Asmussen 1983). The crux of these theories is that the fitness of a genotype is a function of density, with genotype-specific growth rates taken as a surrogate of fitness. Thus, to each genotype can be assigned the genotype-specific parameters *r* (intrinsic

growth rate) and *K* (equilibrium population size). The theory here, which bases itself on models of density-dependent population growth, is that population growth rate, and therefore fitness, is maximized at low densities primarily by a high *r* and at high densities primarily by a high *K*. If one further assumes an *r*-*K* trade-off, then populations adapted to low densities should evolve a higher *r* and a lower *K* than populations adapted to high densities, given sufficient initial levels of additive genetic variation in these populations for the traits that influence *r* and *K*. Another way of stating this is that, through its influence on *r* and *K*, density-dependent selection would be presumed to impact density-specific population growth rates. This prediction was backed up empirically by Mueller & Ayala (1981a), who demonstrated that populations of *D. melanogaster* adapted to high population densities showed higher growth rates at high densities than populations adapted to low population densities, and vice versa when growth rates were measured at low densities.

Given the theory that density-dependent selection acts on population parameters which could in turn be theorized to influence dynamics, it would make sense to propose that density-dependent selection is one avenue through which stability might evolve (Mueller et al. 2000; Mueller & Joshi 2000; Dey et al. 2012). Two sets of studies have been done prior to the current one which examined the effects of adaptation to larval crowding on population stability in *Drosophila*, with varying results. Mueller et al. (2000) did not find any evidence for the evolution of greater stability in populations of *D. melanogaster* adapted to larval crowding, and suggested that if it were possible for stability to evolve it might require a longer period of time for that to occur. However, Dey et al. (2012) found evidence for the evolution of greater stability in populations of *D. ananassae* adapted to larval crowding, and tentative evidence for a corresponding *r*-*K* trade off.

As discussed by Dey et al. (2012), the contrast in the above results may be a consequence of the two sets of crowding-adapted populations in question having followed different evolutionary trajectories in response to larval crowding. Crowding at the larval stage produces not one but

multiple selection pressures, and subtle differences in the ecology of the selection regimes involving larval crowding may cause different sets of pressures to dominate. Consequently, responses to larval crowding can vary in fundamental ways. Details of the diverging arrays of traits witnessed in populations of *Drosophila* subjected to crowding will be discussed in the concluding chapter. However, for now it can be said that the populations of *D. melanogaster* under current study (the MCU populations) have been subjected to larval crowding under a regime very similar to that used on the *D. ananassae* ACU populations described in Dey et al. (2012), and have thus far shown evidence of a similar evolutionary trajectory to those populations.

The current study aimed to examine whether greater population stability had evolved in the MCU populations, whose background and maintenance was described in the first chapter. The logic behind this experiment was twofold. One is that, as mentioned above, the MCU populations demonstrate evidence of having followed a similar evolutionary trajectory to the ACUs (Dey et al. 2012) in their response to larval crowding. In both ACU and MCU populations feeding rates did not evolve, waste tolerance showed uneven patterns of partial evolution in some assays but not others, and development time has evolved to become shorter, as compared to controls (Archana N. 2010; A. Mital, G. Vaidya & A. Joshi, unpublished data). Given that the ACUs did evolve greater stability (Dey et al. 2012), it is natural to ask if the MCUs might have as well. Beyond that, however, the MCUs show further evidence of conforming to classic scenarios of evolution under density-dependent selection. The MCUs appeared to have evolved higher food-to-biomass conversion efficiency at the larval stage (D. Ravi Teja, S. Dey & A. Joshi, unpublished data) and also a reduced body size at eclosion (M. Sarangi, S. Dey & A. Joshi, unpublished data), both of which might result in higher *K*, which even in the absence of a lower *r* can lead to greater stability (Dey et al. 2012). The question to be asked, therefore, was whether the MCUs had evolved a lower *r* and a higher *K* than their controls. The study described in this chapter pursued this question by looking at the population

dynamics of the MCU and MB populations under a destabilizing maintenance regime. In the next chapter, patterns of density-dependence in life-history traits that might influence stability in the MCUs will be examined.

Materials and Methods

Population Dynamics Experiment

Ten single-vial populations were derived from each of the four MB and MCU populations, and their dynamics studied for ten generations under a food regime which has been demonstrated to be destabilizing, resulting in relatively large fluctuations in population size (Mueller & Huynh 1994; Sheeba & Joshi 1998). Under this regime, termed the LH regime (**l**ow larval food levels, **h**igh adult food levels) larvae are provided low food levels and adults are provided normal food levels, with supplementary yeast paste provided for three days prior to egg laying. Here, the food level chosen for the larval stage was 2 mL of corn meal food.

Vial populations were each initiated with 8 adult females from the given MB or MCU population which had been standardized for one generation at normal food levels and egg densities (~60-80 eggs/6 mL of food) in order to eliminate non-genetic parental effects. After laying eggs for 24 hours in vials with exactly 2 mL of food, adults were discarded. In all subsequent generations, population number was not controlled. From the 8^{th} to 17^{th} days post-egg collection, freshly eclosed adults were transferred from egg collection vials into corresponding adult collection vials with ~4 mL of food, with vial identities maintained across generations. Adults were transferred to fresh food every alternate day. On the 17^{th} day, egg collection vials were discarded in order to avoid collecting any eclosing flies from the next generation. On the 18^{th} day post-egg collection, adults were transferred into yeast vials, with ~4 mL of food and a dab of yeast paste on the vial wall. They remained in these vials until the 21^{st} day post-egg collection, when adults were transferred to egg collection vials with 2 mL of food

in each vial. For each generation after the first, adults were allowed to lay eggs for 16 hours, in order to ensure that any adults which might have died were removed before any larvae hatched.

At the end of 16 hours, adults were transferred to empty vials and frozen for counting and dry weight measurement. The census included all flies that had died during the egg laying period. After being censused, dead flies were kept at 64°C for 36 hours and then weighed. All of the flies from a given vial were weighed together, and then average dry weights calculated based on census data. Time series analyses were then performed on the census and dry weight data.

Measures of Stability

No extinctions were observed over the course of this experiment, so stability in terms of persistence (or the probability of extinction) could not be assessed. Rather, stability was measured in terms of constancy stability (Grimm & Wissel 1997), for which two indices were used.

The coefficient of variation (CV) was calculated as the ratio of the standard deviation in the time series data to the population mean, for a given vial population. Here σ = standard deviation and μ = population mean.

$$\mathrm{CV} = \frac{\sigma}{\mu}$$

The fluctuation index (FI) (Dey & Joshi 2006) was calculated as the sum of the absolute step-wise differences between subsequent time series data points, scaled by the population mean and the number of generations. Here T = total number of generations in the time series, $\bar{N}$ = population mean, N_{t+1} = population number in generation t+1 and N_t = population number in generation t.

$$\mathrm{FI} = \frac{1}{(T\bar{N})}\sum_{i=0}^{T-1}\mathrm{abs}(N_{t+1} - N_t)$$

Persistence stability was measured as the probability of extinction, taking the ratio of the number of extinctions across generations in a given vial population to the number of generations.

Calculating population growth parameters

The parameters *r*, *K* and α (= $\frac{r}{K}$) from the Ricker (1954) model of population growth were indirectly estimated by taking a linear regression of the plot of ln $\left(\frac{N_{t+1}}{N_t}\right)$ versus N_t where N_t = population number at generation *t*, and N_{t+1} = population number at generation *t*+1. Given a linear regression of this plot, the x-intercept was taken as *K* (population size when growth rate = 0), the y intercept was taken as a reflection of *r* (growth rate when population size = 0) and the slope of the line was taken as α, representing the strength of density-dependence.

Statistics

Statistical analyses were carried out using STATISTICA v.5 (Statsoft Inc, Tulsa, OK, USA). All estimated descriptors or parameters of population growth were subjected to a mixed model analysis of variance (ANOVA) treating selection regime as a fixed factor and block as a replicate factor.

Results

No difference in constancy stability after ten generations

CV and FI were compared separately between the MB and MCU populations. The MB and MCU populations showed no significant difference either in terms of CV (p = 0.947) or FI (p = 0.525) (Figure 2.1).

Evolution of a higher K, possible trend towards a lower r

Based on values of r, K and α estimated from the linear regression of $\ln(N_{t+1}/N_t)$ versus N_t, an ANOVA was performed to compare the MBs and MCUs. The MCU populations had a significantly higher K than the control MB populations ($p = 0.004$), a significantly less negative α ($p = 0.010$)) and no difference in r ($p = 0.341$). There was, however, a trend towards a lower r in the MCUs (Figure 2.2).

It was observed that the graph of $\ln(N_{t+1}/N_t)$ versus N_t was not approximately linear but appeared to have different slopes at low versus high values of N_t (Figure 2.3). Consequently, a second analysis was done in which two separate linear regressions were fitted to the data and the slopes (α) calculated separately low and high values of N_t separately, and then compared. The data were split roughly into these two categories by fitting a second order polynomial to the graphs for individual block × selection regime combinations and taking the root of the equation as the approximate N_t cut off (Figure 2.3).

An ANOVA done on values of α estimated from low density data found a non-significant trend towards a less negative α in the MCUs ($p = 0.060$). At high densities, α did not differ significantly between the MCU and MB populations, and neither were there any clear trends ($p > 0.4$) (Figure 2.4).

Higher overall realized growth rate in the MCUs

Realized growth rate ((N_{t+1}/N_t) was compared between the MB and MCU populations by performing an ANOVA with selection and population size bin as fixed factors and block as a random factor. Realized growth was found to be significantly higher overall for the MCUs than the MBs ($p = 0.033$) when calculated across six population size bins of 50 individuals each, with the last bin including all values over 250 (Figure 2.5). Bins of 50 individuals were used so that all bins could encompass all blocks, which was not possible at lower bin sizes due to the scarcity

of data points in some regions. However, there was a concern that using bins of fifty might average out the actual differences between the populations, since large changes in realized growth rate might occur over intervals of N_t lower than 50. Additionally, at low population densities (< 40) the majority of data points for the MCUs belonged to the first generation, in which populations were started off with 8 females (counted as a census of 16), and there was a worry that this predominance of very low values might be biasing results towards a higher realized growth rate for the MCUs than was typical.

Consequently, the test was repeated with bins of 20, which eliminated some parts of the population distribution (< 40, 160 – 190, > 250). Results are shown in Figure 2.6, below. Taking all nine bins together, realized growth rate is significantly higher for the MCUs than the MBs ($p = 0.008$), and a significant interaction effect of selection × bin appears ($p = 0.0002$), with the difference between the MB and MCU realized growth rates more distinct at low than at high densities (Figure 2.6).

MCUs maintain a higher average population size than the MBs

The average population size for the MCUs was significantly higher than that of the MBs ($p = 0.0011$) (Figure 2.7a). This can be seen quite clearly in the population size distribution for the two populations (Figure 2.7b). *(Note that the population size distribution disregards the first generation, in which size was controlled.)* The MCUs regularly reached population sizes greater than 300, whereas the MBs seldom went above 250. Additionally, population troughs tended to be lower for the MBs than the MCUs. Overall, however, the population size distribution is broader for the MCUs than it is for the MBs.

Figure 2.1 – a) Coefficient of variation across blocks for MB and MCU time series data (MCU ~ MB, $p = 0.947$), b) Fluctuation index across blocks for MB and MCU time series data (MCU ~ MB, $p = 0.525$). Error bars show standard deviations calculated across the 10 replicate vial populations in each block × selection regime combination. Light grey = MB, dark grey = MCU.

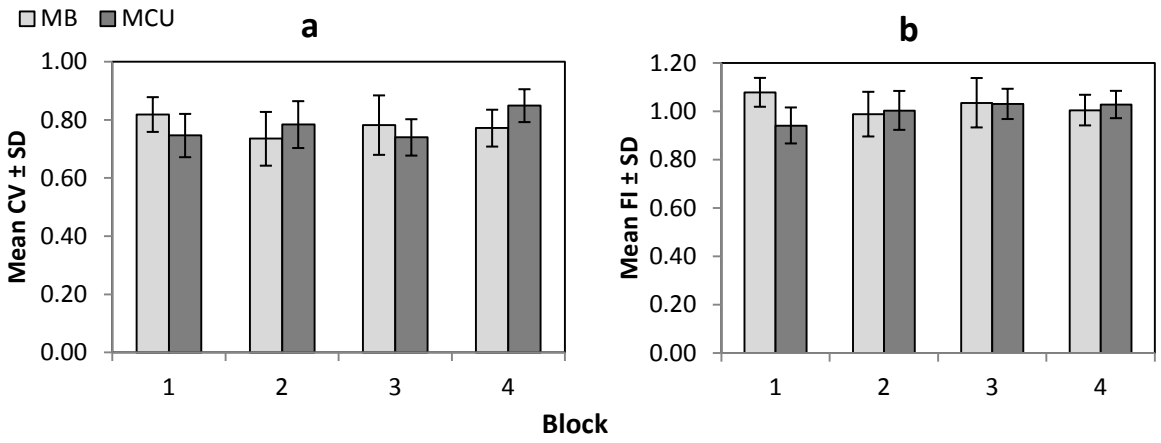


Figure 2.2 – The population growth parameters *r*, *K* and α as estimated from linear regressions of the plot of N_t versus $\ln(N_{t+1}/N_t)$. a) *r* calculated across four blocks for the MB and MCU populations (MCU ~ MB, $p = 0.341$), b) *K* calculated across four blocks for the MB and MCU populations (MCU > MB, $p = 0.004$), c) α calculated across four blocks for the MB and MCU populations (MCU > MB, $p = 0.010$). Light grey = MB, dark grey = MCU.

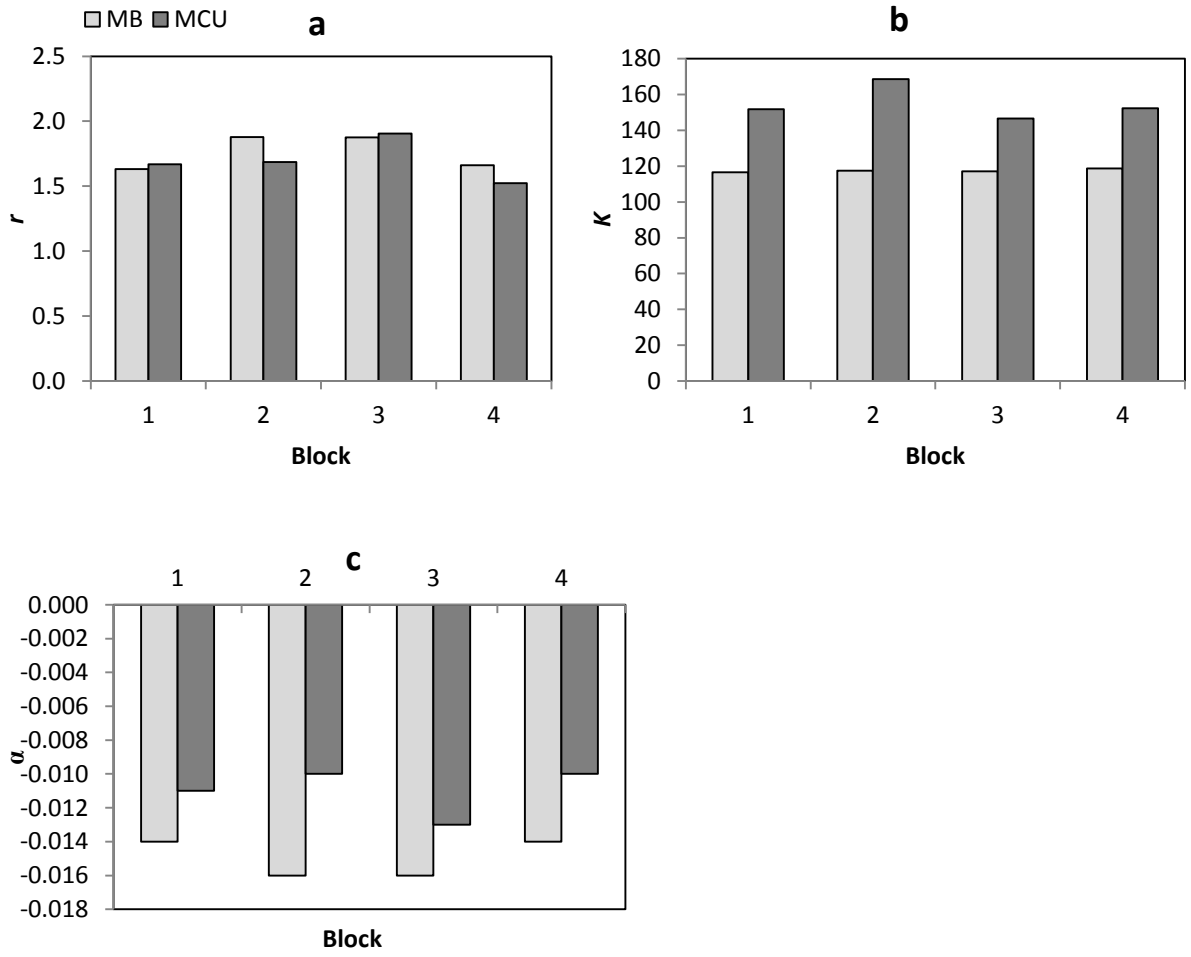


Figure 2.3 – The plot below was divided into high and low densities by taking the root of a second order polynomial regression as a cut off for N_t. Slopes were then calculated for either density by taking linear regressions.

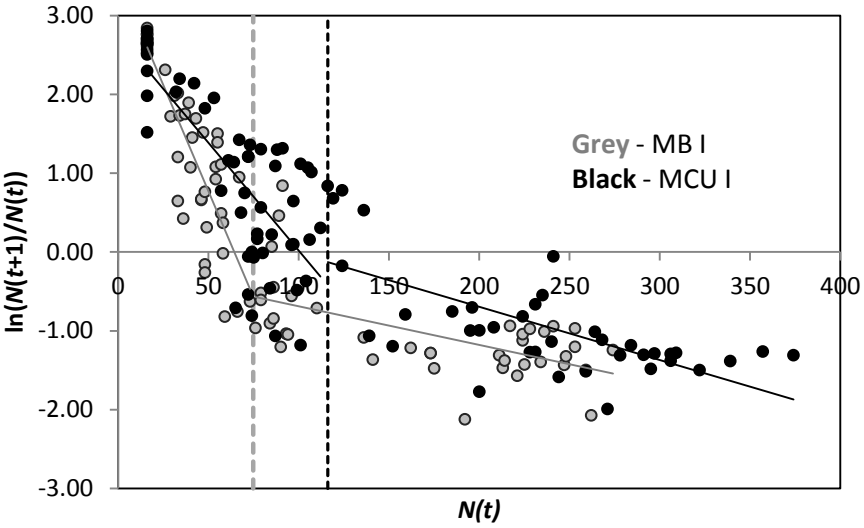


Figure 2.4 – The strength of density dependence (α) calculated from growth rates at low density (a), and high density (b), respectively. Light grey = MB, Dark grey = MCU.

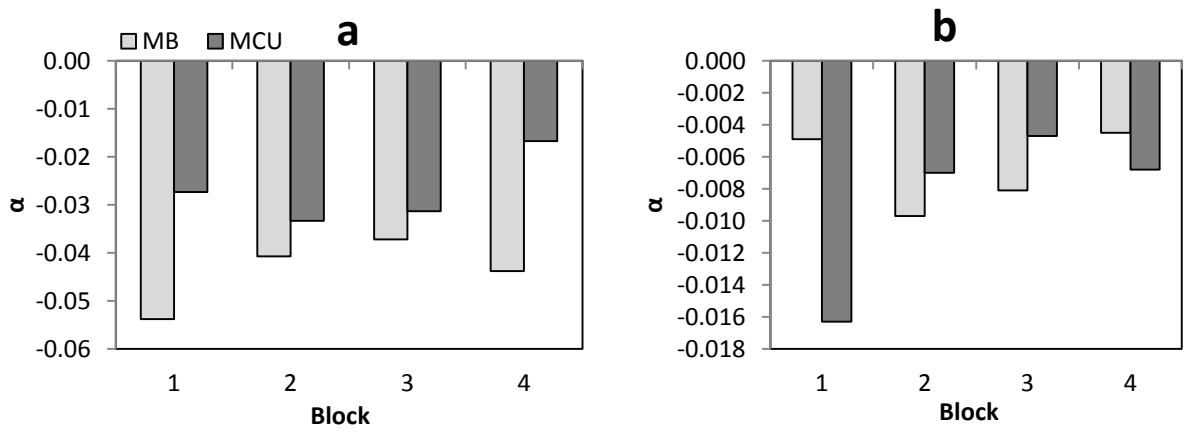


Figure 2.5 – Realized growth rate (N_{t+1}/N_t) plotted against population size in bins of 50, from 0 to > 250. Error bars show standard deviations calculated across the 10 replicate vial populations in each block × selection regime × bin combination. Light grey = MB, Black = MCU. (MCU > MB, $p = 0.033$)

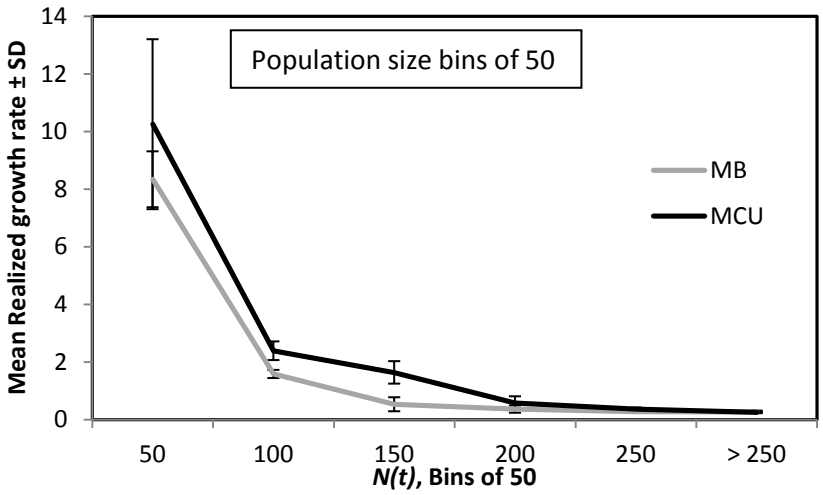


Figure 2.6 - Realized growth rate (N_{t+1}/N_t) plotted block-wise against population size in bins of 20, from 40 – 160, and from 190 - 250. Numbers on the x axis give the upper limit of each bin. Error bars show standard deviations calculated across the 10 replicate vial populations in each block × selection regime × bin combination. Light grey = MB, Black = MCU. The zero growth line ($N_{t+1} = N_t$) is plotted as a dotted line. (MCU > MB, $p = 0.008$; Selection × bin significant at $p = 0.0002$)

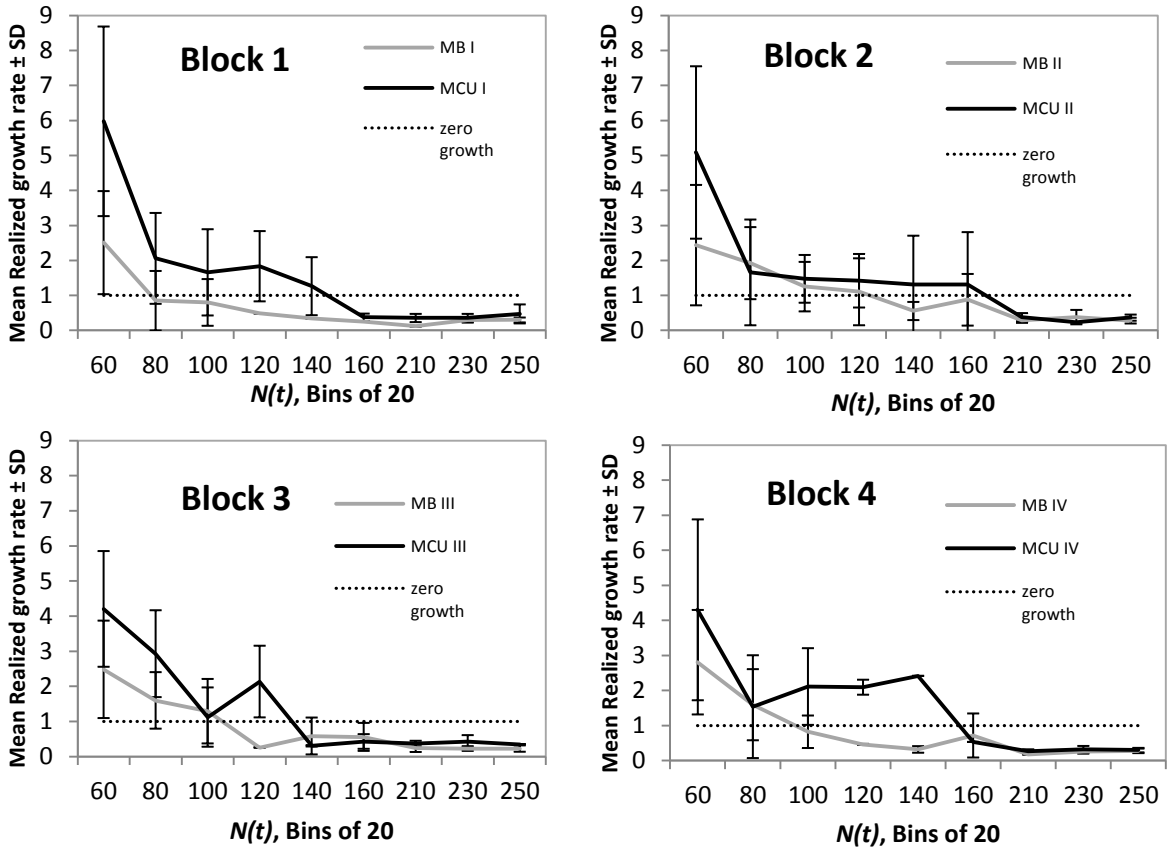
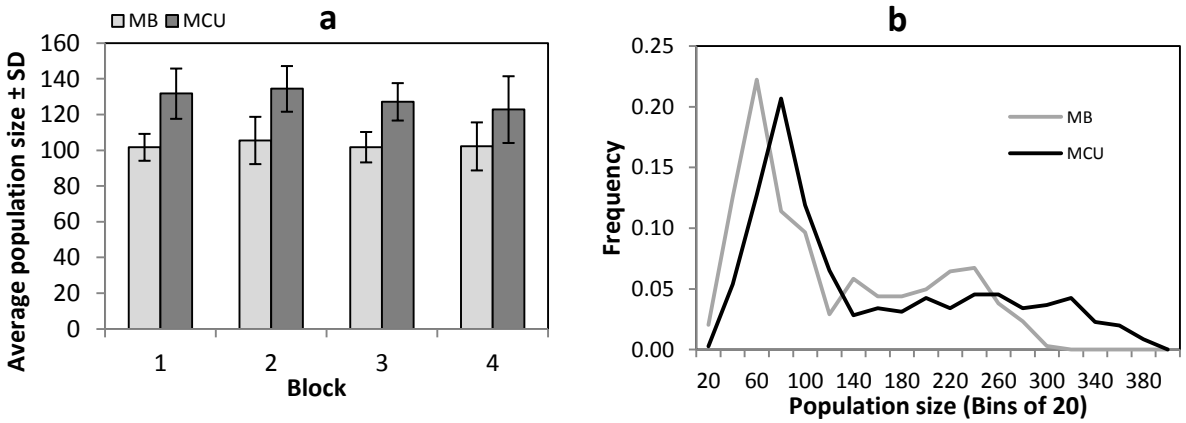


Figure 2.7 – a) Average population size , b) population size distribution. Error bars show standard deviations calculated across the 10 replicate vial populations in each block × selection regime combination. Light grey = MB, Dark grey = MCU.



Discussion

Based on the results above, some correlates of stability appear to have evolved in the MCUs, but in the given LH regime with 2 mL of food these do not appear to have led to detectable changes in stability. In terms of constancy stability, the dynamics of the MCUs showed no significant difference from the MBs. Further, the differences that did exist in CV and FI between the two populations showed no clear trend. Persistence could not be measured for the time series data collected here, since neither type of population showed any extinctions. One could speculate that given more than ten generations extinctions might have occurred, but if one looks at the histogram of population size distributions across both the MBs and the MCUs (Figure 2.7b), it can be seen quite clearly that no near-extinctions were encountered by either type of population.

An explanation for the above results becomes somewhat complicated if one tries to integrate it with the rest of the findings. The MCUs show a significantly higher *K* than the MBs (Figure 2.2b), and a correspondingly high average population size (Figure 2.7a). The MCUs were able, in other words, to maintain a higher population size overall than the MBs, regardless of the degree of their population size fluctuations. The reason why this would not necessarily lead to higher constancy stability can be seen in Figure 2.7b: as the distribution of population sizes shows, although the average population size was higher for the MCUs than the MBs (given both higher troughs and higher peaks), the absolute difference between the upper and lower bounds of population size was higher for the MCUs than it was for the MBs. The strength of density dependence (α) was significantly less negative for the MCUs than the MBs (Figure 2.2c), indicating that realized growth rate dropped at a slower rate with increasing density for the MCUs than for the MBs. There was no significant difference in *r* between the two populations (Figure 2.2a), but there was a trend towards a slightly lower *r* in the MCUs.

This curious division in the rate of change of growth with density between low and high densities can be seen qualitatively in both Figure 2.3 (which demonstrates the method by which

dynamics were split into high and low densities) and in Figures 2.5 and 2.6, which show how realized growth rate fell with increasing density. In each case, a change is found at a point which corresponds roughly with the estimated carrying capacity, or zero growth zone. Before that point, at low densities, realized growth rate is higher for the MCUs than it is for the MBs, and it falls at a somewhat more gradual rate with increasing density than it does for the MBs (Figure 2.4a). This observation shows up qualitatively in Figure 2.6, and can also be explained in terms of the higher K and less negative α for the MCUs. After that point, at high densities, the two populations behave in a statistically indistinguishable manner, with no trends or significant differences in α. (Figure 2.4b)

It is somewhat beyond the scope of the present study to give a complete explanation for the above results. However, there are several points worth noting which might shed some light on the apparent contradictions described above.

Given the results that there were no extinctions and no near-extinctions in either population, it is possible that the regime used was not destabilizing enough for differences in persistence stability to show up. As will be discussed in chapter 4, a time series experiment done in parallel to this one found that the larval food level used here (2 mL) was possibly at one end of a zone in which dynamics change dramatically, with 1 mL of larval food resulting in unstable dynamics with frequent extinctions while 2 and 3 mL are statistically indistinguishable and largely stable (see chapter 4). Also, here adults were only given 16 rather than 24 hours to lay eggs, potentially reducing r and thus enhancing constancy stability. In a more truly LH type environment, it is possible that differences in stability would become statistically detectable.

Secondly, given the non-linear plot of $\ln(N_{t+1}/N_t)$ versus N_t , it appears that the Ricker Model (based upon which it is assumed that the plot of $\ln(N_{t+1}/N_t)$ versus N_t can be approximated by a line from which r, K and α are calculated) does not contain enough complexity to capture the actual dynamical differences between the MBs and the MCUs. The Ricker Model allows for a very basic type of density dependence: the relationship between growth rate and density is

determined by two factors which are fixed and intrinsic to a population, that is to say, *r* and *K*. As will be outlined in chapter 3, how growth rate changes with density in the *Drosophila* system is actually a function of more than one age-specific, density-dependent feedback loop that impacts life-history traits. In that light, the fact that a population adapted to larval crowding might show more distinct responses to increased density from its control at one density and less distinct at another is not, in fact, entirely surprising. It may simply be that the model of population growth used here is not complex enough to allow for those differences in dynamics to be thoroughly characterized.

It cannot be said from these results if stability has evolved in the MCUs or if it has not. Rather, what the results imply is that a few more complications need to be added to the question itself. Under LH conditions with 2 mL of larval food, MCUs are indistinguishable from the MBs in terms of constancy stability, calculated either as CV or FI. However, the MCUs appear to possess several characteristics which would lend themselves to more stable dynamics given a stronger perturbation. They demonstrated the ability to maintain a higher average population size, shallower troughs than the MBs, and a more moderate response in growth rate to density at densities below *K*. The fact that differences in sensitivity to density in terms of realized growth rate were significantly more marked at low than at high densities is worthy of note, and suggests that if it is possible for stability to evolve via density-dependent selection, it may do so in ways that do not necessarily conform to predictions based on simple models of density-dependent population growth.

The next chapter will expand upon several of the questions raised in this and the preceding chapter, and look at density dependent relationships of life-history traits which influence dynamics in the MBs and the MCUs.

Chapter 3: Dynamics and life-history traits.

Introduction

The intrinsic stability of a population depends in part on a range of age-specific, density-dependent feedback loops which impact life-history traits. Simple models of population growth such as the Ricker or Logistic model compress the effects of these feedback loops into a single hump-shaped function with two parameters *r* (intrinsic growth rate) and *K* (carrying capacity). As seen in the previous chapter, such a compression of multiple density-dependent relationships (which may themselves trade off with each other) may be too much of an oversimplification for these purposes. Given a population subjected to density-dependent selection over many generations, one could hypothesize that some of the factors underlying a change in dynamics may be density-dependent themselves.

As described comprehensively by Prout & McChesney (1985), larval crowding may have non-linear effects on both fecundity and survivorship in *Drosophila*. Combined with this, adult crowding may also have non-linear effects on fecundity (Mueller & Huynh 1994) and survivorship (Joshi et al. 1998). Mueller (1988), later expanded upon by Tung (2012), incorporated some of these non-linear relationships into models of *Drosophila* population growth. The density-dependent and –independent factors which were thought to impact growth were included in these models as follows:

1. Egg to larval survivorship (density-independent)
2. Larval to adult survivorship (a function of larval density)
3. Mean female fecundity (a function of female body size and therefore larval density)
4. Effect of adult density on female fecundity.

Of these relationships, the last one (the effect of adult crowding on female fecundity) was found to be most relevant to the dynamics of a population (Mueller 1988). In other words, if female fecundity dropped swiftly with adult density, dynamics would tend to be more stable. Consequently, if one reduced the sensitivity of fecundity to adult density with the application of yeast, instability would increase (Mueller & Huynh 1994).

The above model is relevant to crowding adapted populations in several ways. One could hypothesize that adaptation to crowding could involve changes in the density-dependent relationships between crowding, survivorship and fecundity. Earlier theoretical work suggested that stability might evolve through density-dependent selection via a trade off between fecundity and ability to survive at high densities. (Mueller & Ayala 1981b) Additionally, some evidence exists of a trade off between adaptations to larval and adult crowding (Joshi et al. 1998). Consequently, one could theorize that adaptation to larval crowding might increase the deleterious effects of adult crowding on fecundity and survivorship and thereby lead to the evolution of greater stability.

For the purposes of the present study, the non-linear effects of density on life-history traits have been broken down into four chief areas, summarized in the schematic in Figure 3.1.

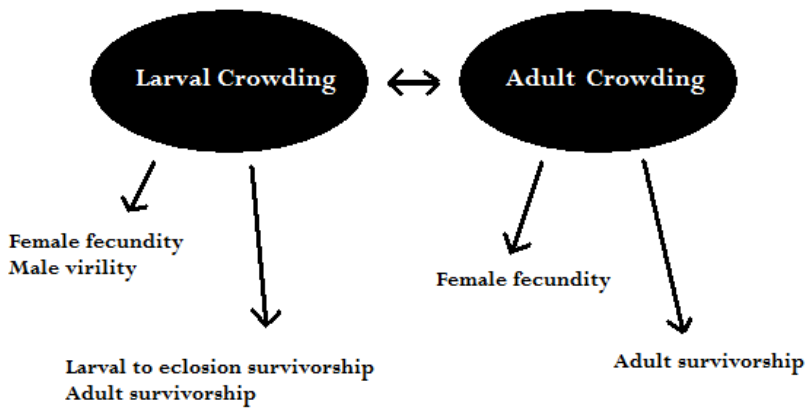
1. **Effect of larval crowding on survivorship:** This relationship has already been looked at in the MCUs across a wide range of larval densities (M. Sarangi, S. Dey & A. Joshi, unpublished data). At high larval densities, MCU survivorship is significantly higher than MB survivorship. Some evidence exists, however, that this advantage disappears or even reverses at low larval densities (G. Vaidya, A. Mital, M. Sarangi, S. Dey & A. Joshi, unpublished data).
2. **Effect of adult crowding on survivorship:** A possible trade off between adaptation to larval crowding and adult survivorship in the face of adult crowding has been witnessed in two previous instances, in the *D. melanogaster* CU populations (Joshi et al. 1998) and the *D.*

ananassae ACU populations (Snigdhadip Dey, personal observation).

3. **Effect of larval crowding on fecundity:** Initial studies (at the 40th generation of MCU selection) of the relationship between larval crowding and fecundity in the MCUs and MBs found no difference in fecundity between the two populations (Archana N. 2010), but it was hypothesized that a difference might have developed in the subsequent 70 generations of selection.
4. **Effect of adult crowding on fecundity:** Previous studies of populations adapted to larval crowding found no difference in the relationship between adult crowding and fecundity between the *D. melanogaster* CU populations and their controls (Mueller et al. 2000). However, given the range of differences in the evolutionary trajectories that the CU populations and the MCU populations have followed (see concluding chapter), it was thought that this relationship might be worth looking into here.

Ultimately, two relationships were examined between life-history traits and density: the effect of larval crowding on 21st day female fecundity and the effect of adult crowding on adult survivorship from days 11 to 21 post-egg collection.

Figure 3.1 – Potential damaging impacts of larval and adult crowding on life-history traits. There may be trade offs between adaptation to larval and adult crowding.



Materials & Methods

Effect of larval crowding on fecundity

The effects of larval crowding on fecundity were examined across three blocks for the MCU (~ Generation 113) and MB populations (~ Generation 125), after one generation of standardization under normal rearing conditions.

Three levels of larval crowding were used: 75 eggs in 2 mL of food, 150 eggs in 2 mL of food and 300 eggs in 2 mL of food. Eggs were counted individually onto thin agar sheets, which were then cut and placed into egg collection vials. For each population, 75 eggs were collected into 10 vials, 150 eggs into 5 vials and 300 eggs into 5 vials each. Adults were collected daily for fecundity and dry weight from the 9^{th} to 11^{th} day post-egg collection. The rest of the distribution was not looked at, since it was observed that the majority of the flies in the 75 and 150 e/v conditions had eclosed by the 10^{th} day, and likewise for the 300 e/v condition by the 11^{th} day. Further, one concern was that collecting late emerging flies might have introduced age-related complications into measurements of fecundity.

At the time of collection, all flies which had emerged over the course of the previous day were partitioned into two: one set for fecundity counts, and another for dry weight measurements. Details of both are given below.

Fecundity Counts

From the 9^{th} – 11^{th} day post-egg collection, a total of 12 vials with ten flies each (5 female, 5 male) were collected per condition for later fecundity measurements. Since these twelve vials were collected over the course of three days, attempts were made to partition the vials according to the distribution of emergence. This was done by making a rough estimate of what percentage of the total had emerged on a given day, based on the number of flies emerged, a rough ratio of

eclosed:darkened pupae, and predicted survivorship in a given condition (higher for MCUs than MBs). Naturally, this was still a very rough estimate, but it was reasoned that such an estimate was sufficient based on the fact that there were no visible size differences between flies emerging in the first three days, and the peaks of the distributions were relatively narrow for all (~24 hours).

Flies were maintained from the 9^{th} – 17^{th} days post-egg collection in vials with ~ 4 mL of corn food. Food was changed every alternate day. On the 18^{th} day post-egg collection, flies were shifted into vials with a dab of yeast paste on the wall, in which they remained until the evening of the 20^{th} day. On the evening of the 20^{th} day, flies were transferred to vials for fecundity counts, in which a thin (~ 0.5 mL) layer of food had been poured for the purpose of egg counting. Flies in a given condition were first collected into a single vial in order to thoroughly mix replicates, and then separated out into male/female pairs under CO_2 anaesthesia. For each condition, 12 replicate vials with 1 pair of flies each were used for fecundity counts. Flies were allowed to lay eggs for 16 hours and then removed on the morning of the 21^{st} day, after which adults were removed and discarded, and fecundity vials kept at -20°C for later egg counting. The above schedule and method for egg collection was used in order to replicate conditions used for the time series experiment described in chapter 2, since the experiment was meant to specifically address the MB/MCU dynamics observed there.

Dry weight measurement

From the 9^{th} – 11^{th} day post-egg collection, flies remaining after a sufficient number had been collected for later fecundity counts were frozen for dry weight measurement. Flies were sorted for dry weight measurement as follows: all flies collected across vials for a given condition were pooled and mixed thoroughly, out of which 10 replicates of either 10 males or 10 females were each set up at random. Flies were dried at 64°C for 36 hours, after which each replicate was

weighed to the nearest milligram. Individual fly weights were then calculated by dividing the value for each replicate by ten. Note therefore that there was no one-to-one correspondence between dry weight replicates and fecundity count replicates. Average dry weight measurement for specific selection regime × larval density combinations indicate the pooled average for the 3 day distribution over which flies were also collected for fecundity counts.

Effect of adult crowding on adult survivorship

The effect of adult crowding on adult survivorship from day 11 to 21 post-egg collection was examined across four blocks for the MCU (~ Generation 113) and MB populations (~ Generation 125), after one generation of standardization under normal rearing conditions. The protocol used here partly mirrors that used by Joshi et al. (1998), since one aim was to allow for a comparison with previously studied crowding-adapted populations derived from the same initial stock.

Eggs were collected from standardized cage populations of the MB and MCU populations at a density of ~60-80 eggs/6 mL corn food. On the 11th day post-egg collection, adults were collected from these vials to set up two adult crowding regimes. These were 7 replicates each of *low* adult density (50 adults) and *high* adult density (150 adults), both collected with a 1:1 sex ratio. Attempts were made to maintain a similar volume across adult collection vials. Vials were chosen of similar height, thickness and diameter, and marked at a consistent height from the bottom so that plugs could be maintained at similar heights. Note that this could not be exact, however, since the shape of the cotton plugs could not be completely controlled. Additionally, food levels in adult collection vials were maintained at exactly 3 mL per vial.

Statistics

Statistical analyses were carried out using STATISTICA v.5 (Statsoft Inc, Tulsa, OK, USA). All estimated life-history trait values were subjected to a mixed model analysis of variance (ANOVA) treating selection regime and crowding (larval/adult) as fixed factors and block as a

replicate factor.

Results

Trend towards lower fecundity in MCUs

There was no significant effect of selection regime on fecundity ($p = 0.156$) and no significant selection regime × larval density effects ($p = 0.570$). There was a fairly consistent trend, however, towards lower fecundity in the MCUs than in the MBs (Figure 3.2). This trend persisted when block means for fecundity counts were plotted against dry weight, as did the lack of interaction between selection and density (Figure 3.3).

Dry weight across selection lines, sexes and densities

Since the differences in fecundity across larval densities were presumed to be largely a function of body size, further analyses were also performed on dry weight measurements. An ANOVA was performed on dry weight block means with selection regime, sex and larval density as fixed factors, and block as a random factor. Selection was not found to be significant ($p = 0.959$), with dry weight measurements overall similar between the MBs and MCUs. However, there were close to significant ($p < 0.06$) interaction effects of selection regime × sex ($p = 0.056$) and selection regime × larval density ($p = 0.058$) (Figure 3.4).

Significantly lower adult survivorship in MCUs than MBs

Adult survivorship from day 11 to 21 post-egg collection was found to be significantly lower across densities for the MCUs for both arcsine transformed ($p = 0.016$) and non-transformed ($p = 0.015$) data. For non-transformed data, there was a close to significant effect of selection regime × adult density ($p = 0.057$) which disappeared upon transformation ($p = 0.223$) (Figure 3.5).

Figure 3.2 – 21st day fecundity counts versus larval rearing density across blocks 2, 3 and 4 of the MBs and the MCUs. Error bars are standard deviation across replicate vials for the given block × selection regime x larval density combination. Grey = MB, Black = MCU. (MCU ~ MB, $p = 0.156$)

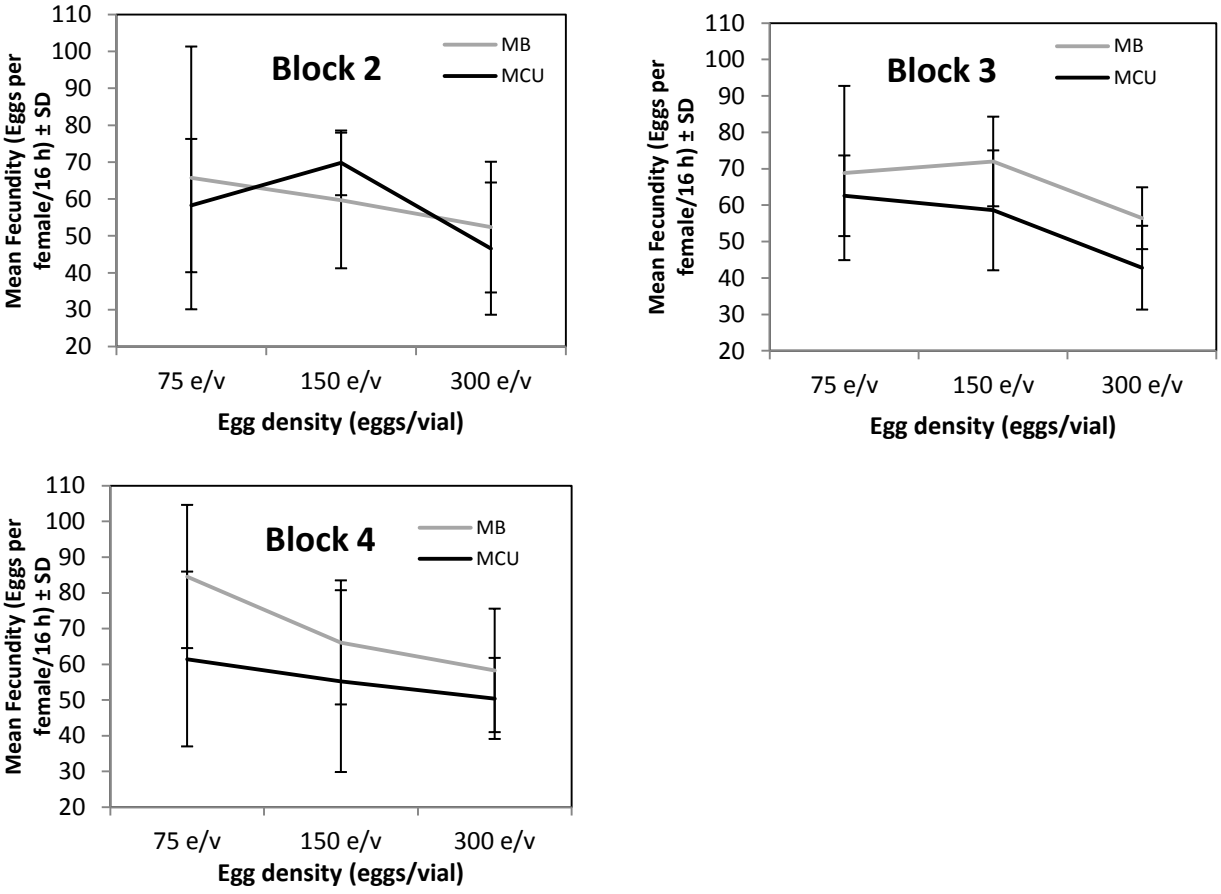


Figure 3.3 – 21st day fecundity counts versus female dry weight. Individual points represent block means across blocks 2, 3 and 4. Log regressions were taking by grouping block means. ($R^2 > 0.5$). Grey = MB, Black = MCU.

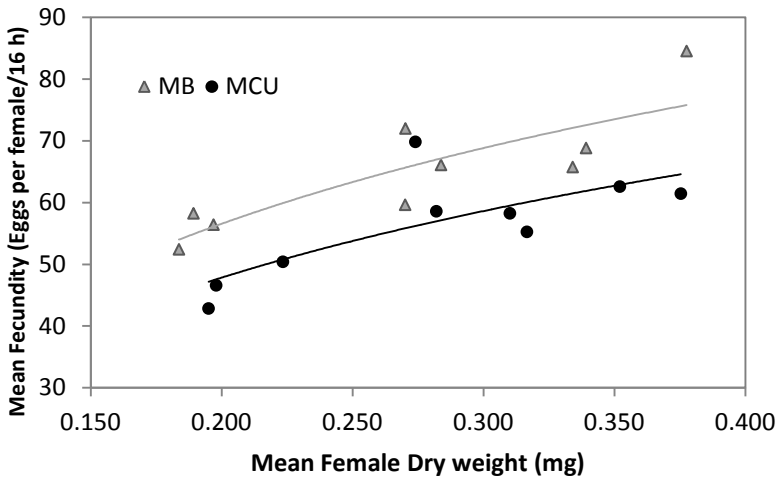


Figure 3.4 - Dry weight (mg) versus larval density across males and females of blocks 2, 3 and 4 of the MBs and MCUs. Error bars are standard deviation across replicate vials for a given block × selection regime × larval density × sex combination. Grey = MB, Black = MCU, dotted line = male, solid line = female.

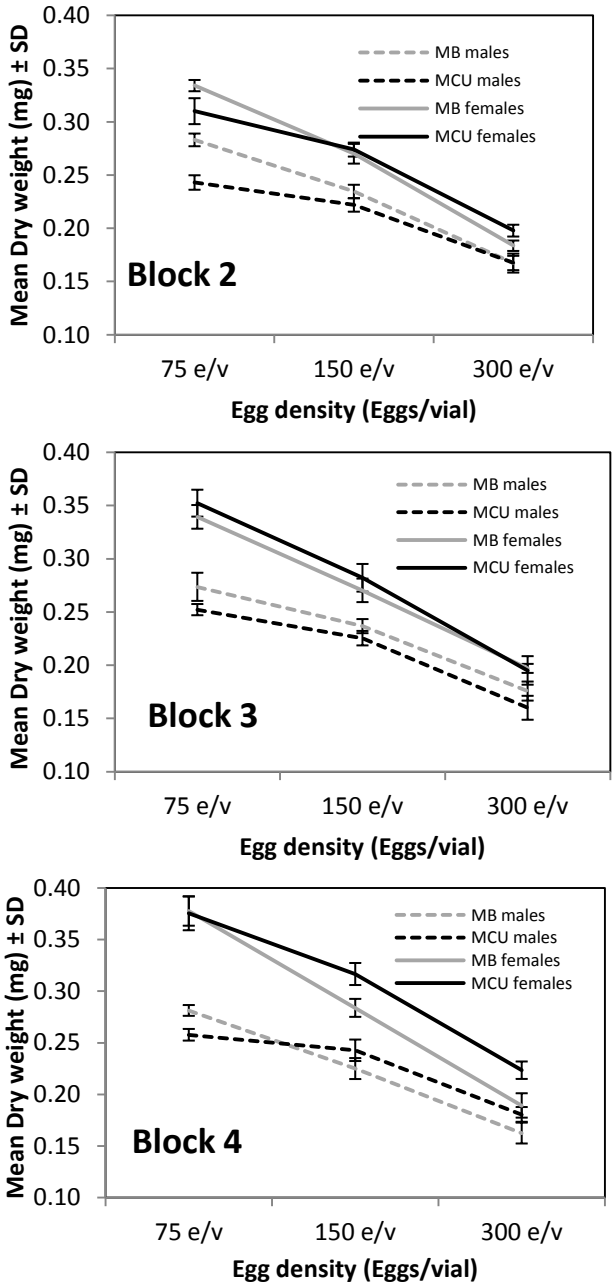
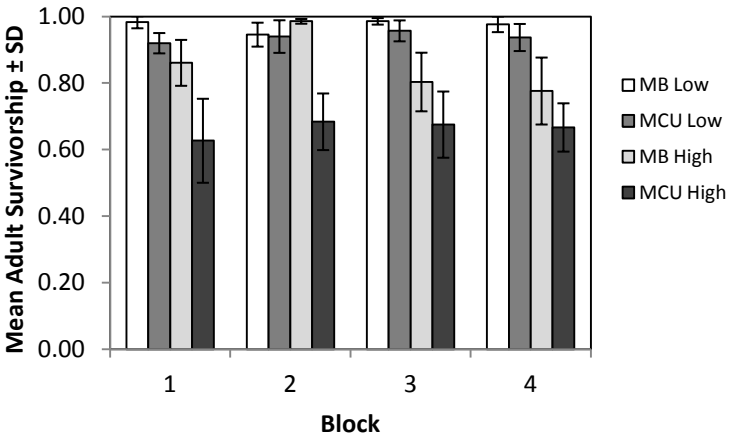


Figure 3.5 – Adult survivorship from day 11 to 21 post-egg collection across high (150 adults/vial) and low (50 adults/vial) densities. Error bars are standard deviations across replicate vials for the given block × selection regime × adult density combination. White/light grey = MB low/high density, Grey/dark grey = MCU low/high density.



Discussion

The MCUs show a trend towards lower 21st day fecundity overall than the MBs (Figure 3.2), as well as significantly lower adult survivorship from day 11 to 21 post-egg collection (Figure 3.5). The adult survivorship data is also backed up by previous results which found that the MCUs had significantly lower 21st day adult survivorship than the MBs at densities below 30 (M. Sarangi & A. Joshi, unpublished data).

Dry weight measurements were taken from the same populations that were used for fecundity counts with the reasoning that the effect of larval density on fecundity is essentially the effect of a reduced body size. There was some concern that this might complicate fecundity results, since the MBs and MCUs would not necessarily demonstrate the same average body weight for a given larval density. Earlier results had found higher larval survivorship for the MCUs (M. Sarangi, S. Dey, A. Mital, G. Vaidya & A. Joshi, unpublished data) as well as a lower body weight at eclosion for the MCUs, in particular the males (M. Sarangi, S. Dey & A. Joshi, unpublished data). The results here partly mirror the latter results, with male MCUs consistently smaller than male MBs for a given density (Figure 3.3). Females, on the other hand, tended to be slightly larger for the MCUs. It is possible that the reason why dry weight was not significantly different between the populations was that these two results cancelled each other out, and that with the addition of one more block the near-significant ($p < 0.06$) effect of selection regime × sex will become significant. Speculation aside, however, the results show that female MCUs were certainly not *smaller* than the MBs, and that therefore their lower fecundity cannot be explained in terms of a lower dry weight. Indeed, when fecundity was plotted as a function of dry weight the trend towards lower fecundity in the MCUs remained clear (Figure 3.4).

However, there was no significant interaction effect on fecundity of selection regime × larval density. Further, the near-significant effect on adult survivorship of selection × adult

density ($p < 0.06$) disappeared after performing an arcsine transformation on the survivorship data. A strong case cannot therefore be made from this data that any of the non-linear relationships between crowding and fecundity / survivorship have changed in the MCUs as a result of adaptation to larval crowding. However, it does appear that the baseline fecundity and adult survivorship have dropped, and it would be interesting to speculate why this might be the case, and why that change is not showing up as a difference in overall stability.

Previous studies have shown that adaptation to larval crowding trades off with adult survivorship at high densities in the *D. melanogaster* CU populations (Joshi et al. 1998). This drop in adult survivorship was not sufficient to effect a change in stability, however (Mueller et al. 2000). The CU populations showed no difference in fecundity from their controls (Mueller et al. 2000), and no other crowded-adapted population has been tested simultaneously for stability and fecundity. However, Prasad et al. (2003) found that *D. melanogaster* populations selected for faster development and early reproduction (the FEJ populations) showed an increase in stability relative to their controls, alongside a corresponding 35% drop in fecundity (Joshi et al. 2001). It was presumed that in the FEJ populations there was a trade off between faster development and fecundity, as well as body weight and pre-adult survivorship. The trend towards lower fecundity in the MCUs shown above is not, however, comparable to a 35% drop, and does not in fact show up as statistically significant.

One possibility, therefore, is that the drop in adult survivorship and fecundity in the MCUs is not sufficient to produce a difference in stability. At the very least, the drop in adult survivorship is not enough to bring average population sizes in the MCUs down to MB levels. The drop in fecundity is not so extreme as to bring MCU realized growth rate below MB levels at population densities below the carrying capacity. And yet differences were seen in the previous chapter between characteristics of the dynamics of the MBs and MCUs. The fact that the MCUs are able to maintain a higher average population size overall than the MBs, and that realized growth rate drops at a slower rate with density in the MCUs than the MBs, suggests that

perhaps the MCUs are able to respond to perturbations in density in a more moderate manner than the MBs. One could speculate that drops in fecundity and survivorship, in moderate amounts, might mean that higher growth rates and population sizes may be maintained overall.

This can be explained, in part, by imagining scenarios in which a population might crash. For the population numbers described in the previous chapter (which seldom went below 30 individuals), every subsequent generation size represents a crash from the number of eggs which are actually laid, given that even 8 flies might lay over 400 eggs (personal observation) and 21st day census numbers seldom came close to that level. Every fluctuation is – in terms of egg number – a crash, and therefore the question that must be introduced is: how large must the egg number be in order for the realized growth rate (in terms of adults) to slow and then finally dip below zero? The *adult* number (as opposed to egg number) at which this occurs can be increased in one of two ways: a reduction in fecundity (which postpones that peak egg number), and an increase in larval survivorship (which increases the peak egg number). The MCUs demonstrate both. A reduction in adult survivorship may also contribute, particularly if such a reduction increases with density, since that would make it more difficult for populations to reach levels at which they might crash.

The above scenario is, of course, a slightly simplified picture. In actual fact, the multiple age-specific, density-dependent feedback loops which act upon life-history traits (described in part in the introduction) exist in a slightly more complicated balance with each other, and subtle differences in the nature of these density-dependent relationships can cause large differences in outcome. In order to see if the predictions made here were correct, two pieces of information would be helpful, which are unfortunately not available at the present time. The first of these is how fecundity changes with adult density in the MBs versus the MCUs, as this density-dependent effect is predicted by the model in Mueller (1988) to be a large factor in the determination of stability. The second is how dynamics might differ between the MBs and the MCUs at 1.5 mL of larval food which, based on results from the next chapter, would possibly be

sufficiently destabilizing for any actual differences between the two populations to be brought out.

Chapter 4: Larval Resource Availability and Dynamics.

Introduction

The dynamics of populations can be impacted by both genetic and environmental factors. Previous chapters spoke chiefly of genetic factors, with attempts made to decouple the intrinsic dynamics of crowding-adapted populations from outside influences. The study that will be described below addresses the issue of the dynamics of crowded populations from the environmental perspective. Here, resource availability was manipulated manually across populations with similar genetic backgrounds. The JB populations (described in chapter 1) are populations of *D. melanogaster* adapted to normal lab rearing conditions on a 21-day discrete generation cycle, and are not under conscious selection for any life-history related traits. In this experiment, small populations derived from one of the JB populations were exposed to three variations on the LH food regime described in chapter 2, which is destabilizing at sufficiently low levels of larval food (Mueller & Huynh, 1994; Sheeba & Joshi, 1998). One could predict that an increase in food levels would cause a corresponding increase in stability, but as seen in the preceding chapters the array of factors which influence constancy and persistence do not always correspond with each other in a predictable manner. Consequently, the current study aimed to look at how constancy, persistence and population growth parameters varied with increasing food levels in the LH regime.

Materials and Methods

Population Dynamics Experiment

Thirty single-vial populations were derived from one of the JB populations (JB – 1), and their dynamics were studied for ten generations under variations of a food regime which has been

demonstrated earlier to be destabilizing, resulting in relatively large fluctuations in population size (Mueller & Huynh 1994; Sheeba & Joshi 1998). Under this regime, termed the LH regime (**l**ow larval food levels, **h**igh adult food levels) larvae are provided low food levels and adults are provided normal food levels, with supplementary yeast paste provided for three days prior to egg laying. In this experiment, the food was maintained at three different levels for the larval stage, each with 10 replicate populations: 1 mL, 2 mL and 3 mL of banana food. Five additional vial populations were maintained as backups at 6 mL of larval food and run in parallel with the 30 experimental vials.

Vial populations were each initiated with 20 eggs for the first generation. In all subsequent generations, population number was not controlled. From the 9^{th} to 17^{th} day post-egg collection, freshly eclosed adults were transferred from egg collection vials into corresponding adult collection vials with ~4 mL of food, with vial identities maintained across generations. Adults were transferred to fresh food every alternate day. On the 17^{th} day post-egg collection vials were discarded in order to avoid collecting any eclosing flies from the next generation. On the 18^{th} day post-egg collection, adults were transferred into yeast vials, with ~4 mL of food and a dab of yeast paste on the vial wall. They remained in these vials until the 21^{st} day post-egg collection, when adults were transferred to egg collection vials with the corresponding level of food (1 mL, 2 mL or 3 mL) in each vial. For each generation after the first, adults were allowed to lay eggs for 16 hours, in order to ensure that any adults which might have died were removed before any larvae hatched. If the vial had faced an extinction (less than at least one male, one female), then it was reset from the backup vials with 2 males and 2 females.

At the end of 16 hours, adults were transferred to empty vials and frozen for counting and dry weight measurement. The census included all flies that had died during the egg laying period. After being censused, dead flies were kept at 64°C for 36 hours and then weighed. All of the flies from a given vial were weighed together, and then average dry weights calculated

based on census data. Time series analyses were then performed on the census and dry weight data.

Measures of Stability

Constancy stability was measured in terms of two indices: the coefficient of variation and the fluctuation index, described below.

The coefficient of variation (CV) was calculated as the ratio of the standard deviation in the time series data to the population mean, for a given vial population. Here σ = standard deviation and μ = population mean.

$$\mathrm{CV} = \frac{\sigma}{\mu}$$

The fluctuation index (FI) (Dey & Joshi 2006) was calculated as the sum of the absolute step-wise differences between subsequent time series data points, scaled by the population mean and the number of generations. Here T = total number of generations in the time series, $\bar{N}$ = population mean, N_{t+1} = population number in generation t+1 and N_t = population number in generation t.

$$\mathrm{FI} = \frac{1}{(T\bar{N})}\sum_{i=0}^{T-1}\mathrm{abs}(N_{t+1} - N_t)$$

Persistence stability was measured as the probability of extinction, taking the ratio of the number of extinctions across generations in a given vial population to the number of generations.

Calculating population growth parameters

The parameters r, K and α ($= \frac{r}{K}$) from the Ricker (1954) model of population growth were indirectly estimated by taking a linear regression of the plot of $\ln\left(\frac{N_{t+1}}{N_t}\right)$ versus N_t where N_t = population number at generation t, and N_{t+1} = population number at generation t+1. Given a

linear regression of this plot, the x-intercept was taken as *K* (population size when growth rate = 0), the y intercept was taken as reflecting *r* (growth rate when population size = 0) and the slope of the line was taken as α, representing the strength of density-dependence.

Statistics

Statistical analyses were carried out using STATISTICA v.7 (Statsoft Inc, Tulsa, OK, USA). All estimated descriptors or parameters of population growth were subjected to a mixed model analysis of variance (ANOVA) treating selection regime as a fixed factor.

Results

Measures of Constancy Stability

A significant difference was found between food levels for mean CV ($p < 0.001$), and a post-hoc analysis using Tukey's HSD found significant differences between 1 and 2 mL ($p < 0.001$) and 1 and 3 mL ($p < 0.001$), but not between 2 and 3 mL ($p = 0.514$) (Figure 4.1a). Similarly, a significant difference was found between food levels for mean FI ($p < 0.001$), and a post-hoc analysis using Tukey's HSD found significant differences between 1 and 2 mL ($p < 0.001$) and between 1 and 3 mL ($p < 0.001$), but not between 2 and 3 mL ($p = 0.384$) (Figure 4.1b).

Measures of Persistence Stability

A significant difference was found in persistence between food levels ($p < 0.001$), and a post-hoc analysis done using Tukey's HSD found that differences in persistence were significant for 1 and 2 mL ($p < 0.001$) and 1 and 3 mL ($p < 0.001$), but not between 2 and 3 mL, in both of which conditions there was precisely one extinction (Figure 4.1c).

Measures of population growth parameters

A one-way ANOVA was performed on values of r, K and α estimated from the linear regression of $\ln(N_{t+1}/N_t)$ versus N_t. No significant difference was found in r across food levels ($p = 0.517$) (Figure 4.2a). A significant difference was found in K across food levels ($p < 0.001$), with a post-hoc analysis using Tukey's HSD finding significant differences between all three food levels ($p < 0.05$) (Figure 4.2b). Likewise, a significant difference was found in α across food levels ($p < 0.001$). Here, a post-hoc analysis using Tukey's HSD found significant differences between 1 and 2 mL ($p < 0.001$) and between 1 and 3 mL ($p < 0.001$), but not between 2 and 3 mL ($p = 0.428$) (Figure 4.2c).

Analyses of the strength of density-dependence (α) were repeated after dividing time series data into low and high densities, due to observations that the graph of $\ln(N_{t+1}/N_t)$ versus N_t was not approximately linear and appeared to have different slopes at high and low values of N_t. The cut off for high versus low densities was taken as the root of a second order polynomial regressed onto the graph of $\ln(N_{t+1}/N_t)$ versus N_t for a given condition. This method and its rationale are described in more detail in chapter 2 (Figure 2.3). Values of α were then calculated as above for linear regressions of low and high density data, taking data for each replicate vial separately.

At low densities, a significant difference was also found in α across replicate vials and food levels ($p < 0.001$), and a post-hoc analysis using Tukey's HSD for unequal N found significant differences between 1 and 2 mL ($p < 0.001$) and between 1 and 3 mL ($p < 0.001$) but not between 2 and 3 mL ($p = 0.997$) (Figure 4.3a). At high densities, no significant difference was found across food levels for α ($p = 0.182$), although the mean slope was most negative at 1 mL (Figure 4.3b).

Realized growth as a function of density across food levels

Realized growth rate was calculated as N_{t+1}/N_t for the first nine generations of time series data. It was averaged across population size bins of 10, with that value chosen so as not to average out the rapid changes of behaviour shown by 1 mL populations at low densities. A graph of realized growth rate against population bin is shown in Figure 4.4. Statistical analyses could not be performed between food levels, due to the concern that the zone in which they appear to be behaving quite differently (< 30 individuals) is also a zone in which sample sizes were extremely low for 2 mL and 3 mL, and very high for 1 mL (see Figure 4.5b). It can be seen visually, however, that above N_t = 30 individuals, 2 mL and 3 mL are similar in appearance. 1 mL shows clearly distinct behaviour from both 2 mL and 3 mL, with an early and steep drop in realized growth rate which plateaus out at about 40 individuals (Figure 4.4).

Increase in average population size across food levels

A significant main effect of food level on average population size was found ($p < 0.001$). Post-hoc comparisons with Tukey's HSD found significant differences between 1 and 2 mL ($p < 0.001$), between 1 and 3 mL ($p < 0.001$) and between 2 and 3 mL ($p = 0.032$) (Figure 4.5a). These results are reflected in the frequency distribution of population sizes across food levels (Figure 4.5b). With increasing food level, the mode of the population size distribution appears to increase as well. Moreover, it is only the 1 mL treatment that the population size distribution has the characteristic L-shape of LH populations with 1 – 1.5 mL of larval food (Dey & Joshi 2013). For both the 2 mL and 3 mL treatments, the population size distribution does not have any signature of the close to two-point cycles characteristic of LH populations with very low larval food levels.

Figure 4.1 – a) Mean coefficient of variation (CV), b) Mean fluctuation index (FI), c) Persistence (calculated as extinction probability), each calculated at three larval food levels. Letters indicate significant differences between population size levels based on Tukey's HSD ($p < 0.05$). Error bars are standard deviations calculated across the 10 replicate vial populations at each food level.

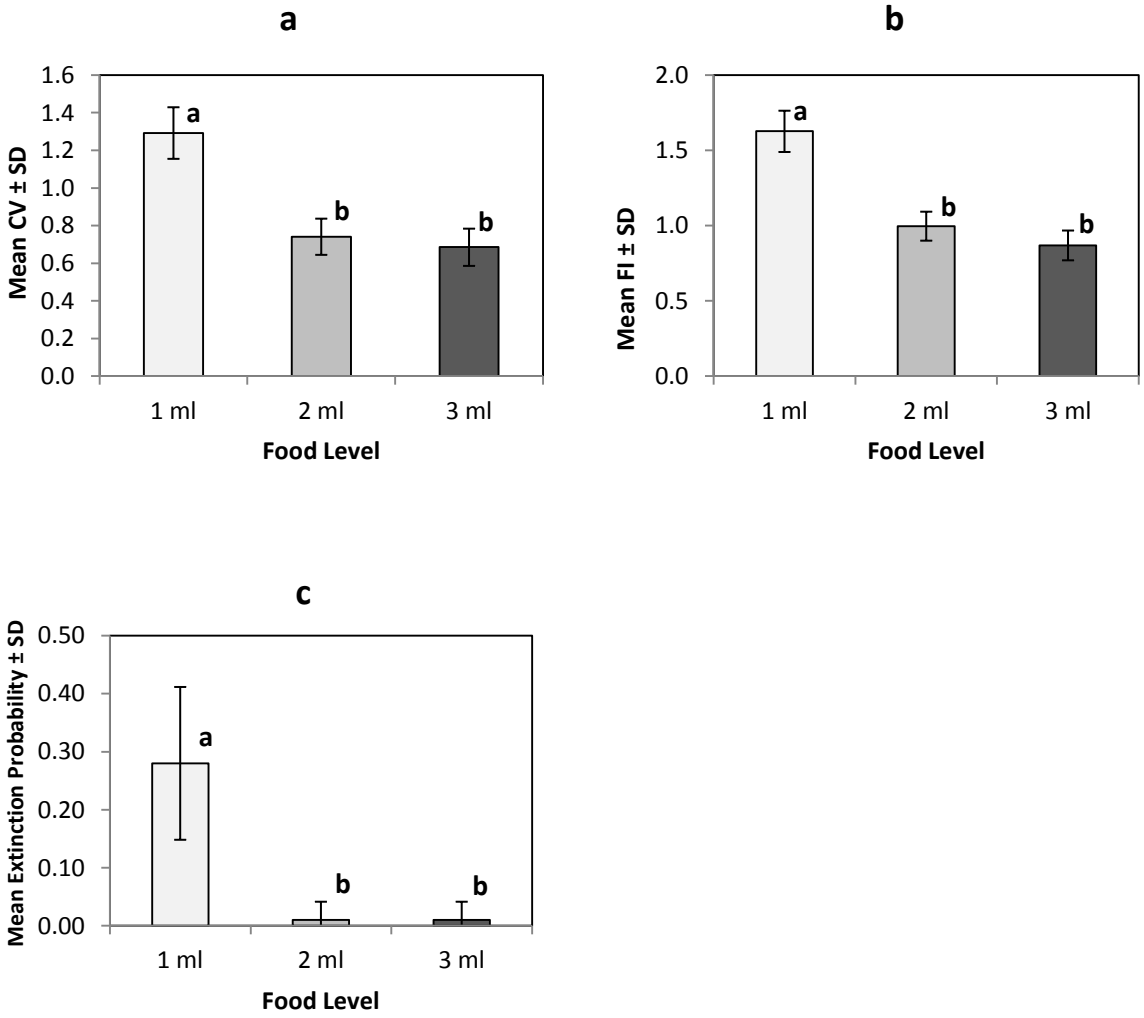


Figure 4.2 – Population growth parameters at different larval food levels estimated from the linear regression of $\ln(N_{t+1}/N_t)$ versus N_t. Letters indicate significant differences between population size levels based on Tukey's HSD ($p < 0.05$). Error bars are standard deviations calculated across the 10 replicate vial populations at each food level. a) r (intrinsic growth rate), b) K (carrying capacity), c) α (strength of density dependence). White = 1 mL, Grey = 2 mL, Dark grey = 3 mL.

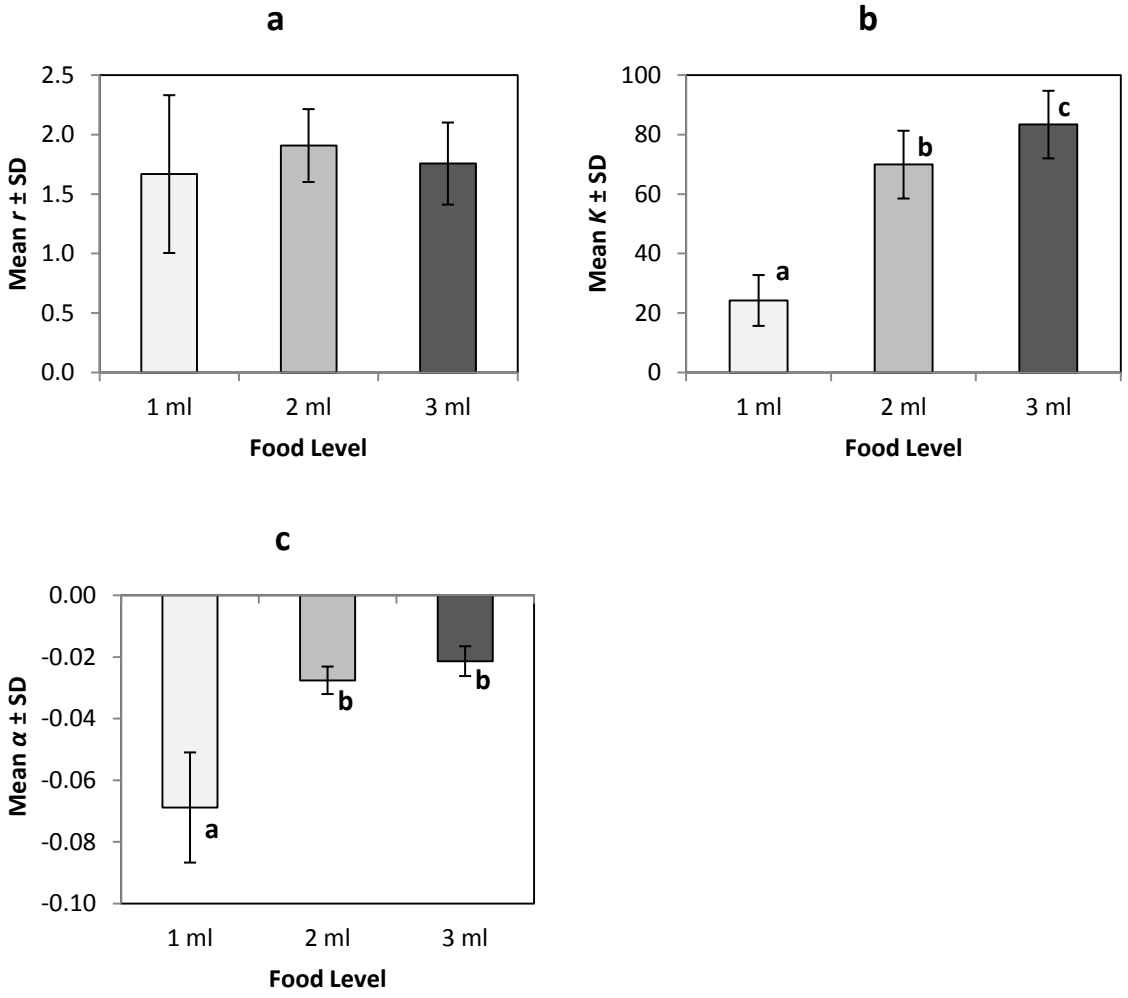


Figure 4.3 – Values of α (strength of density dependence) calculated across food levels for low densities (*left*) and high densities (*right*) from the linear regression of $\ln(N_{t+1}/N_t)$ versus N_t. Letters indicate significant differences between population size levels based on Tukey's HSD for unequal N ($p < 0.05$). Error bars are standard deviations calculated across the 10 replicate vial populations at each food level. a) α at low densities, b) α at high densities. White = 1 mL, Grey = 2 mL, Dark grey = 3 mL.

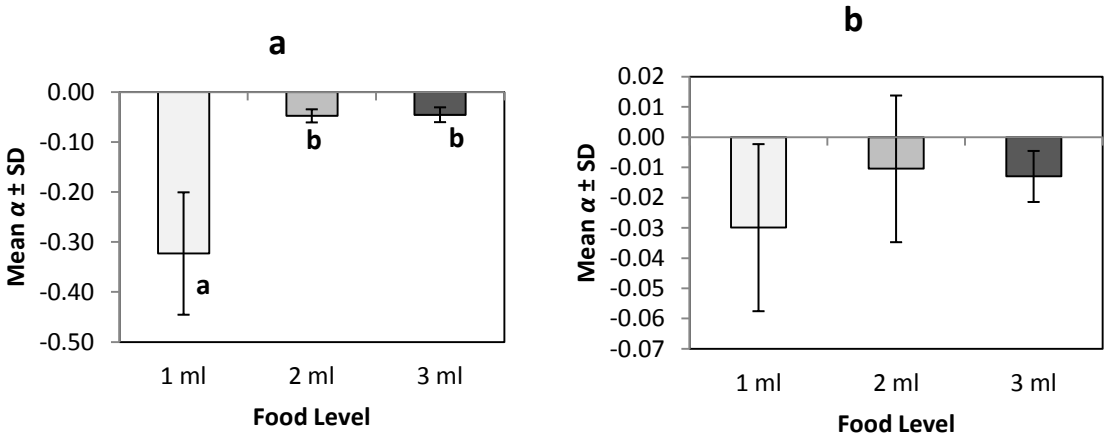


Figure 4.4 – Realized growth rate (N_{t+1}/N_t) as a function of density, calculated across population size bins of 10 individuals each. Dashed grey = 1 mL, Grey = 2 mL, Black = 3 mL. The dotted black line at realized growth rate equal to one represents zero growth.

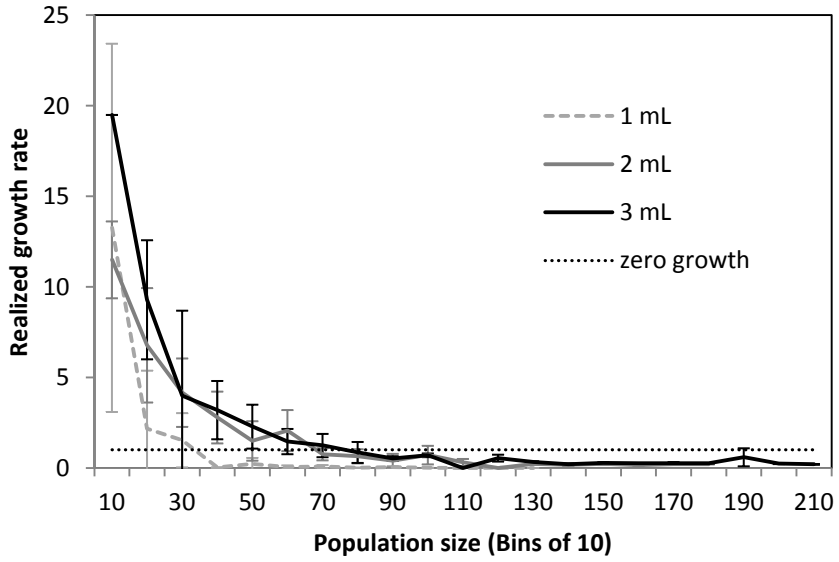
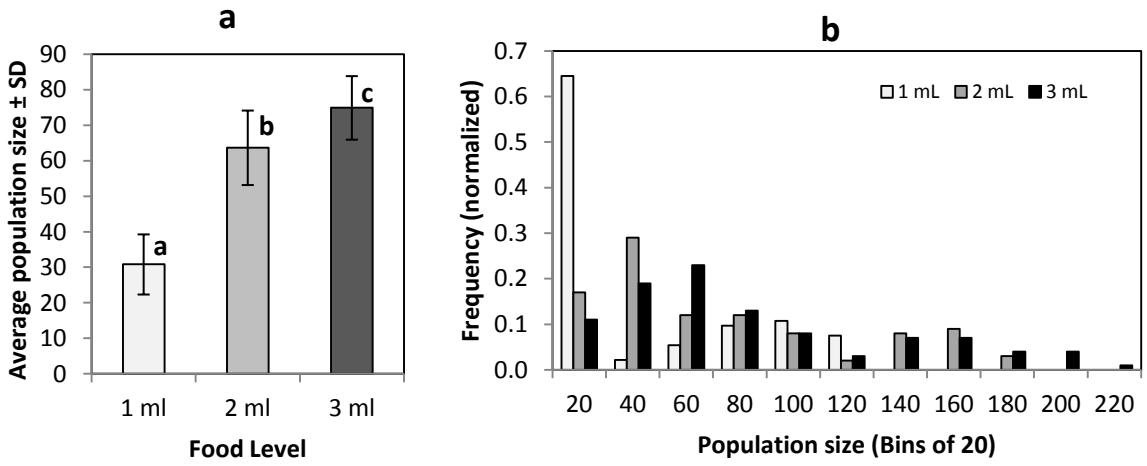


Figure 4.5 – a) Average population sizes across food levels. Letters indicate significant differences between population size levels based on Tukey's HSD ($p < 0.05$). Error bars represent standard deviation across replicate populations. b) Frequency distribution of population sizes across food levels, with population in bins of 20. Light grey = 1 mL, Grey = 2 mL, Dark grey = 3 mL.



Discussion

With increasing larval food levels from 1 to 3 mL, both constancy and persistence stability changed in a consistent and non-linear manner. In the case of constancy, both CV and FI showed a sharp and significant change from 1 to 2 mL, and then were statistically indistinguishable between 2 and 3 mL (Figure 4.1 a-b). In the case of persistence, extinctions were very frequent at 1 mL (occurring with a probability of approximately 30% each generation), but did not occur at 2 mL and 3 mL after the first generation (Figure 4.1c).

Population growth parameters showed patterns across food levels which were consistent with the above results. There was no difference in *r* between the three food levels (Figure 4.2a), which would make sense given that all three conditions were sourced from the same population and adults were yeasted in all three treatments, and therefore no difference in intrinsic growth rate would be expected. Rather, the sharp increase in constancy and persistence stability between 1 and 2 mL occurs alongside a significantly higher *K* and less negative α for populations at 2 mL compared to 1 mL (Figure 4.2 b – c). Dey et al. (2012) have suggested that greater values of *K* can stabilize population dynamics on a quantitative scale, even in the absence of a decrease in *r*, although qualitative changes in the nature of the dynamics need changes in *r*. When data were divided into high and low densities, both sets of data demonstrated a drop in α from 1 to 2 mL and then no change from 2 to 3 mL (Figure 4.2). These patterns in *K* and α further manifest themselves in the plot of realized growth rate against density (Figure 4.4), with realized growth rate dropping far more swiftly with density at 1 mL than it does in either 2 or 3 mL, and consequently falling to zero and then negative growth at much lower population sizes.

The change in constancy, persistence, *K* and α between 1 mL and 2 mL does not run contrary to expectations. With increasing resource availability, a population will be less likely to crash and go extinct. The sharp difference between the 1 – 2 mL and 2 – 3 mL transitions is slightly more counter-intuitive, however. From 2 to 3 mL, there is no significant difference in

CV or FI (Figure 4.1), and each showed precisely one extinction, and that too in the first generation when the population size had been artificially set at 20 eggs. Realized growth rate drops with density for 2 and 3 mL in ways that are visibly almost indistinguishable, apart from at lower bins which had very few samples and are therefore difficult to compare (Figure 4.4). Neither is there a significant difference in α between the two conditions (Figure 4.2c), although there is a slight trend for a less negative α for the populations at 3 mL which could hypothetically create a biologically relevant difference, given that slight differences in slope could create large ultimate differences in the intercepts of the given linear regressions. Such a speculation would make sense given that the only quantities measured which showed up as significantly different between 2 and 3 mL were K (Figure 4.2b) and average population size (Figure 4.5a). The latter results are also supported by the fact that the mode of the population size distribution falls at a higher bin for 3 mL than it does for 2 mL (Figure 4.5b).

Based on the results above, one could suggest that the pressure that limited resource availability exerts on population survival and consequently stability is higher at 1 mL than it is at 2 mL. By 2 mL, however, it is possible that resource pressure is low enough that increasing resources more makes little difference to stability. Populations at 3 mL exhibit a higher K than those at 2 mL and are able to maintain a larger population size overall, but these differences do not show up in terms of either constancy or persistence, which have both apparently reached a plateau.

This result is especially interesting given the manner in which it mirrors otherwise slightly paradoxical results from chapter 2. The MCUs demonstrated a higher K than the MBs and a less negative α (Figure 2.2), as well as a higher average population size and right-shifted mode in the population size distribution (Figure 2.7). However, there was no difference in CV and FI between the MBs and MCUs (Figure 2.1), with CV and FI values similar to what is seen here for the 2 and 3 mL conditions (a CV of ~ 0.7 – 0.8 and an FI of ~ 1.0). Likewise, neither the MBs nor the MCUs faced extinctions. Given the two scenarios, stripped of background, the only

differences are that α is not significantly different for 2 and 3 mL, and that the MBs and MCUs are both at a food level of 2 mL for the larval stage.

Similarities in FI and CV between the two very different sets of conditions can be explained partly by the fact that by 2 mL, in the present experiment, levels of stability have possibly already begun to plateau. However, the similarities in the behavior of *K* between the MBs and the MCUs in chapter 2, and between the JBs at 2 mL versus 3 mL in the present experiment are worth dwelling on for a moment. For the present experiment, the reason for that difference in *K* can be explained in terms of the fact that food levels have been artificially manipulated. More resources are available at 3 than 2 mL, and therefore one could predict that a higher average population size can be maintained. In the case of the MBs and MCUs, however, their behavior is as if they were at different food levels, even though technically they are not. Their behavior is as though they are at differing levels of resource availability, without resources being scarce enough for stability to be affected (such as with 2 and 3 mL, in the current chapter). What one is possibly seeing, therefore, are two paths to the same result (the maintenance of a higher population size): one environmental, with food levels directly altered, and another genetic, manifesting itself as a difference in efficiency. In either case, a difference in net resource availability results.

To summarize, levels of stability as measured by constancy and persistence change in a non-linear manner between 1 and 3 mL. 1 to 2 mL appears to be a very sensitive zone, with large changes in population dynamics (both in terms of constancy and persistence stability), in the behavior of realized growth rates and in average population sizes. By 2 mL, however, one could hypothesize that resources are plentiful enough that the addition of more food does not create large changes in dynamics. Rather, further increases in food only manifest themselves in terms of a higher average population size overall, with constancy and persistence having both reached a plateau.

Chapter 5: Conclusions

As described in the preceding three chapters, one can look at the dynamics of crowded populations from a variety of perspectives. In chapter 2, the dynamics of populations adapted to larval crowding were studied over ten generations under relatively destabilizing conditions. In chapter 3, the dynamics of the same crowding-adapted populations were studied in a more indirect manner, by examining the density-dependence of life-history traits which form a biological underpinning for dynamical behaviour. Chapter 4 switched strategies again, studying the impact of environmental rather than genetic changes on the dynamics of populations. Here, the proximate effects of resource manipulation on population dynamics were examined, rather than the ultimate effects of crowding as a selective pressure. The view from each perspective was slightly different, but an attempt will now be made to distil these results into one unified story.

In chapter 2, it was found that the MCU populations, selected for adaptation to larval crowding for over 100 generations, did not show a detectable difference in measures of stability from their control MB populations, when reared in an LH regime with a larval food level of 2 mL. Constancy stability was similar between the MB and MCU populations, with a CV of ~0.6-0.7 and and an FI of ~1 across blocks. An FI of ~1 is characteristic, in fact, of *D. melanogaster* populations in an LL regime with about 1.2 mL of larval food and no yeast supplement for adults (Dey & Joshi 2013). A typical LH regime with 1 or 1.5 mL of food and yeast supplements for adults usually demonstrates FI values of ~1.6 (Dey & Joshi 2006; Dey & Joshi 2013). The LH regime with 2 mL of food, thus, did not appear to be too destabilizing. No extinctions occurred in either the MCU or MB populations, and therefore persistence stability could not be measured. It was expected that a change in stability might have resulted from a trade off between the population growth parameters r and K. A trend towards a lower r was found in the MCU populations, along with a significantly higher K and less negative α, relative to the MB controls.

Similarly, the MCUs were able to maintain a significantly higher average population size than the MBs, with higher peaks and troughs overall in their time series data (data not shown).

In chapter 3, it was found that the MCUs showed a trend towards lower fecundity on the 21st day post-egg collection than the MBs, with no difference between the MCUs and MBs in how fecundity dropped with increased larval crowding. The MCUs also demonstrated significantly lower adult survivorship from the 11th – 21st day post-egg collection than the MBs, with a near-significant trend towards survivorship differences between the two populations increasing with adult density.

Chapter 4 saw that stability increases in a non-linear manner with larval food level in the LH regime, with both constancy and persistence stability showing a sharp increase between 1 and 2 mL of food, and then not demonstrating a significant change from 2 and 3 mL of food. No difference in *r* was found between food levels, which was an expected result given that all vial populations were expected to be genetically similar, sourced from the same block of the JB populations, and adults were given a yeast supplement in all three larval food level treatments. A significant rise in *K* was found with each increase in food, however, from 1 to 2 mL and then from 2 to 3 mL. Likewise, average population sizes showed a significant difference between each food level.

The results from chapter 4 can shed some light on some of the more counter-intuitive results from chapters 2 and 3. First of all, one suggestion here is that an LH regime with larval food levels of 2 mL is not in fact particularly destabilizing. As we go from 1 to 2 mL both constancy and persistence stability increase sharply and then appear to have plateaued by 2 mL, as they show no further change from 2 to 3 mL of larval food. Levels of constancy stability for both CV and FI are also comparable between what is seen for the MBs and the MCUs in chapter 2, and for the JBs at 2 mL in chapter 4. What this might indicate is that if a difference in stability exists between the MBs and the MCUs, they were not tested at a regime which would reveal that difference.

Secondly, there is an interesting parallel that can be drawn between the comparison of stability and stability-related population growth parameters between the MBs and the MCUs in chapter 2 and that between 2 and 3 mL larval food treatments in chapter 4. In either case, no difference was found between constancy and persistence stability. A difference was, however, found in *K* and in average population size. A higher *K* and average population size in 3 mL compared to 2 mL of larval food can be explained by the increase of food. This increase in food, although it occurs at a point past which it can affect stability (since resource levels are high enough that stability appears to have plateaued), still has the effect of increasing the average number of individuals which can be maintained in the population. In terms of the MBs and the MCUs, one could rephrase this to say that at 2 mL of larval food, the MCUs *behave* as though they have more resources available than the MBs even though technically they do not. This would suggest that one effect of selection for adaptation to larval crowding in the MCUs has been that they have evolved higher efficiency than the MBs. Previous work has also suggested that the MCUs are more efficient in terms of food to biomass conversion at the larval stage than the MBs, as described in the introduction to chapter 2.

Both fecundity and adult survivorship appear to have evolved to become lower in the MCUs. Given this and the potential increase in efficiency, it is possible that, given a lower larval food level (for example 1 mL) in an LH regime, the MCUs would demonstrate higher stability than their controls. Higher efficiency is the ability to increase the effective availability of resources. Given that one cause of instability is low resource availability (as demonstrated clearly in the sharp difference in dynamics between 1 and 2 mL, chapter 4), an ability to make fewer resources go farther could be predicted to increase the constancy and persistence of a population.

These results correspond with a larger picture which has developed regarding possible evolutionary trajectories which can result in response to larval crowding (Dey et al. 2012). Selection for adaptation to larval crowding is not the imposition of one pressure on a population

but many, and depending on the balance of these pressures multiple responses might result. Some possible adaptations to larval crowding, such as faster feeding, would mean an increase in competitive ability which would not necessarily correspond with a change in either *r* or *K* (Joshi et al. 2001) and possibly, therefore, no change in stability. If populations adapt through feeding which is not faster but rather more *efficient*, then one might expect to see an increase in *K*, conceivably through a trade off with traits such as fecundity which impact *r*. In consequence, one might see a change in stability as a by-product of adaptation to larval crowding. Which trajectory a population takes may depend on subtle details about how larval crowding is imposed, such as the absolute larval food level and density (M. Sarangi & A. Joshi, unpublished data). Below, in Table 5.1, a brief summary is provided of the findings regarding *Drosophila* populations which have been selected for adaptation to crowded conditions over the past 30 years. The characteristics of these populations show a tendency to cluster, as shown by the difference in shading. These clusters, in turn, correspond to slight differences in rearing conditions, which appear to have resulted in different (and mutually exclusive) strategies in response to crowded conditions.

Future Directions

The current work suggests several projects which could be performed in the future. One is that it would be useful to subject the MBs and the MCUs to an LH regime at 1 to 1.5 mL of larval food, and observe the dynamics that result. It would also be interesting to examine issues of possible lowered fecundity and survivorship in the MCUs further. One inevitable concern, however, given reduced fecundity in a population is that it might be the result of inbreeding depression. It may be useful to check for hybrid vigour between blocks in the MCUs. Current work is involved with examining the effect of adult density on fecundity in the MBs and MCUs.

Table 5.1 – Summary of findings regarding *Drosophila* populations selected for adaptation to crowding. Differences in shading correspond to different possible evolutionary trajectories.

	K *(D. melanogaster)*	**CU** *(D. melanogaster)*	**NCU** *(D. nasuta)*	**ACU** *(D. ananassae)*	**MCU** *(D. melanogaster)*
Faster feeding	Yes[3]	Yes[7]	No[1]	No[10]	No[1]
Waste tolerance	Yes[5]	Yes[11]	No[1]	No[10]	No[13]
Rapid development	-	No[10]	Yes[1]	Yes[10]	Yes[1]
Efficiency	No[6]	No[4]	-	-	Yes[12]
Stability?	-	No[8]	-	Yes[2]	Possible

---

[1] Archana, N. (2010)
[2] Dey et al. (2012)
[3] Joshi & Mueller (1988)
[4] Joshi & Mueller (1996)
[5] Mueller, L.D. (unpublished observation)
[6] Mueller, L.D. (1990)
[7] Mueller et al. (1993)
[8] Mueller et al. (2000)
[9] Santos et al. (1997)
[10] Sharmila Bharathi, N. (2007)
[11] Shiotsugu et al. (1997)
[12] D. Ravi Teja, S. Dey & A. Joshi (unpublished data)
[13] A. Mital, G. Vaidya & A. Joshi (unpublished data)

References

Andrewartha, H. G. and Birch, L. C. (1954). The distribution and abundance of animals. University of Chicago Press, Chicago, IL, USA.

Archana, N. (2010). The genetic architecture of fitness-related traits in populations of three species of *Drosophila* subjected to selection for adaptation to larval crowding. Ph.D. thesis, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India.

Asmussen, M. A. (1983). Density-dependent selection incorporating intraspecific competition. II. A diploid model. Genetics, 103:335–350.

Clarke, B. (1972). Density-dependent selection. Am. Nat., 106:1–13.

Dempster, J. P. (1983). The natural control of populations of butterflies and moths. Biol. Rev. Camb. Phil. Soc., 58:461–481.

Den Boer, P. J. (1968). Spreading of risk and stabilization of animal numbers. Acta Biotheor., 18:165-194.

Dennis, B. and Taper, B. (1994). Density dependence in time series observations of natural populations: Estimation and testing. Ecol. Monogr., 64:205-224.

Dey, S. and Joshi, A. (2006). Stability via asynchrony in *Drosophila* metapopulations with low migration rates. Science, 312:434–436.

Dey, S. and Joshi, A. (2013). Effects of constant immigration on the dynamics and persistence of stable and unstable *Drosophila* populations. Sci. Reports, 3:1405.

Dey, S., Bose, J. and Joshi, A. (2012). Adaptation to larval crowding in *Drosophila ananassae* leads to the evolution of population stability. Ecol. and Evol., 2(5):941-951.

Dey, S., Prasad, N.G., Shakarad, M. and Joshi, A. (2008). Laboratory evolution of population stability in *Drosophila*: constancy and persistence do not necessarily coevolve. J. Anim. Ecol., 77:670–677.

Ebenman, B., Johansson, A., Jonsson, T. and Wennergren, U. (1996). Evolution of stable population dynamics through natural selection. Proc. R. Soc. Lond. B, 263:1145–1151.

Ellner, S. and Turchin, P. (1995). Chaos in a noisy world: new methods and evidence from time-series analysis. Am. Nat., 145:343–375.

Gadgil, M., and Bossert,W. H. (1970). Life-historical consequences of natural selection. Am. Nat., 104:1–24.

Gatto, M. (1993). The evolutionary optimality of oscillatory and chaotic dynamics in simple population models. Theor. Pop. Biol., 43: 310–336.

Grimm, V. and Wissel, C. (1997). Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. Oecologia, 109:323-334.

Guckenheimer, J., Oster, G. and Ipaktchi, A. (1977). The dynamics of density dependent population models. J. Math. Biol., 4:101-147.

Hansen, T. F. (1992). Evolution of stability parameters in single-species population models: stability or chaos? Theor. Pop. Biol., 42:199–217.

Hildenbrandt, H., Müller, M. S., and Grimm, V. (2006). How to detect and visualize extinction thresholds for structured PVA models. Ecol. Model., 191(3):545-550.

Joshi, A. and Mueller, L. D. (1988). Evolution of higher feeding rate in *Drosophila* due to density-dependent natural selection. Evolution, 42:1090-1093.

Joshi, A. and Mueller, L. D. (1996). Density-dependent natural selection in *Drosophila*: trade-offs between larval food acquisition and utilization. Evol. Ecol., 10:463-474.

Joshi, A., Prasad, N. G. and M. Shakarad, M. (2001). *K*-selection, α-selection, effectiveness, and tolerance in competition: density-dependent selection revisited. J. Genet., 80:63–75.

Joshi, A., Wu, W. P. and Mueller, L. D. (1998). Density-dependent natural selection in *Drosophila*: adaptation to adult crowding. Evol. Ecol., 12: 363-376.

Lewontin, R. C. (1969). The meaning of stability. Brookhaven Symposia in Biology, 22:13–23.

Lotka, A. J. (1925). Elements of physical biology. Williams & Wilkins, Baltimore, MD, USA.

MacArthur, R. H., and Wilson, E. O. (1967). The theory of island biogeography. Princeton Univ. Press, Princeton, NJ, USA.

Malthus, T. (1798). An essay on the principle of population. London, UK.

May, R. M. (1974). Biological populations with non-overlapping generations: stable points, stable cycles and chaos. Science, 186:645–647.

Milne, A. (1958). Perfect and imperfect density dependence in population dynamics. Nature, 182:1251-1252.

Mueller, L.D. (1988). Density-dependent population growth and natural selection in food limited environments: the *Drosophila* model. Am. Nat., 132:786-809.

Mueller, L. D. (1990). Density-dependent natural selection does not increase efficiency. Evol. Ecol., 4:290–297.

Mueller, L. D. and Ayala, F. J. (1981a). Trade-off between *r*-selection and *K*-selection in *Drosophila* populations. Proc. Natl. Acad. Sci. USA, 78:1303–1305.

Mueller, L. D. and Ayala, F. J. (1981b). Dynamics of single-species population growth: stability or chaos? Ecology, 62:1148–1154.

Mueller, L. D., Graves Jr., J. L. and Rose, M. R. (1993). Interactions between density-dependent and age-specific selection in *Drosophila melanogaster*. Func. Ecol., 7:469-479.

Mueller, L. D. and Huynh, P. T. (1994). Ecological determinants of stability in model populations. Ecology, 75:430–437.

Mueller, L. D. and Joshi, A. (2000). Stability in model populations. Princeton University Press, Princeton, NJ, USA.

Mueller, L. D., Joshi, A. and Borash, D. J. (2000). Does population stability evolve? Ecology, 81:1273–1285.

Nicholson, A. J. (1933). The balance of animal populations. J. Anim. Ecol., 2:132-178.

Pearl, R. and Reed, L. J. (1920). On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc. Natl. Acad. Sci. USA, 6(6):275.

Prasad, N. G., Shakarad, M., Anitha, D., Rajamani, M. and Joshi, A. (2001). Correlated responses to selection for faster development and early reproduction in *Drosophila* melanogaster: the evolution of larval traits. Evolution, 55:1363–1372.

Prout, T. and McChesney, F. (1985). Competition among immatures affects their fertility: population dynamics. Am. Nat., 126:521–558.

Ricker, W. E. (1954). Stock and recruitment. J. Fish. Res. Board Can., 11:559–623.

Roughgarden, J. (1971). Density-dependent natural selection. Ecology, 52:453–468.

Santos, M., Borash, D. J., Joshi, A., Bounlutay, N. and Mueller, L. D. (1997). Density-dependent natural selection in *Drosophila*: evolution of growth rate and body size. Evolution, 51:420-432.

Sharmila Bharathi, N. (2007). Variation and covariation in life-history related traits in some species of *Drosophila*. Ph.D. thesis, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India.

Sheeba, V. and Joshi, A. (1998). A test of simple models of population growth using data from very small populations of *Drosophila melanogaster*. Curr. Sci., 75:1406–1410.

Sheeba, V., Madhyastha, N. A. A. and Joshi, A. (1998). Oviposition preference for novel versus normal food resources in laboratory populations of *Drosophila melanogaster*. J. Biosci., 23:93–100.

Shiotsugu, J., Leroi, A. M., Yashiro, H., Rose, M. R. and Mueller, L. D. (1997). The symmetry of correlated responses in adaptive evolution: an experimental study using *Drosophila*. Evolution, 51:163–172.

Stokes, T. K., Gurney, W. S. C., Nisbet, R. M. and Blythe, S. P. (1988). Parameter evolution in a laboratory insect population. Theor. Pop. Biol., 34:248–265.

Strong, D. R. (1986). Density-vague population change. Trends Ecol. Evol., 1(2):39-42.

Thomas, W. R., Pomerantz, M. J. and Gilpin, M. E. (1980). Chaos, asymmetric growth and group selection for dynamical stability. Ecology, 61:1312–1320.

Tung, S. (2012). Insights into the mechanistic underpinnings for the evolution of population stability in *Drosophila*, based on modelling of laboratory populations. M.S. Thesis, Indian Institute of Science Education and Research, Kolkata, India.

Turchin, P. and Taylor, A. D. (1992). Complex dynamics in ecological time series. Ecology, 73:289–305.

Turelli, M. (1978). A reexamination of stability in randomly varying versus deterministic environments with comments on the stochastic theory of limiting similarity. Theor. Pop. Biol., 13(2):244-267.

Turelli, M. and Petry, D. (1980). Density-dependent selection in a random environment: an evolutionary process that can maintain stable population dynamics. Proc. Natl. Acad. Sci. USA, 77:7501–7505.

Verhulst, P. (1838). Notice sur la loi que la population poursuit dans son accroissement. Correspondance mathématique et physique, 10:113–121.

Volterra, V. (1926). Fluctuations in the abundance of a species considered mathematically. Nature, 118:558-560.