An Individual-Based Model for Simulating the Ecological and Evolutionary Dynamics of *Drosophila* Cultures Under Different Scenarios of Larval Crowding

A Thesis

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the degree of

Master of Science

By

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Contents

Declaration

I hereby declare that the work embodied in this thesis entitled "An individual-based model for simulating the ecological and evolutionary dynamics of *Drosophila* cultures under different scenarios of larval crowding" has been carried out by me under the supervision of Prof. Amitabh Joshi, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, and that it has not been submitted for any degree or diploma to any other institution.

Following prevalent scientific practice, acknowledgement has been accorded wherever due. Any omission, which might have occurred by oversight or error of judgement, is deeply regretted.

Place: Bengaluru (Srikant Venkitachalam)

March 31, 2017

Certificate

This is to certify that the work embodied in this thesis entitled "An individual-based model for simulating the ecological and evolutionary dynamics of *Drosophila* cultures under different scenarios of larval crowding" has been carried out by Mr. Srikant Venkitachalam under my supervision at the Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, and that the results presented in this thesis have not previously formed the basis for the award of any diploma, degree or fellowship.

(Prof. Amitabh Joshi)

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Chapter 1

Introduction

The motivation for this simulation study is the realization in our laboratory over the past several years that experimental populations of *Drosophila* subjected to larval crowding every generation can actually evolve greater competitive ability via fairly different sets of phenotypes, depending on the ecological details of how exactly the larval crowding was imposed (Sarangi 2013; Nagarajan *et al*. 2016; Sarangi *et al*. 2016; M. Sarangi and A. Joshi, *unpubl*. *data*). Earlier work on multiple sets of selected and control *Drosophila melanogaster* populations had suggested that populations subjected to larval crowding evolve greater competitive ability largely through an increased larval feeding rate and greater tolerance to metabolic wastes (Mueller 1997; Joshi *et al*. 2001; Prasad and Joshi 2003; Mueller and Cabral 2012). The more recent studies, however, indicate that which suite of traits evolves in crowding-adapted *Drosophila* populations is likely dependent on the total volume of food available in the crowded cultures, and not just the density in terms of egg per unit volume of food (Sarangi 2013; Nagarajan *et al*. 2016; Sarangi *et al*. 2016; M. Sarangi and A. Joshi, *unpubl*. *data*). In this context, it will be important to be able to examine the effects of different ecological scenarios of larval crowding implemented through differing protocols in selection experiments using *Drosophila*. Due to logistical constraints, large numbers of selection experiments cannot be carried out. Therefore, computer simulations that enable a systematic examination of the evolutionary dynamics of different suites of fitness-related phenotypes in *Drosophila* populations subjected to larval crowding in different ways can be very useful in narrowing down the

list of possible selection experiments to identify those that will offer the greatest understanding for the effort put in. The present study reports on the development of the kernel of such an individual-based simulation with which one can ascertain the effects of different ways of imposing larval crowding on the distributions of fitness-related traits within a generation, as well as the longer-term evolutionary dynamics of *Drosophila* populations subjected to larval crowding in different ways.

The theory of density-dependent natural selection, initiated formally by MacArthur and Wilson (1967), a body of knowledge aiming to explore the evolutionary consequences of extreme population densities, is a major conceptual bridge connecting ecological and evolutionary dynamics (Mueller 1997; Joshi *et al*. 2001; Dey *et al*. 2012). Since the early phase of the formalization of mathematical models and the verbal theory of density-dependent selection (e.g. Pianka 1970; Roughgarden 1971), laboratory cultures of *Drosophila* have been extensively used to test predictions from the theory (reviewed by Mueller 1997, 2009; Prasad and Joshi 2003).

One of the first rigorous laboratory studies involving empirical testing of predictions of the *r*- and *K*-selection theory about the density-dependent evolution of population growth rates and of life-history traits, was done using six populations of *D*. *melanogaster*. Three of the populations (*r*-selected populations) were reared at low numbers by adult culling each generation, while the other three (*K*-selected populations) were reared at high density, maintained at carrying capacity by serial transfer (Mueller and Ayala 1981). The *r* and *K* prefixes here refer to the maintenance regimes having density-independent and

density-dependent mortalities, respectively, not the *r* and *K* parameters of the logistic equation of population growth (Mueller and Sweet 1986).

As predicted by mathematical formulations of the theory, the *r*-selected populations showed higher population growth rates at low densities compared to the *K*-selected populations, but lower growth rates at high densities (Mueller and Ayala 1981). Also as predicted, the *K*-selected populations showed increased competitive ability in comparison to the *r*-selected populations (Mueller 1988). Subsequent studies on these populations explored the evolved differences between the two sets of populations in various traits that gave rise to this observed *r*-*K* trade-off accompanying the evolution of greater competitive ability in the crowding adapted *K*-populations.

Larval feeding rate, measured as cephalopharyngeal sclerite retraction rate, was thought to be a major contributor to competitive ability (Bakker 1962; Burnet *et al*. 1977). Thus, the *K*-selected populations were expected to have evolved increased larval feeding rate compared to the *r*-selected populations. Indeed, the experimenters did see an increased feeding rate in the *K*-selected populations compared to the *r*-selected populations, suggesting that sclerite retraction rates were reliable indicators of competitive ability in larvae (Joshi and Mueller 1988), an observation subsequently confirmed multiple times in *D*. *melanogaster* populations selected for adaptations to larval crowding, increased parasitoid resistance, and rapid pre-adult development (Joshi and Mueller 1996; Fellowes *et al*. 1999; Joshi *et al*. 2001; Shakarad *et al*. 2005; Rajamani *et al*. 2006).

One of the traits expected to evolve in populations routinely facing crowding was an increased efficiency of food utilization and conversion to biomass (MacArthur and Wilson 1967). However, contrary to expectations, the *K*-selected populations did not evolve an increase in efficiency of food utilization, as demonstrated by experiments for minimum food requirement for pupation, where the larvae of the *K*-selected populations required as much or more food to pupate at low density, compared to larvae of *r*-selected populations, while having similar growth profiles (Mueller 1990; Mueller and Southwood 1991). It was thus proposed, as an explanation to these puzzling results, that a trade-off might exist between efficiency of food conversion versus other traits like feeding rate and waste tolerance that could give larvae substantially greater competitive ability, while adversely affecting the efficiency of conversion of food to biomass (Mueller 1990).

In addition to increased larval feeding rates, the *K*-selected populations also evolved greater pupation height than their *r*-selected control populations (Mueller and Sweet 1986; Joshi and Mueller 1993). It was also seen that body size of adults was greater in the *K*-selected populations compared to the *r*-selected populations, but only at high densities, with no differences between the two types of population observed at low densities (Bierbaum *et al*. 1989). Pre-adult viability also showed a similar pattern, suggesting that genotype × density interactions were very important in the considerations of density-dependent evolutionary theories, an aspect that theoreticians had largely ignored until then (Bierbaum *et al*. 1989).

Adult traits such as fecundity and survivorship were also studied to discover any genetic differences that could have evolved as a result of crowding in *K*-selected populations. Even though no differences were found, the assays were only conducted at low density and thus genotype × density differences could not be ruled out (Bierbaum *et al*. 1989).

As the low population size of the *r*-selected populations put them at the risk of having deleterious alleles fixed in the populations due to random genetic drift, the *r*-selected populations were mixed to form the *rxr* populations, which had the combined genetic variation of the three populations. Matched *rxrK* populations and *rK* populations were then started at high densities from *rxr* and *r*-selected populations respectively, and were subsequently tested to confirm the robustness of the results with respect to the phenotypic differences found in earlier high versus low density comparisons between the *r*- and *K*-selected populations. Similar trade-offs in growth rate were seen in the derived populations as observed earlier in the original low- and high-density populations. Larval trait differences of increased competitive ability, larval feeding rate and pupation height were also found in these new *rK* and *rxrK* populations when compared to their low-density matched populations, enabling drift to be ruled out as a cause of observed differences between the two sets of populations (Mueller *et al*. 1991, Guo *et al*. 1991, Joshi and Mueller 1993).

Although the *r*- and *K*-selected populations allowed the rigorous study of the evolution of larval traits under low and high densities, as well as the testing of density-dependent selection models, the study was not without many confounding factors, making

unequivocal interpretations of some results difficult. As the *r*-selected populations were maintained at very small population size, the evolved traits could always be affected by random genetic drift. Additionally, the *r*-selected populations had discrete generations and a very short breeding life, whereas the *K*-selected populations had overlapping generations and could breed throughout life. Besides larval density, the selection pressures faced by these populations were, thus, also different along the axes of adult density and time of reproduction. To avoid these confounding factors, a new set of populations was started in the same lab, this time with more modular control of the selection pressures at different life stages. The UU (**U**ncrowded as larvae, **U**ncrowded as adults), CU (**C**rowded as larvae, **U**ncrowded as adults) and UC (**U**ncrowded as larvae, **C**rowded as adults) populations were started, each population block ancestrally matched and derived from *D. melanogaster* populations from a different geographical origin than the *r*- and *K*-selected populations (Mueller *et al*. 1993).

The CU populations evolved a greater mean larval feeding rate compared to the low larval density UU populations, similar to the results seen in *K*- and *r*-selected populations (Joshi and Mueller 1996). Another result that was robust enough to be replicated in the new set of populations was the food to biomass conversion efficiency of the larvae, with the CU populations having lower efficiency, taking more food to complete development to become similar sized adults, when compared to matched UU populations (Joshi and Mueller 1996). The possible explanation of faster food passage in the larvae of CU populations leading to lower efficiency was also tested, and rejected. Thus, it was proposed that a trade–off between larval food acquisition and efficiency of food to

biomass conversion was a possible general phenomenon in organisms exhibiting scramble competition (Joshi and Mueller 1996). One difference seen in case of the *r*- and *K*-selected populations that was not replicated in case of the later set of populations was that of pupation height, with no differences being observed between the pupation heights of the UU and CU populations (Joshi and Mueller 1996). Possible reasons for this were cited to be the shift of the new populations to the more moist banana food medium compared to the previous cornmeal medium, and maintenance differences between the two sets of populations, with the *r*-selected populations escaping selection for pupation height by pupating on the inserted tissue (Joshi *et al*. 2003).

On further inspection of the feeding behaviour of the larvae reared for many generations in low or high density environments, it was found that the feeding rate in larvae from both the UU and CU populations increased with age (Santos *et al*. 1997). However, from early third instar on, CU larvae were consistently heavier than UU larvae. Despite this larval body size difference, CU larvae eclosed as adults of the same size, suggesting that higher feeding rate in the CU larvae might have elevated energy costs and thus lowered food to biomass conversion efficiency. Additionally, no changes were seen in the development time of CU larvae compared to the UU larvae, when reared at low density (Santos *et al*. 1997).

Another interesting discovery was the finding of a genetic polymorphism in the CU populations, with different larval phenotypes being favoured at different times in the course of one generation of rearing in crowded culture vials, likely due to deterioration of

food over time by larval and microbial activity (Borash *et al*. 1998). The larvae that developed from egg to adult stage relatively early evolved faster feeding rate and suffered a cost to pre-adult viability, especially in food treated with ammonia or urea, which are thought to be the components of waste that builds up over time in larval cultures (Botella *et al.* 1985; Borash *et al.* 1998). Conversely, larvae developing towards the end of the eclosion distribution over time were found to have higher viability in crowded conditions as well as at low density, and were not faster feeders. The larvae of late eclosing flies also had higher viability in food treated with ammonia or urea. These results could be explained by the two types of larval morphs having two different feeding strategies – the early eclosing larvae would feed faster and largely avoid the period of food deterioration, whereas the larvae eclosing late would have to largely feed and grow at a later stage in the larval cultures, with relatively low levels of food and a high metabolic waste concentration. Such late eclosing larvae would then be selected for higher viability in the presence of waste products, whereas the early eclosing larvae would be selected for faster feeding rate (Borash *et al*. 1998). Subsequent work suggested that inadvertent assortative mating for development time, due to the handling protocol, may have facilitated the maintenance of this early-late larval polymorphism (Nagarajan 2010).

Further tests of this apparent genetic trade–off between feeding rate and tolerance to urea/ammonia discovered in the CU populations were also carried out. In a different study, populations of *D*. *melanogaster* from the same ancestral populations as the UU and CU populations were selected for faster development (Chippindale *et al.* 1997), and the larvae of these were taken as substitutes for the 'early' larval morphs seen in the CU

cultures. As expected, the larvae from faster developing populations displayed higher larval feeding rates and low egg to adult viability especially in the presence of ammonia/urea when assayed at low density (Borash *et al*. 2000b).

In order to test if the genetically based tolerance to high levels of toxic compounds like ammonia or urea could evolve in populations of *D. melanogaster*, the AX and UX populations were started, which were directly selected for ammonia and urea tolerance respectively. The larvae of these ammonia/urea tolerant populations evolved slower feeding rates compared to their untreated controls, and had greater viability in toxic media of ammonia/urea respectively (including some cross tolerance – see Borash *et al*. 2000a), similar to the 'late' phenotypes of the CU larval cultures (Borash *et al.* 2000b).

Additionally, adults of the CU populations also evolved higher time to death by starvation as well as higher lipid content in their bodies (Borash and Ho 2001). This is similar to earlier studies performed on *K*-selected populations, which were also seen to evolve greater starvation resistance compared to *r*-selected populations (Mueller *et al.* 1993). Other larval traits like greater foraging path length evolved in the CU larvae compared to UU larvae as well (Sokolowski *et al.* 1997, greater foraging path length was also observed in case of larvae of *K*-selected populations compared to *r*-selected populations). It was also seen that larval foraging path length and larval feeding rate evolved in a positively correlated manner (Mueller *et al.* 2005).

By the close of the 20th century, a clear picture had emerged of the evolution of competitive ability in populations of *D. melanogaster* reared at high larval densities. Larvae from selected populations could feed faster and eclose earlier than others (at high but not low density) at the cost of both food to biomass conversion efficiency and pre-adult viability (Mueller 1997; Prasad and Joshi 2003). Alternatively, the larvae could stay longer in the food by being slower feeders, but enjoy greater viability in the relatively more toxic and lower levels of food (Mueller 1997; Prasad and Joshi 2003). Further theoretical work, using simple optimization models, explored this issue further and predicted reduced feeding rate to be optimal when larvae were faced with increased energy expenditure of ammonia detoxification in food with high levels of metabolic waste (Mueller *et al.* 2005; Mueller and Barter 2015).

Within a decade after the crystallization of this canonical understanding of adaptation to larval crowding in *Drosophila*, it began to become apparent that the story was more nuanced. Long-term studies were carried out in our laboratory to see whether *D. ananassae* and *D. nasuta* also evolved adaptations to crowding in a manner similar to that seen in the earlier studies on *D. melanogaster* (Sharmila Bharathi 2007). It was found that the evolutionary trajectory of these population was very different from that seen earlier in *D. melanogaster*, with the evolution of a completely different set of larval trait than expected (Nagarajan *et al*. 2016). Rather than evolving higher larval feeding rates at the cost of larval food to biomass conversion efficiency, or evolving higher nitrogenous waste tolerance, these crowding adapted populations of *D. ananassae* and *D. nasuta* evolved shorter pre-adult development times, even when assayed at low density, and

reduced minimum feeding time to pupation (Nagarajan *et al*. 2016). Like in the previous studies, though, these crowded larval populations still evolved greater competitive ability and pre-adult viability at high density compared to their matched low larval density control populations. Thus, at least in some species related to *D*. *melanogaster*, it became clear that the evolution of competitive ability due to larval crowding could take trajectories different from those predicted by the then-canonical view (Nagarajan *et al*. 2016). Reasons proposed to explain such unexpected evolutionary changes in these related species included species-specific differences in genetic architecture of traits comprising competitive ability, a shorter duration of laboratory domestication, or different, supposedly minor, details of crowding ecology of larvae leading to large differences in how they experienced competition (Nagarajan *et al*. 2016). While the CU larvae were crowded in regimes of 1200-1500 eggs in 5-6 mL banana-molasses food, the larvae of *D. ananassae* and *D. nasuta* were crowded with 600 eggs in 1.5 mL cornmeal food and 350-400 eggs in 2 mL cornmeal food, respectively.

To further study these differences in the traits that evolved under crwoding in different studies, a new set of populations of *D*. *melanogaster* descended from the UU populations was started, with the crowding regime of 600 eggs in 1.5 mL cornmeal food in the MCU (**M**elanogaster, **C**rowded as larvae, **U**ncrowded as adults) populations and the low larval density of 70 eggs in 6 mL cornmeal food in the MB (**M**elanogaster **B**aseline) populations (for more details on ancestry and maintenance regime, see Sarangi *et al.* 2016). These populations were, thus, subjected to crowding at high density but low levels of food, like in the *D*. *ananassae* and *D*. *nasuta* studies, but unlike the earlier *D*.

melanogaster studies that used high density, but with relatively high total amount of food (Sarangi *et al*. 2016).

Compared to the MB controls, the MCU larvae evolved higher egg to adult survivorship and competitive ability at high densities, this result being similar to all the previous crowding selected populations. However, the MCU populations also evolved shorter pre-adult development times compared to the MB populations at both densities. The MCU larvae showed higher larval survivorship than the MB larvae when both were measured at various time points of feeding from egg collection, and also had higher dry weight than their MB counterparts at most of these time points. The higher body weight of the larvae of MCU populations were limited mainly to the pre-critical size phase, and by the wandering stage the MCU larvae were lighter than the MB larvae (Sarangi 2013). Adult MCU males and females were lighter in dry weight than MB males and females, respectively. No differences were observed in larval feeding rates, pupation height or foraging path lengths for the MB and MCU populations, nor did they show any difference in tolerance to ammonia or urea (Nagarajan 2010, Sarangi 2013, Sarangi *et al.* 2016). Thus, it was clear that like in the *D*. *ananassae* and *D*. *nasuta* populations, the MCU populations had evolved greater competitive ability via a shortened mean pre-adult development time and reduced minimum critical feeding time to pupation, suggesting that differences in details of larval crowding were probably responsible for the divergent results compared to the CU populations (Sarangi *et al.* 2016). Additional evidence for this line of thought came from the fact that in larval competition assays, the lowest overall survivorship was observed in the 1200 eggs in 6 mL culture (‘CU’ type) rather

than the 600 eggs in 1.5 mL culture ('MCU' type), and this suggested that the dynamics of competition were probably governed by factors other than just density defined by the number of eggs per unit volume food (Sarangi 2013). A single generation monotypic culture experiment involving MB and MCU larvae was set up to explicitly test this, with the cultures replicating MB (70 eggs in 6 mL food), MCU (600 eggs in 1.5 mL food), and CU (1200 eggs in 6 mL food) types of environments. Another culture of 1200 eggs in 3 mL food (henceforth called 'CCU' or **C**ontrol **CU** type culture) was also included as it had the same eggs/ mL food density as the MCU type cultures, but had egg number similar to CU type cultures (Sarangi 2013). The results from this experiment showed that the highest mean egg to adult development time was seen in case of the CU type of culture, with higher egg/mL food density treatments having lower pre-adult development times (Figure 1). The MB type culture had the highest adult dry weight, and in case of the crowded larval cultures, the dry weight showed a trend of increasing with the level of food provided (Figure 2). Interestingly, pre-adult survivorship was lowest in case of the CCU type of culture, with the MCU and CU type cultures having higher pre-adult survivorship (but much lower than MB type) (Figure 3). On studying the distribution of adult dry weight along the pre-adult development time axis, the initially eclosing flies in all the crowded cultures showed a trend of being heavier than the ones eclosing later in the distribution (but appeared smaller than the adults at any time of the MB type culture) (Figure 4). While the MB type culture had eclosions lasting for around two days, this figure was five days in case of MCU type culture, and over 20 days (500 hours) in case of the CU type of cultures. Initial as well as later eclosions in case of the CU type of culture had a trend of heavier adults compared to the middle eclosions (Figure 5). The CCU type

cultures had eclosion distributions similar to MCU type, but also had some flies eclosing after 500 hours, which appeared to be heavier than the adults that emerged in the middle of the eclosion distribution (Sarangi 2013).

Thus, a conclusion could be made that the level of food column in the vial (longer food columns might potentially act as a 'sink' for metabolic waste to diffuse into), as well as the density of larvae at the feeding surface (the 'feeding band' of larvae) are also very important factors in the ecology of a high density culture vial, besides the number of eggs per unit volume food (Sarangi 2013). Indeed, a recent study did find some diffusion of nitrogenous waste at the lower levels of 8 mL food columns after four days of 1200 egg larval cultures. The level of waste in the feeding band was found to plateau at around the four- to five-day mark as well (M. Sarangi, S. Dey and A. Joshi, *unpubl. data*).

Along with the MB and MCU populations, two more sets of populations are now undergoing larval crowding selection in our laboratory to explore the evolution of traits contributing to competitive ability under different ecologies of crowding (M. Sarangi and A. Joshi, *unpubl. data*). The LCU (**L**aurence Mueller type **CU**) populations have a selection regime of larval crowding involving 1200 eggs in 6 mL cornmeal food in 6 dram vials, whereas the CCU populations have larval crowding of 1200 eggs in 3 mL cornmeal food in 8 dram vials, acting as exact density but not food level controls to the MCU populations. The LCU populations were started in order to control for the food medium differences between CU and MCU populations (banana and cornmeal medium respectively), as well as remove the daily vial transfer aspect of the CU populations'

maintenance regime, which was thought to promote assortative mating, leading to the stark patterns of genetic polymorphism seen in the CU larvae (Nagarajan 2010). Studies involving the two new populations have been conducted for about two years before the time of writing.

After almost 30 generations of selection, the larvae of LCU and CCU populations showed an overall trend of greater survival in competition assays in comparison to the MB populations at high-density environments, with significantly greater pre-adult survivorship than the MB larvae in their respective selection environments. In the monotypic culture competition experiments as well, the LCU and CCU populations showed a greater trend of survival than the MB populations at high densities. These results suggest that the two populations are probably evolving greater competitive ability and survivorship at high densities. As to the traits that are evolving in order to increase competitive ability, the results are still relatively preliminary. In case of the single larval feeding experiment like in the earlier crowding experiments, the LCU populations do show the highest larval feeding rate, just like the CU populations. However, this difference disappears when there are groups of up to 20 larvae feeding on a plate of larger diameter. If feeding rate is measured directly in the culture vials via video recordings, then the MCU larvae appear to feed faster than MB larvae, a result that is not observed in single larval assays. Additionally, no consistent results have been seen in case of nitrogenous waste tolerance of any larval crowding selected population (M. Sarangi and A. Joshi, *unpubl*. *data*).

In light of all these studies, suggesting subtle effects of crowding ecology on the manner in which increased competitive ability in high-density populations evolves, further exploration of these ideas is required. Although extremely useful, long-term selection experiments like the CCU populations and the monotypic culture competition assays are also logistically limited in their scope of exploration due to their demands of resources and manpower. One way more ideas of larval crowding can be explored is via an individual based simulation which takes into account all the traits thought to be related to competitive ability and systematically explores their distributions in different ecologies in isolation, and in combination with other traits, ultimately tracing their evolutionary patterns. Although not exactly representative of the real world scenarios observed in the experiments, such a modelling exercise would be useful in streamlining the thoughts of experimenters on the avenues and kinds of studies that may be useful to conduct in the future.

In this thesis, I have presented the preliminary work on such an individual based simulation study. The first half attempts to study the larval feeding ecology with variations in different traits thought to contribute to competitive ability, using different combinations of egg numbers and food volume. The second half explores the evolutionary consequences of different details of larval crowding.

Figure 1. Mean pre-adult development time in monotypic culture experiments (with permission from M. Sarangi, *unpubl. data*)

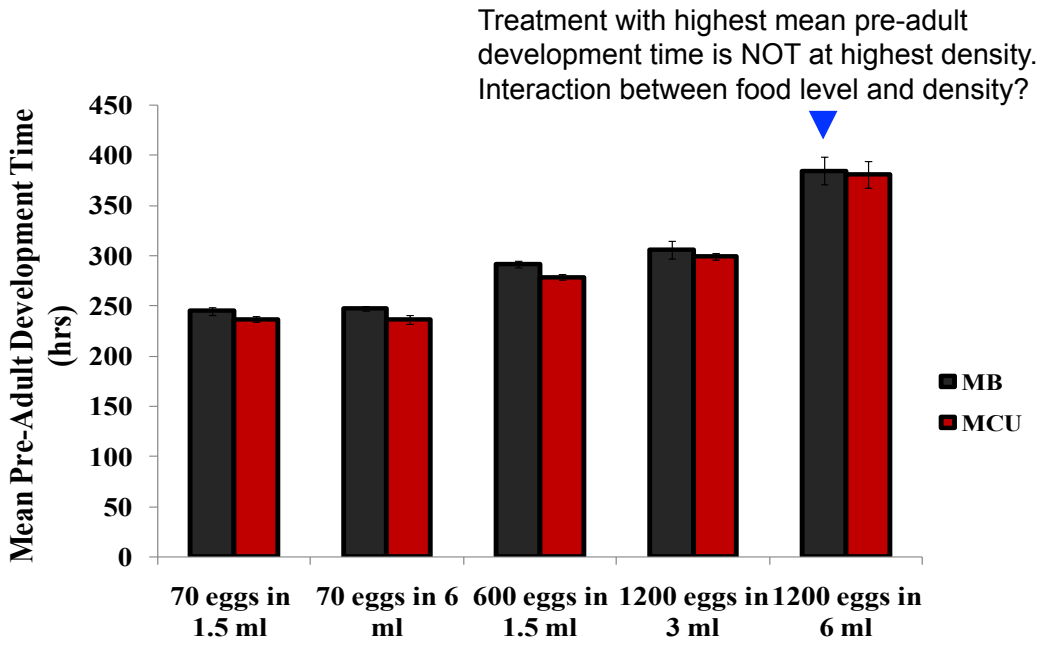


Figure 2. Mean adult dry weight in monotypic culture (with permission from M. Sarangi, *unpubl. data*)

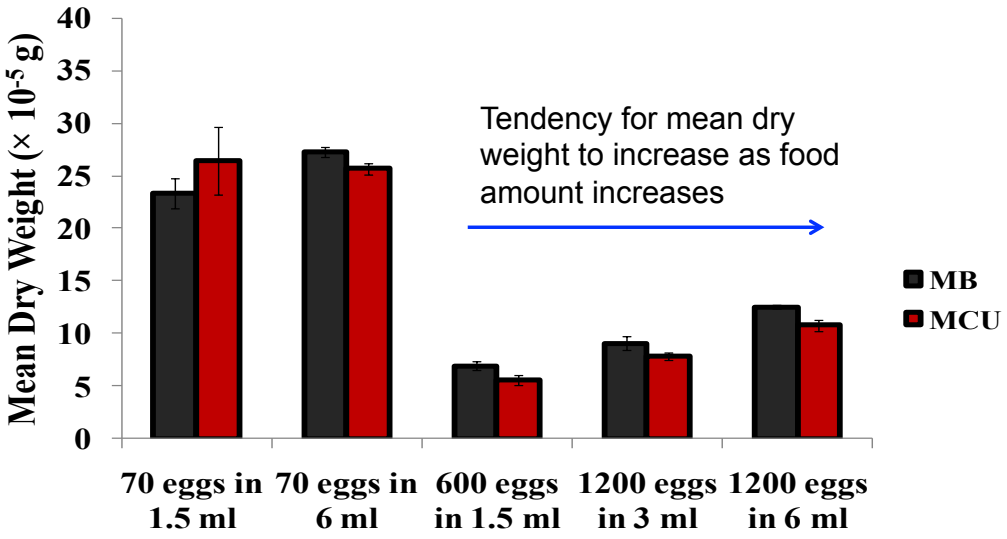


Figure 3. Mean pre-adult survivorship in monotypic culture experiments (with permission from M. Sarangi, *unpubl. data*)

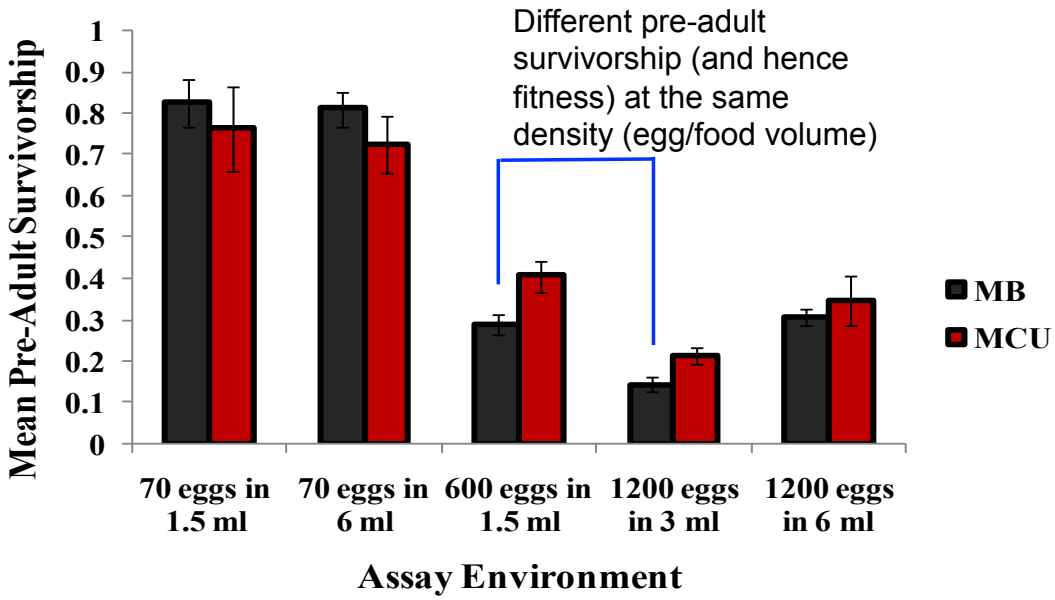
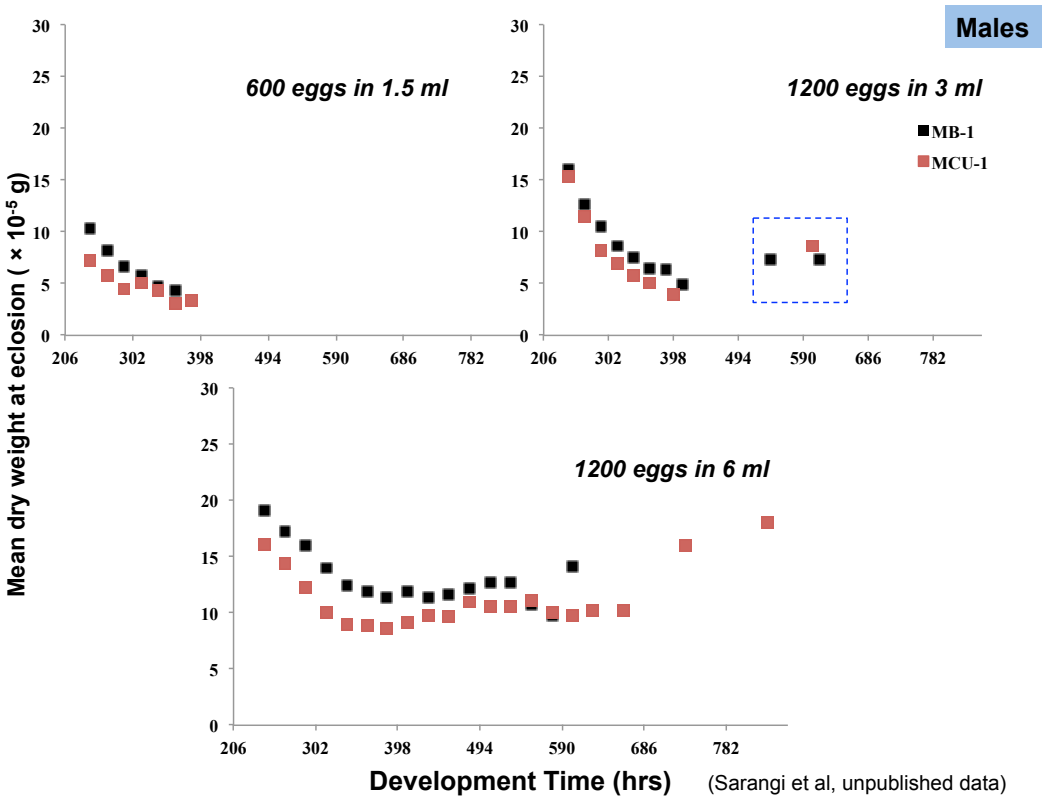
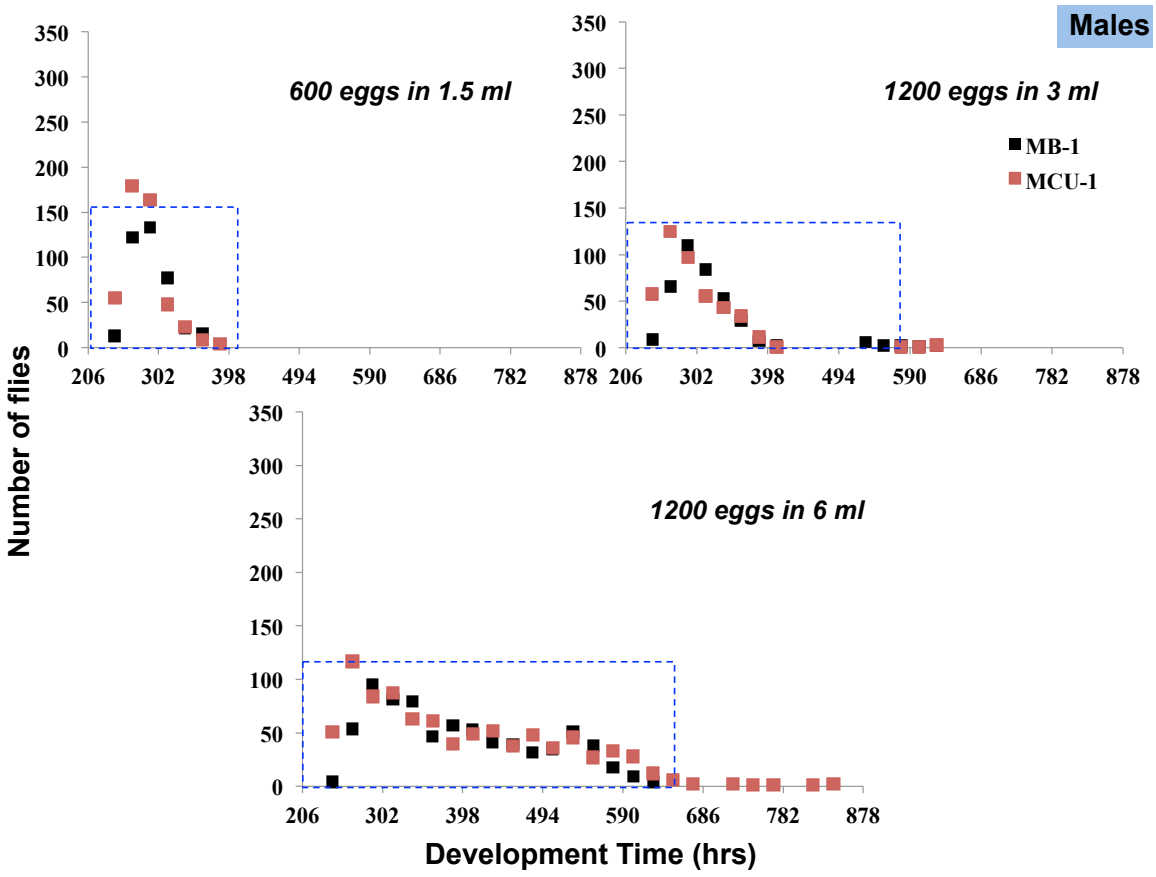


Figure 4. Dry weight vs. pre-adult development time of males in high-density monotypic cultures (with permission from M. Sarangi, *unpubl. data*)



(Sarangi et al, unpublished data)

Figure 5. Number of male adult flies eclosing over time in high-density monotypic cultures (with permission from M. Sarangi, *unpubl. data*)



Chapter 2

Modelling the Ecology of Larval Culture vials – I

Primary objective and description of the model

In capturing the essence of a real biological system akin to the *Drosophila* larval feeding environment in a culture vial, perhaps the most fundamental step is to replicate the feeding ecology of the vials to the greatest extent possible. In case of experimental set-ups such as those employed in our laboratory, the important factors to incorporate in the vial ecology are growth due to feeding, excretion of metabolic waste, and the feedback of increasing waste concentrations on the future feeding of larvae (Prasad and Joshi 2003). Experiments using single species cultures, of the kind described in the previous chapter, offer a system that can be readily represented in a simulation with similar egg/food ratios to those used in the experiments.

In one such experiment (Sarangi 2013), five types of culture vials were used to examine the effects of different types of crowding on fitness-related traits (Figure 1) –

1) 70 eggs, 6.0 mL food (Henceforth called MB type)
2) 70 eggs, 1.5 mL food
3) 600 eggs, 1.5 mL food (Henceforth called MCU type)
4) 1200 eggs, 3.0 mL food (Henceforth called CCU type)
5) 1200 eggs, 6.0 mL food (Henceforth called LCU type)

Of these, 70 eggs in both 1.5 mL and 6 mL food are classified as low-density cultures, but results are primarily focused on the MB type. As for the experiments themselves, the results have been discussed in chapter 1.

The ultimate goal of the current model is to see if one can reproduce patterns similar to what is seen in case of the MB populations in each of the monotypic cultures, with the further aim of predicting survivorship, pre-adult development time and body size distributions across a wide range of possible culture conditions not experimentally studied yet.

In the description of the model, terms such as eggs, larvae, food, and metabolic waste are used. By these, one only means to represent certain facets of the entity, such as volume in model units, or specific behaviours, rather than the actual entity.

Additionally, although actual values of food volume may not be represented in the simulation, it is assumed that the ratios of egg/food provide a reasonable basis for comparison between the model and empirical results.

Base model

Every simulation experiment was recorded over a certain number of discrete time steps, chosen to encompass the entire assumed period of each larva's activity, from hatching to its ultimate fate at leaving the food in the culture. Common to each run, the starting point consisted of a given number of eggs of equal size, based on the culture type being

simulated. These eggs hatched after the first time step and the larvae began feeding on the given amount of food immediately, following certain feeding rules (Figure 4). Each larva ate food according to a given bite size scaled by its current body size as well as a given feeding rate (number of bites per time step) (Equations 1, 2). Not all of the food eaten by each larva in a time step was translated to growth (Equation 3), due to some loss in potential growth caused by its given food to biomass conversion efficiency (Equation 4). Additionally, a fraction (half) of the food lost to efficiency by all the larvae was excreted as metabolic waste, which built up over time as the larvae exponentially grew in size (Equation 6). The concentration of metabolic waste in remaining food also reduced the potential growth a larva could achieve in a given time step (Equation 5).

Even though time steps were discrete, the volume of food eaten per larva in a time step decreased the total food available to the next larva in the feeding order in that time step. The waste pool also increased in a similar fashion over the time step. The feeding order of larvae at every time step was randomized.

As the larvae increased in size over time, they crossed an arbitrarily set minimum critical size for pupation (Chiang and Hodson 1950; Bakker 1962). After attaining critical size, the larvae could only eat until reaching a maximum size (equal to five times the minimum critical size), or completing a post-critical-size feeding period, before committing to the wandering stage, eventually leading to pupation (Robertson 1963) (Figures 2, 3).

In the base model, multiple larval traits could be varied by drawing each of the trait values from a normal distribution with a given mean and standard deviation, in order to study their effects on the survivorship, time to wandering and final body size distributions in high- or low-density scenarios. The complete code with comments is given in Appendix 1.

Food Eaten by an Individual per Bite at a time step = Individual's Bite Size =

Current Body Size * Scaling Factor **(Equation 1)**

Food Eaten per Time Step = Bite Size * Feeding Rate **(Equation 2)**

Growth per Time Step = Food Eaten per Time Step - Potential Growth Lost to Food to Biomass Conversion Efficiency - Potential Growth Lost to Feedback Based on Current Total Metabolic Waste Concentration **(Equation 3)**

Potential Growth Lost to Food to Biomass Conversion Efficiency = Food Eaten per Time Step * (1 - Food to Biomass Conversion Efficiency) **(Equation 4)**

Potential Growth Lost to Feedback Based on Current Total Metabolic Waste Concentration =

Food Eaten * Current Total Metabolic Waste /Current Total Food * Feeding Rate **(Equation 5)**

Metabolic Waste Excreted = Percentage of Potential Growth Lost to Food to Biomass Conversion Efficiency **(Equation 6)**

Waste sensitivity as a variable

Earlier empirical evidence has shown that competitive ability in *Drosophila melanogaster* larvae can evolve via increased waste tolerance (Borash *et al.* 1998). A term of waste sensitivity (the inverse of waste tolerance) was incorporated for each individual in the feedback term (Equation 7), adding to the repertoire of manipulable traits. Larger waste sensitivity values would result in reduced potential growth of larvae.

Potential Growth Lost to Feedback Based on Current Total Metabolic Waste Concentration = Current Total Metabolic Waste /Current Total Food * Feeding Rate * Waste Sensitivity (Equation 7)

The phenomenon of larval stop

Larval stop (described by Mensua and Moya 1983) is the stopped development of third instar larvae when deprived of food. These 'stopped' larvae can resume feeding and pupate after over 300 hours of food deprivation. In the simulation, larval stop was incorporated by giving larvae an additional delay in time to wandering if they were forced to stop feeding due to shortage of food or build up of excessive metabolic waste in the culture (See Figures 5 and 6 for the altered algorithm).

Figure 1. The differing ecology in the monotypic culture experiment: photos taken 119 hours after egg collection (with permission from M. Sarangi, *unpubl. data*)

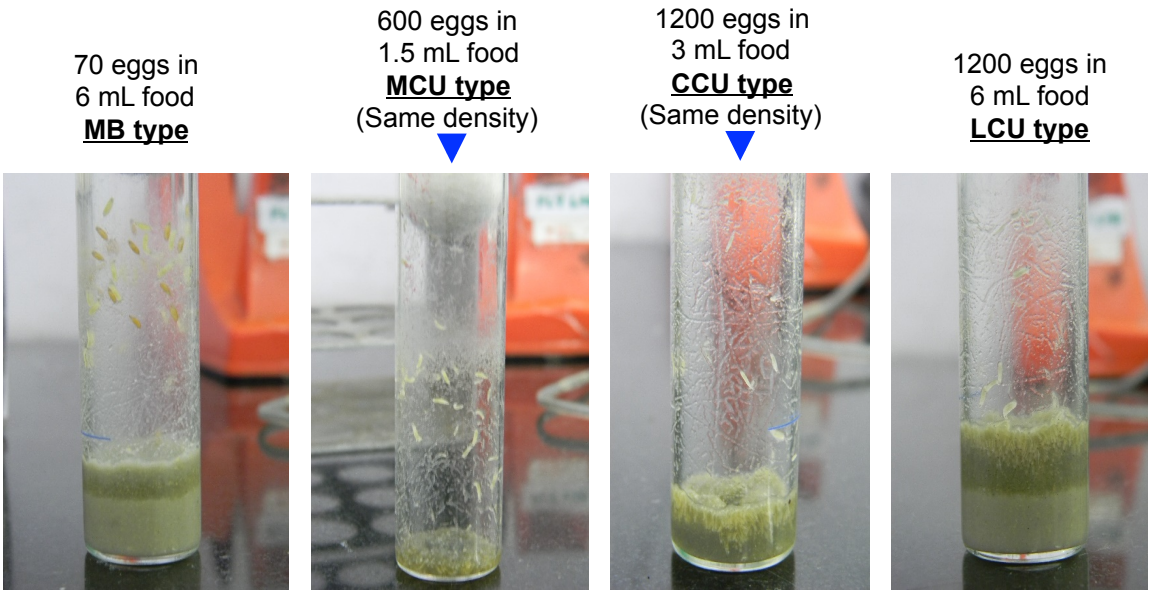


Figure 2. Increase of larval body size over time in the simulation for a single larva. The dashed red line denotes the minimum critical size for pupation

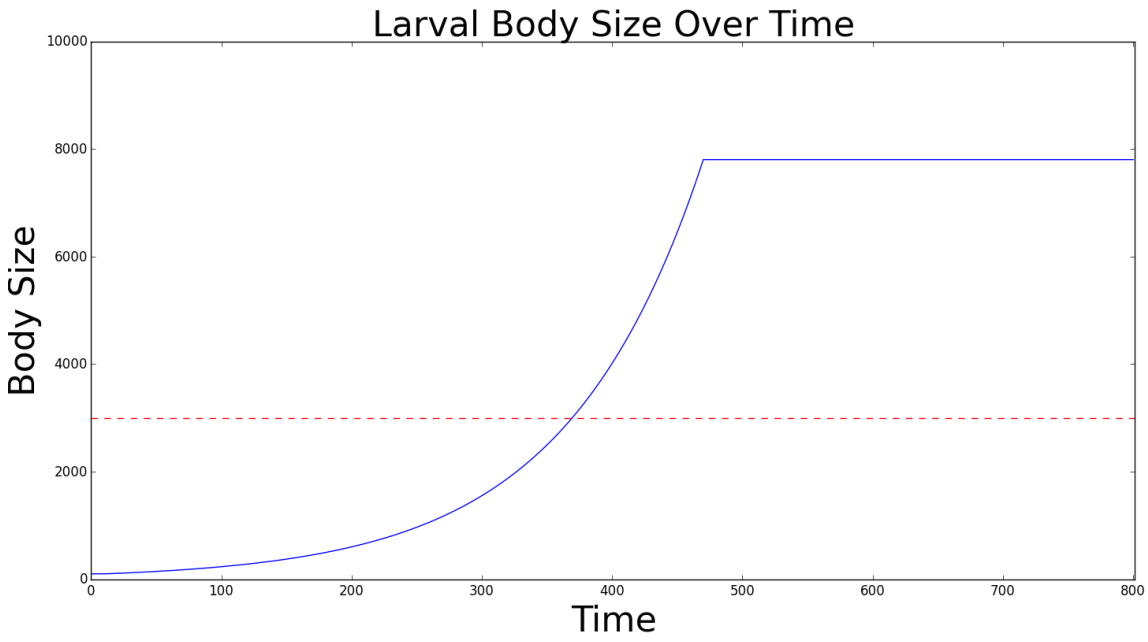


Figure 3. Increase of larval body size over time: multiple larvae, with among-individual feeding rate variation, drawn from a normal distribution. The dashed red line denotes the minimum critical size for pupation

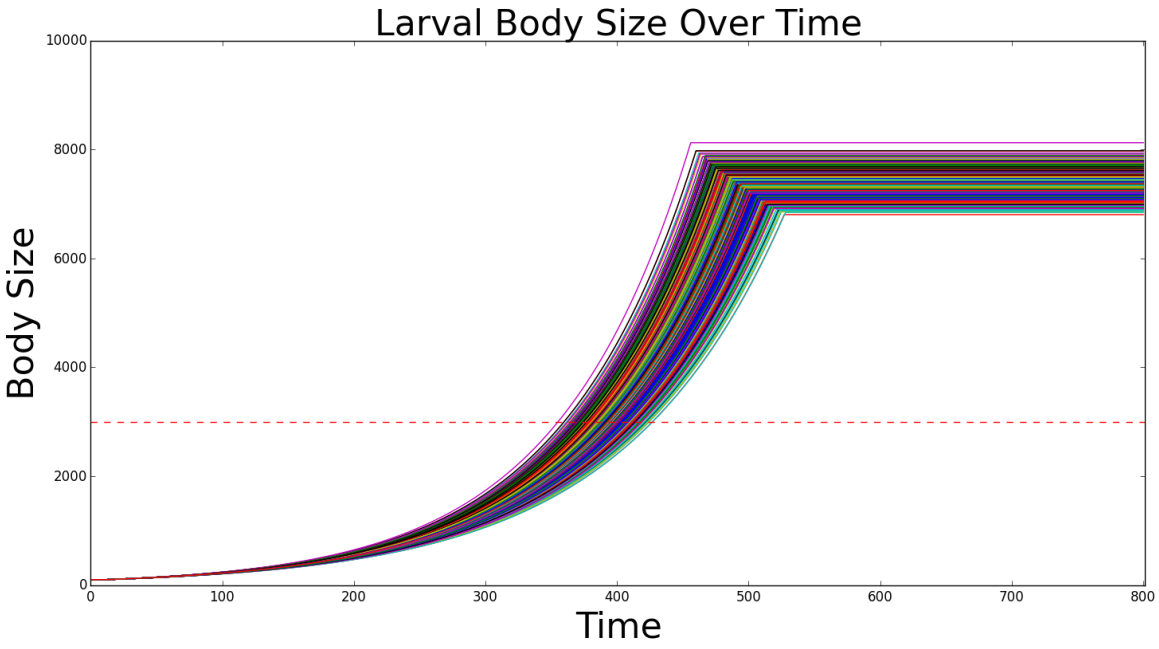


Figure 4. Base model algorithm; (PCT = Post Critical Feeding Time)

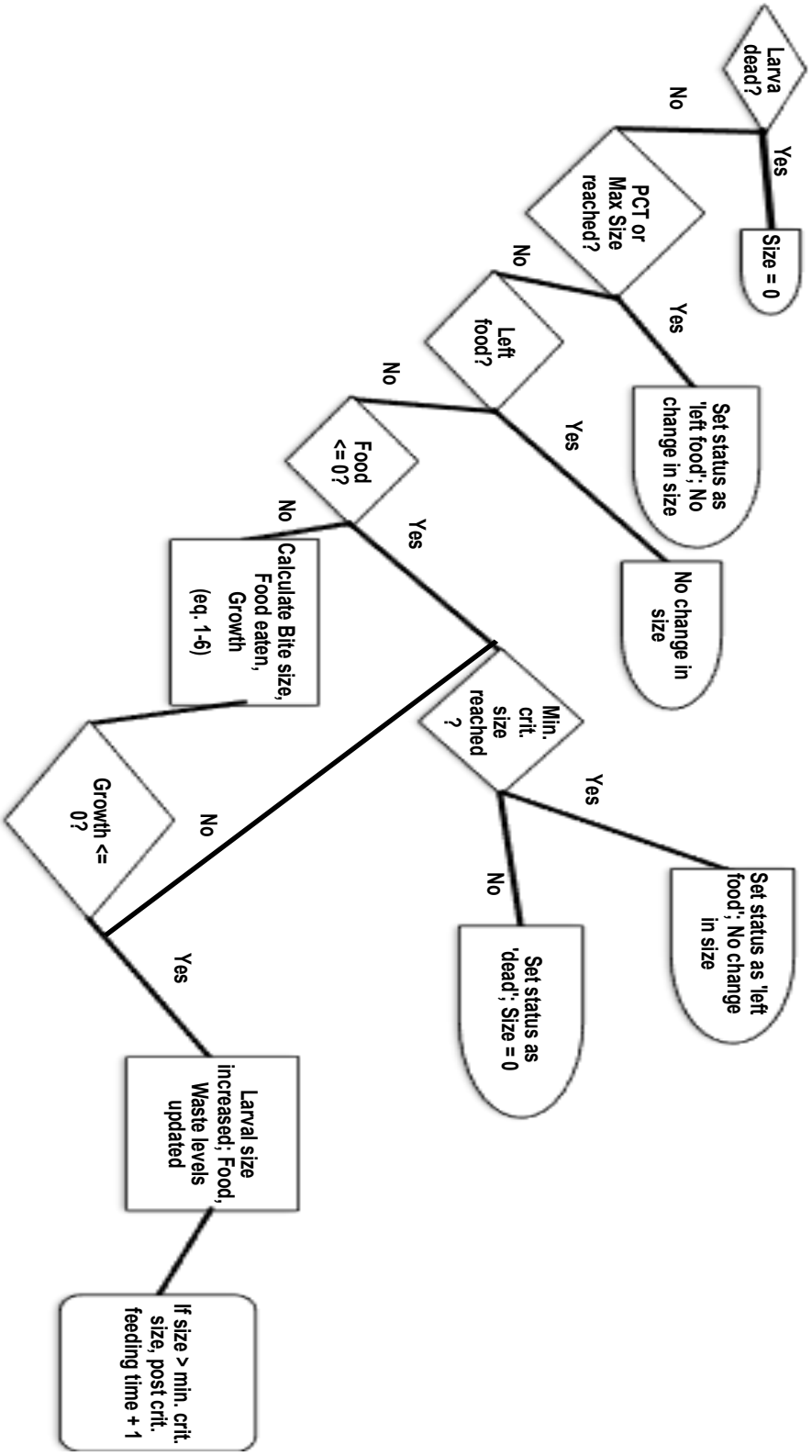


Figure 5. Alterations to base model due to larval stop (i)

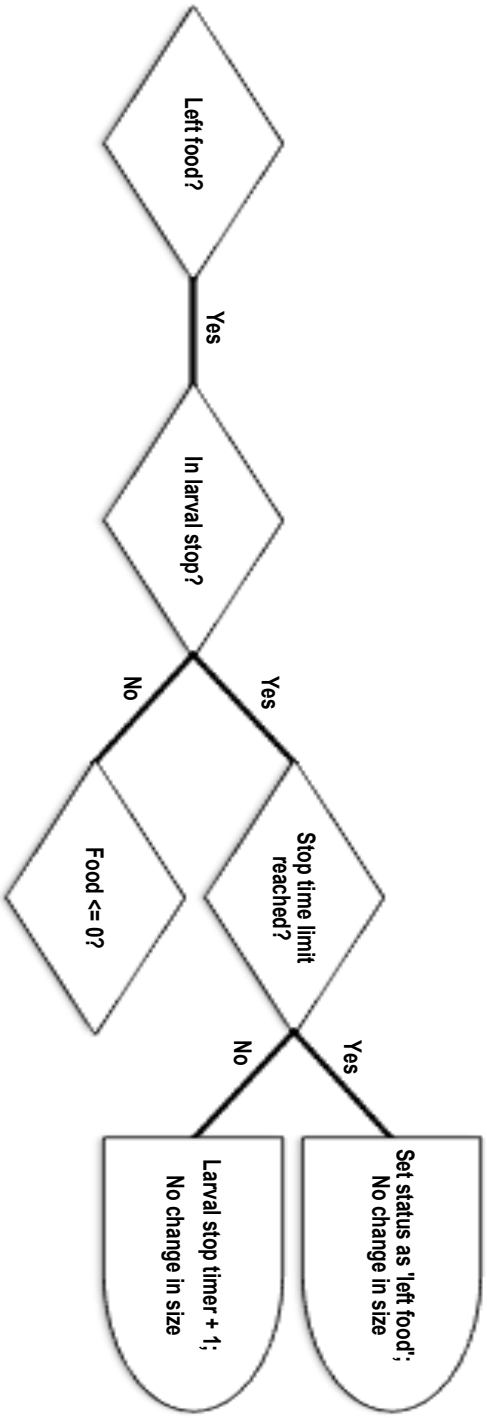
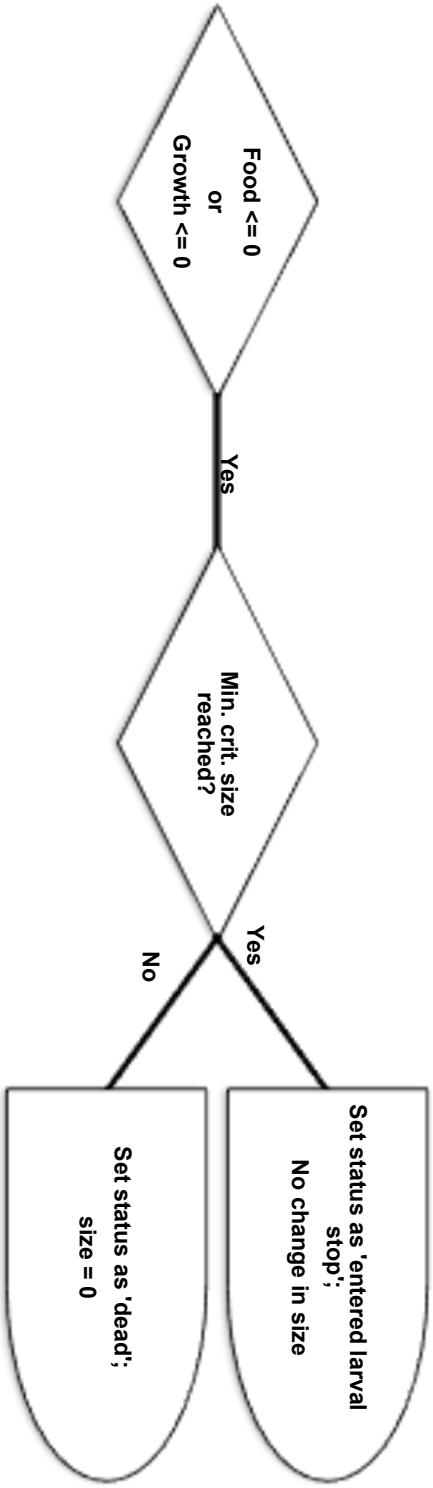


Figure 6. Alterations to base model due to larval stop (ii)



Chapter 3

Modelling the Ecology of Larval Culture Vials – II

Preliminary results from the model

In order to compare patterns of the adult body size and pre-adult development time distributions seen in experimental results to those seen in simulations, it was assumed that larvae reaching the wandering stage would translate their final body size to their adult forms, and that there would be no variation in the wandering or pupation times between individuals.

Food levels were set such that cultures in the simulations with 70 eggs (MB types), taken as low density, would have complete survivorship, while the MCU and CCU type cultures would suffer a large amount of mortality (about 75-85%).

Variation in the trait value distribution from which individual trait values were assigned to larvae was set such that it resulted in small perturbations in the low-density cultures while having greater effects on the high-density cultures. Thus, for the base model, only a small amount of variation was chosen for the feeding rate trait, while keeping all other trait values constant, in order to examine preliminary patterns in the results.

The final output of body size and time to wandering distributions were obtained as box plots for each egg/food volume ratio of the monotypic culture experiment – MB, MCU, CCU and LCU types (Figure 1). Additional plots were also generated for comparison

between size and time to wandering, size and varying trait(s), and varying trait(s) and time to wandering.

Base model

In the base model, feeding rate was varied among individuals using samples drawn from a normal distribution with standard deviation equal to 2.5% of the mean for each of the four culture types, resulting in inter-culture body size variation seen in Figure 2a and time to wandering variation seen in Figure 2b.

Body size of the MB type culture larvae had the highest mean value, with LCU type larvae having intermediate body size, and MCU and CCU types having the lowest body size values (Figure 2a). In terms of the spread of body sizes, the MCU and CCU types had very narrow spreads in body size, whereas MBs had intermediate spread and LCUs had the largest spread (Figure 2a). The MB type of culture had 100% survivorship, with the MCU and CCU type cultures both having around 15% survivorship. LCU type cultures had 100% survivorship (Figure 2a).

Time to wandering of the MCU and CCU type cultures had the lowest mean value (i.e. they were the fastest developers). The MB and LCU types had similar time to wandering mean values. The spread of the LCU type time to wandering was the largest, with the MB types having slightly lower spread of time to wandering. Both the MCU and CCU type cultures had extremely narrow spread in their time to wandering (Figure 2b).

In both the MB and LCU type cultures, the largest larvae appeared to have the lowest time to wandering and the highest feeding rate values. The relationship between size and feeding rate was similar in the MCU and CCU type cultures, although the distributions of both size and time to wandering were too narrow to distinguish any trends (Figures 2c, 2d, and 2e).

Adding waste sensitivity variation

Three levels of waste sensitivity variation were studied, with trait values drawn from normal distributions with standard deviations equal to 10%, 20% and 30% of the mean, respectively. The larval size and time to wandering distributions for the three levels of variation are shown in Figures 3, 4 and 5 respectively. Most notably, there is little to no change in the body size distributions of the MB type larvae in all three scenarios, whereas the spread in body size increases in all three crowded cultures with increasing waste sensitivity variation (Figures 3a, 4a, 5a).

The time to wandering distributions of both MB and LCU type cultures showed little change on increasing waste sensitivity variation. However, the MCU and CCU type larvae had greater time to wandering spread with higher waste sensitivity variation. Additionally, the mean value of the MCU and CCU time to wandering also increased with increasing trait variation (Figures 3b, 4b, 5b).

While the size versus time to wandering plots for MB type larvae are similar to those seen in the base model, the data look very noisy in case of the crowded cultures (all the

waste sensitivity variants showed this trend, although only the 30% trait variation plots are shown; Figure 5c).

Larval stop

Larval stop durations of 20, 40 and 60 time steps were added in three different simulation runs, with 30% waste sensitivity variation. For all cases of larval stop, there was no change the body size distributions compared to the case without larval stop. This was an expected result, as the larval stop modifications in the algorithms had no interaction with the actual feeding process.

However, development time spread in all three crowded cultures increased with increasing larval stop, with no change being seen in case of the low-density cultures (Figures 6, 7, 8). The change was made clear in the size versus time to wandering distributions (Figure 9), where the data that seemed noisy in case of 30% waste sensitivity variation with no larval stop, showed a clear split in two groups of individuals in the population, one showing larval stop and one not showing larval stop. The individuals that showed larval stop were also seen to have incomplete post minimum critical size feeding time, verifying that they were forced to leave the food before completing their feeding period. Individuals having reached the post critical size time limit did not show any larval stop. Additionally, there was also an emerging pattern of waste sensitivity versus time to wandering seen in case of the individuals undergoing larval stop, with the earliest developing individuals having the greatest amount of waste sensitivity amongst those showing larval stop. This was in contrast to the individuals who

did not undergo larval stop, who consistently had the lowest waste sensitivity. Individuals who developed later while facing larval stop had intermediate waste sensitivity values. They also completed a greater period of their post critical feeding period and had a greater body size compared to earlier developers facing larval stop (Figure 10).

Discussion

Preliminary results suggest that the kernel of the single generation model is robust with regard to expectations in its basic predictions about the ecology of the *Drosophila* culture vials. The low-density culture typically maintains a narrow body size distribution with the highest mean when density-dependent traits are varied, for example waste sensitivity and larval stop. The MCU and CCU type of cultures have the smallest body size, as also seen in the experiments, with the LCU type larvae showing an intermediate body size mean with a large spread. Thus, in very basic form, the simulation is able to reasonably capture gross aspects of patterns seen in the experimental data.

However, the pre-adult development time distributions seen with the base model are not in agreement with the experimental results. While the distributions of the MCU and CCU type cultures are typically far more spread out in the experiments, with a greater mean than the low-density culture, the simulation shows them as having the lowest mean time to wandering, with an extremely small spread. This is likely due to an exponential increase in metabolic waste coupled with a large decrease in food in the model, causing almost all larvae to stop feeding at the same time. LCU type larvae, however, have

enough food to feed till completion, even though their body size is lower due to higher accumulation of waste retarding growth over time.

The results for survivorship in different cultures are a mixed bag in terms of their agreement with experimental data. The MB types having higher survivorship than the MCUs and CCUs is in accord with experimental data, but the LCUs also show lower survivorship experimentally (they show complete survivorship in the simulations, probably due to higher food volume letting all larvae leave the food before it runs out or gets too toxic). Furthermore, there is also difference in MCU and CCU survivorship, although capturing that trend is outside the scope of the base model, which does not incorporate any aspects of the ecological differences between cultures with the same egg/food volume density but different total food amount.

On adding waste sensitivity variation, the time to wandering distributions of MCU and CCU type cultures expand, with an increase in mean value as well. Body size variation in the high-density cultures also increases, becoming more similar to the trends seen in the experiments. Low-density distributions stay unchanged, confirming that waste sensitivity is a trait that expresses its phenotypic effects mainly at higher densities. Other anomalies with development time correspondence to empirical results, however, remain: chiefly that the mean value of time to wandering in the crowded cultures stays the same or lower as compared to the low-density culture, and the first larvae leaving the food in MCU and CCU type cultures do so long before those in the MB type. Survivorship values of MCU and CCU type cultures also increase with increasing waste sensitivity variation, with a

larger number of less sensitive individuals able to stay in the toxic food for longer time periods. Another confusion is the noise observed in the size versus time to wandering distributions of the crowded cultures with high waste sensitivity variation.

The phenomenon of larval stop solves this confusion, and shows that high waste sensitivity variation leads to a split in terms of development time, with the individuals having lowest sensitivity to metabolic waste completing their development early, while those having highest sensitivity leave as soon as they cross their respective minimum critical size. The larvae with intermediate waste sensitivity stay on in the food and feed for longer periods of time, leaving the food last.

Even though these initial results give fairly good size and development time trend approximations in low- and high-density cultures with the interaction of waste sensitivity and larval stop alone, earlier empirical evidence suggests that there are some major disagreements. Work on the CU populations showed that the earliest eclosing flies had low waste tolerance and higher feeding rate, while late eclosing flies had higher waste tolerance and lower feeding rate (Borash *et al*. 1998). While the simulation data suggests that early eclosing individuals would have higher feeding rate in the simulations as well, it is in clear disagreement with the empirical results in its prediction that the early individuals also have the lowest waste sensitivity (highest tolerance).

One of the fundamental problems required to be addressed by this model is the discrepancy between the size and pre-adult development time distributions of the MCU

and CCU cultures. Even though these two cultures have the same egg/food volume density, their body size, development time distributions as well as survivorship values are quite different in experiments. In its current form, the model does not incorporate any potential for differences between different details of crowding at the same density, and thus all results obtained till now show no differences between the MCU and CCU type distributions.

Figure 1. Key to box plots showing size and time to wandering distributions in the following figures. The boxes denote the interquartile range (25% of data above and below the median, for a total of 50% data covered). The whiskers cover up to 1.5 times the interquartile range. Any data beyond the whiskers are outliers. The x-axis denotes the culture type, with the number next to the label denoting survivorship in percentage.

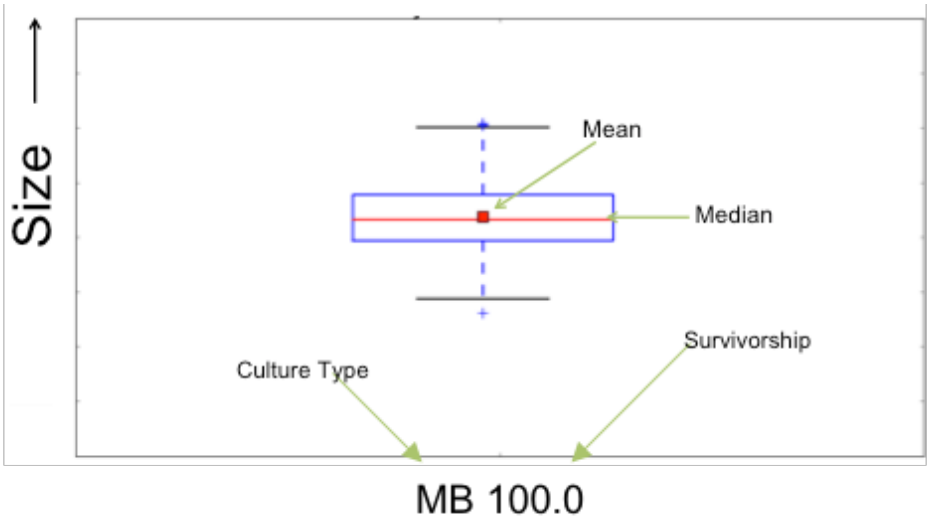
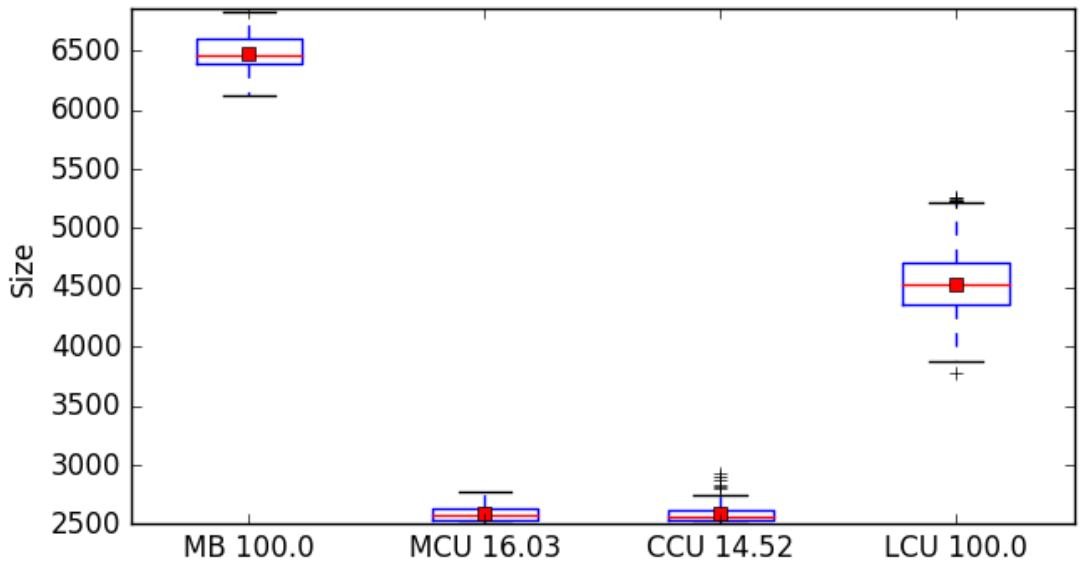
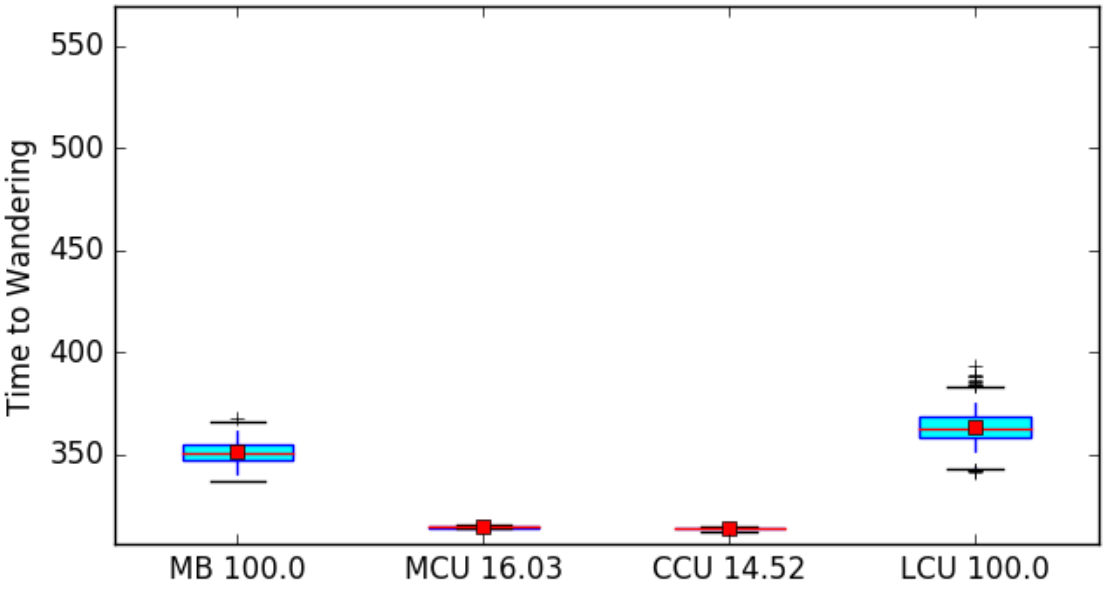


Figure 2. Base model

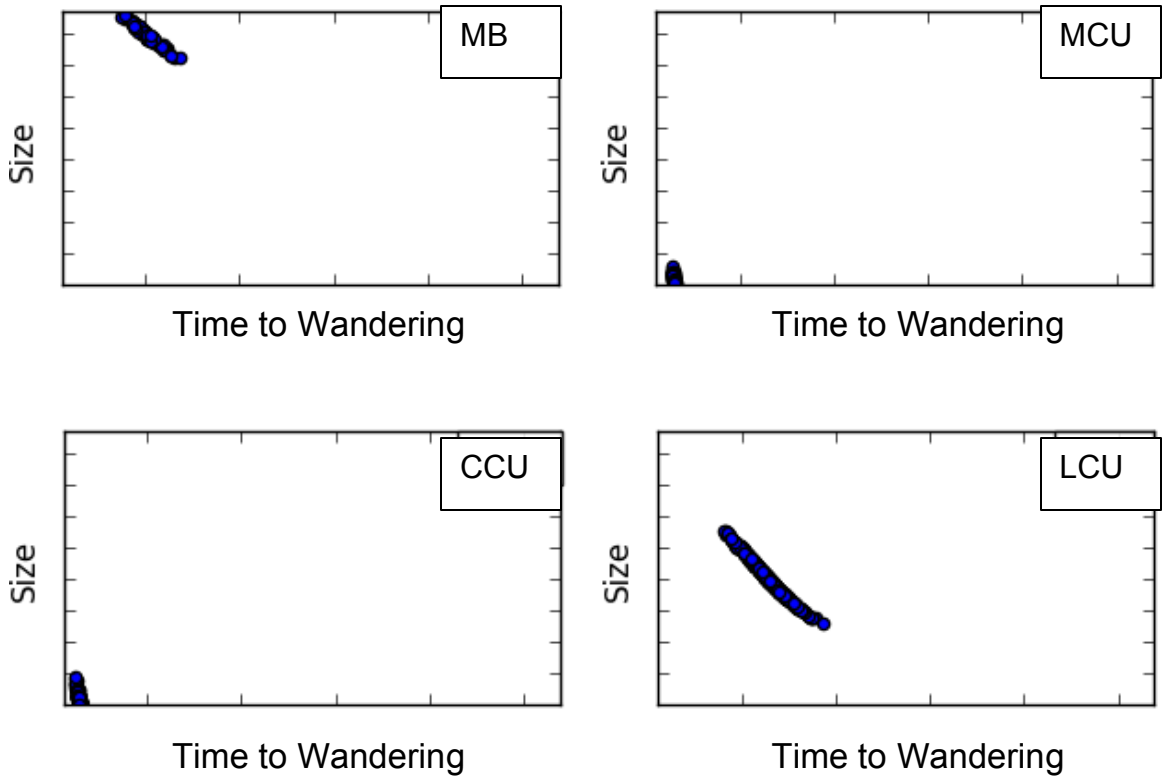
a) Larval size distribution at wandering stage



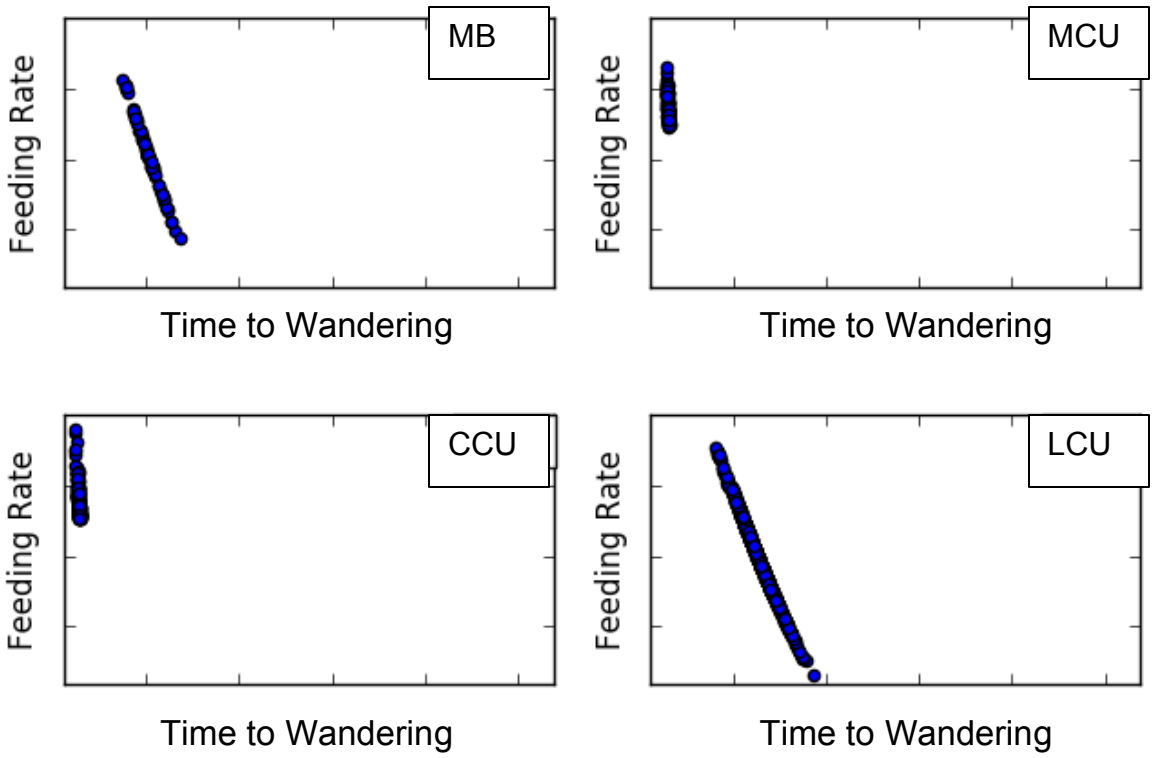
2.b) Time to wandering distribution



2.c) Larval size vs. time to wandering



2.d) Feeding rate vs. time to wandering



2.e) Larval size vs. feeding rate

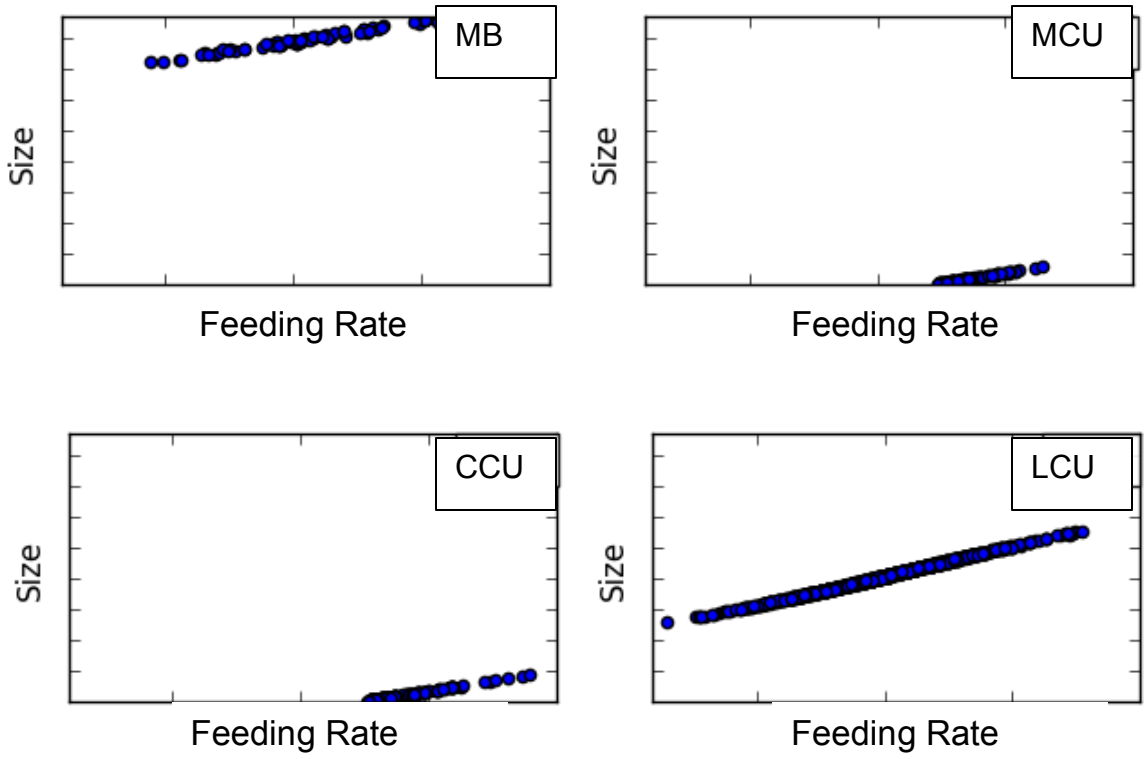
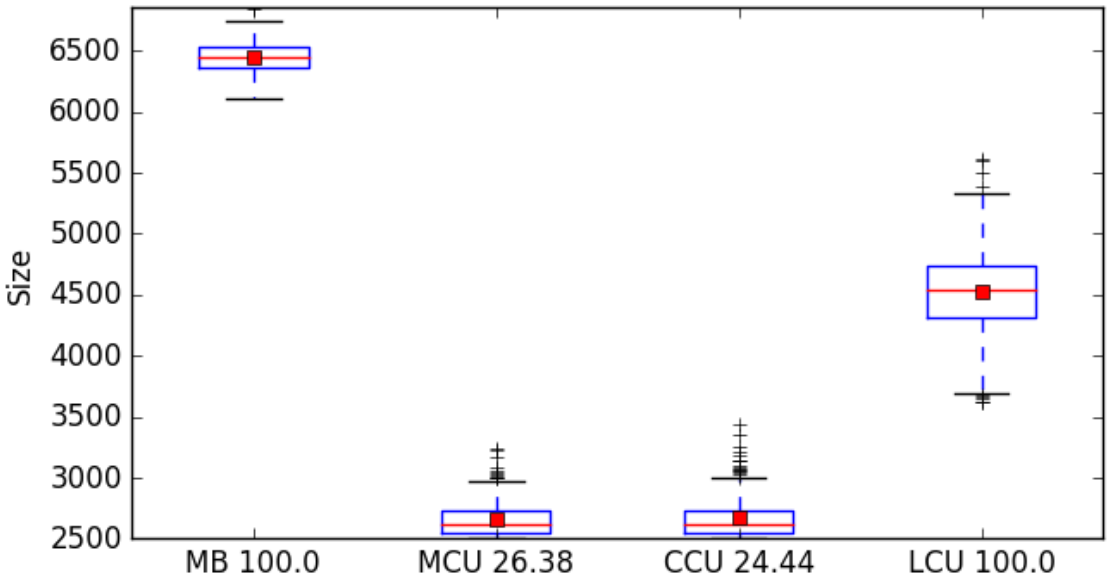


Figure 3. Waste sensitivity variation = 10% of the mean

a) Larval size distribution at wandering stage



3.b) Time to wandering distribution

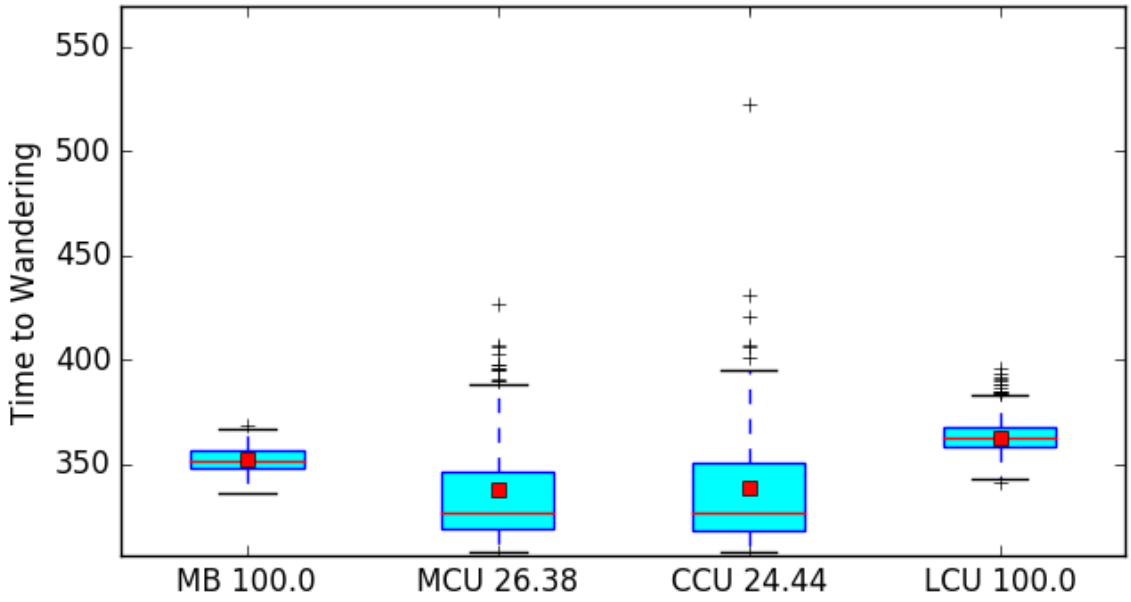
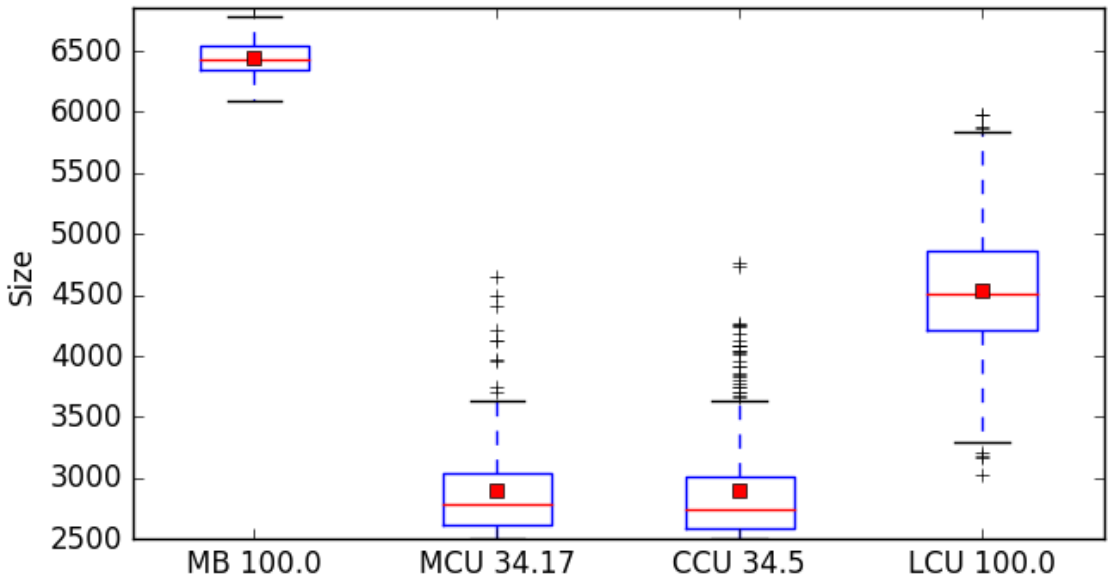


Figure 4. Waste sensitivity variation = 20% of the mean

a) Larval size distribution at wandering stage



4.b) Time to wandering distribution

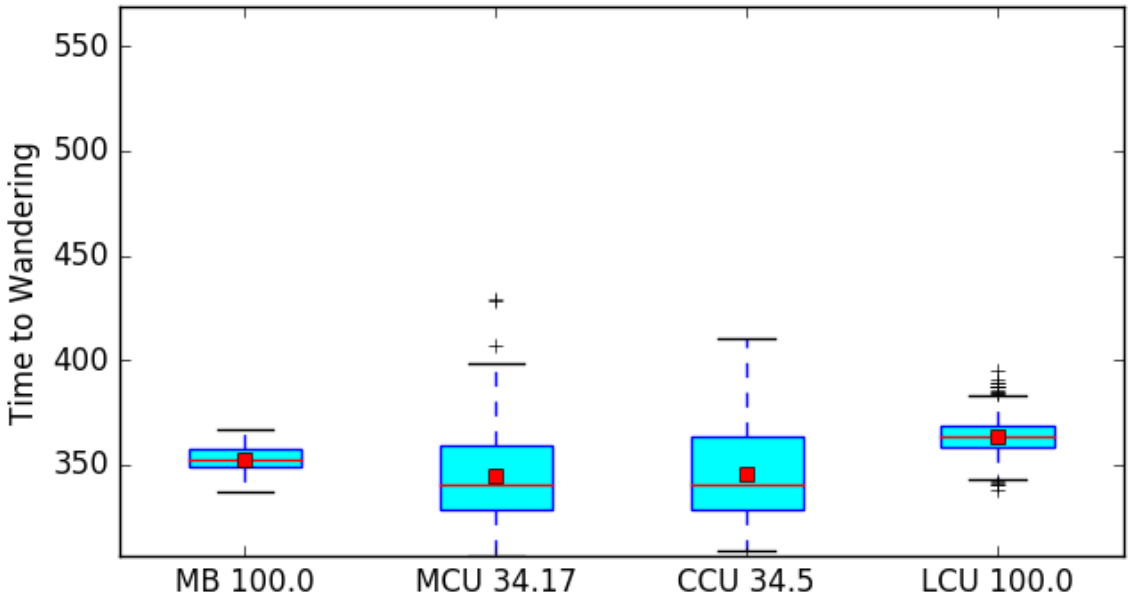
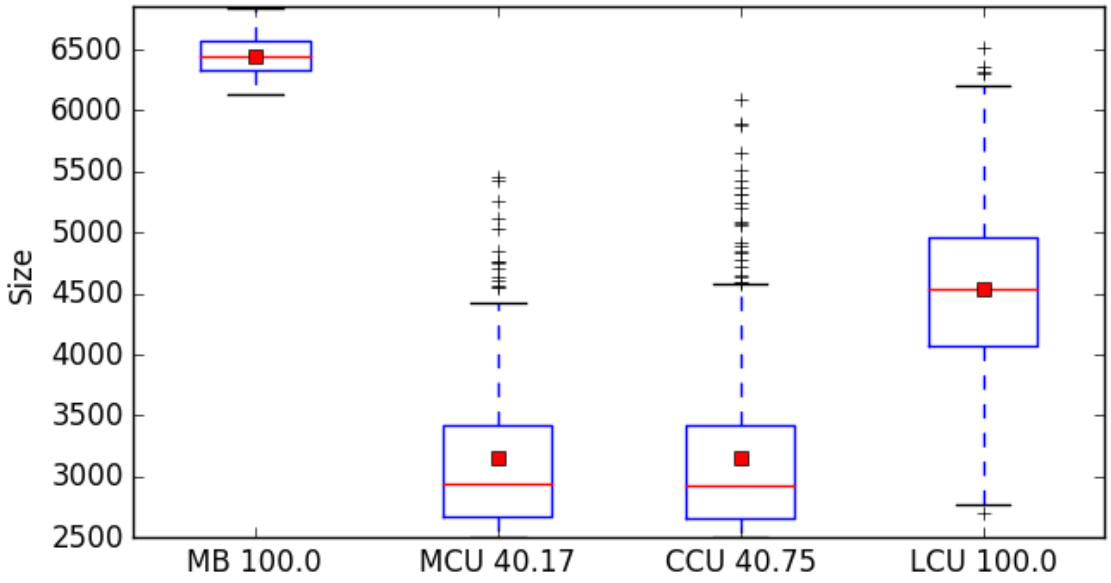
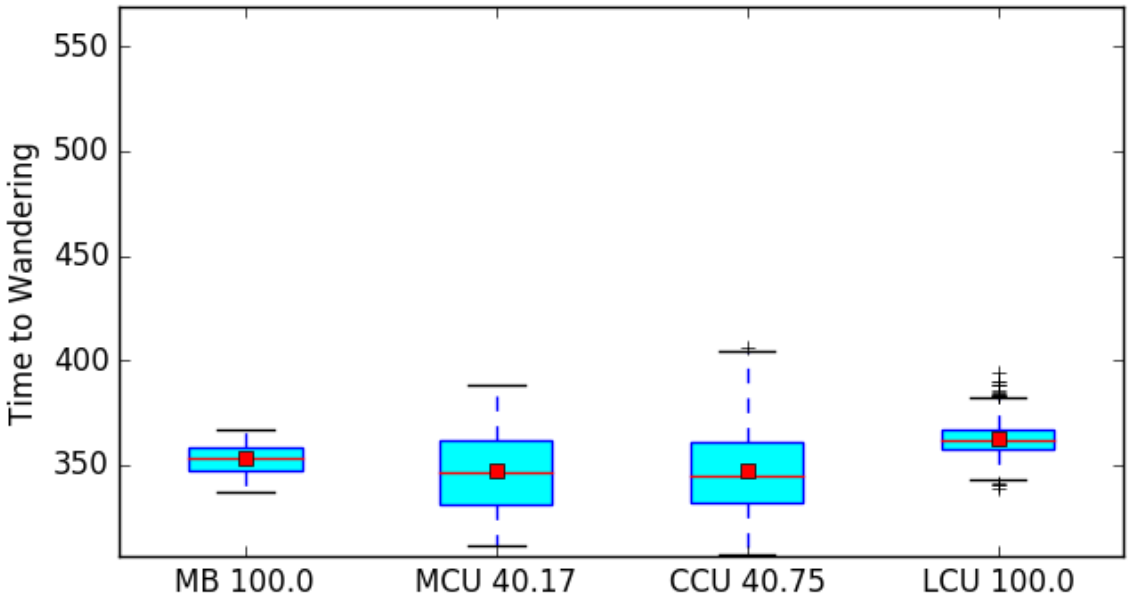


Figure 5. Waste sensitivity = 30% of the mean

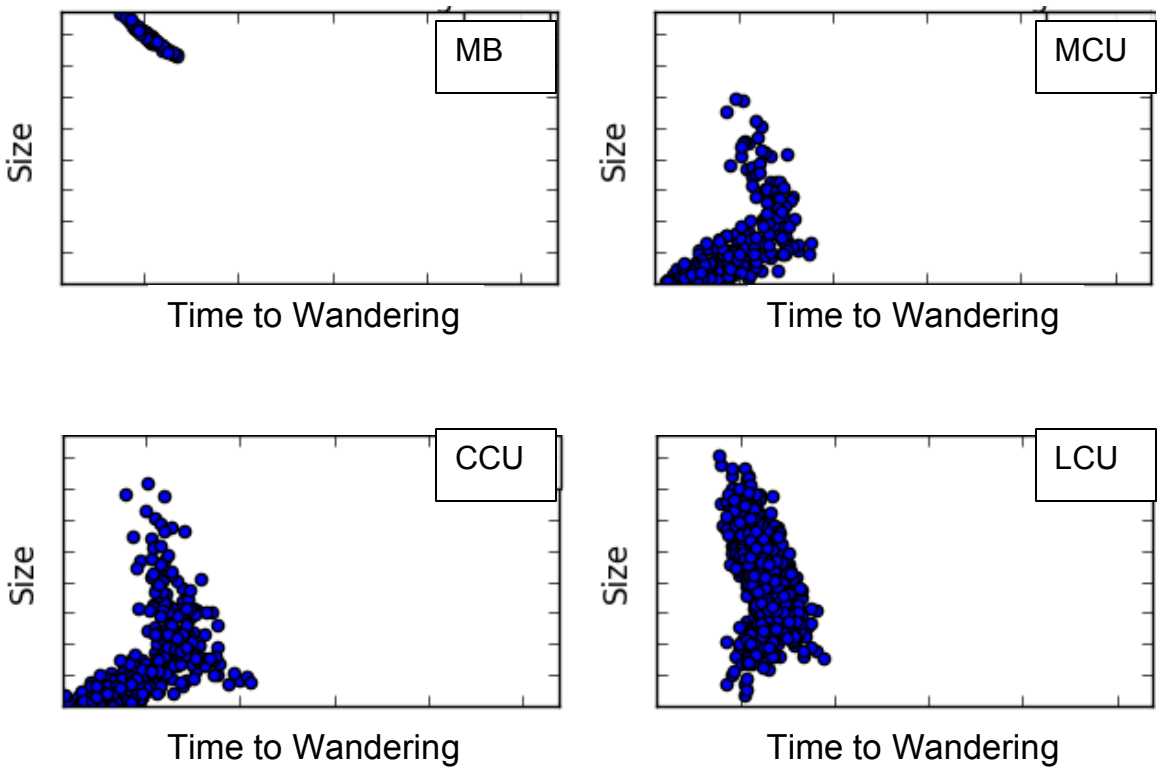
a) Larval size distribution at wandering stage



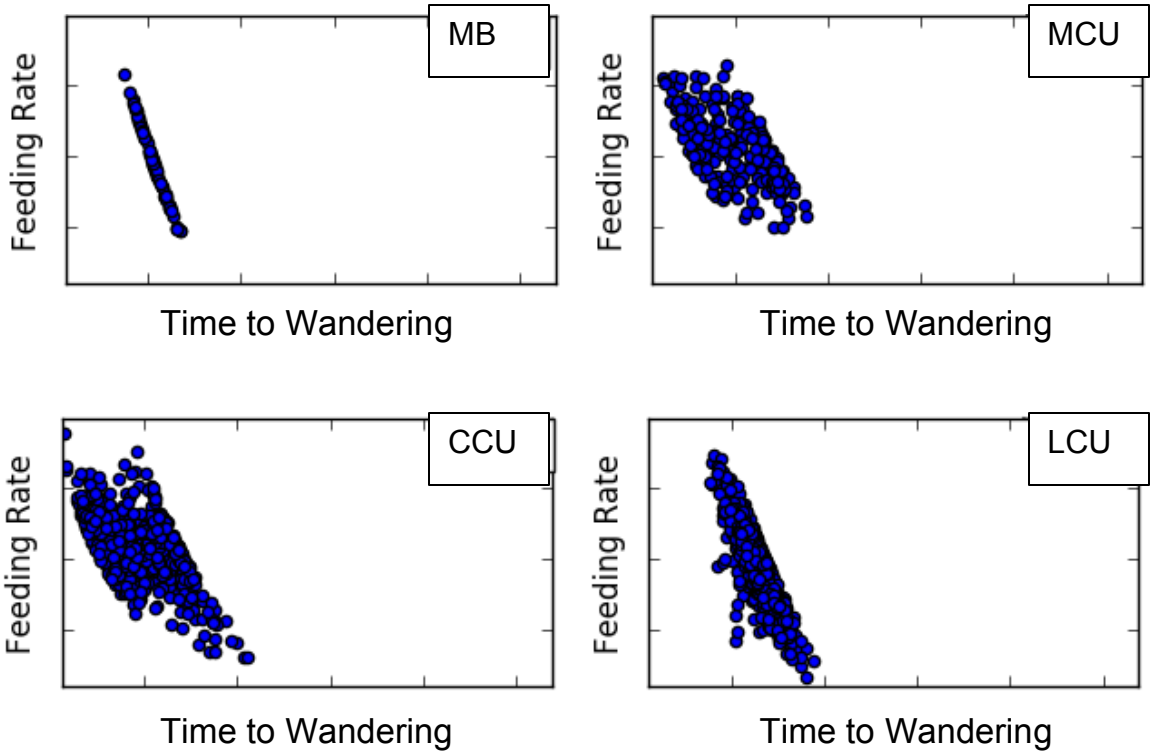
5.b) Time to wandering distribution



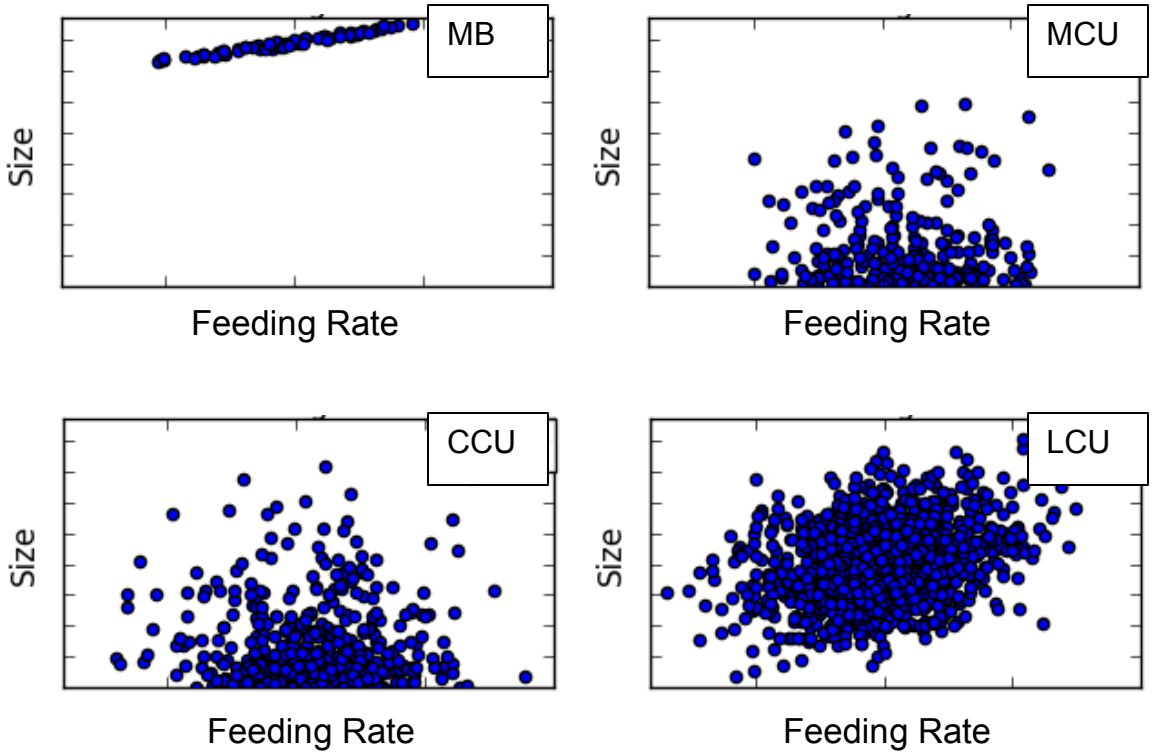
5.c) Larval size vs. time to wandering



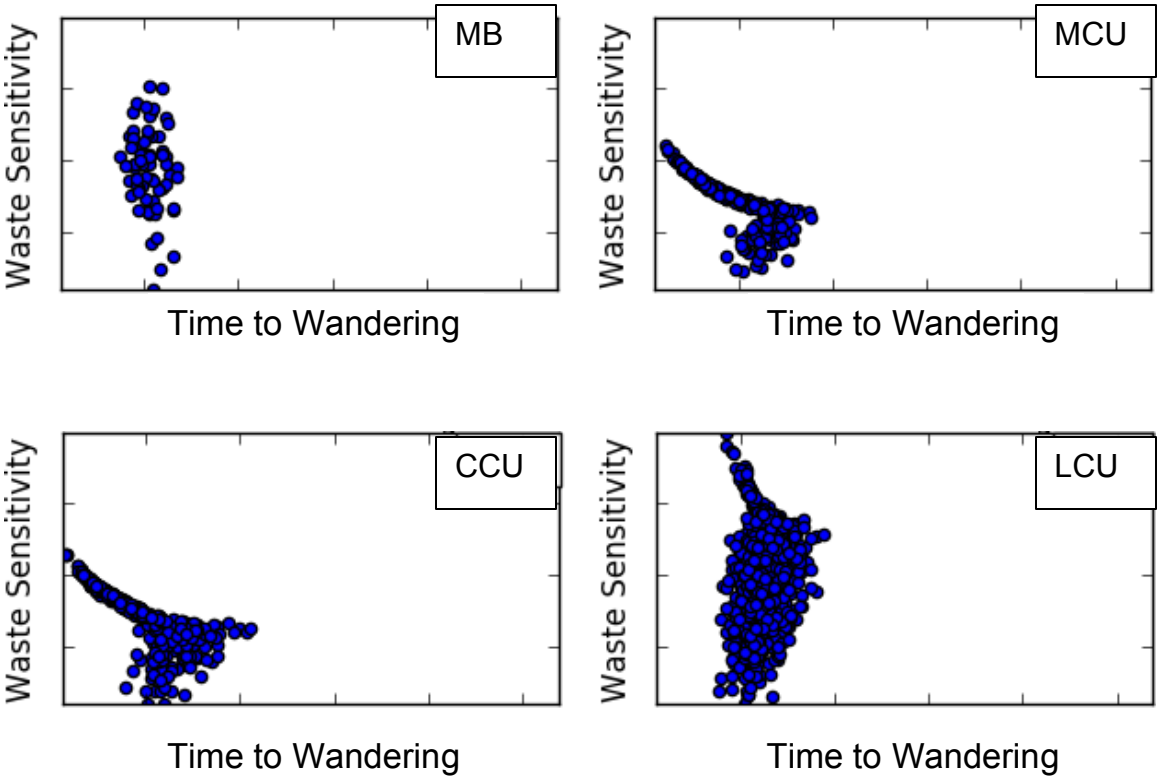
5.d) Feeding rate vs. time to wandering



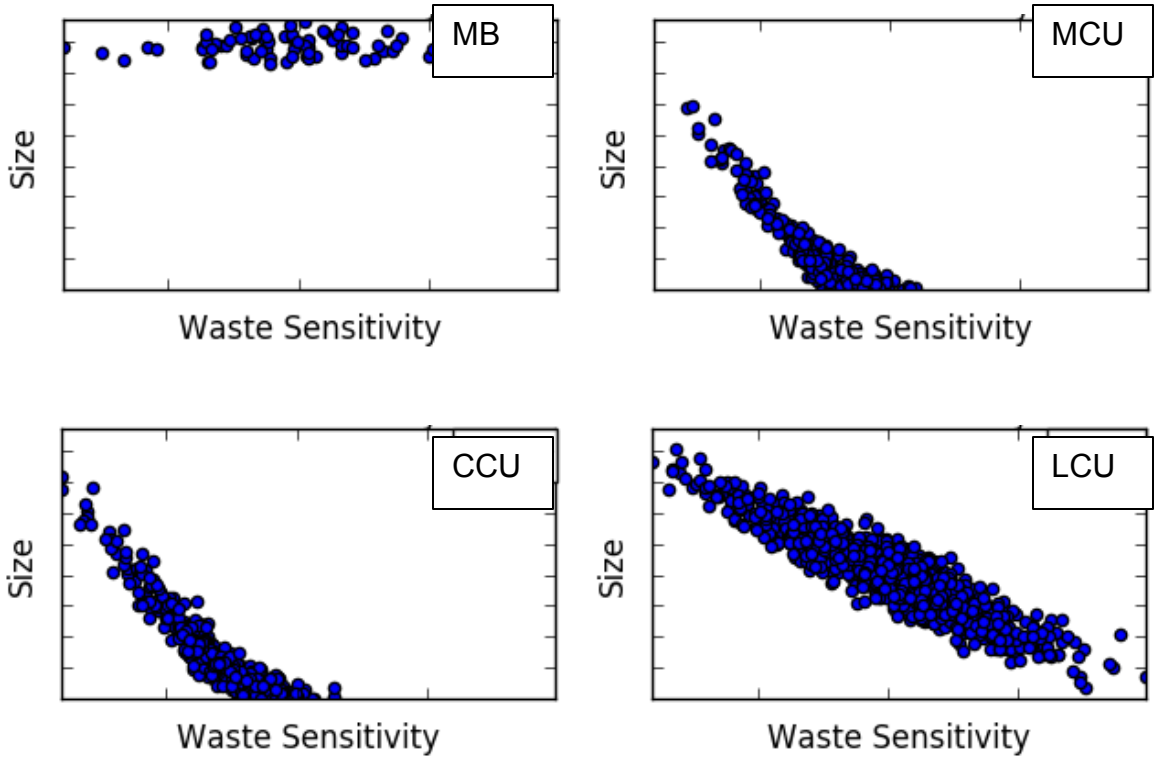
5.e) Larval size vs. feeding rate



5.f) Waste sensitivity vs. time to wandering



5.g) Larval size vs. waste sensitivity



5.h) Feeding rate vs. waste sensitivity

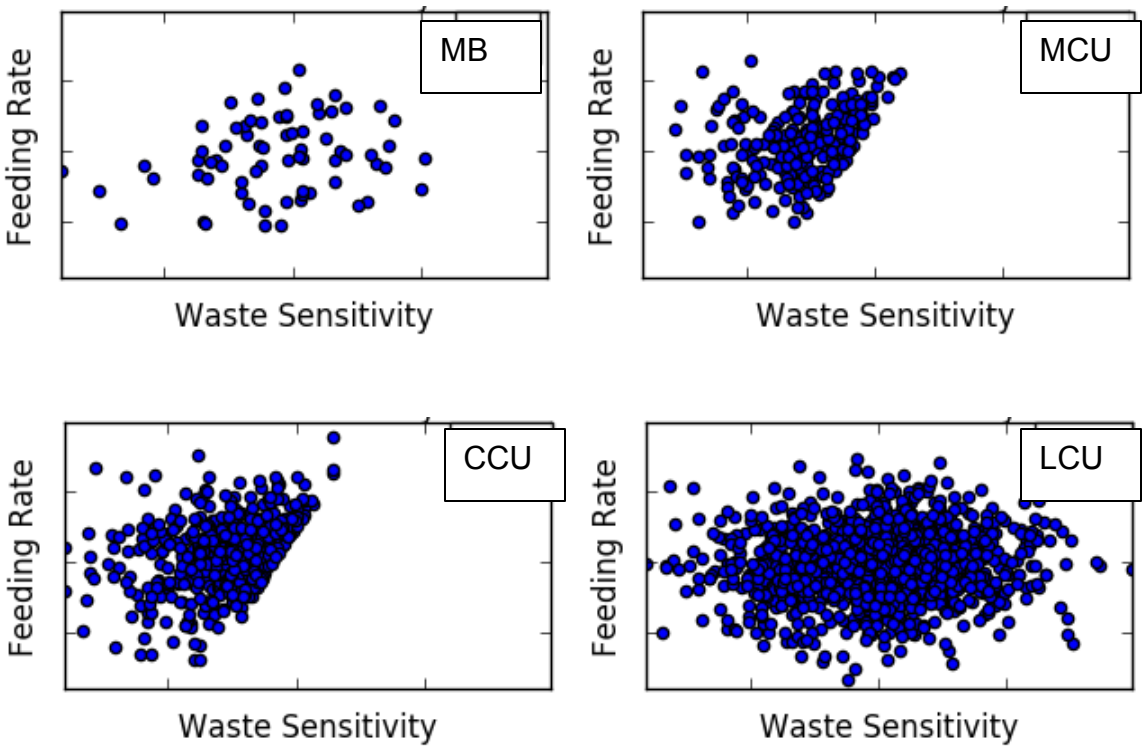


Figure 6. Larval stop = 20 time steps; Time to wandering distributions

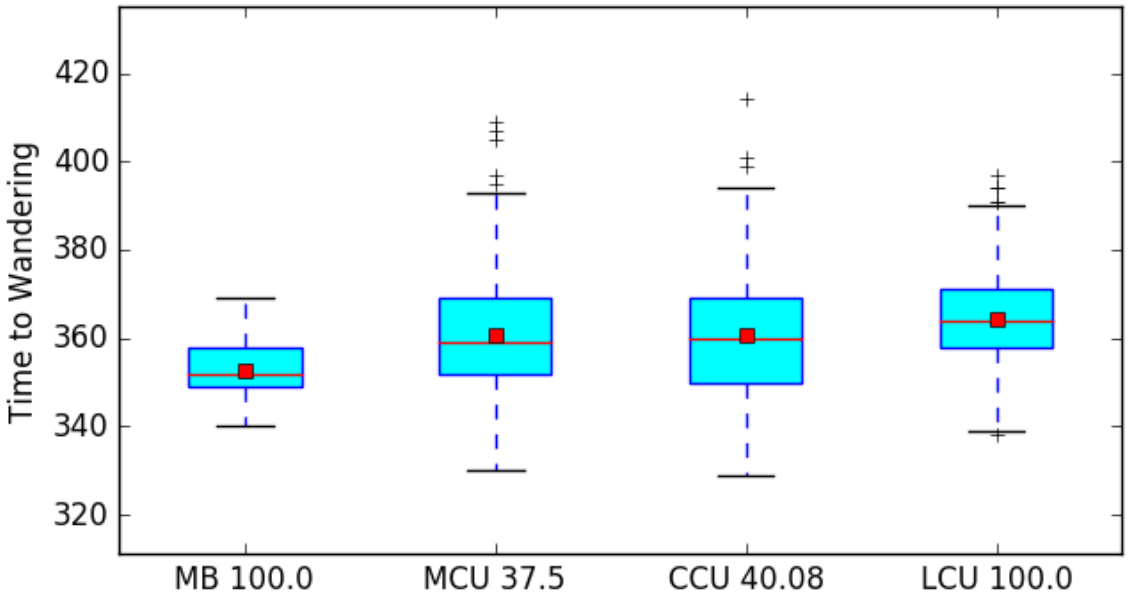


Figure 7. Larval stop = 40 time steps; Time to wandering distributions

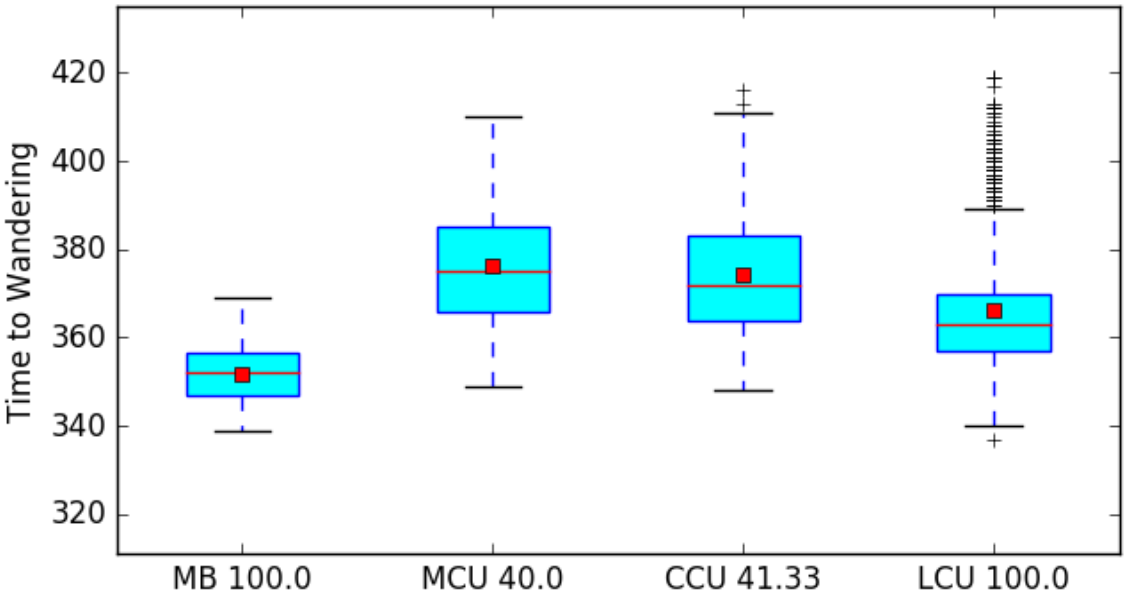


Figure 8. Larval stop = 60 time steps; Time to wandering distributions

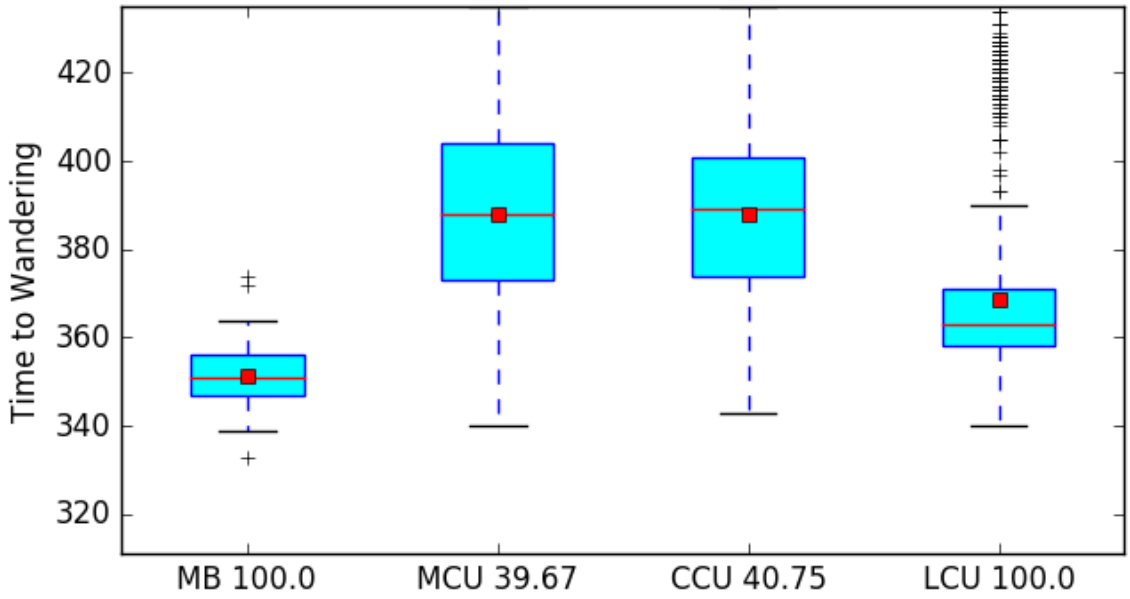


Figure 9. Larval size vs. time to wandering distributions –

a) 60 larval stop; b) 40 larval stop; c) 20 larval stop; d) 0 larval stop

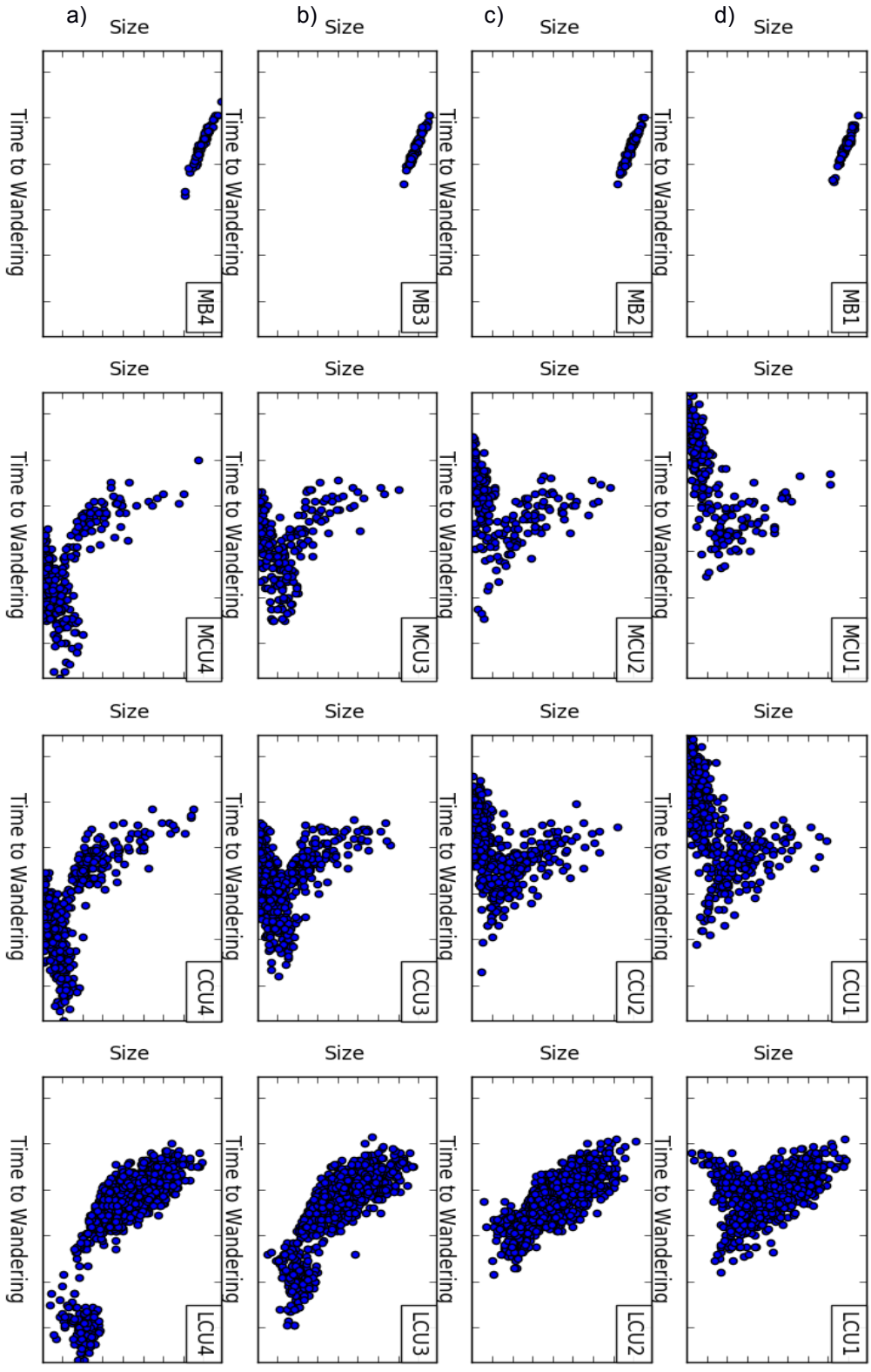
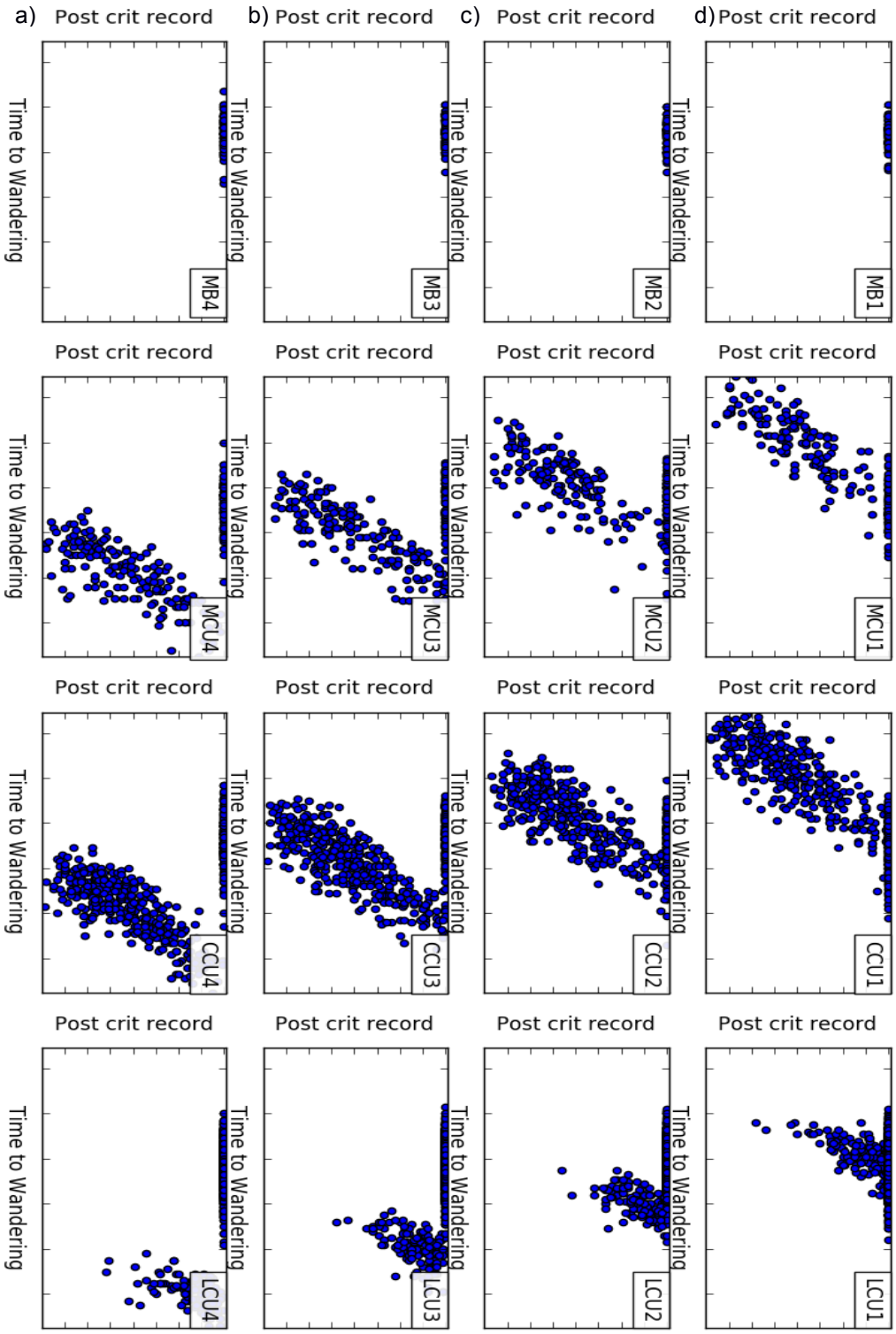


Figure 10. Post critical size feeding time vs. time to wandering distributions – a) 60 larval stop; b) 40 larval stop; c) 20 larval stop; d) 0 larval stop



Chapter 4

An Evolutionary Extension – I

Description of the model

Having established that preliminary results from the single generation monotypic larval culture simulations behave according to basic assumptions about which traits impact competitive ability, the next step is to study the change in the trait distributions over multiple generations, in order to further test the robustness of the model with respect to assumptions about the evolution of traits related to competitive ability under different scenarios of larval crowding. The model described below is an extension of the single generation model to multiple generations.

Given that most traits relevant to competitive ability are known to be polygenic and complex phenotypes, we incorporate simple rules of inheritance in our model extension that mimic the inheritance of polygenic phenotypes. Thus, the evolutionary extension to the base model presented here used an arbitrary set of simple inheritance rules to check for changes in the distribution of traits related to competitive ability over multiple generations under different scenarios of crowding.

My approach broadly followed two lines of attack. First, I tried slightly different variants of an inheritance rule and studied the effects on the trait variance distributions in the absence of any developmental, feeding or mating-related interference, in order to

ascertain which inheritance rule yielded the least change in trait distribution over time in the absence of evolutionary forces acting on the trait.

Next, I used the chosen inheritance variant to extend to multiple generations the different monotypic cultures seen in the single-generation simulations. The complete code with comments is given in Appendix 2.

Inheritance rule used

It was assumed that individuals in the simulation underwent sexual reproduction in a random mating regime, without any fecundity differences among mating pairs. At the start of any generation, individuals were randomly sorted into sets of two without replacement, in order to form mating pairs. In case there was an odd number of total individuals, one randomly chosen individual was excluded from the mating pairs and thus did not produce any offspring. Each mating pair gave rise to offspring equal in number to the defined fecundity. The trait values of these offspring were drawn randomly from a normal distribution with a mean equal to the mid-parent trait value and variance as a fraction, varied across different runs of the simulation, of the total population variance for each trait. The number of offspring produced was, thus, the total adult population times half the fecundity value, which meant that the population would grow if the fecundity was greater than two. In order to adhere to the given density of the culture every generation, a number of individuals equal to the culture density were picked from the total offspring pool at random to survive to the next generation. This also ensured that the number of breeding adults every generation was constant, all else being equal.

Variants of this inheritance rule could be generated along the axis of the fraction of total parental variance used to generate offspring trait distributions from mating pairs. Each simulation was limited to a single fraction value equal for each mating pair across all generations.

Applying the inheritance rule to the single generation model

The fraction of total adult trait variation that produced the least generation-to-generation variation was used on the single generation simulation. All surviving larvae from the culture (those whose time to wandering got recorded) were assumed to become adults with their final body size. All adults were randomly sampled into mating pairs, producing offspring according to the given fecundity values and inheritance rules. A number of offspring (the 'eggs') were sampled from the total offspring pool according to the given density of the culture. Any changes in the trait distribution over generations were studied in the low- and high-density cultures.

Checking for the evolution of competitive ability

In order to test if any changes in the distributions of traits in populations simulated in high larval density regimes affected competitive ability (as a selection response to density-dependent mortality), single generation competition simulations were conducted on populations that had earlier been allowed to evolve for 20 generations under low-density or high-density conditions. A competition simulation had equal number of eggs from two populations, one kept under high-density conditions (MCU type) for 20 generations and the other maintained at low-density conditions (MB type) for the same

number of generations. Both the populations were initiated with feeding rate variation of 2.5% of the mean, and waste sensitivity variation of 30% of the mean. Two types of larval competition simulations were run, one at high-density (MCU type of culture, 300 eggs from each population) and another at low-density (MB type of culture, 35 eggs from each population). The survivorships of both populations were compared in the two competition regimes. Results from these simulations are presented and discussed in Chapter 5.

Chapter 5

An Evolutionary Extension – II

Results from the model

Testing the inheritance rule's effects on cross generational variation

In implementing trait inheritance, first different levels of stochasticity in how closely individual offspring matched the mid-parental trait value were examined. The fecundity per mating pair was set to 20 offspring, with random culling of offspring with respect to trait value being imposed to keep adult population size constant. When stochasticity was low, with the variance of the offspring from a mating pair being set at 10% of the total phenotypic variance in the population, the phenotypic variation of adults over generations quickly collapsed into approximately the population mean value (Figure 1a), which is not surprising since this assumed mechanism of inheritance is essentially a stochastic version of blending (largely additive) inheritance. On increasing the level of stochasticity, with the variance of the offspring from a mating pair being set up to 70% of the total phenotypic variance in the population, the phenotypic variation in the population continued to collapse into the mean value of the trait, albeit at a slower rate as the percentage value increased (Figures 1 b,c,d). On taking per mating pair offspring variation as 71% of total population phenotypic variation and above, the phenotypic variation in the population across generations started increasing its spread about the mean over generations, with the extent of spread per generation increasing with increasing percentage of total parental variation taken (Figures 1 e,f,g). The increasing cross-

generational variation, with offspring variation being 80% or more of the total population variation, caused the population mean phenotypic value to increase as well, if the minimum value of the trait was bound to zero (Figure 2). At values of per mating pair offspring variation around 71% of total parental phenotypic variation, the cross-generational variation seemed to be relatively unchanged, at least for the first 50 generations. For lower values of density, using the same inheritance rule, there was considerably more noise in the mean and variation over generations. At the low-density equivalent of 70 individuals, 73% parental variation produced the least noisy cross-generational change in trait value, while also not showing any increasing or decreasing trends for the majority of the runs (Figure 3). As large changes in a trait's distribution over generations in the absence of selection may confound the directional changes that occur due to selection, the 73% variant of the inheritance rule was chosen for extending the single-generation model to multiple generations, to correspond to a form of inheritance maintaining phenotypic trait distribution in equilibrium in the absence of selection.

The multi-generational model

Traits assumed to be related to competitive ability were varied using values drawn randomly from a normal distribution with standard deviation equal to a given percentage of the mean, in order to observe the evolutionary trajectory of the trait values over generations.

Feeding rate variation

A 2.5% variation in feeding rate gave rise to no changes in the MB and LCU types of regimes (Figures 4 and 5, respectively), but caused an increase in the trait value by ~4% in case of MCU and CCU types of culture (Figure 6). Over the period of about 10 generations, the percentage survivorship in the latter two cultures also increased from ~20% to ~100% survivorship, and the increase in mean value over generations appeared to stop after nearly complete survivorship had been achieved in the culture. As the MB and LCU type of regimes had 100% survivorship initially itself, no selection response was observed.

The food level used in these simulations was 10% greater than that used in the single generation assay. The latter amount of food was low enough, and the feeding rate increase in all individuals was high enough, that the food got depleted really fast and caused most larvae to die before reaching minimum critical size.

Minimum critical size variation

A 5% variation in minimum critical size for pupation gave no trends in changes of the mean trait value in MB and LCU types of regimes due to complete survivorship in those cultures (Figures 4 and 5, respectively). However, in case of the MCU and CCU type of cultures, a decrease in the mean value of the trait of about 10% was consistently seen over the period of about 10 generations, with the response slowing down after that. Survivorship in these cultures became nearly 100% after the decrease in critical size mean (Figure 7).

Waste sensitivity

The response here was similar to that seen in case of the minimum critical size, with a ~40% decrease in the mean over a period of around 10 generations in the cultures with initially low survivorship (Figure 8).

Competitive ability

In the high-density competition simulation, complete survivorship was consistently observed for the eggs raised for several generations at high density (MCU). Survivorship in the eggs taken from the low-density rearing environment (MB) showed relatively low survivorship (typically ranging from 0-20% across runs) (Figure 9). In case of the low-density competition simulation, both the MB and MCU populations showed 100% survivorship (Figure 10).

Discussion

Relatively simple rules of inheritance have been shown to give rise to interesting patterns of cross-generational trait variation. Consistently low offspring variation for every mating pair tends to collapse the trait variation to its approximate mean value, whereas high offspring variation for every mating pair causes the trait variation to spread out over generations, leaving the mean relatively unchanged. For a small range of fractions of parental variation, in this case between 0.7-0.71 for 1000 individuals and around 0.73 for 100 individuals, the collapse or expansion behaviour (if it occurs at all) presumably takes so long as to not be observed for up to a few hundred generations, although over longer time periods the mean and variance of the distribution shift considerably from their initial

positions. Thus, values taken from this range of fractions mentioned above may be used to produce relatively unchanged trait distributions for up to a few tens of generations.

Setting the fraction to a large value, such that the distribution expands over generations, while keeping the minimum limit to zero, causes the mean value to inflate along with the variance, suggesting that a directional shift in mean trait value is possible in the absence of selection, in a hypothetical scenario with large cross generational trait variation and a one-sided bound on the trait values.

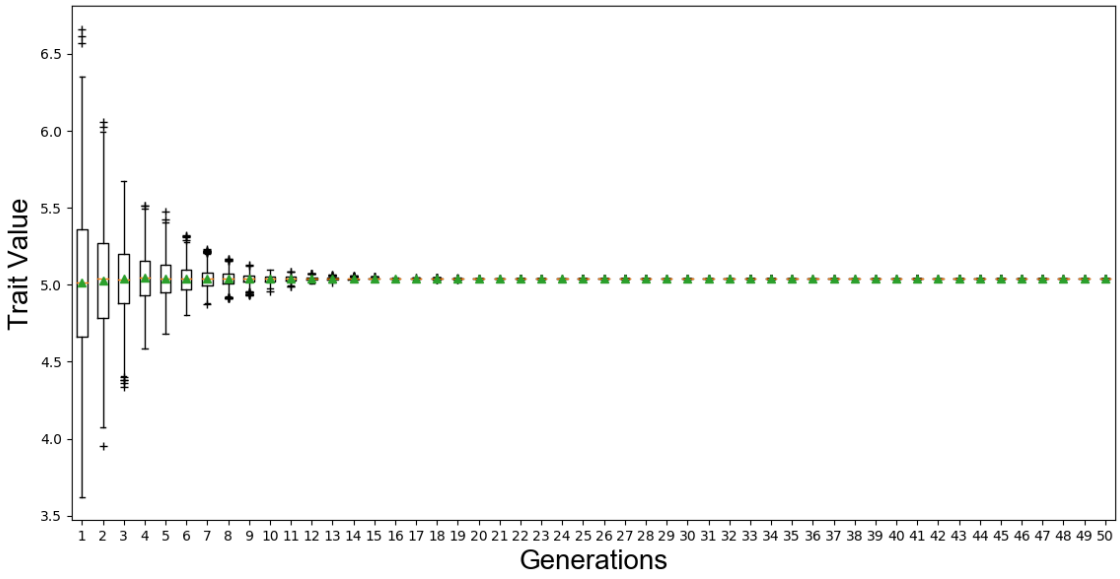
The multi-generational larval culture simulations have demonstrated that even under such relatively simple and arbitrary rules of inheritance, along with the simple single generation framework mentioned in the previous chapters, a change in mean values of traits thought to comprise competitive ability is possible, such that the survivorship in the culture increases drastically over a few generations. Empirical evidence suggests that higher feeding rate (Joshi and Mueller 1988, 1996), higher waste tolerance (or lower waste sensitivity) (Borash *et al.* 1998) and smaller minimum critical size (Sarangi 2013) can all independently cause evolution of competitive ability. As seen in these simulations, cultures subjected to high density-dependent mortality evolve higher mean values of feeding rates, or lower values of minimum critical size, or lower values of waste sensitivity (higher waste tolerance) in independent simulations – results that are consistent with existing experimental data.

Similar to experimental evidence, greater competitive ability also evolved in the simulated high larval density cultures such as MCUs, as seen in the competition simulations. Under high-density competition, larvae from cultures maintained at high-density had 100% survivorship (likely due to evolution of higher feeding rate and lower waste sensitivity values). In contrast, eggs from cultures reared in the absence of density-dependent mortality did not show any consistent changes in trait values, and had relatively very low survivorship values in competition. In low-density competition cultures, there was no decrease in survivorship in larvae from either population, suggesting that the evolved traits in the high-density populations only gained relevance under conditions of resource limitations, i.e. when competitive ability became an important factor.

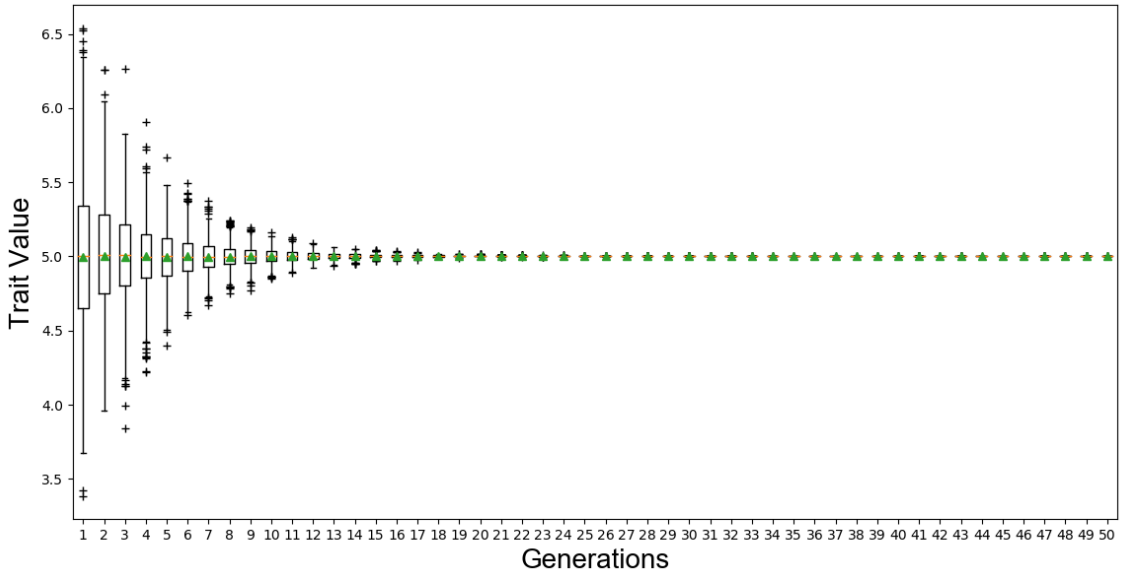
Thus, with respect to the evolution of certain traits related to competitive ability, the current simulation framework is broadly in agreement with experimental evidence.

Figure 1. The inheritance rule variants (Starting trait distribution – normal distribution with mean = 5.0 and standard deviation = 0.5; 1000 individuals taken). Offspring of each mating pair are generated from a normal distribution using mid-parent value as the mean and the following percentages of total parental variation as the standard deviation –

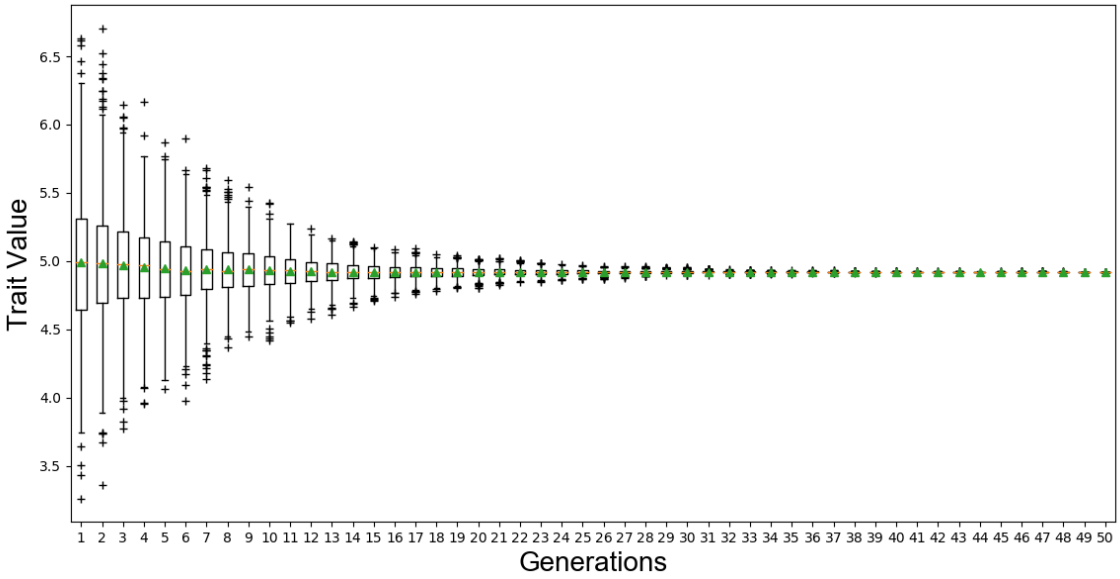
a) 10% of total parental variation



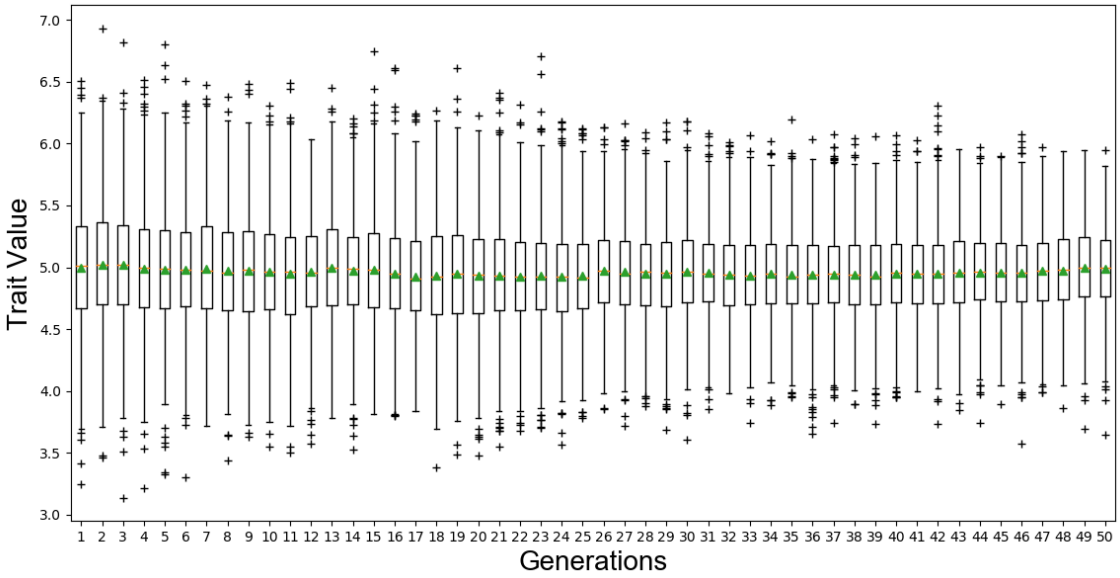
1.b) 30% of total parental variation



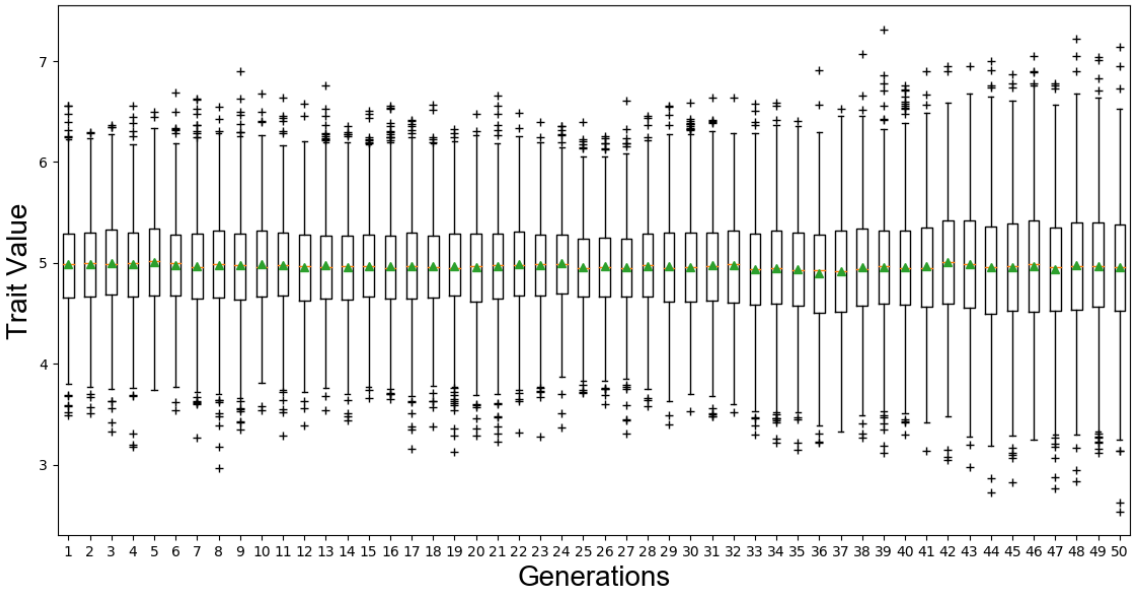
1.c) 50% of total parental variation



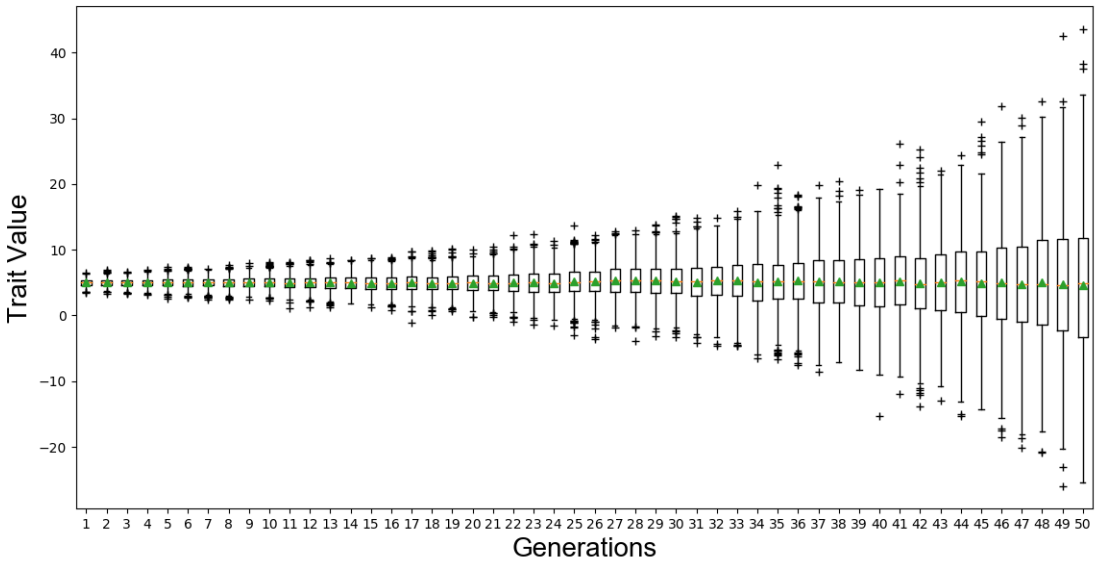
1.d) 70% of total parental variation



1.e) 71% of total parental variation



1.f) 80% of total parental variation



1.g) 100% of total parental variation

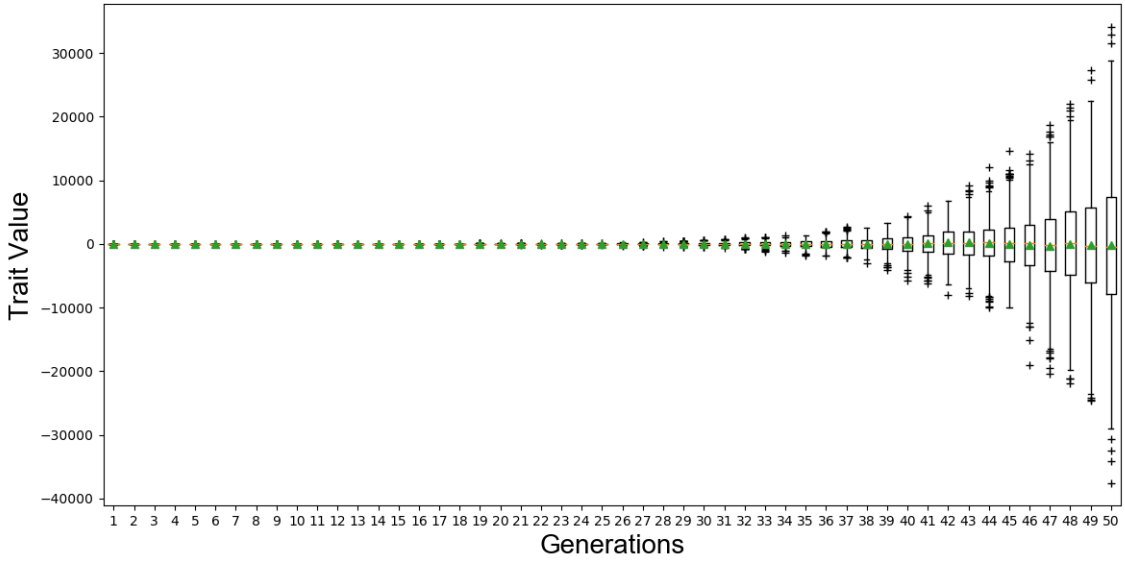


Figure 2. 100% total variation with a lower limit bound to 0

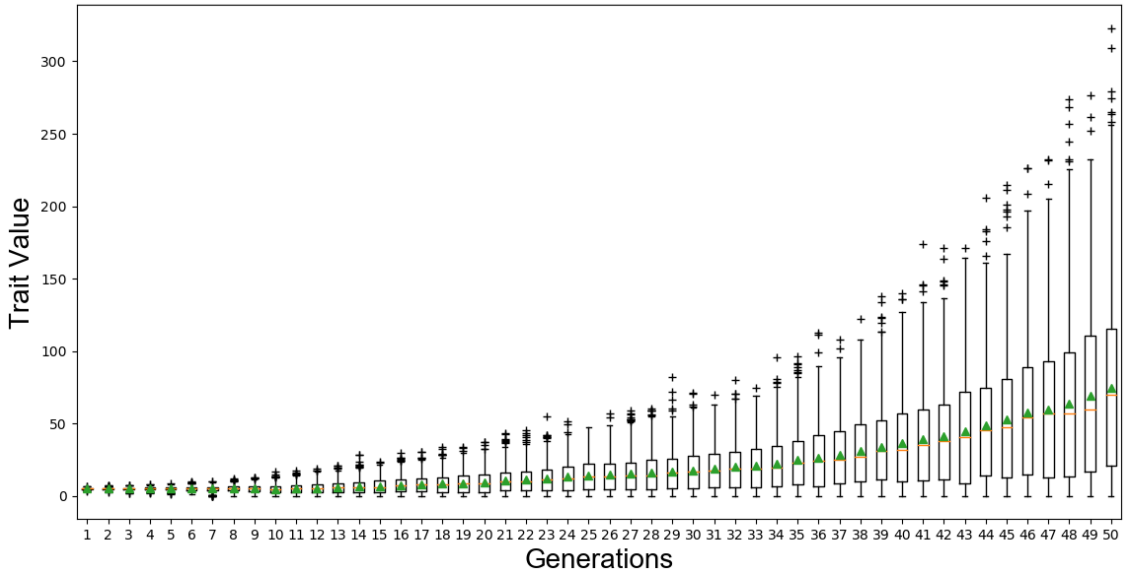


Figure 3. 73% of total parental variation, 100 individuals

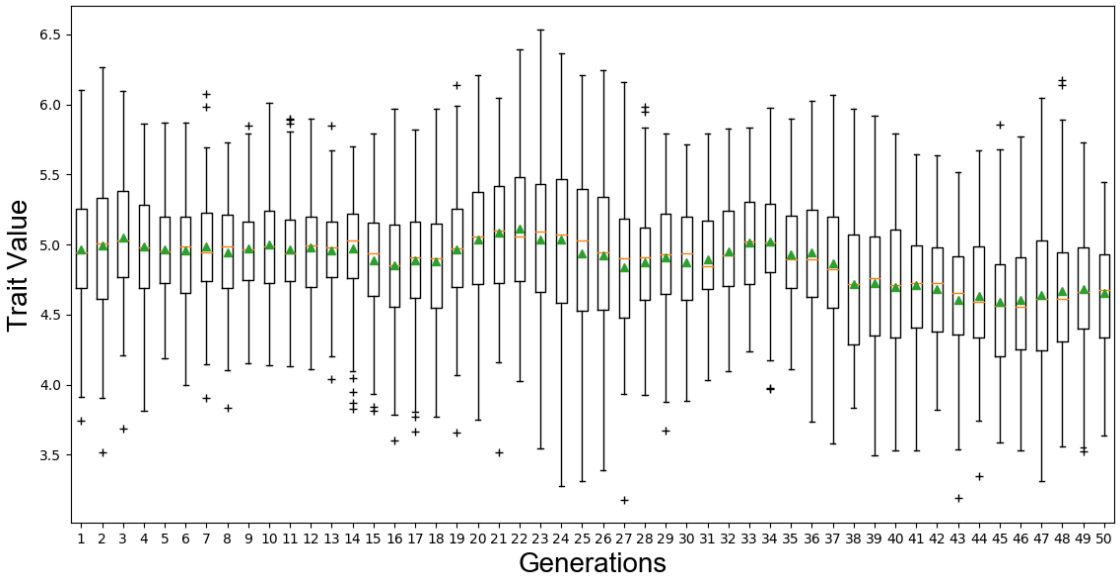
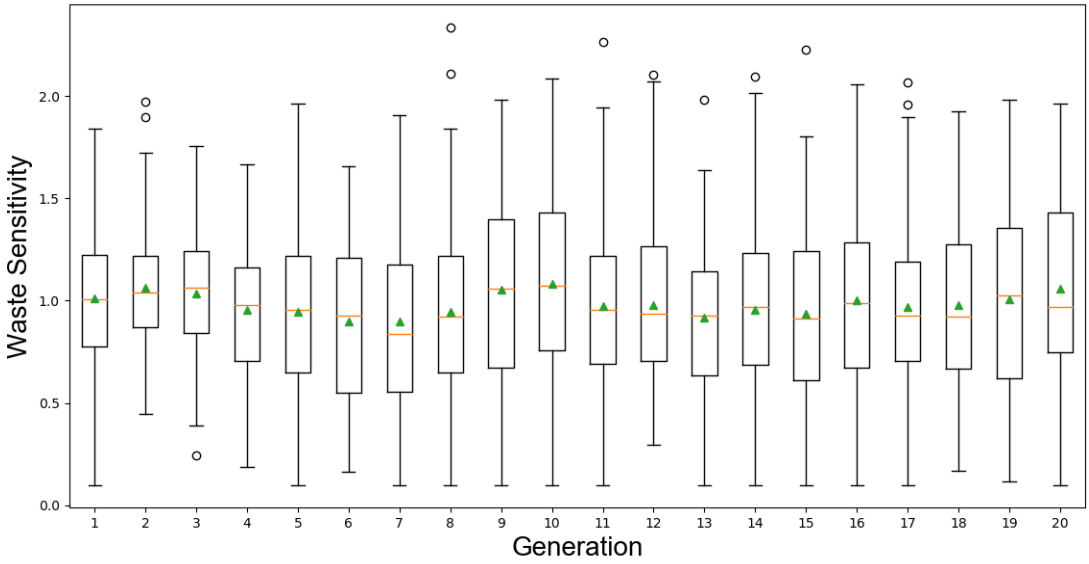


Figure 4. Waste sensitivity variation in MBs over generations (similar patterns seen for minimum critical size and feeding rate)

a) Waste sensitivity (generation 0 – mean = 1.0; s.d. = 30% of mean)



4.b) Survivorship over generations

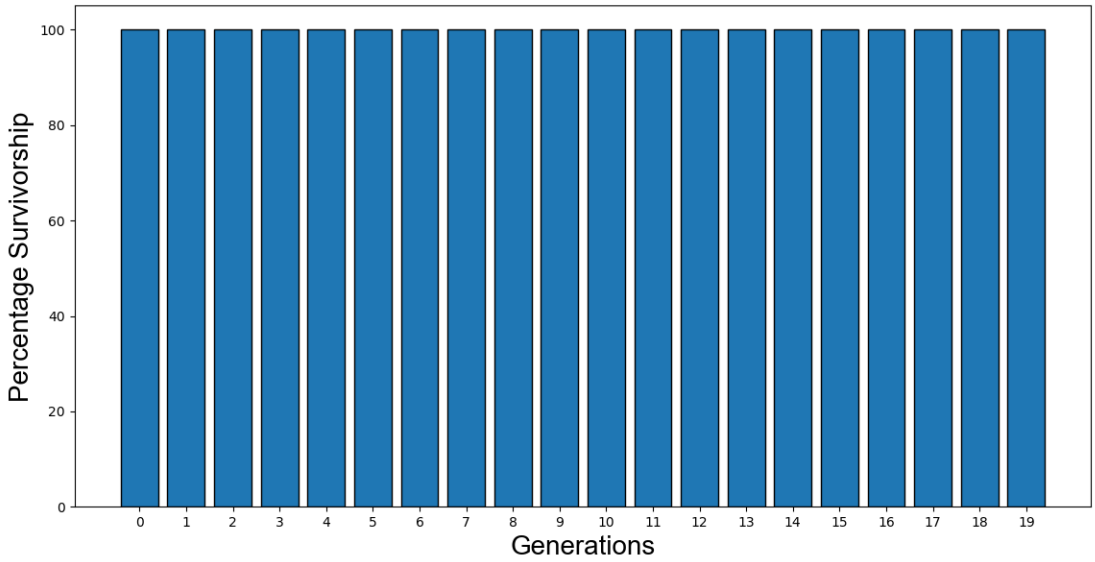
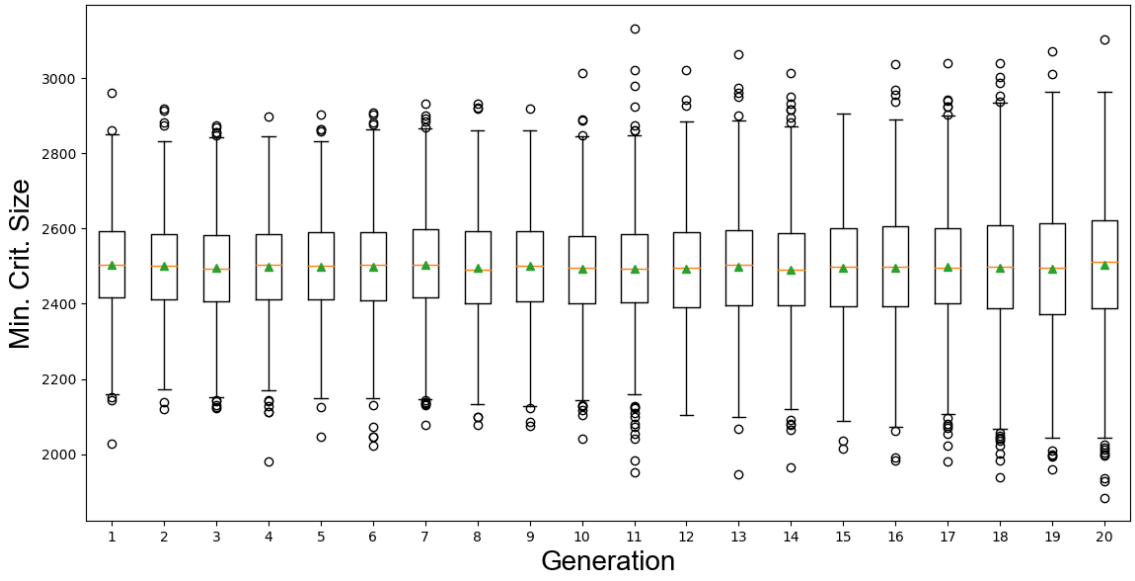


Figure 5. Minimum critical size variation in LCUs over generations (similar patterns seen for waste sensitivity and feeding rate)

a) Minimum critical size (generation 0 – mean = 2500; s.d. = 5% of mean)



5.b) Survivorship over generations

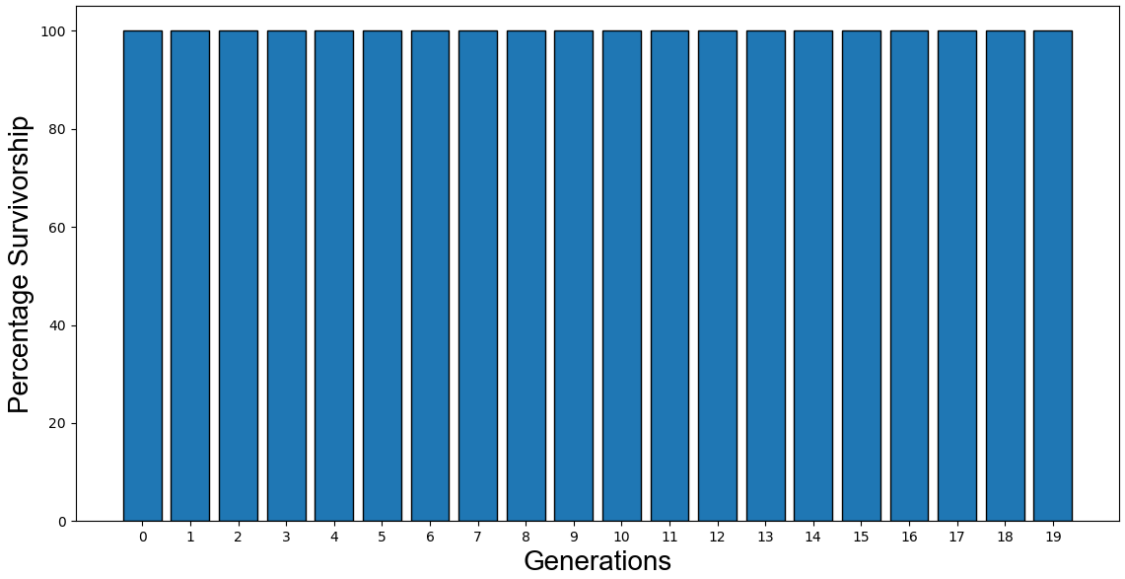
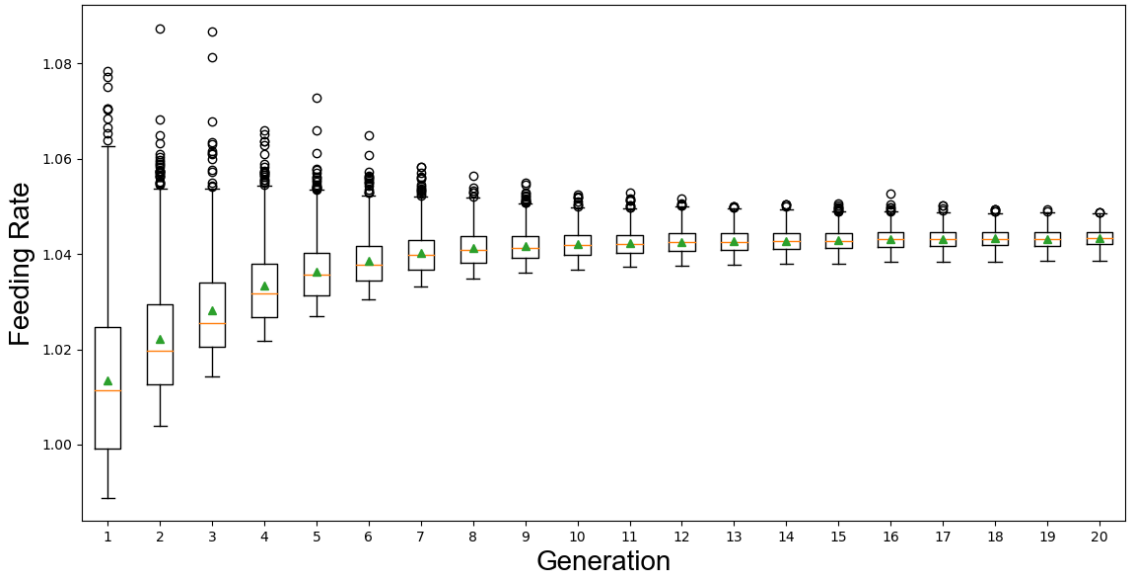


Figure 6. Feeding rate variation in CCUs over generations (similar patterns seen in the MCUs)

a) Feeding rate (generation 0 – mean = 1.0; s.d. = 2.5% of mean)



6.b) Survivorship over generations

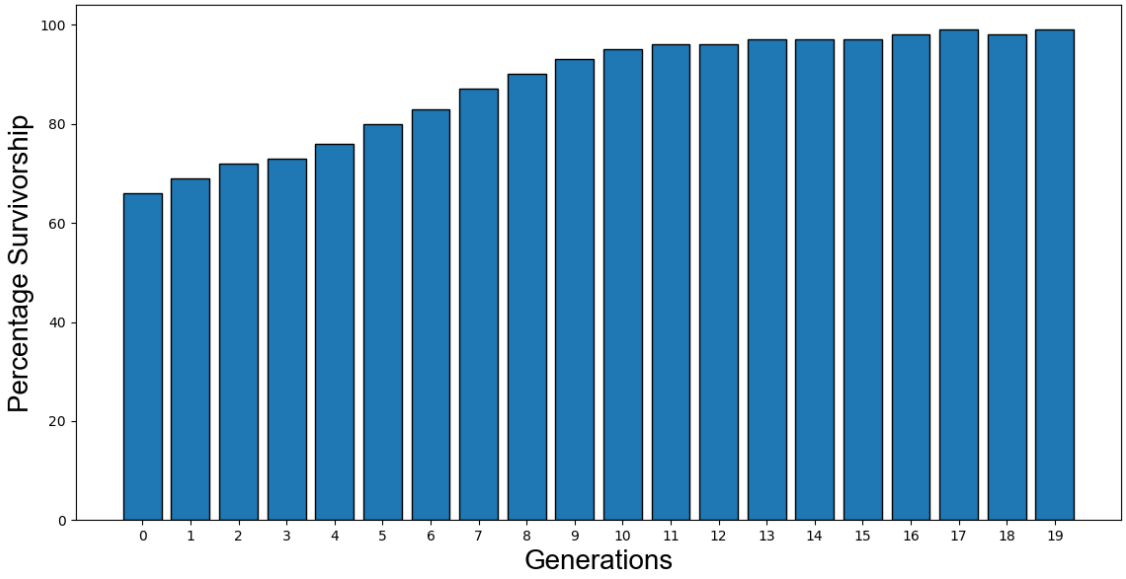
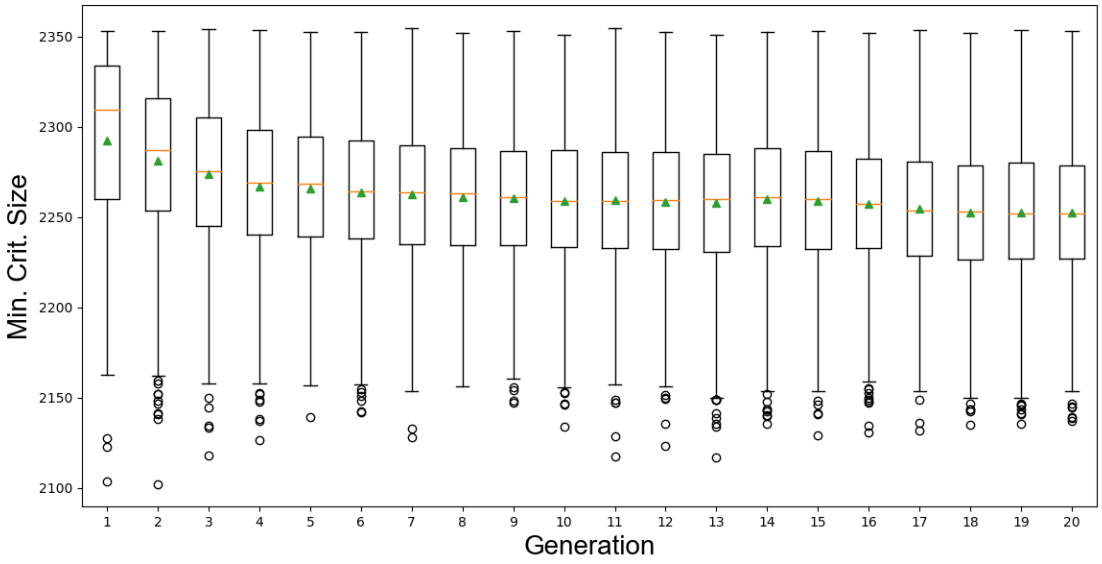


Figure 7. Minimum critical size variation in CCUs over generations (similar patterns seen in the MCUs)

a) Minimum critical size (generation 0 – mean = 2500; s.d. = 5% of mean)



7.b) Survivorship over generations

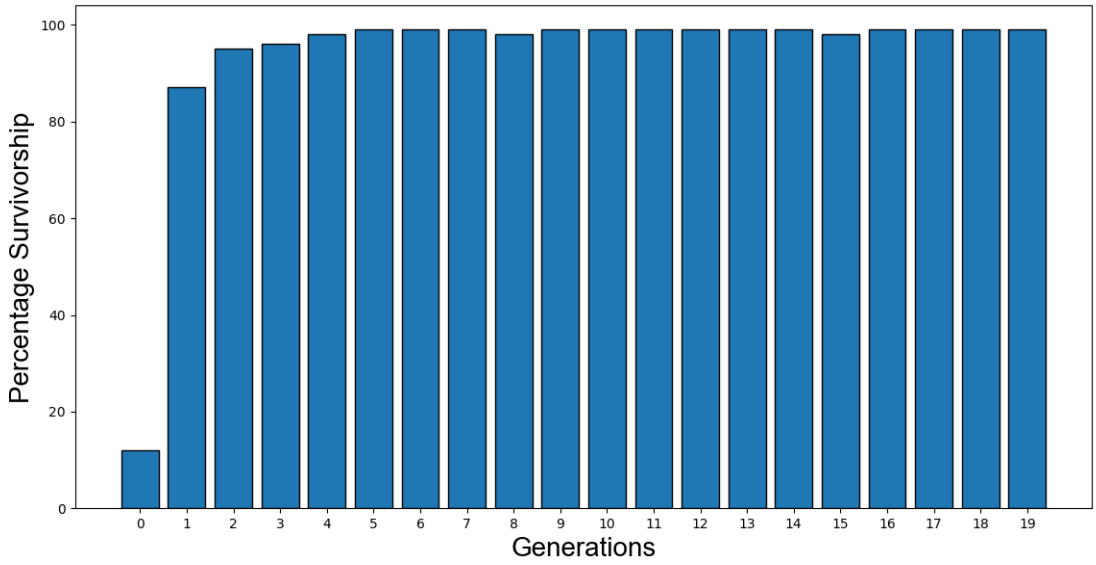
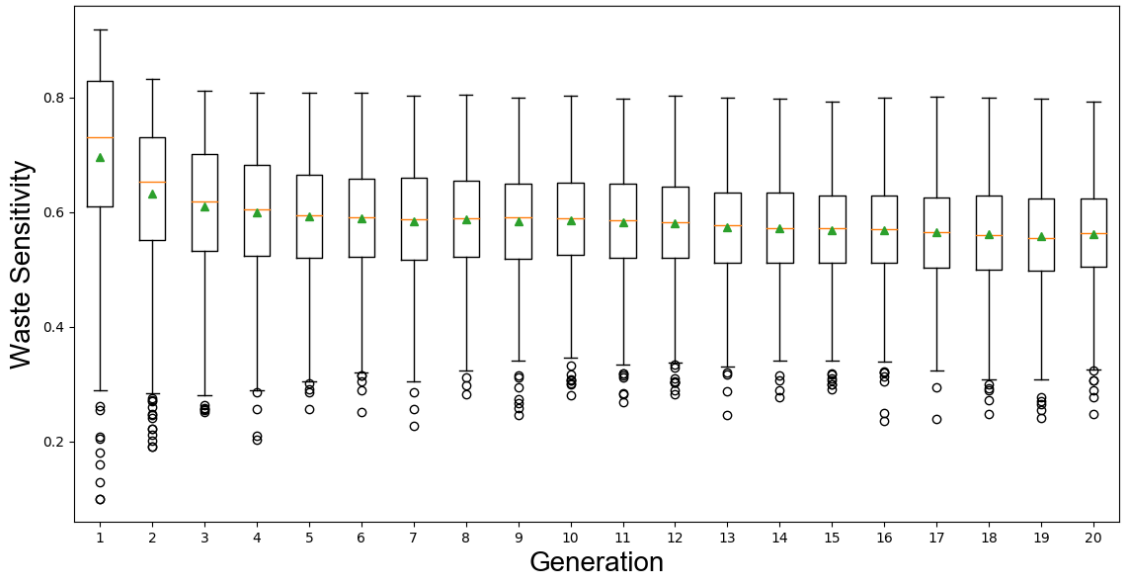


Figure 8. Waste sensitivity variation in CCUs over generations (similar patterns seen in the MCUs)

a) Waste sensitivity (generation 0 – mean = 1.0; s.d. = 30% of mean)



8.b) Survivorship over generations

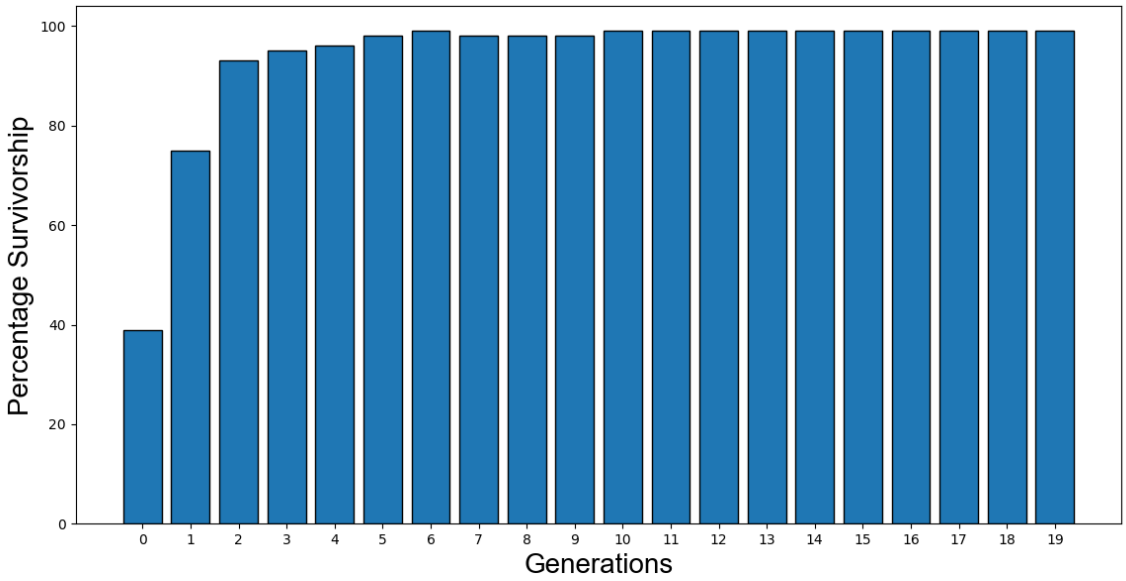


Figure 9. Survivorship difference in the competition experiment at high-density (600 eggs in MCU level of food)

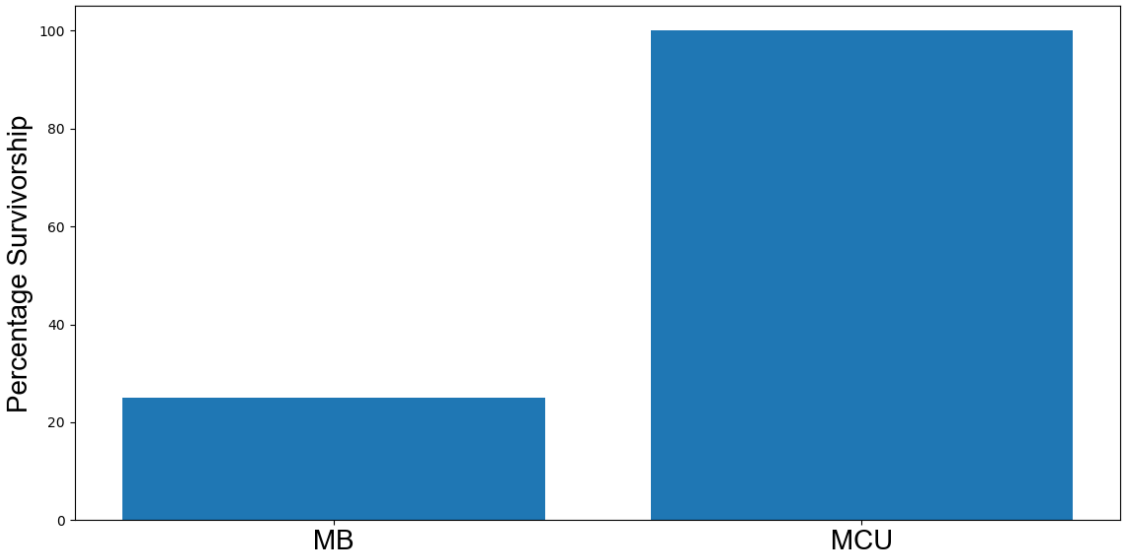
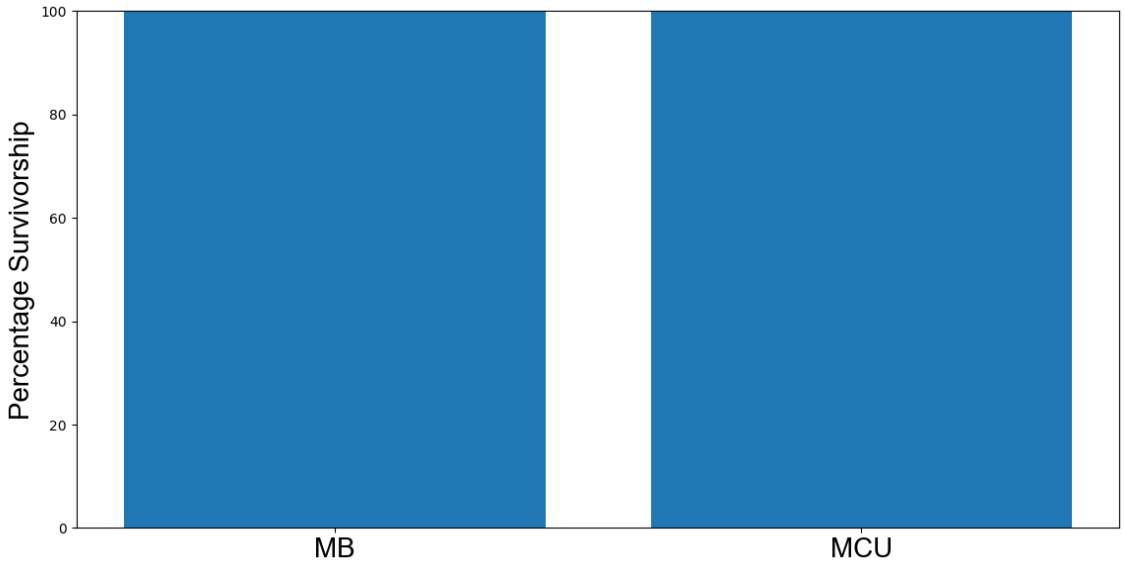


Figure 10. Survivorship difference in the competition experiment at low-density (70 eggs in MB level of food)



Chapter 6

Conclusions And Future Directions

Preliminary results from the individual based simulations of both the ecological and evolutionary dynamics of *Drosophila* larvae in cultures subjected to different modes of crowding are presented in this thesis, in a single-generation and multi-generation framework, respectively.

In the single-generation larval culture simulations, the base model with feeding rate variation alone was able to show capture some of the gross patterns in body size distribution trends seen in the monotypic culture experiment of Sarangi (2013). However, this base model failed to capture even gross trends in the development time distributions of the cultures subjected to different combinations of density and food level. Incorporating an interaction of waste sensitivity variation among-larvae with the larval stop behaviour, however, was able to capture well the patterns of density effects on development time distributions in experiments. As promising as this simple interaction seemed to be in its power to explain the gross effects of different density regimes, recent results (M. Sarangi and A. Joshi, *unpubl*. *data*) suggest that waste tolerance may not have evolved in the different crowded populations maintained in our laboratory. Thus, waste tolerance may only be relevant to certain individuals in the larval culture, such as those that pupate later, similar to what was observed in the CU populations (Borash *et al.* 1998).

In the evolutionary extension of the model, it was seen that simple rules of stochastic blending (additive) inheritance could lead to directional change in some of the traits thought to comprise competitive ability, in the direction expected under the evolution of adaptations to larval crowding. These changes included increase in feeding rate, decrease in waste sensitivity, and lowering of minimum critical size, each being seen in independent simulations. Experimentally, all three of these traits have been considered important to competitive ability in *Drosophila* larvae over the years, with the observed directions of change being similar to those seen in the simulations. However, the evolutionary changes in the simple simulations thus far are only seen as a response to high levels of density-dependent mortality, due to absence of assortative mating in the model. Furthermore, the trait evolution in the simulations is on an extremely rapid scale compared to empirical results, with response peaking and then plateauing out after less than 10 generations.

Overall, the kernel for the current framework seems to be in agreement with empirical results on several basic assumptions about crowding and competitive ability, while also leaving a lot of room for improvement and/or refinement.

The most pressing change required for the single-generation larval culture model is some metric which introduces change between cultures with the same egg/volume food density but different absolute values of eggs and food volume, as this was the primary reason for starting a model of this kind. Though some preliminary work has been done, it is as yet not clear exactly how to approach this problem most effectively.

There are several ways in which this change can be brought about. In any larval culture with a sufficiently long food column, larvae tend to feed primarily on the surface, forming a 'feeding band'. It is reasonable to assume that larvae probably do not experience the toxic effects of any metabolic waste outside of this feeding band, just as they do not have access to fresh food further down from the surface. A recent study on the build up of nitrogenous waste in crowded larval cultures has shown that waste on the surface tends to plateau after a few days, whereas the level of diffused waste in the food column increases (M. Sarangi, S. Dey and A. Joshi, *unpubl*. *data*). Thus, incorporating waste dynamics across the feeding-accessible and non-accessible parts of the food column in the simulation using a feeding band volume of food and a food volume in which waste diffuses over time could establish differences between the MCU, CCU and LCU types of cultures, which have increasing food column lengths, respectively. There would be no diffusion of waste in the MCU type culture, whereas some diffusion would take place in the CCU type culture, leading to different ratios of waste experienced by the larvae within the feeding band in the two cultures.

Another possibility is to incorporate a surface volume in which only a limited volume of larvae can feed at any given time. The 1200 eggs regimes typically have a large number of larvae outside the food even in the early stages of feeding (S. Venkitachalam, *pers*. *obs*.), suggesting that they may not have enough space to eat in the feeding band at any given time. Such a surface capacity, being the same for MCU and CCU type cultures, would lead to very different feeding dynamics – with a much greater proportion of MCU type larvae being able to feed at any given time. This may be expected to lead to large

differences in the body size and time to wandering distributions of the two types of cultures.

Differences in the cultures with distinct details of crowding may also be brought about by the incorporation of density-based feeding rate increase, a trait whose importance has been only recently brought to the fore by experimental evidence (M. Sarangi and A. Joshi, *unpubl. data*). Briefly, while the MCUs have not evolved feeding rate, when assayed in isolation as was typically the case in all feeding rate assays in the past, they appear to be faster feeders than the MB ancestral controls, when assayed at high density in vials, suggesting that increasing the upper limits of a plastic, density-based response to feeding rate may also increase competitive ability.

Another factor that the present versions of the model ignore is the large amount of pupal mortality seen in real crowded cultures. This may possibly be occurring due to excessive waste ingestion by larvae, and can only be incorporated by changing the way excreted waste interacts with larval feeding and survival in the current version of the simulations.

As for the evolutionary model, the obvious next step is to modify the rules of inheritance used such that they allow for greater realism by incorporating factors like dominance, epistasis etc. Also, while the current inheritance rules assume the same degree of offspring variation regardless of the trait values of the parents chosen, it may be closer to reality if this variation increases the closer the mid-parent value is to the overall parental mean, and vice versa, assuming greater heterozygosity at polygenic loci affecting these

traits in parents close to the population mean phenotypic value. Offspring variation would be expected to decrease for parents having large differences in their trait values. In case of small parental difference in the trait value, the offspring variation would be large if the parents are found closer to the mean and small if the parents are away from the mean. Thus, adding a condition to the previous statement, offspring variation would be expected to increase the closer the mid-parental value is to the overall parental mean, only if the difference between the parents is small.

The current evolutionary model also ignores assortative or other forms of non-random mating, or fecundity differences of any kind. In the real high-density larval populations such as the LCUs, there is a large spread in the distribution of body size as well as pre-adult development time. It is also very likely that assortative mating with respect to development time was responsible for the dimorphism between the 'earlies' and the 'lates' of the (similar to LCUs) CU populations of Borash *et al.* (1998) (Nagarajan 2010). Thus, assortative mating with respect to size and development time in the evolutionary model may allow us to better explore these ideas. Female size based fecundity differences, with larger females being able to lay more eggs compared to the smaller females, would favour more offspring for individuals having trait values allowing them to reach larger sizes. Thus, patterns of assortative mating and fecundity differences may lead to different evolutionary dynamics of traits related to competitive ability.

Another direction in which the evolutionary model can be further extended is into the realm of population dynamics. The alteration to be made here would be to remove the

sampling of the offspring according to the given culture density at every generation, instead letting all the eggs laid by each mating pair stay in the culture for the subsequent generation, such that the dynamics of the population can be simulated. This is important given the links between adaptation to crowding and population dynamics and stability (Dey *et al*. 2012).

Overall, the simulation framework developed and described in this thesis has explored some basic ideas related to the evolution of competitive ability in larval culture vials with different modes of imposed larval crowding. More importantly, it has laid the groundwork for further incorporation of ecologically meaningful details, as well as more realistic inheritance rules, and it is hoped that these extensions to the work reported here will eventually enable a far fuller understanding of the ecological and evolutionary responses to crowding in organisms with predominantly scramble competition, like *Drosophila* species.

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Appendix 1

This appendix contains code written for the single generation monotypic culture simulations. All code is written in Python 2.7 (http://www.python.org)

Part 1. Main code

```
import numpy as np # import numpy module
import Total_iterator # import python file with iterator function (see part 2)

def LAJ(Density, Time_steps, Food,
        ES = 100, ES_v = 0, FR = 1., FR_v = 0.025, MC = 2500, MC_v = 0,
        PC = 80, PC_v = 0, WS = 1, WS_v = 0, EF = 0.6, EF_v = 0,
        LS = 0, LS_v = 0,
        Start_waste = 0., LStop_status = 0):
    '''''
    This is the function that runs the single generation monotypic culture
    simulation (informally called LAJ, which is the name of the function).
    The inputs are explained as follows-
    Density = number of individuals
    Time_steps = number of time steps
    Food = starting food amount
    The next few terms are abbreviations of larval traits used in the
    simulation, the two letter term is the mean value of the trait,
    the _v suffix is the fraction of the mean value taken as standard deviation.
    The terms are-
    ES = Egg Size; FR = Feeding Rate; MC = Min. Crit. Size; PC = Post-Crit. Time;
    WS = Waste Sensitivity; EF = Efficiency (Food to biomass); LS = Larval Stop
    Start_waste = Starting waste value in the simulation, set to 0
    LStop_status = 0 denotes simulation without larval stop
    LStop_status = 1 denotes simulation WITH larval stop
    '''

    #Starter arrays -

    Larvae = np.zeros((Time_steps, Density))
    # An array containing the information of larval size through the time steps

    Status = np.zeros(Density)
    # An array that tracks the current status of the larva
    # Status legend -
    # 0 = Egg to minimum critical size
    # 1 = Crossed minimum critical size, can still feed
    # 1.5 = Larval stop
    # 2 = Left food
    # 2.5 = Left food after larval stop
    # 3 = Dead
    # 4 = Killed if larva is still status < 2 at the last time step
```

```
    Post_crit_record = np.zeros(Density)
    # Array recording the post critical feeding time of the larvae

    Food_record = np.zeros(Time_steps)
    # Food record over time
    Food_record[0] = Food
    # First time step in food record is input food

    Waste_record = np.zeros(Time_steps)
    # Waste record over time
    Waste_record[0] = Start_waste
    # First time step in waste record is input starting waste = 0
    Waste = np.copy(Start_waste)
    # Waste value that gets updated, similar to food value

    Dev_time = np.zeros(Density)
    # An array for recording time to wandering (status[i] = 2 or status[i] = 2.5)
    Mortality = np.zeros(Density)
    # An array for recording time of death (status[i] = 3)
    KIA = np.zeros(Density)
    # In case larvae fail to complete development by end of simulation,
    # they are Killed In Action, given the status[i] = 4, to account for all larvae
    Stop_timer = np.zeros(Density)
    # Array for recording time spent in larval stop

    Food_eaten = np.zeros((Time_steps, Density))
    # Array for recording food eaten per larva, per time step
    Feedback = np.zeros((Time_steps, Density))
    # Array for recording waste feedback per larva, per time step
    Waste_ind = np.zeros((Time_steps, Density))
    # Array for recording waste excreted per larva, per time step
    Growth = np.zeros((Time_steps, Density))
    # Array for recording growth per larva, per time step

    Bite_scale = np.zeros(Density) + 0.02
    # Bite scale, set to 0.02 for all larvae

    # Trait arrays –

    if ES_v == 0:
        Egg_size = np.zeros(Density) + ES
        # If egg size variation = 0, then all larvae have egg size
        # equal to ES
    elif ES_v > 0:
        Egg_size = np.random.normal(ES, (ES * ES_v), Density)
        # Egg size for each larva, drawn from a normal distribution
        # with mean = ES, standard deviation = ES * ES_v (fraction of ES)
    Egg_size[Egg_size<0] = 0
    # Lower cap on egg size set to 0
    Larvae[0, :] = Egg_size
    # Size of all larvae in the first time step set equal to egg size

    if FR_v == 0:
        Feeding_rate = np.zeros(Density) + FR
        # If feeding rate variation = 0, then all larvae have feeding rate
        # equal to FR
    elif FR_v > 0:
        Feeding_rate = np.random.normal(FR, (FR * FR_v), Density)
        # Feeding rate for each larva, drawn from a normal distribution
        # with mean = FR, standard deviation = FR * FR_v (fraction of FR)
```

```
    Feeding_rate[Feeding_rate<0] = 0
    # Lower cap on feeding rate set to 0

    if MC_v == 0:
        Min_crit = np.zeros(Density) + MC
        # If min. crit. size variation = 0, then all larvae have min. crit. size
        # equal to MC
    elif MC_v > 0:
        Min_crit = np.random.normal(MC, (MC * MC_v), Density)
        # Min. crit. size for each larva, drawn from a normal distribution
        # with mean = MC, standard deviation = MC * MC_v (fraction of MC)
    Min_crit[Min_crit<0] = 0
    # Lower cap on min. crit. size set to 0

    if PC_v == 0:
        Post_crit = np.zeros(Density) + PC
        # If post-crit. time variation = 0, then all larvae have post crit. time
        # equal to PC
    elif PC_v > 0:
        Post_crit = np.random.normal(PC, (PC * PC_v), Density)
        # Post-crit. time for each larva, drawn from a normal distribution
        # with mean = PC, standard deviation = PC * PC_v (fraction of PC)
    Post_crit[Post_crit<0] = 0
    # Lower cap on Post_crit set to 0
    Post_crit = np.round(Post_crit)
    # All values of Post_crit rounded to whole numbers

    if WS_v == 0:
        Waste_sen = np.zeros(Density) + WS
        # If waste sensitivity variation = 0,
        # then all larvae have waste sensitivity equal to WS
    elif WS_v > 0:
        Waste_sen = np.random.normal(WS, (WS * WS_v), Density)
        # Waste sensitivity for each larva, drawn from a normal distribution
        # with mean = WS, standard deviation = WS * WS_v (fraction of WS)
    Waste_sen[Waste_sen<0.1] = 0.1
    # Lower cap on Waste_sen set to 0.1

    if EF_v == 0:
        Efficiency = np.zeros(Density) + EF
        # If efficiency variation = 0, then all larvae have efficiency
        # equal to EF
    elif EF_v > 0:
        Efficiency = np.random.normal(EF, (EF * EF_v), Density)
        # Efficiency for each larva, drawn from a normal distribution
        # with mean = EF, standard deviation = EF * EF_v (fraction of EF)
    Efficiency[Efficiency<0] = 0
    # Lower cap on Efficiency set to 0.1

    if LS_v == 0:
        Larval_stop = np.zeros(Density) + LS
        # If larval stop variation = 0, then all larvae have larval stop
        # equal to LS
    elif LS_v > 0:
        Larval_stop = np.random.normal(LS, (LS * LS_v), Density)
        # Larval_stop for each larva, drawn from a normal distribution
        # with mean = LS, standard deviation = LS * LS_v (fraction of LS)
    Larval_stop[Larval_stop<0] = 0
    # Lower cap on Larval_stop set to 0.1
    Larval_stop = np.round(Larval_stop)
    # All values of Larval_stop rounded to whole numbers
```

```
    Max_size = Min_crit * 5
    # The maximum possible size achievable by a larva set to 5 x min. crit. size

    for i in range(1, Time_steps):
        # From 2nd to final time step, iterates over all time steps
        Larvae[i, :], Status, Dev_time, Mortality, KIA, Post_crit_record, \
        Stop_timer, Food, Waste, Food_eaten[i, :], Feedback[i, :], \
        Growth[i, :] \
         = Total_iterator.Checks(
            Larvae[i - 1, :], Status, Post_crit_record, Post_crit,
            Mortality, KIA, Dev_time, Food_record[i - 1], i, Time_steps,
            LStop_status, Stop_timer, Larval_stop, Feeding_rate, Waste_sen,
            Efficiency, Min_crit, Bite_scale, Waste, Max_size)

         # Passes all relevant arrays through the Checks function in
         # Total_iterator file (see part two of this appendix)
         # The Larvae, Status, Dev_time, Mortality etc. arrays are all updated
         # every time step through this function


        Food_record[i] = Food
        # Food_record for ith time step updated to current food
        Waste_record[i] = Waste
        # Waste_record for ith time step updated to current waste

    # The function returns all the output arrays that can be further studied
    # by plotting or analysis

    return Larvae, Status, Dev_time, Mortality, KIA, Post_crit_record, \
    Food_record, Waste_record, Growth
```

Part 2. Iterator function

```
import numpy as np

def Checks(Larvae_current, Status, Post_crit_R, Post_crit, Mortality, KIA,
    Dev_time, Food, Time, Time_steps, LStop_status, Stop_timer, Larval_stop,
    Feeding_rate, Waste_sen, Efficiency, Min_crit, Bite_scale, Waste, Max_size):
    '''
    Checks is the iterator function for the single generation monotypic culture
    simulation. It takes various arrays as input from the main function
    described in part 1, and iterates over all the larvae for one time step

    Larvae_current = Larval array in the current time step
    Post_crit_R = Post critical feeding time record
    (See part 1 for more info. about the remaining terms)
    '''

    New_larvae = np.zeros(len(Larvae_current)) # Output larval array
    Food_eaten = np.zeros(len(Larvae_current)) # Output food eaten array
    Feedback = np.zeros(len(Larvae_current)) # Output feedback array
    Growth = np.zeros(len(Larvae_current)) # Output growth array
    Waste_ind = np.zeros(len(Larvae_current)) # Output waste excreted array

    Shuffler = np.arange(len(Larvae_current))
    # Array with integers from 0 to (Density - 1) in ascending order
```

```
    np.random.shuffle(Shuffler)
    # Randomly shuffle elements of Shuffler

    for j in range(len(Larvae_current)):
        # For all larvae in the simulation
        i = Shuffler[j] # Shuffle the order of larvae in feeding order

        Condition = False
        # Condition being that max size or Post_crit time is reached

        if Status[i] == 1 and Post_crit_R[i] == Post_crit[i]:
            Condition = True

        elif Status[i] == 1 and Larvae_current[i] >= Max_size[i]:
            Condition = True

        # If ith larva has not left food, but reached minimum critical size
        # and has finished post critical feeding time, or reached
        # maximum size possible, then Condition is set to True

        if Status[i] >= 3:
            New_larvae[i] = 0
            # If ith larva is dead, set its output size to 0

        elif Condition == True:
            # If post critical feeding time or max size is reached
            Status[i] = 2 # set status as left food
            New_larvae[i] = Larvae_current[i]
            # Output larval size set to current larval size
            # i.e. larval size remains unchanged
            if Dev_time[i] == 0:
                Dev_time[i] = Time
                # Record time to wandering


       elif Time == (Time_steps - 1) and Status[i] < 2:
            # If final time step of simulation is reached,
            # and larva hasn't left food
            Status[i] = 4 # Status set to KIA
            New_larvae[i] = 0 # Output larval size set to 0
            if KIA[i] == 0:
                KIA[i] = Time # KIA time recorded (should be Time_steps - 1)

        elif Status[i] == 2 or Status[i] == 2.5:
            # If larva has left food,
            #  and time to wandering has been previously recorded
            New_larvae[i] = Larvae_current[i]
            # Output larval size set to current larval size

        elif Status[i] == 1.5:
            # If larva is in larval stop
            if Stop_timer[i] < Larval_stop[i]:
                # If larval stop time limit has not been reached
                Stop_timer[i] += 1
                # larval stop timer for ith larva is increased by 1
                New_larvae[i] = Larvae_current[i]
                # Output larval size set to current larval size
```

```
            elif Stop_timer[i] == Larval_stop[i]:
                # If larval stop time limit reached
                Status[i] = 2.5 # Set status as left food after larval stop
                New_larvae[i] = Larvae_current[i]
                # Output larval size set to current larval size
                if Dev_time[i] == 0:
                    Dev_time[i] = Time
                    # Record time to wandering

        elif Food <= 0:
            # If no food is remaining
            if Status[i] == 0:
                # If ith larva has not crossed min. crit. size
                Status[i] = 3
                # Status set to 'dead'
                New_larvae[i] = 0
                # Output larval size set to 0
                if Mortality[i] == 0:
                    Mortality[i] = Time
                    # Time of death recorded

            elif Status[i] == 1:
                # If ith larva has crossed min. crit. size
                if LStop_status == 1:
                    # If larval stop is active in the current simulation
                    Status[i] = 1.5 # Larva enters larval stop
                    New_larvae[i] = Larvae_current[i]
                    # Output larval size set to current larval size
                    if Stop_timer[i] == 0:
                        Stop_timer[i] += 1
                        # Larval stop timer is updated

                elif LStop_status == 0:
                    # If larval stop is not active in the current simulation
                    Status[i] = 2 # set status as left food
                    New_larvae[i] = Larvae_current[i]
                    # Output larval size set to current larval size
                    if Dev_time[i] == 0:
                        Dev_time[i] = Time
                        # Time to wandering is recorded

        elif Food > 0:
            # If food is remaining
            # See chapter 2 for the following terms (equations 1-7)
            Bite_size = Bite_scale[i] * Larvae_current[i]
            # Bite size of current larva
            Food_eaten[i] = Feeding_rate[i] * Bite_size
            # Food eaten by ith larva
            Feedback[i] = (((Waste_feed + Fresh_waste)/Food) * Waste_sen[i]
                           * Feeding_rate[i])
            # Feedback experienced by ith larva
            Growth[i] = Food_eaten[i] * (1 - (1 - Efficiency[i]) - Feedback[i])
            # Growth of ith larva
```

```
            if Growth[i] <= 0:
                # If growth of ith larva is not positive
                if Status[i] == 0:
                    # If larva has not crossed min. crit. size
                    Status[i] = 3 # set status as dead
                    New_larvae[i] = 0 # Output larval size set to 0
                    if Mortality[i] == 0:
                        Mortality[i] = Time
                        # Time of death recorded

                elif Status[i] == 1:
                    # If larva has crossed min. crit. size
                    if LStop_status == 1:
                        # If larval stop is active in the current simulation
                        Status[i] = 1.5 # Larva enters larval stop
                        New_larvae[i] = Larvae_current[i]
                        # Output larval size set to current larval size
                        if Stop_timer[i] == 0:
                            Stop_timer[i] += 1
                            # Larval stop timer is updated

                    elif LStop_status == 0:
                        # If larval stop is not active in the current simulation
                        Status[i] = 2 # set status as left food
                        New_larvae[i] = Larvae_current[i]
                        # Output larval size set to current larval size
                        if Dev_time[i] == 0:
                            Dev_time[i] = Time
                            # Time to wandering is recorded

            elif Growth[i] > 0:
                # If growth of ith larva is positive
                New_larvae[i] = Larvae_current[i] + Growth[i]
                # Output larval size is updated according to growth
                Waste_ind[i] = Food_eaten[i] * (1 - Efficiency[i]) * 0.5
                # Waste excreted by the larva is recorded
                Waste += Waste_ind[i]
                # Waste level updated
                Food -= Food_eaten[i] # Food level updated
                if New_larvae[i] >= Min_crit[i]:
                    # If output larva has size >= its min. crit. size
                    if Status[i] == 0:
                        # If status is set to 'not crossed min. crit. size'
                        Status[i] = 1
                        # Set status as 'crossed min. crit. size'

                    elif Status[i] == 1:
                        # If status is set to 'crossed min. crit. size'
                        Post_crit_R[i] += 1
                        # Post critical feeding time is updated

    # Function returns all relevant arrays for the main code

    return New_larvae, Status, Dev_time, Mortality, KIA, Post_crit_R, \
            Stop_timer, Food, Food_eaten, Feedback, Waste, Growth
```

Appendix 2

This appendix contains code written for the evolutionary extension of the monotypic culture simulation. All code is written in Python 2.7 (http://www.python.org)

Part 1. Inheritance rule

```
import numpy as np # import numpy module

def Inheritance(Traits, child_var = 0.1, fecundity = 20, output = 70,
    lower_bound = True):
    '''
    The inheritance function is home to the inheritance rule in the simulations,
    which is used both with and without the feeding behaviour
    (See chapters 4, 5)
    Traits is a 2D array, each row represents a trait, each column an individual
    child_var is the fraction of total standard deviation used to generate
    offspring
    fecundity is the number of offspring produced by each mating pair
    output is the number of individuals selected for the next generation
    lower_bound = True, Trait values can't go below 0
    lower_bound = False, Trait values CAN go below 0
    '''
    Ind = len(Traits[0, :]) # Number of individuals from the previous generation

    if Ind%2 == 1: # If number of individuals is odd
        Traits = np.delete(Traits, -1, 1)
        # individual at the last index is removed from consideration
        Ind = len(Traits[0, :])

    Ind_order = np.arange(Ind)
    # Array with integers from 0 to (Ind - 1) in ascending order,
    # each number represents a parent
    Pick_status = np.ones(Ind)
    # Array of ones with length = Ind,
    # status is set to 0 for each individual picked into a mating pair
    Mating_pairs = np.zeros((Ind/2, 2))
    # Array with Ind/2 rows, 2 columns, for recording mating pairs

    Total_var = np.std(Traits, axis=1)
    # Overall parental variation for each trait
    Total_mean = np.mean(Traits, axis=1)
    # Overall parental mean for each trait

    for i in range(Ind/2):
        # For all potential mating pairs
        E = np.random.choice(Ind_order, 1, p=Pick_status/np.sum(Pick_status))
        Pick_status[E] = 0
        # E is parent 1 in ith row,
        # status of picked individual set to 0 to avoid repeated picking
```

```
        F = np.random.choice(Ind_order, 1, p=Pick_status/np.sum(Pick_status))
        Pick_status[F] = 0
        # F is parent 2 in ith row
        Mating_pairs[i, 0] = E # ith row, parent 1
        Mating_pairs[i, 1] = F # ith row, parent 2
    Parent_traits = range(Ind/2)
    # Array for traits of each parent in the mating pairs

    Mid_parent = range(Ind/2) # Mid-parent array
    Off_var = range(Ind/2) # Offspring variation array
    Offspring = range(Ind/2) # Offspring array

    for j in range(Ind/2):
        # For all mating pairs
        Parent_traits[j] = [Traits[:, Mating_pairs[j, 0]],
                            Traits[:, Mating_pairs[j, 1]]]
        Mid_parent[j] = range(len(Traits[:, 0]))
        Off_var[j] = range(len(Traits[:, 0]))
        Offspring[j] = range(len(Traits[:, 0]))

        for k in range(len(Traits[:, 0])):
            # For all traits
            # Parent_traits[j][P][k]:
            # j = mating pair number; P = 0 or 1 for either parent;
            # k = each of the individual's trait values, as an array

            Mid_parent[j][k] = np.mean([Parent_traits[j][0][k],
                                        Parent_traits[j][1][k]])
            # Mid-parent value of jth mating pair, kth trait
            Off_var[j][k] = Total_var[k] * child_var
            # Offspring variation of the jth mating pair, kth trait

            if Off_var[j][k] > 0:
                # If kth trait variation > 0
                Offspring[j][k] = np.random.normal(Mid_parent[j][k],
                                                   Off_var[j][k], fecundity)
                # Offspring equal in number to fecundity
                # drawn from normal distribution with
                # mean = mid-parent value, S.D. = offspring variation

            elif Off_var[j][k] == 0:
                # If kth trait variation = 0
                Offspring[j][k] = np.zeros(fecundity) + Mid_parent[j][k]
                # Offspring equal in number to fecundity,
                # with trait value equal to the mid-parent value

            if lower_bound==True:
                for l in range(fecundity):
                    if Offspring[j][k][l] < 0:
                        Offspring[j][k][l] = 0
                        # If lower_bound is True,
                        # all trait values < 0 are set to zero

    Off_array = np.array(Offspring) # Making Offspring data type into ndarray

    Off_flat = np.zeros((len(Traits[:, 0]), np.size(Off_array[:, 0, :])))
    # Rearranging the offspring array along the axis of Traits, such that
    # each row represents a trait,
    # each column represents an individual
```

```
    for m in range(len(Off_flat)):
        # For each trait
        Off_flat[m, :] = np.ndarray.flatten(Off_array[:, m, :])
        # The array is flattened for each trait,
        # making each row represent the particular trait for all individuals
        # (Individual order is preserved)
    Chosen_indices = np.random.choice(np.arange(np.size(Off_flat[0, :])),
        output, replace=False)
        # Choose individuals equal to the output value
        # from the total offspring pool

    Chosen_offspring = Off_flat[:, Chosen_indices]

    # The function returns trait values for all the chosen offspring

    return Chosen_offspring
```

Part 2. Evolutionary extension

```
import numpy as np # import numpy module
import LAJ_Gen0 # import gen 0 variant of LAJ
# (same as LAJ code, with different output)
import LAJ_evo_iterator # import LAJ variant for evolutionary extension of model
# Similar to LAJ code, but without trait array generation
# Instead the function in this module accepts trait arrays from
# surviving adults of the previous generation
import Offspring # import module with inheritance rule function

def Evolve(Density, Generations, Food, CV, Time_steps):
    '''
    The Evolve function iterates the monotypic culture simulation for multiple
    generations, with survivors passing their offspring to the next generation
    according to the inheritance rule in the Offspring function
    Density = Number of individuals in the culture
    Generations = Number of generations over which the simulation is run
    Food = Food in the culture
    CV = Fraction of parental variation that offspring are generated from
    Time_steps = Number of time steps for each culture
    '''

    MS = [100, 1., 2500, 80, 1, 0.6, 60]
    # Mean values of each trait for the 0th generation
    # Order of traits - egg size, feeding rate, min. crit. size,
    # post-crit. time, waste sensitivity, efficiency, larval stop
    VS = [0, 0, 0, 0, 0, 0, 0] # Fraction of mean values for standard deviation
    # Same order in VS as in MS
    SS = [0] # Status for larval stop
    Misc = [0.]
    # Start_waste

    Size_adult = range(Generations)
    # Records the sizes of surviving adults every generation
    Traits = range(Generations)
    # Records the trait values of surviving adults every generation
```

```
    Dev_time = range(Generations)
    # Records the development time of surviving adults every generation
    Mortality = range(Generations)
    # Records the time of death of dead larvae every generation


    Size_adult[0], Traits[0], Dev_time[0], Mortality[0] = \
    LAJ_Gen0.LAJ(Density, Time_steps, Food,
        ES = MS[0], FR = MS[1], MC = MS[2], PC = MS[3], WS = MS[4], EF = MS[5],
        LS = MS[6],
        ES_v = VS[0], FR_v = VS[1], MC_v = VS[2], PC_v = VS[3], WS_v = VS[4],
        EF_v = VS[5], LS_v = VS[6],
        LStop_status = SS[0], Start_waste = Misc[0])

    # The first generation in the simulation is similar to the monotypic culture
    # function. The output includes size, trait values and
    # development time of surviving adults, as well time of death of dead larvae

    for i in range(Generations - 1):

        CT = Offspring.Inheritance(Traits[i], child_var = CV, output = Density)
        # Offspring trait values generated
        Size_adult[i + 1], Traits[i + 1], Dev_time[i + 1], Mortality[i + 1] = \
        LAJ_evo_iterator.Culture(Density, Time_steps, Food,
            CT[0], CT[1], CT[2], CT[3], CT[4], CT[5], CT[6],
            LStop_status = SS[0], Start_waste = Misc[0])
        # The Culture function is similar to LAJ, but the trait arrays are
        # added as input into the function rather than being generated with
        # given mean and standard deviation values as in LAJ
        # Adult size, traits and development time, as well as death record for
        # dead larvae are recorded for the ith generation

    # The function returns adult size, traits, development time and larval death record
    # for all the generations
    return Size_adult, Traits, Dev_time, Mortality
```