Theoretical and empirical investigations on population stability and dispersal evolution using laboratory populations of *Drosophila melanogaster*

A thesis

Submitted in partial fulfilment of the requirements

Of the degree of

Doctor of Philosophy

By

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DEDICATED TO

My family,

Father (Ganesh Chandra Tung),

Mother (Manjushree Tung),

Sister (Sudipa Tung Bera)

&

Brother-in-law (Anirban Bera)

CERTIFICATE

Certified that the work incorporated in thesis titled 'Theoretical and empirical investigations on population stability and dispersal evolution using laboratory populations of *Drosophila melanogaster*', submitted by Sudipta Tung was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.

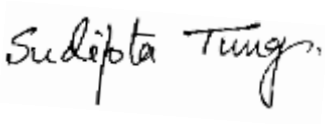
Dr. Sutirth Dey

(Supervisor)

Date: 31/12/2017

DECLARATION

I declare that this written submission represents my ideas in my own words and where others ideas have been included, I have adequately cited and referred to original sources. I also declare that I have adhered to all principles of academic honesty and integrity and I have not misinterpreted or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above can cause disciplinary action by the institute and evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.



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CONTENTS

SYNOPSIS

Title: Theoretical and empirical investigations on population stability and dispersal evolution using laboratory populations of *Drosophila melanogaster*

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1. Introduction

Rapid changes in climatic conditions and habitat degradation are posing alarming threats of extinction to a number of species (Crooks et al., 2017; Didham et al., 2012; Thomas et al., 2004). Unfortunately, but not surprisingly, not all species are equal in their ability to respond to such new challenges and maintain a steady abundance over the long run (Parmesan, 2006). For some of these species human intervention is necessary to avoid extinction, whereas others might survive either by adapting to the new conditions or by dispersing to more favorable areas. In this thesis, I have examined potential strategies for increasing stability of extinction-prone populations and investigating evolutionary consequences of dispersal evolution. For this purpose, I have used computer simulations and experiments using laboratory populations of the fruit-fly, *Drosophila melanogaster*. Here I present an overview of the various studies and the corresponding findings presented in this thesis.

2. A Comparison of Six Methods for Stabilizing Population Dynamics (Chapter 2)

Here I compare six different control methods with respect to their efficiency at inducing a common level of enhancement (defined as 50% increase) for two kinds of stability (constancy and persistence) under four different life history/ environment combinations. I show that for these six methods, even when the magnitude of stabilization attained is the

same, other aspects of the dynamics like population size distribution can be very different. Consequently, correlated aspects of stability, like the amount of persistence for a given degree of constancy stability (and vice versa) or the corresponding effective population size (a measure of resistance to genetic drift) vary widely among the methods. Moreover, the number of organisms needed to be added or removed to attain similar levels of stabilization also varies for these methods, a fact that has economic implications. Finally, I compare the relative efficiency of these methods through a composite index of various stability related measures. My results suggest that Lower Limiter Control (LLC) (Hilker and Westerhoff, 2005) seems to be the optimal method under most conditions, with the recently proposed Adaptive Limiter Control (ALC) (Sah et al., 2013) being a close second.

The content of this chapter has been published as the following research article:

***Tung, S.**, Mishra, A. and Dey, S. 2014. A comparison of six methods for stabilizing population dynamics. Journal of Theoretical Biology 356, 163-173.*

3. Population stability through Upper Limiter Control (ULC) and Lower Limiter Control (LLC) (Chapter 3)

Although a large number of methods exist to control the dynamics of populations to a desired state (Dattani et al., 2011; Hilker and Westerhoff, 2005; Sah et al., 2013; Tung et al., 2014), few of them have been empirically validated, which limits the scope of using these methods in real-life scenarios. To address this issue, I tested the efficacy of two well-known control methods in enhancing different kinds of stability in highly fluctuating, extinction-prone populations of *Drosophila melanogaster*. The Upper Limiter Control (ULC) method (Hilker and Westerhoff, 2005) was able to reduce the fluctuations in population sizes as well as the extinction probability of the populations. On the negative side, it had no effect on the effective population size and required a large amount of effort. On the other hand, Lower Limiter Control (LLC) enhanced effective population size and reduced extinction probability at a relatively low amount of effort. However, its effects on population fluctuations were equivocal. I also show that biologically-realistic simulations, using a very general population dynamics model, are able to capture most of the trends of my data. This suggests that my results are likely to be generalizable to a wide range of scenarios.

The content of this chapter has been published as the following research article:

Tung, S., *Mishra, A., Dey, S. 2016. Stabilizing the dynamics of laboratory populations of Drosophila melanogaster through upper and lower limiter controls. Ecological Complexity 25, 18-25.*

4. Population stability through Both Limiter Control (BLC) and Target Oriented Control (TOC) (Chapter 4)

Here, I investigated the effects of two other well-studied control methods (Both Limiter Control and Target-Oriented Control) on the dynamics of unstable populations of *Drosophila melanogaster*. In contrast to the methods investigated in the previous chapter, here I show that both BLC and TOC (Dattani et al., 2011) can significantly reduce population fluctuations, decrease extinction probability and increase effective population size simultaneously. I use the distribution of population sizes to derive biologically intuitive explanations for the mechanisms of how these two control methods attain stability. Finally, I show that non-*Drosophila* specific biologically realistic simulations are able to capture almost all the trends of my data, indicating that these results are likely to be generalizable over a wide range of taxa.

The content of this chapter has been published as the following research article:

Tung, S., *Mishra, A., Dey, S. 2016. Simultaneous enhancement of multiple stability properties using two-parameter control methods in Drosophila melanogaster. Ecological Complexity 26, 128–136.*

5. Understanding *Drosophila* dynamics through a stage-structured individual-based model (Chapter 5)

The amount of resources available to different life-stages can affect the dynamics of stage-structured populations. I investigate this through a stage-structured individual-based model of *Drosophila* that incorporates life-history parameters common to many holometabolous insect populations. I also compare my study with experiments and models on the dynamics of various other species to understand which aspects of dynamics are generalizable. I then use this model to explore how the interaction between nutrition levels and various facets of unequal number of males and females affect population dynamics. I show that the effects of unequal sex-ratio and sex-specific culling are greatly influenced by fecundity but not by levels of juvenile nutrition. I also demonstrate that the efficiency of a widely-used pest

control method (Sterile Insect Technique) depends on a complex interaction between the levels of juvenile nutrition and the density-independent adult fecundity.

The content of this chapter has been submitted as the following research article and it is currently under review:

***Tung, S.**, Rajamani, M., Joshi, A., Dey, S. 2017. Understanding the dynamics of laboratory populations of Drosophila melanogaster: Long-term experiments meet individual-based modelling. bioRxiv 138446.*

6. Simultaneous evolution of multiple dispersal components and kernel in laboratory populations of Drosophila melanogaster (Chapter 6)

Global climate is changing rapidly and is accompanied by large-scale destruction of habitats. Since dispersal is the first line of defense for mobile organisms to cope with such adversities in their environment, it is important to understand the causes and consequences of evolution of dispersal. Using four large (N~2500) outbred populations of *Drosophila melanogaster*, subjected to artificial selection for increased dispersal, I show that different components of dispersal, such as propensity and ability, can evolve rapidly and simultaneously. The response to selection persisted even in the absence of proximate drivers for dispersal. The dispersal kernel evolved to have significantly greater standard deviation and reduced values of skew and kurtosis, which ultimately translated into a 67% greater spatial extent. I also found that although sex-biased dispersal exists in this species, its expression can vary depending on which dispersal component is being measured and the environmental condition under which dispersal takes place. Interestingly though, there was no difference between the two sexes in terms of dispersal evolution.

The content of this chapter has been published as the following research article:

***Tung, S.**, Mishra, A., Shreenidhi, P. M., Sadiq, M. A., Joshi, S., Sruti, V. S., Dey, S. 2017. Simultaneous evolution of multiple dispersal components and kernel. Oikos 127, 34–44.*

7. Evolution of dispersal syndrome and its corresponding metabolomic changes (Chapter 7)

In this chapter, I explore the the behavioral, life-history and metabolic consequences of dispersal evolution in *Drosophila*. In terms of life history, the dispersal selected populations had similar values of body size, fecundity and longevity as the controls. However, in terms of behavior, the selected populations evolved significantly greater locomotor activity,

exploratory tendency, and aggression. These observations led to predictions about putative mechanisms that were confirmed through untargeted metabolomic fingerprinting using NMR spectroscopy. The selected flies had evolved greater amounts of glucose, AMP, and NAD, suggesting elevated cellular respiration. At the same time, levels of neuropeptides, such as octopamine, serotonin and dopamine, related to aggression and exploration had increased in the dispersal selected flies.

The content of this chapter has been submitted as the following research article and it is currently under review process:

Tung, S., *Mishra, A., Gogna, N., Sadiq, M. A., Shreenidhi, P. M., Sruti, V. S., Dorai, K., Dey, S. 2017. Evolution of dispersal syndrome and its corresponding metabolomics changes. bioRxiv 178715.*

8. Conclusion

In the final chapter (Chapter 8), I have summarized the main results of the previous chapters, discussed their potential implications and mentioned possible avenues for further work.

Apart from the published/ submitted research articles mentioned above, I am also associated with the following manuscripts, which are at different stages preparation:

i. Mishra, A., **Tung, S.**, Shree Sruti, V. R., Sadiq, M. A., Srivathsa, S., and Dey, S. Pre-dispersal conditions and presence of opposite sex modulate density dependence and sex bias of dispersal. bioRxiv (2017) 146605. [submitted]
ii. **Tung, S.**, Mishra, A., Shreenidhi, P. M., Sadiq, M. A., Sruti, V. S., Dey, S. Evolution of larval and adult life-history traits as a correlated response to selection for increased dispersal in *Drosophila melanogaster*. [under preparation]
iii. **Tung, S.**, Mishra, A., Shreenidhi, P. M., Sadiq, M. A., Sruti, V. S., Dey, S. Selection for condition-dependent dispersal leads to the evolution of phenotypic-dependent dispersal in *Drosophila*. [under preparation]
iv. Mishra, A.*, **Tung, S.***, Shreenidhi, P. M., Sadiq, M. A., Sruti, V. S., Chakraborty, P. P., and Dey, S. Sex differences in dispersal syndromes are modulated by environment and evolution (2018) *Equal contribution. [submitted]

CHAPTER 1

Introduction

Millions of life forms co-exist on this planet and are responsible for the sustenance of each other, often in a completely non-intuitive manner. This bond of dependency among the species is often so strong that elimination of even one species can affect the composition, structure, and function of an entire ecosystem (Schmitz et al., 2000). Unfortunately, this extraordinary diversity of life on earth is threatened today by, *inter alia*, massive over-exploitation, habitat degradation and habitat destruction (reviewed in Pereira et al., 2010; Rands et al., 2010). Natural phenomena like flood and soil erosion, coupled with human activity, are constantly remodeling the natural landscape, which, in turn, has negative consequences for the biota that relies on it (Didham et al., 2012; Wilson et al., 2016). Similarly, habitat fragmentation, another prominent reason for habitat degradation, is reducing animal movement and gene flow, thereby increasing the risk of local extinction (Crooks et al., 2017). To make matters worse, global climate, in particular, temperatures and patterns of precipitation, are changing rapidly (IPCC, 2007). As these environmental conditions have strong effects on most organisms, such changes, not surprisingly, are not only affecting the life-history, behaviour and abundance / distribution of species worldwide, but also threatening the survival of many species (Thomas et al., 2004).

Organisms can cope with such dynamic and stressful environmental scenarios, either by dispersing to areas with favorable environments or by adapting to the new local environmental conditions (Bellard et al., 2012; Berg et al., 2010; Travis et al., 2013). Unfortunately, but not surprisingly, not all species are equal in their ability to respond to such new challenges (Parmesan, 2006). In particular, the ability to shift and expand their geographical range is found to be crucially dependent on attributes of the local population dynamics, including abundance and stability (Mair et al., 2014). Therefore, understanding the key factors that affect the various attributes of population dynamics, and the nature of adaptive evolution due to contemporary habitat degradation and global climate change, are some of the most crucial issues in biology today. Consequently, the nature of the problem is not only limited to its mere scientific interest, but also it has attracted the attention of the practitioners of biodiversity conservation and resource management (Allen-Wardell et al., 1998; Butchart et al., 2010; Gray, 1997; Noss, 1990).

The size of any biological population typically fluctuates over time in the wild (Turchin and Taylor, 1992 and the references therein) and even under controlled laboratory conditions (Becks and Arndt, 2008; Desharnais et al., 2001; Dey and Joshi, 2007; Sah et al., 2013). Whenever population sizes reach small values, the population has a high risk of going extinct

due to increased demographic stochasticity (Gabriel and Bürger, 1992). Small perturbations in population sizes can prevent such crashes- a notion that has been extensively investigated in theoretical ecology (Corron et al., 2000; Dattani et al., 2011; Hilker and Westerhoff, 2005; McCallum, 1992; Sah et al., 2013). Although notionally a part of the vast literature on controlling non-linear systems (for reviews see Andrievskii and Fradkov, 2003, 2004; Schöll and Schuster, 2008), methods that can be actually used to control real populations are quite few in number. This is primarily due to the ways these methods typically operate and certain properties of most ecological time series. Most chaos-control methods rely on small time-dependent changes in the key parameters of the equations controlling the nonlinear system, which turns a chaotic trajectory into a stable periodic motion (Ott et al., 1990). However, this strategy is unsuitable for real biological populations as, in most cases, the exact equations underlying the dynamics remain unknown. Moreover, even when a rough idea about the equations are available, precise estimation of the parameters of the equations are difficult, since most ecological time series are short in length and are invariably noisy. Finally, the system parameters that determine the observed dynamics (e.g. growth rate and carrying capacity) are mostly estimated post-facto through model-fitting, and thus, are not available for real-time perturbations. Over the last two decades, a number of control methods have been proposed which do not require a real-time estimation of parameter values and work over large parameter ranges, thus being relatively robust to the noise in parameter estimation (Dattani et al., 2011; Hilker and Westerhoff, 2005; Sah et al., 2013). These methods typically work by perturbing the number of individuals in the population which is a more easily maneuvered empirical quantity.

Unfortunately, despite a substantial amount of theoretical work, there is little interest in practitioners of conservation to try out these methods on field. There can be several reasons for this reluctance. Firstly, these methods have typically been shown to work using a variety of population dynamics models and in the context of very different concepts of stability (e.g. Corron et al., 2000; Güémez and Matías, 1993; McCallum, 1992). This makes it difficult to compare the relative efficiencies of these methods and determine 'a suitable method' in the context of a real biological population. Secondly, only a few of the proposed methods have been empirically validated even under laboratory conditions, let alone in nature. Given that the survival of threatened species is at stake, it is understandable that the practitioners of conservation are unwilling to try out untested methods in the field.

While experiments with external perturbation methods and insights from the associated generic phenomenological models are important for controlling a biological population, it is also crucial to identify the intrinsic and extrinsic determinants of population stability and understand how these factors interact. This is even more critical for organisms with stage-structured life cycles. This is because the different developmental stages can potentially experience different environments (for example in insects where the larvae and adults have very different niches) or the same environmental factor can have different effects across multiple developmental stages (Payne, 1933). Unfortunately, the interaction of life-history and environment in shaping the dynamics of stage-structured populations is poorly understood. For example, despite a rich body of work on *Drosophila* population dynamics (Dey and Joshi, 2006; Mueller and Joshi, 2000; Nunney, 1983; Prout and McChesney, 1985; Rodriguez, 1989), it is still not clear if and how various life-history traits interact with nutritional availability at larval and adult stages in affecting its population stability (although see Nicholson, 1957; Mueller, 1988; Mueller and Huynh, 1994). Moreover, there is little theoretical or empirical understanding of how these nutritional regimes affect the various aspects of the population size distribution (mean, skewness and the position of the various quantiles). Apart from the obvious academic interest, such insights, wherever generalizable, can be invaluable for a better long-term management of already unstable populations.

It is unfortunate but self-evident fact that the above-mentioned kinds of population management are implementable only for a small number of species, both in terms of implementation and economic viability. What about the species that need to fend for themselves? In order to survive in the rapidly changing contemporary climate and landscape, organisms can either adapt to the new conditions or disperse to places with favorable environmental conditions. Since adapting to the new stressful environments can often be a slow process, dispersal is more likely to be the first line of defense, at least for those organisms that are capable of it. Moreover, by distributing offspring over different environmental conditions, dispersal acts as a useful bet hedging strategy to cope with spatio-temporal heterogeneity (Matthysen, 2012). Thus, not surprisingly, the evolution of dispersal and its consequences have been a major focus of research in evolutionary ecology for the last few decades (reviewed in Bowler and Benton, 2005; Clobert et al., 2012; Ronce, 2007). In terms of the process, dispersal is often subdivided into three stages – emigration from the natal habitat, inter-patch movement and immigration into the destination patch (Bowler and Benton, 2005). Although all these three stages are part of the same phenomenon, the

behavioural and physiological attributes necessary to tackle them can be very different. For example, emigration involves behavioural traits related to judging the natal habitat quality and complex decision-making, whereas traits related to physical stamina, endurance, etc. might be more important for the movement stage. Consequently, dispersal is a composite trait that consists of multiple components, like dispersal propensity (i.e. the fraction of dispersers leaving the current habitat), which is mostly related to the emigration stage, and dispersal ability (i.e. the mean distance travelled), which is mostly related to the movement stage. Thus, in the course of dispersal evolution, the component(s) of dispersal which will eventually evolve becomes contingent upon the nature of the selection pressure faced by each component, the costs associated with them, the manner these costs interact with each other and the ways they are countered by the organisms (Bonte et al., 2012). Therefore, evolution of a given component does not necessarily make an organism better in terms of another component. For example, in spider mites, artificial selection can increase dispersal propensity (Yano and Takafuji, 2002) but not dispersal ability (Bitume et al., 2011). In the same organism, when selection is imposed in the form of spatially correlated extinctions, the frequency of long-distance dispersers (LDDs) increases but dispersal propensity is reduced (Fronhofer et al., 2014). However, from these studies, it is not clear whether different dispersal components can evolve simultaneously or evolution of one actually imposes an intrinsic constraint over the evolution of other components. Also, despite several theoretical propositions, the way in which the evolutionary response to the individual components will interact to affect dispersal distance distribution or dispersal kernel remains unclear.

To complicate matters further, dispersal is interrelated with a number of other life-history and behavioural traits, either functionally or through cost/benefit relationships (Bonte et al., 2012; Clobert et al., 2009; Stevens et al., 2012). That is why, often certain suites of behavioural and life-history traits are found to be closely associated with more dispersive phenotype (reviewed in Clobert et al., 2009). This suite of traits associated with dispersal are called dispersal syndromes and have been investigated in some detail (Clobert et al., 2009; Stevens et al., 2014). For example, in terms of behaviour, dispersers typically exhibit greater exploratory tendencies (Cote et al., 2010; Korsten et al., 2013) and are more aggressive (Duckworth and Badyaev, 2007). Similarly, in terms of life-history, dispersers are often larger in size (Dingle et al., 1980) and have greater fecundity (Ebenhard, 1990). Although, dispersal syndromes are important to understand the genetic and demographic consequences of dispersal (Clobert et al., 2009; Ronce and Clobert, 2012), such trait correlations cannot

reliably predict which trait(s) will evolve as a correlated response to dispersal evolution. This is because dispersal evolution is also a function of the nature of the genetic variation at the disposal of the organism, including the underlying trade-off structures among the traits. This automatically calls for a detailed investigation of the evolutionary robustness of the dispersal syndromes (Ronce and Clobert, 2012).

In this context, another important issue is the underlying mechanism of dispersal evolution. Compared to the anatomical or physiological changes associated with dispersal traits (reviewed in Zera and Brisson, 2012), we have relatively lesser understanding regarding the causal molecular mechanisms. So far, two genes, Phosphoglucose isomerase (*Pgi*) (Niitepõld et al., 2009) and the cGMP-dependent protein kinase called foraging (*for*) gene (Osborne et al., 1997) have been shown to be related to dispersal in insects. In *C. elegans*, three genes, namely G-protein coupled receptors *npr-1* (de Bono and Bargmann, 1998), and *tyra-3* (Bendesky et al., 2011) and *rol-1* (Friedenberg, 2003) have also been shown to have a connection with dispersal phenotypes. Although these are valuable insights, it is not always clear whether these genes would be the ones whose frequencies would change during dispersal evolution (Saastamoinen et al., 2017; Turner et al., 2015). More critically, given the complexity and polygenic nature of dispersal (Zera and Brisson, 2012), it is natural to assume that in any given species, dispersal evolution will probably involve changes in a relatively large number of genes and metabolic pathways (Saastamoinen et al., 2017). Therefore, a promising approach would be to look at the changes at the level of the metabolome and correlate those with corresponding behavioural and life-history changes. Although this approach has been successfully used in the context of adaptation to various stresses (Sørensen et al., 2017) or circadian profiles of metabolites (Gogna et al., 2015), to the best of our knowledge, it has rarely been attempted in the context of dispersal evolution (although see De Roissart et al., 2016; Matsumura et al., 2016).

In this thesis, I investigate some of the above-mentioned issues.

Chapter 2 describes a theoretical comparison of the efficiencies of six different control methods in inducing population stability under four different life-history/environment combinations using a unifying framework. Here, I used biologically realistic simulations, incorporating noise in growth rates and carrying capacity, as well as the lattice effect, to study how these control methods alter the distributions of population sizes before and after the methods are applied in a generation. From this, I demonstrate how multiple aspects of

stability like extinction probabilities, fluctuations in population sizes and effective population size are affected by the six control methods.

Next, in Chapter 3 and 4, I present an empirical validation of the effects of four well-known control methods of the limiter family (Upper Limiter Control, Lower Limiter control, Both Limiter Control and Target-Oriented Control) in stabilizing extinction-prone, widely fluctuating populations of *Drosophila melanogaster*. Using the distributions of the population sizes, I derive a biologically intuitive understanding of how these methods affect five different aspects of *Drosophila* dynamics, namely constancy, persistence, effective population size, average population size and effort magnitude. In the process, I have also validated a number of existing theoretical predictions from the literature. Finally, I demonstrate that Ricker-based simulations, under biologically realistic assumptions, capture most of the empirical trends obtained here. Since the Ricker model is not *Drosophila*-specific and is applicable over a wide range of taxa (*inter alia*, bacteria (Ponciano et al., 2005), fungi (Ives et al., 2004), ciliates (Fryxell et al., 2005), insects (Dey and Joshi, 2006; Sheeba and Joshi, 1998) and fishes (Denney et al., 2002; Ricker, 1954)), these results indicate that the empirical insights obtained here are likely to be generalizable.

In Chapter 5, I present a novel Individual-Based model of *Drosophila* dynamics, which includes parameters that are common to the life history of several holometabolous insects. This model successfully captured most of the aspects of the dynamics observed in a previously reported 49-generation (~3 years) long population-dynamics experiment under four contrasting nutritional regimes. Upon validation of the model using these data, I investigated how the various parameters of the model, which represent different life-history attributes, interact with the nutritional environments to affect the dynamics of the populations. Next, I compared my results with those of the experimental and theoretical studies in various taxa including dipterans, crustaceans, fishes, birds and mammals, to understand which aspects of dynamics are generalizable. Further, I extend the model to investigate the interaction between the amount of food and an unequal number of males and females on the population dynamics. Specifically, I addressed the following three issues: the effects of various sex ratios, sex-specific culling and sterile-insect technique. These studies reveal that the interaction is quite complex and certain aspects of nutrition affect the dynamics more than others, which implies that it is important to take nutrition into account while modelling these phenomena.

In Chapter 6, I report the results of a selection experiment on laboratory populations of *Drosophila melanogaster*, using a setup analogous to increasing habitat- fragmentation over generations. I demonstrate that multiple components of dispersal can evolve rapidly and simultaneously, which can lead to the evolution of the dispersal kernel. I then show that, once evolved, these traits can express themselves even in the absence of any proximal cues, indicating that the individuals have become intrinsically more dispersive. In the process, I also empirically demonstrate sex-biased dispersal (SBD) in *D. melanogaster* and investigate the selection × sex interaction in this species.

Chapter 7 extends the study in the previous chapter and focuses on finding out the consequences of dispersal evolution in terms of three behavioural traits- locomotor activity, exploration and aggression, and three life-history traits- dry body weight, fecundity, and longevity. The observation of these phenotypic assays led to predictions about putative mechanisms that were confirmed through untargeted metabolomic fingerprinting using NMR spectroscopy with the help of a collaborator. Comparing the metabolome of the selected and the control flies, I found that the former had greater amounts of glucose, AMP, NAD and citric acid in their body, suggesting the evolution of an elevated level of cellular respiration. At the same time, the levels of neuropeptides that play a crucial role in the expression of the above-mentioned behavioural traits were found to be higher in the dispersal-selected flies.

In the final chapter, I summarize the salient results obtained in chapters 2-7 and discuss their academic and potential practical implications. I also mention how the results presented here can stimulate future empirical and theoretical investigations in the field. Some of the chapters in this thesis are extended/modified forms of already published (Chapters 2, 3, 4, 6) or submitted (Chapters 5, 7) manuscripts.

CHAPTER 2

A Comparison of Six Methods for Stabilizing Population Dynamics

Highlights

- Methods that involve culling promote persistence more than constancy stability.
- The converse is true for methods that involve only restocking steps.
- Efficacies of the methods depend upon growth rates and carrying capacities.
- Overall, restocking to a fixed lower threshold is the optimal control method.

Adapted from: **Tung, S.**, Mishra, A. and Dey, S. 2014. A comparison of six methods for stabilizing population dynamics. Journal of Theoretical Biology 356, 163-173.

1. INTRODUCTION

1.1 Background

Since the seminal work of Ott, Grebogy and Yorke (Ott et al., 1990), a large number of methods have been proposed to stabilize the dynamics of unstable non-linear systems (for reviews see (Andrievskii and Fradkov, 2003, 2004; Schöll and Schuster, 2008)). Many of these methods work by manipulating the parameters of the system in real time, such that the trajectory of the system can be stabilized to the desired kind of dynamics (stable point or cycles of appropriate periodicity). However, such methods are unsuitable for controlling real biological populations in which the precise equations governing the system are typically unknown and parameters (e.g. intrinsic growth rate, carrying capacity etc.) can only be estimated *a posteriori*, through model-fitting. Control of biological populations is more easily achieved through methods that stabilize the dynamics through perturbations to the state variable, (i.e. the population size) and require relatively less system-specific information. Over the last two decades, many such methods have been proposed (Corron et al., 2000; Dattani et al., 2011; Hilker and Westerhoff, 2005, 2007; McCallum, 1992; Sah et al., 2013) and at least a few of them have also been empirically verified (Becks and Arndt, 2008; Desharnais et al., 2001; Dey and Joshi, 2007; Sah et al., 2013).

This proliferation of biologically relevant control methods has created some interesting problems of its own. In ecology, there are multiple notions about the concept of stability (Grimm and Wissel, 1997) and ideally one would not like to opt for a method that enhances one kind of stability (say reduction in fluctuation in population size) at the cost of another (say long term persistence). However, studies on control methods often focus on enhancement of only one type of stability, without investigating how other aspects of the dynamics get affected (e.g. Corron et al., 2000; Güémez and Matías, 1993; McCallum, 1992). Recent empirical studies indicate that induction of one kind of stability may (Sah et al., 2013) or may not (Dey et al., 2008) translate into the enhancement of other kinds of stability. Therefore it is important to investigate how different control methods affect multiple kinds of stability simultaneously.

Such comparisons can be quite complex as most theoretical studies employ different models of population growth and evaluate the efficacies of the control methods in different parameter ranges, some of which can even be biologically unrealistic. Thus, for meaningful comparison, these methods need to be investigated under common conditions, i.e. for the same model and similar levels of enhancement of stability. Moreover, since it has been empirically shown that

the effects of perturbation can vary depending on the intrinsic growth rates or the environment of the population (e.g. Dey and Joshi, 2013), it is conceivable that the efficacy of control methods can also be affected by these factors. Thus, any comparison of the control methods also needs to take into account multiple combinations of intrinsic growth rate and carrying capacity values. Finally, any real world scenario typically involves an economic component (Hilker and Westerhoff, 2005), which might play a significant role in deciding which control method is best suited to a given scenario. Our study aims to compare the performance of six well-known control methods in population dynamics under the above-mentioned set of conditions.

Here, owing to logistic constraints, we restrict our analyses to six control methods which were selected based on two criteria. Our primary selection criterion was the relative ease with which the methods could be implemented in real, biological populations. This ruled out some of the well-known, empirically verified control methods that require extensive knowledge of the equations governing the system and the corresponding parameter values (Becks et al., 2005; Desharnais et al., 2001). Our second criterion was the extent of information already available about the control methods in the population dynamics literature. Barring one (Both Limiter Control, see section 1.2), for which we found no prior reference in the literature, all the methods that we chose have been extensively investigated both analytically and numerically, and have been shown to be robust to at least some degree of noise. We realize that there might be other control methods that fit these two criteria and therefore do not claim that our coverage is comprehensive.

1.2 Description of six control methods

The mathematical expressions for the six control methods and the corresponding ranges investigated in the exploratory analysis are given in Table 2.1. Here we present a brief description of how these methods stabilize population dynamics. Among the six, constant pinning (CP), also referred to in the literature as constant immigration / feedback, is perhaps the most well studied (McCallum, 1992; Sinha and Parthasarathy, 1995; Solé et al., 1999) and involves the influx of a constant number of individuals (from some external source) into the population in every generation. In its general form, CP involves both immigration and emigration from a population (Sinha and Parthasarathy, 1995), but here we concentrate solely on immigration which has been shown to enhance stability for populations governed by the Ricker (Ricker, 1954) dynamics (McCallum, 1992; Stone, 1993). The reason for this is best understood graphically. For models that have single-humped first-return maps (also known as

the stock-recruitment curve) with at most one inflection point to the right of the maximum, the nature of the dynamics depends upon how negative the slope of the first-return map is at the point where it intersects the 45° line. Since constant immigration shifts the entire return map upwards (see Fig 2 of Stone and Hart, 1999), the slope at this point is reduced, which can convert chaotic dynamics into periodic oscillations or even stable points, depending upon the magnitude of the reduction (Sinha and Parthasarathy, 1995). For those models, such as the logistic, where moving up the first-return map increases the slope at the intersection point with the 45° line, CP destabilizes the dynamics by making it more complex (Sinha and Parthasarathy, 1995). Biologically, CP creates a "floor" and does not allow the population to hit values below the constant immigration threshold. This method has been empirically demonstrated to reduce fluctuations in sizes for spatially-unstructured (Dey and Joshi, 2013) but not spatially structured populations (Dey and Joshi, 2007).

Table 2.1. Details of the six control methods compared in this study*

Sl. No.	Control Method	Mathematical expression	Control parameter constants	Control parameter range(s) for Fig 2.2- 2.7	Step size
1.	Constant Pinning (CP)	$a_t = b_t + p$	Pin (p)	1 to k-1	1
2.	Lower Limiter Control (LLC)	$a_t = max\ [b_t\ ,\ h]$	Lower limit (h)	1 to k-1	1
3.	Adaptive Limiter Control (ALC)	$a_t = max\ [b_t\ ,\ c \times a_{t-1}]$	ALC intensity (c)	0.05 to 0.95	0.05
4.	Upper Limiter Control (ULC)	$a_t = min\ [b_t\ ,\ H]$	Upper limit (H)	k+1 to 3k	1
5.	Both Limiter Control (BLC)	$a_t = max\ [h,\ min[\ b_t\ ,\ H]]$	Lower limit (h)	1 to k-1	1
			Upper limit (H)	k+1 to 3k	1
6.	Target Oriented Control (TOC)	$a_t = max\ [0,\ c_d \times T + (1 - c_d) \times b_t)]$	Target, T	k	NA
			c_d	0.05 to 0.95	0.05

* b_t and a_t are the population sizes before and after perturbation in the t^{th} generation, such that b_{t+1} = FUNC(a_t), where FUNC stands for the population recruitment function (Ricker model, in this study). For BLC, $H > h$. NA denotes not applicable.

One of the issues with constant pinning is that the population sizes are augmented even when they are not low. This problem is avoided with the so called hard 'limiter control from below' (Hilker and Westerhoff, 2005), or Lower Limiter Control (LLC) in this study, which prescribes that each time the population size falls below a pre-determined lower threshold, it is brought back to that value through restocking. Graphically, LLC truncates some part of the lower end of the return map, which in turn makes part of the upper end unavailable to the system (Hilker and Westerhoff, 2005). This constrains the range of values that the system can take, finally leading to stabilization of the dynamics (Hilker and Westerhoff, 2005, 2006; Wagner and Stoop, 2000). A similar truncation of the return map can also be obtained by bringing the population size back to a given upper threshold (through culling) every time it crosses the threshold (Middleton et al., 1995). This is the control strategy known as hard 'limiter control from above' (Hilker and Westerhoff, 2005) and referred to as Upper Limiter Control (ULC) in this study. A logical extension of the LLC and the ULC scheme is to combine both and bring the population to a lower or a upper threshold each time the size goes respectively below or above those limits. This is what we term as Both Limiter Control (BLC), a scheme that has not been proposed earlier to the best of our knowledge.

One of the drawbacks of all the four methods described above is that the value of the control parameter (i.e. the various thresholds, or the fixed number of immigrants for CP) is hard set *a priori*, as a result of which, the methods are unable to adjust when there are underlying increasing or decreasing trends in the time series. This problem is alleviated in a class of control methods that set the magnitude of the perturbation as a function of the population size (Güémez and Matías, 1993; Pyragas, 1992), leading to methods such as proportional feedback control (Carmona and Franco, 2011). In these methods, although the proportion of the feedback is set *a priori*, the magnitude of the change (i.e. number of individuals added or removed) is dependent on the actual population sizes. We decided to investigate one such recently proposed method called Adaptive Limiter Control or ALC which has been theoretically and empirically demonstrated to reduce the fluctuation in size of both spatially structured and un-structured populations (Sah et al., 2013). Unlike the previous methods, ALC is not capable of turning a chaotic trajectory into a stable point equilibrium, but "traps" the dynamics into a region around the carrying capacity (Franco and Hilker, 2013). The range of this trapping region is determined by the growth rate, the carrying capacity and the value of the controlling parameter (Franco and Hilker, 2013) and the nature of the dynamics can be either periodic cycles or chaos (Sah et al., 2013). For situations where it is desirable to guide

the system towards a particular value of the state variable rather than a range of values, the so-called Target-Oriented Control or TOC (Dattani et al., 2011) seems to be of greater use. In this method, the system is guided towards a particular target value (set *a priori* based on whatever is the desired usage) by introducing individuals whenever the population size is below the target and removing individuals when the population size is above the target. The number of individuals to be added or removed is proportional to the difference between the target and the current value of the population size. It has been analytically shown that for high enough values of this proportion, TOC will always lead the system to a positive stable point (Franco and Liz, 2013). In this study we fixed the target at carrying capacity, which is expected to minimize the magnitude of interventions for attaining a given level of stability (Dattani et al., 2011).

In this study, we compare the efficiency of these six methods in inducing a common level (50%) of reduction in either fluctuation in population sizes or extinction frequencies under four different life history/ environment combinations. Since these control methods have been extensively investigated (analytically and numerically) in the literature, we focus on an intuitive understanding of how these methods change the distributions of population sizes over time, thereby affecting fluctuations, extinctions, effective population sizes and the amount of perturbation required to attain a defined stability goal. We use Ricker model–based, biologically realistic simulations incorporating parameter noise, stochastic extinctions and lattice effect (*sensu* Henson et al., 2001). We show that for these six methods, even when the degree of stability attained is similar, the resulting population size distributions can be very different. Consequently, for a given degree of stability attained, the correlated features of dynamics (e.g. extinction probability, ability to resist genetic drift etc.) vary widely among the methods. The magnitude of perturbation needed to attain similar levels of stability was also different for these methods, which is likely to have economic consequences. Finally, we computed a composite index of various stability related measures to compare the relative efficiency of these methods. The major insight from our study is that there is no single method that is optimal under all circumstances. The performances of the methods are likely to depend upon which aspect of the dynamics is being controlled and what are the life-history / environment of the habitat. However, under many conditions, Lower Limiter Control, i.e. restocking to a constant lower threshold, seems to be an optimal strategy.

2. METHODS

2.1 Population growth model

We used the well-studied Ricker model (Ricker, 1954) for representing the population dynamics. This map is given as $N_{t+1}=N_t*exp(r*(1-N_t/K))$, where r, K and N_t denote intrinsic growth rate, carrying capacity and population size at time t, respectively. Due to its simplicity and lack of species specific features, this model has been extensively investigated theoretically (May and Oster, 1976). First principle derivation suggests that populations exhibiting scramble competition and random spatial distribution should exhibit Ricker dynamics (Brännström and Sumpter, 2005). Since populations of several species are expected to exhibit these properties, the model has been widely used to describe the dynamics of, *inter alia*, bacteria (Ponciano et al., 2005), fungi (Ives et al., 2004), ciliates (Fryxell et al., 2005), insects (Dey and Joshi, 2006; Sheeba and Joshi, 1998) and fishes (Denney et al., 2002; Ricker, 1954). Therefore, simulation results obtained here from this model are expected to be broadly applicable across a large number of taxa.

2.2 Life history / environmental regimes

The aim of this study was to compare the efficacy of various ecologically meaningful control algorithms under biologically relevant common conditions. However, the intrinsic growth rates and carrying capacities of species under different environments vary widely. Thus, it is conceivable that control algorithms might differ in terms of their efficacy and/or cost of implementation under various environments. Since it is not possible to represent every growth rate-carrying capacity combination, we arbitrarily chose two levels of intrinsic growth rate r (Low = 2.8 and High = 4.0) crossed with two levels of carrying capacity K (Low = 60 and High = 300). Thus, we investigate four combinations HrHk, HrLk, LrHk and LrLk, where HrHk denotes a combination of r = 4.0 and K = 300 and so on. Since we aimed to study the stabilizing effects of the control methods, we explicitly chose r-values that led to stochastic extinctions and large amplitude oscillations in population sizes in the unperturbed cases. The value of r = 2.8 is representative of the growth rates of some laboratory insect populations (Dey and Joshi, 2006; Sheeba and Joshi, 1998) whereas the value of r = 4.0 is well within the limits of Ricker growth rates estimated from natural populations of fishes (Denney et al., 2002). In the context of the Ricker model, r = 2.8 represents a value just after the onset of chaotic dynamics (which happens at r = 2.697) while r = 4.0 represents highly chaotic dynamics (May and Oster, 1976). The value of Lk (= 60) was chosen to be similar to

a previous analysis (Sah et al., 2013) while Hk was arbitrarily chosen as 5 times Lk. Thus, the four regimes represented dynamics that seem well within the ranges of real biological populations in the laboratory and nature. In all cases, the initial population size (N_0) was taken to be 20.

2.3 Transients and lattice effect

Most studies in the control literature tend to investigate the dynamics of the system under steady states. However, many factors that determine the dynamics of a population (e.g. the environment of its habitat or the distributions of various life-history traits) are unlikely to remain constant for long (Hastings, 2004). Moreover, due to the time scales involved, most available ecological time series are short and, therefore, unsuitable for checking the predictions on steady-state dynamics. Therefore, following earlier work (Franco and Hilker, 2013; Sah et al., 2013) we explicitly concentrated on the transient dynamics by restricting our simulations to the first 50 iterations. Moreover, we rounded off the number of organisms and the magnitude of the perturbations to the nearest integer values. This accounted for the fact that real organisms always come in integer numbers (lattice effect; Henson et al., 2001), which is known to significantly affect the dynamics in simulation studies (Domokos and Scheuring, 2004).

2.4 Extinctions and resets

In the absence of lattice effect, a Ricker-generated time-series can never take a zero value (i.e. become extinct) when initiated with non-zero population sizes. However, in simulations incorporating the lattice-effect, the Ricker dynamics permits extinctions whenever the population size goes below 0.5. We call this kind of extinction as Lattice Effect Extinction (LEE) which happens when $INT[FUNC(N_{t-1})] = 0$, where INT is a function rounding off the population size to the nearest integer and FUNC represents the population recursion function (here the Ricker model). Following previous empirical studies (Dey and Joshi, 2006; Dey and Joshi, 2013; Sah et al., 2013), we also incorporated extinction due to demographic stochasticity (EDS) in the form of a 50% probability of extinction whenever the population size went below four. Mathematically, this can be represented as $P(N_t = 0 \mid N_t' <= 4)) = 0.5$ where N_t denotes the population size in generation t after the extinction step and N_t' denotes the population size in generation t immediately after the application of the Ricker model on N_{t-1} (i.e. $FUNC(N_{t-1})$). Biologically, EDS occurs due to chance realizations of probabilistic events in a population, e.g. when all the members of a population are of the same sex, or are infertile, or fail to reach adulthood (Hunter and Gibbs, 2007). Prior simulations using this

level of EDS have been seen to give good fits to trends from experimental time series of laboratory populations of *Drosophila melanogaster* (Dey and Joshi, 2006; Sah et al., 2013). For unperturbed populations and those controlled by ULC, the population size was reset to a value of eight after an extinction event (Dey and Joshi, 2006; Sah et al., 2013). In the case of all other control methods, the extinct populations were automatically reset by the respective control schemes.

For representational purposes, we scaled the extinction probability for all control methods in a given regime by the average (over 50 replicates, see below) extinction probability of the unperturbed population in that regime. This allowed us to directly compare the efficacies of the methods across regimes.

2.5 Stochasticity and replication

Since noise is known to have a major impact on the dynamics of perturbed populations (Dey and Joshi, 2007), we incorporated noise in both r and K in each iteration of the simulations. Thus, the stochastic intrinsic growth rate was given as $r + \varepsilon$ (where $\varepsilon \in U[-0.2, 0.2]$) and the stochastic carrying capacity as λK (where $\lambda \in U[0.9, 1.1]$). All simulations were repeated 50 times and the corresponding mean values (of FI, effort magnitude etc.) and standard errors around the mean are reported here. The small error bars indicated that 50 replicates were enough for the purpose of our study. However, we repeated a subset of our simulations with 1000 replicates and found no qualitative difference with our results (data not shown).
Thus, the complete sequence of steps in the simulation is given as:

$$a_{t-1} \rightarrow [\text{FUNC}] \rightarrow [\text{INT}] \rightarrow [\text{EXT}] \rightarrow b_t \rightarrow [\text{CTRL}] \rightarrow a_t$$

where a_t and b_t are the population sizes after and before application of control in the t^{th} generation, and FUNC, INT, EXT and CTRL stand for the population recursion (here Ricker model), integerization, stochastic extinction and control (here CP, LLC etc.) functions respectively. Henceforth, the time series of b_t and a_t are referred to as the pre- and post-perturbation time series respectively.

2.6 Effort magnitude and Effective Population Size (N_e)

The cost of implementation of the six control methods was quantified as the effort magnitude, defined as the average number of individuals added or removed per generation from the population in order to attain the desired control level (Hilker and Westerhoff, 2005). It can be computed as:

$$\left(1/\left(T\times\overline{N}\right)\right)\times\sum_{t=1}^{T}\left|b_t-a_t\right|,$$

where b_t and a_t are the population sizes before and after perturbation in the t^{th} generation and $\overline{N}$ and T denote the average population size and total length of the simulation time series respectively (Sah et al. 2013). Following previous studies (Dey and Joshi, 2007; Sah et al., 2013), $\overline{N}$ was computed on the time series of a_t values. Note that effort magnitude defined this way is dimensionless, being a fraction of the corresponding mean population size, thus permitting direct comparisons across different regimes. Following earlier work (Hilker and Westerhoff, 2005; Sah et al., 2013), we assumed that lower values of effort mean less expense and therefore are economically more viable.

We quantified Effective population Size (N_e) as the harmonic mean of the post-perturbation time series (Allendorf and Luikart, 2007):

$$N_e = T\Big/\sum_{t=1}^{T}1/N_t$$

where, N_t is the population size in generation t and T is the length of the time series. In any fluctuating population, the loss of genetic diversity is accelerated each time the population size hits low numbers but is relatively unaffected by high numbers. Due to this asymmetry, average population size, which puts equal weight on population troughs and peaks, is a poor indicator of the loss of genetic diversity. On the other hand, N_e gets more adversely affected by small population sizes, and hence is a better index for the rate of loss of genetic diversity. For representational purposes, we scaled the N_e for all control methods in a given regime by the average N_e of the unperturbed population in that regime. This allowed us to directly compare the efficacies of the methods across regimes.

2.7 Measures of stability

Populations with relatively large amplitude of fluctuations in population size are considered to have lower 'constancy' stability and vice versa (Grimm and Wissel, 1997). The constancy stability of the simulated time series was quantified using the Fluctuation index, FI (Dey and Joshi, 2006) which represents the average one-step fluctuation in population size, scaled by the population mean:

$$FI=\left(1/\left(T\times\overline{N}\right)\right)\times\sum_{t=0}^{T-1}\left|N_{t+1}-N_t\right|$$

where, $\overline{N}$ is the average population size, N_t is the population size in generation t and T is the length of the time series. Persistence stability is the converse of the extinction probability of a population (Grimm and Wissel, 1997). Mathematically, extinction probability = E/T; where E represents the total number of extinction events during T iterations.

2.8 Composite Performance Score

One of the aims of this study was to compare the overall performance of the six control methods against each other. Therefore we devised a composite performance score which combined the relative performance of each control method w.r.t FI, extinction probability (EP), effort magnitude (EM) and effective population size (N_e). Since the ranges of values taken by these quantities are very different, we normalized each quantity in each regime, by the highest value in that regime. So for example, in the HrLk regime, since ULC had the highest effort magnitude, the corresponding values for all the methods were divided by the effort magnitude of ULC. This normalizes all values to a scale of 0 to 1, with 0 being the best performance and 1 being the worst. This works well for fluctuation index, extinction probability and effort magnitude where lower values are more desirable. Since higher values of N_e are more preferable, we subtracted the normalized N_e values from 1. The composite performance score for each method in a given regime was simply the sum of the three normalized scores, which implies equal weightage to each score. In other words, for 50% reduction in FI, the performance score is given as $EM' + EP' + (1- N_e')$ and for 50% reduction in extinction probability, the corresponding expression is $EM' + FI' + (1- N_e')$, where prime denotes the normalization operation. Clearly lower values of this score for a given method in a particular scenario indicate better performance than higher values.

3. RESULTS AND DISCUSSION

3.1 Dynamics of unperturbed populations

We first explored the dynamics of the four regimes in the absence of any perturbation. The two Hr regimes (i.e. HrHk and HrLk) showed higher FI (Fig 2.1A) and greater extinction probability (Fig 2.1B) compared to the corresponding Lr regimes. This difference between Hr and Lr is not surprising in terms of constancy, as a higher value of r implies greater amplitude of fluctuation in the time series and hence a greater FI. A larger FI is also expected to increase the frequency with which a population crashes to very low values, and thus reduce

persistence. Interestingly, although HrLk had the same *r* and a marginally higher FI than HrHk (Fig 2.1A), the corresponding extinction probability was approximately double (Fig 2.1B). This is intuitive since increasing the habitat capacity of a population (which is analogous to *k* here) is expected to increase the time to extinction (Grimm and Wissel, 2004). This also highlights the point that constancy and persistence of a population need not necessarily have a simple relationship and it is risky to try and extrapolate one from the other, an observation that seems to have a fair bit of empirical support of late (Dey and Joshi, 2013; Dey et al., 2008; Sah et al., 2013).

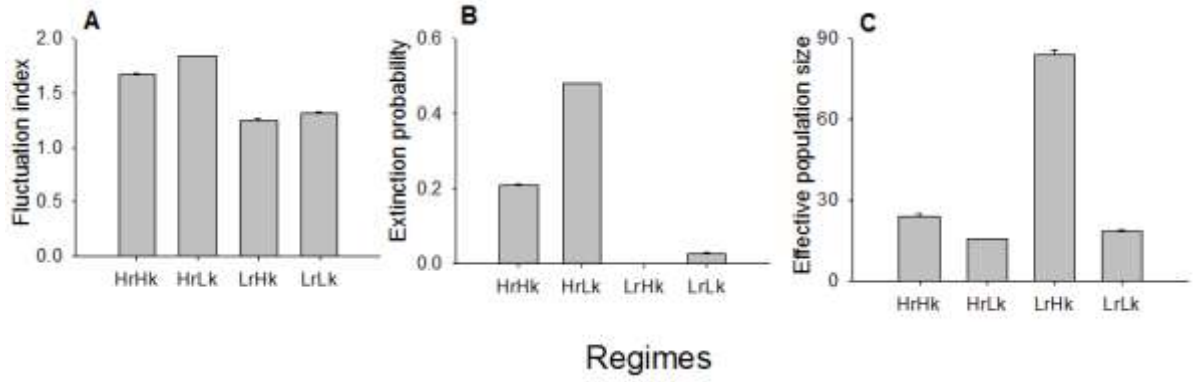

Figure 2.1. Dynamics of unperturbed populations in four regimes. (A) Average fluctuation index, (B) average extinction probability, and (C) average effective population size of the unperturbed populations calculated over 50 replicate runs for each of the four regimes. In this and all subsequent figures, HrHk stands for High *r* (= 4.0) and High *k* (= 300), LrLk denotes a combination of Low *r* (= 2.8) and Low *k* (= 60), and so on for the other two regimes. Error bars represent standard error of the mean (SEM). The two Hr regimes had higher FI and extinction probability compared to the two Lk regimes. Although the effective population sizes of the two Hr regimes were comparable to that of the LrLk regime, this was an artifact of the simulation protocol (see section 3.1 for more details). Some of the error bars are too small to be visible.

Another important factor that increases the chance of population extinction is the erosion of genetic diversity through genetic drift. Although the importance of drift is well recognized in evolutionary (Wright, 1931) and conservation biology literature (Hare et al., 2011), most studies in population control have ignored the effects of a control method on genetic drift. Here we investigate this phenomenon by studying the effective population size N_e (Fig 2.1C), which is defined as the corresponding size of an ideal population that has an equivalent rate of loss of heterozygosity as the population under study (Allendorf and Luikart, 2007). The rate of loss of heterozygosity increases with decrease in population sizes or bottlenecks (Gillespie, 1998). More interestingly, even the rate of generation of genetic variation, as measured by the average mutation rate, scales negatively with N_e among the major

phylogenetic groupings (Lynch, 2010). This suggests that higher values of N_e are more desirable since they are expected to cause lesser loss of genetic variation. When the N_e of a series is measured as the corresponding harmonic mean, it tends to get much more negatively affected by the presence of low values than the corresponding arithmetic mean. This suggests that the two Hr regimes (HrHk and HrLk) are expected to have much lower values of N_e compared to the two Lr regimes. However, the N_e of HrHk and HrLk were found to be roughly similar to that of LrLk. This discrepancy is explained by the fact that both Hr regimes were marked by large number of extinctions (Fig 2.1B) which were followed by resets to a value of eight (see section 2.4). Consequently, all the zero values in the population sizes in these two regimes were replaced by eight, which significantly increased the N_e. We verified this line of reasoning by simulating the unmodified Ricker model (i.e. in the absence of lattice effect, noise or extinction) and found that the N_e under HrHk and HrLk regimes were actually orders of magnitude less compared to the two Lr regimes. Therefore, the high values of N_e in the two Hr regimes should be considered an artefact of the extinction-reset protocol.

Thus, even though the dynamics of all four regimes are chaotic, we find important differences in their properties in the absence of any perturbation. We next investigated how the six different control methods affected these properties.

3.2 Exploratory Analysis

Literature survey indicated that given high enough values of the corresponding control parameter, most of the methods are expected to turn chaotic dynamics into stable points or low amplitude periodic oscillations. However, the relative efficiencies of these methods are hard to compare since they have typically been studied in the context of different kinds of stability properties. For example, TOC has been primarily investigated in the context of ameliorating chaotic dynamics to a stable point (Dattani et al., 2011), whereas ALC does not ameliorate chaos to begin with (Franco and Hilker, 2013; Sah et al., 2013). Therefore in order to make the comparisons meaningful, we began by exploring the efficacy of all six methods in enhancing the constancy and persistence stability over a wide but biologically / realistically meaningful control parameter range (Fig 2.2-2.7). Here, we stress upon the meaningfulness of the parameter ranges, since some times the methods perform the best under parameter values that are realistically unfeasible or biologically undesirable. For example, for CP, we only considered number of immigrants up to *k*, since we feel that

perturbation sizes greater than the carrying capacity are impractical. Similarly, for cases where the population size is not allowed to go below a fixed threshold (Lower Limiter Control or LLC), we only considered values less than the carrying capacity, since setting a lower threshold above *k* is biologically unrealistic.

From Fig 2.2-2.7, it is clear that although there were differences in terms of their performance, all six methods were able to reduce the fluctuation index under all four regimes. To create a common platform for comparison, we extracted the values of the control parameters from Fig 2.2-2.7 that lead to a 50% reduction in Fluctuation Index (FI) and Extinction Probability w.r.t the unperturbed dynamics as in Fig 2.1. For those methods where the desired level of stability (constancy or persistence) is attained for multiple control parameter values, we have arbitrarily chosen the one which had the lowest effort magnitude. Table 2.2 summarizes these values of control parameters. We then compared these six methods in terms of their effort magnitude, resulting effective population size (N_e) and the corresponding extinction probability or fluctuation index. Finally, we combined the performance of all these methods to come up with a common score that would help us to choose one method over another in a given scenario. It should be noted here that we do not seek to establish mathematically rigorous results on how these methods actually stabilize the dynamics. We have explicitly chosen methods for which such information already exists (see section 1.2 for the relevant references). The current study aims to create an intuitive understanding of how the different control methods alter the distributions of pre- and post-perturbation population sizes. The reasons for focussing on population size distributions are two-fold. Firstly, although it has been shown that in response to some control methods, the pre-and post-perturbation population sizes can have quantitatively (Franco and Liz, 2013) or qualitatively (Franco and Hilker, 2007) different dynamics, the phenomenon has not been explored extensively**.** Secondly, as we demonstrate here, various aspects of population dynamics like effort magnitude and the probability of extinction can be ultimately thought of as an interaction between the pre-and post- perturbation population sizes.

Table 2.2. Parameter values of the six control methods leading to 50% enhancement of stability*

	Regimes	CP	LLC	ALC	ULC	BLC		TOC	
		p	h	c	H	*h*	*H*	T	c_d
50% reduction in Fluctuation Index	HrHk	266	236	0.47	367	193	468	300	0.6
	HrLk	52	46	0.42	75	34	93	60	0.59
	LrHk	225	229	0.49	373	225	478	300	0.4
	LrLk	47	46	0.47	75	45	104	60	0.4
50% reduction in Extinction Probability	HrHk	264	222	0.52	751	15	753	300	0.51
	HrLk	54	44	0.44	141	26	126	60	0.54

*Note that these values represent the thresholds of population sizes / values of control parameters set for attaining 50% reduction in FI or extinction probability, and not the number of organisms to be added or subtracted. In case of BLC, since multiple parameter combinations satisfied the criteria of 50% reduction in FI and extinction probability, we chose the combination with the lowest effort magnitude.

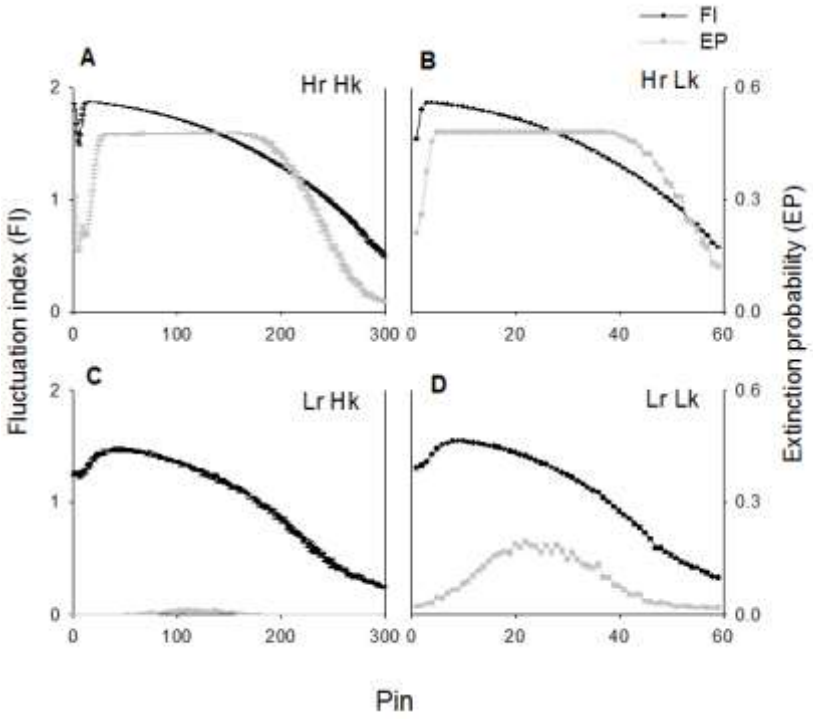

Figure 2.2: Average fluctuation index (± SE; black) and extinction probability (± SE; grey) for different levels of immigration under the Constant Pinning (CP) method. A, B, C and D represent HrHk, HrLk, LrHk, and LrLk regimes respectively. Error bar at each point is too small to be visible clearly.

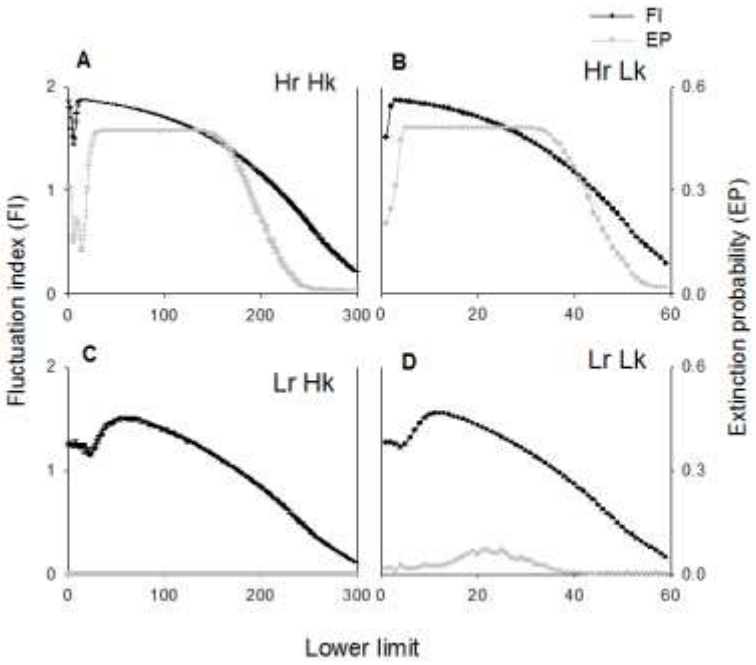

Figure **2.3**: Average fluctuation index (± SE; black) and extinction probability (± SE; grey) for different levels of lower threshold under the Lower Limiter Control Method (LLC). A, B, C and D represent HrHk, HrLk, LrHk, and LrLk regimes respectively. Error bar at each point is too small to be visible clearly.

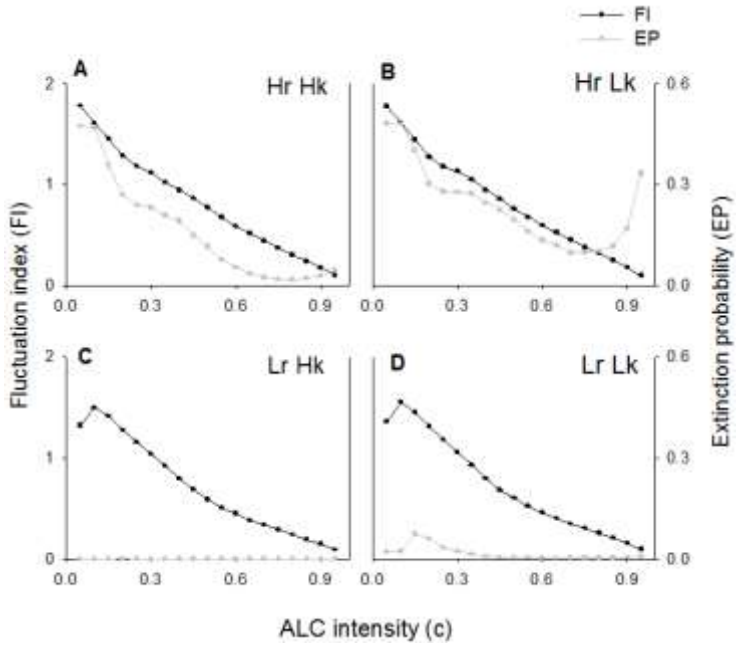

Figure **2.4**: Average fluctuation index (± SE; black) and extinction probability (± SE; grey) for different levels of ALC intensity (*c*) under the Adaptive Limiter Control Method (ALC). A, B, C and D represent HrHk, HrLk, LrHk, and LrLk regimes respectively. Error bar at each point is too small to be visible clearly.

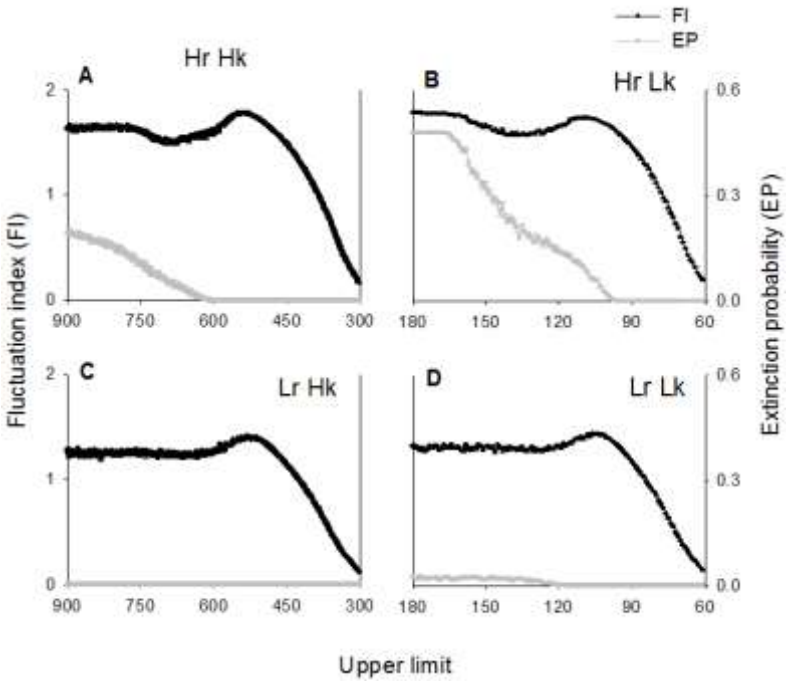

Figure **2.5**: Average fluctuation index (± SE; black) and extinction probability (± SE; grey) for different levels of upper threshold under the Upper Limiter Control Method (ULC). A, B, C and D represent HrHk, HrLk, LrHk, and LrLk regimes respectively. Error bar at each point is too small to be visible clearly.

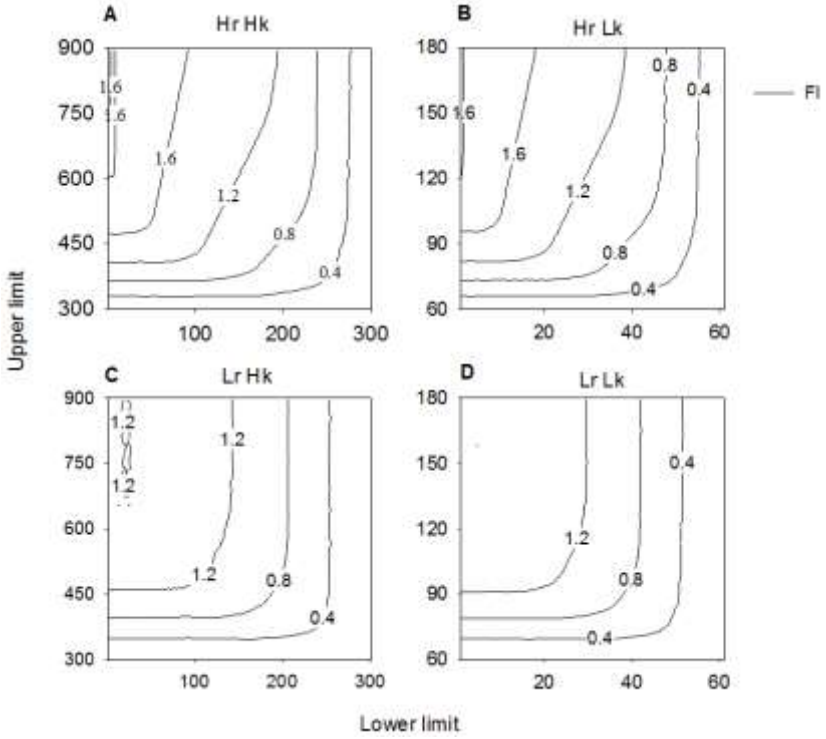

Figure 2.6.1: Average fluctuation index for different combinations of upper and lower threshold under the Both Limiter Control Method (BLC). A, B, C and D represent HrHk, HrLk, LrHk, and LrLk regimes respectively.

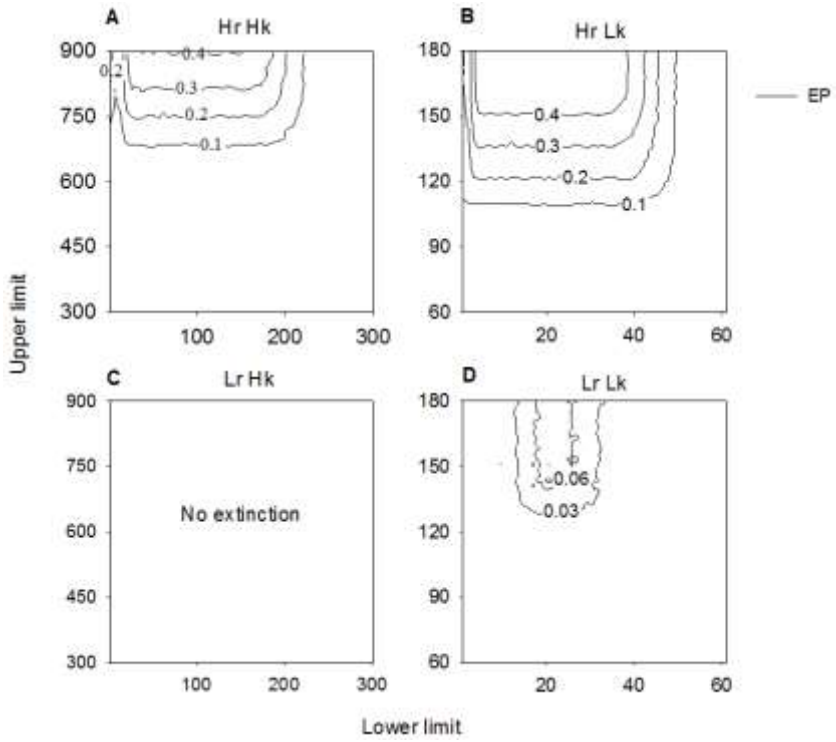

Figure 2.6.2: Average extinction probability for different combinations of upper and lower threshold under the Both Limiter Control Method (BLC). A, B, C and D represent HrHk, HrLk, LrHk, and LrLk regimes respectively.

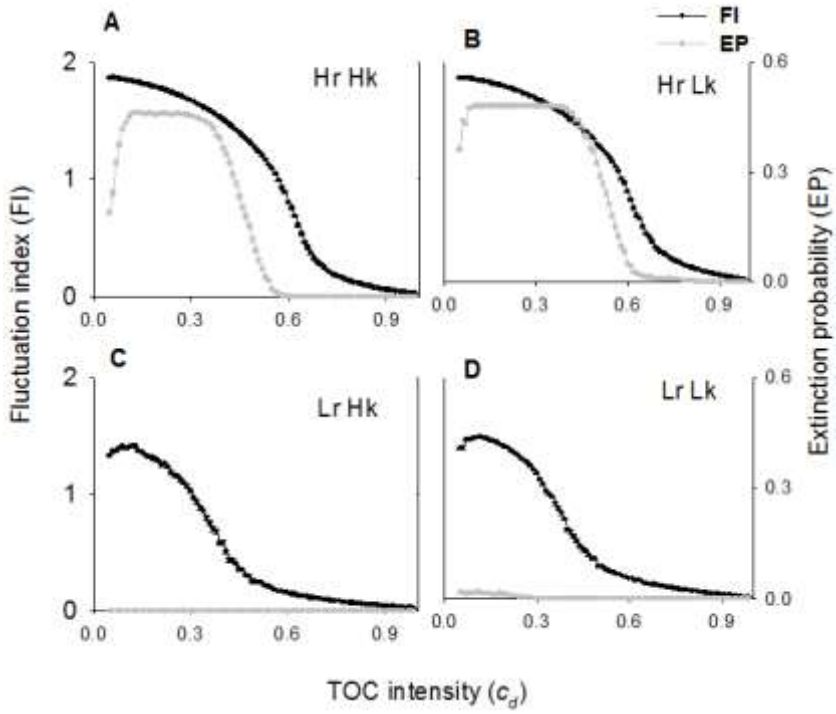

Figure 2.7: Average fluctuation index (± SE; black) and extinction probability (± SE; grey) for different levels of TOC intensity (c_d) under the Target Oriented Control Method (TOC). A, B, C and D represent HrHk, HrLk, LrHk, and LrLk regimes respectively. Error bar at each point is too small to be visible clearly.

3.3 50% reduction in Fluctuation Index (FI)

3.3.1 50% reduction in FI: Persistence stability

Although extinction has been extensively studied theoretically and empirically (Griffen and Drake, 2008 and references therein), few studies on control methods have explicitly considered enhancement of persistence (although see Dey and Joshi, 2013; Hilker and Westerhoff, 2007; Sah et al., 2013). This is perhaps because many studies on controlling single species biological populations come from the tradition of chaos control in non-linear dynamics, and primarily focus on the attainment of simpler dynamics (Dattani et al., 2011; McCallum, 1992; Sinha and Parthasarathy, 1995) or reducing the variation in population size, i.e. constancy (Hilker and Westerhoff, 2005). Therefore, we first investigated how achieving a given level of constancy affects the corresponding persistence stability.

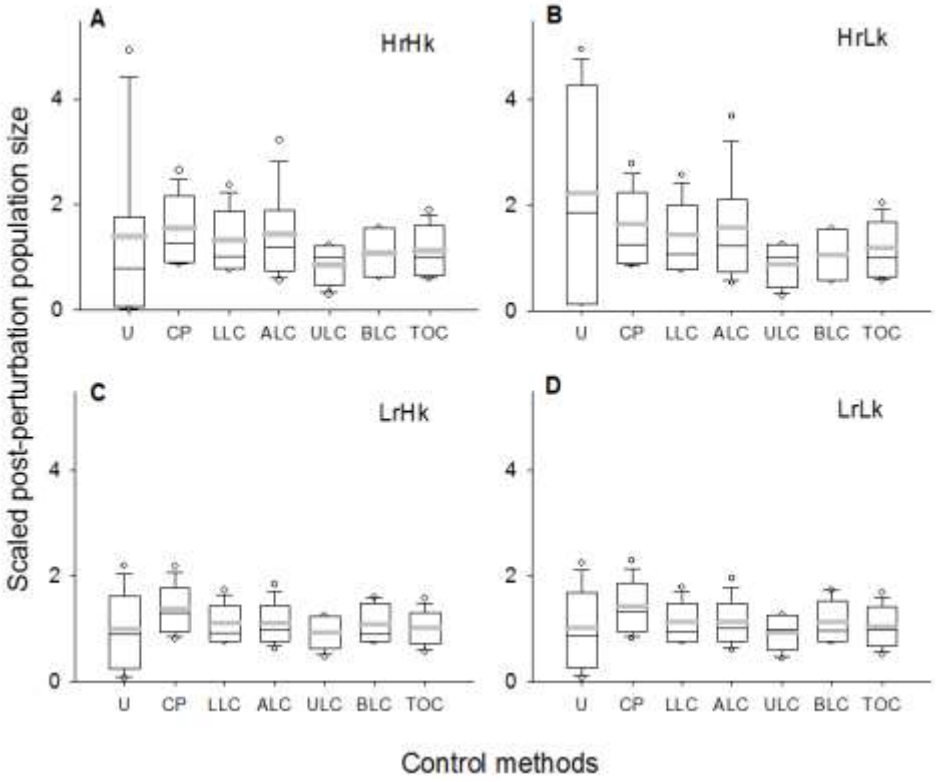

Figure 2.8. **Box-plots of the post-perturbation population size distributions for 50% reduction in Fluctuation Index**. A, B, C, and D correspond to the HrHk, HrLk, LrHk and LrLk regimes respectively. Thick grey lines = means, thin black lines in the box = medians. Lower and upper limits of the box represent 25th and 75th percentiles, lower and upper whiskers denote 10th and 90th percentiles while the lower and upper dots stand for 5th and 95th percentiles. U stands for the unperturbed population while the other abbreviations denote the six methods. All the values have been scaled by the corresponding carrying capacity (k) of the regime to facilitate comparison. ULC always had the lowest post-perturbation population size, since it did not involve any restocking.

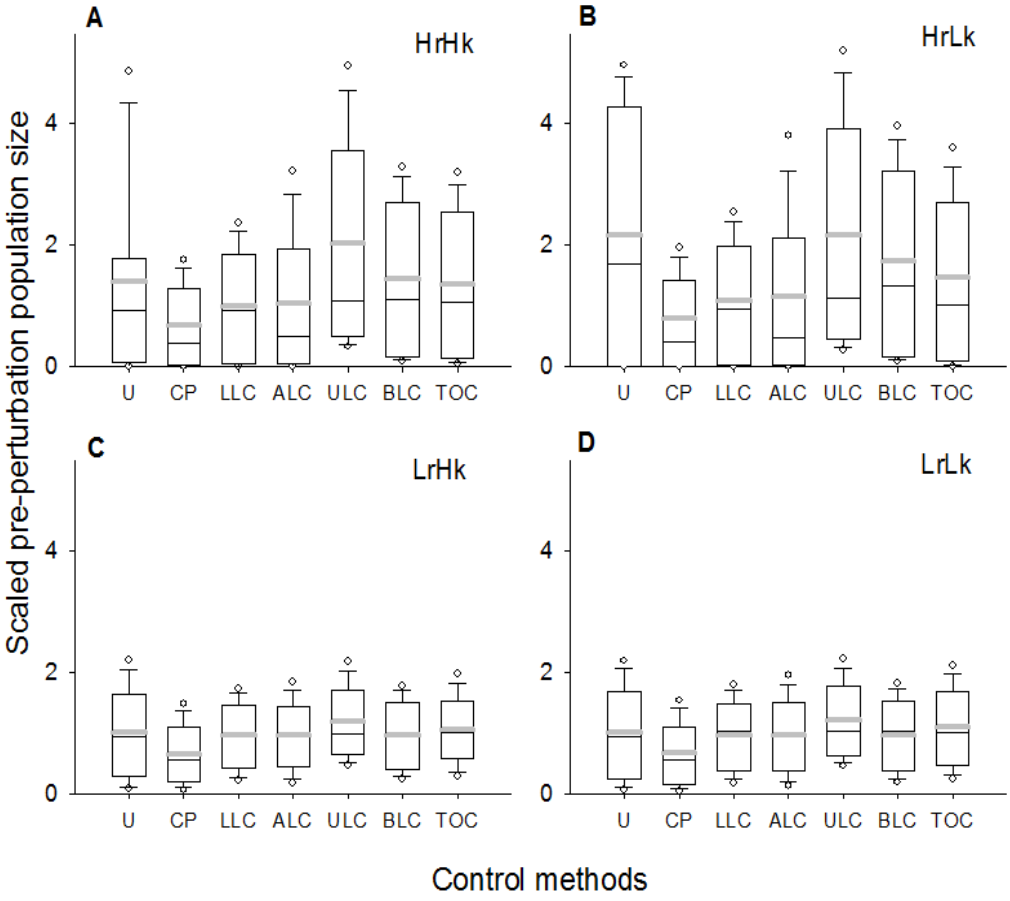

Figure 2.9. Box-plots of the pre-perturbation population size distributions for 50% reduction in Fluctuation Index. A, B, C, and D correspond to the HrHk, HrLk, LrHk and LrLk regimes respectively. Thick grey lines = means, thin black lines in the box = medians. Lower and upper limits of the box represent 25th and 75th percentiles, lower and upper whiskers denote 10th and 90th percentiles while the lower and upper dots stand for 5th and 95th percentiles. U stands for the unperturbed population while the other abbreviations denote the six methods. All the values have been scaled by the corresponding carrying capacity (*k*) of the regime to facilitate comparison. ULC had the highest pre-perturbation population size, which indicated that it would also have the least amount of extinctions. Note that the size distribution of the unperturbed (U) populations is asymmetric in the two Hr regimes (A,B), but not in the two Lr regimes (C,D). This has consequences for the corresponding effort magnitudes.

For a similar level of reduction in FI (i.e. 50% of the unperturbed population), the corresponding population size distributions were found to be very different for the six methods, particularly in the two Hr regimes (Fig 2.8 and 2.9). The lower percentiles (5th, 10th and 25th) of post-perturbation population sizes are higher than the corresponding values for the unperturbed for all the control methods in all four regimes (Fig 2.8). This is not surprising for all methods that include a restocking component (i.e. except ULC). In the case of ULC, the lower percentiles take higher values due to prevention of crashes from high numbers in the previous generation. This becomes apparent from the distribution of the pre-perturbation population sizes (Fig 2.9) which shows that ULC maintains the lower percentiles of

population sizes higher than all the other methods. This leads to the prediction that ULC is expected to have the smallest probability of extinction for this level of enhancement of constancy and indeed we found no extinctions at all under ULC in any of the four regimes (Fig 2.10A). This result apparently contradicts an earlier study that found that the imposition of an upper limiter is actually expected to increase the extinction probability of a population (Middleton et al., 1995). This discrepancy is due to the fact that the earlier study assumed density-independent, randomly distributed growth rates, due to which the chances of an extinction in the next generation (*t+1*) increased monotonically with any kind of reduction in population size in generation *t* (Middleton et al., 1995). However, in this study, we explicitly assumed growth rates to be density-dependent, as a result of which, the extinction probability in *t +1* actually goes down with reduction in population size in *t*. As long as the ULC threshold is not set at extremely low levels (i.e. so low as to become extinction pre-images) and there are no Allee effects (which was not considered in this study), reducing the ULC upper limit is expected to promote persistence (Fig 2.5). This discrepancy between the results of the two studies highlights that the effects of a particular control method can be conditional upon the nature (i.e. density-dependent vs density-independent) of the population growth rates.

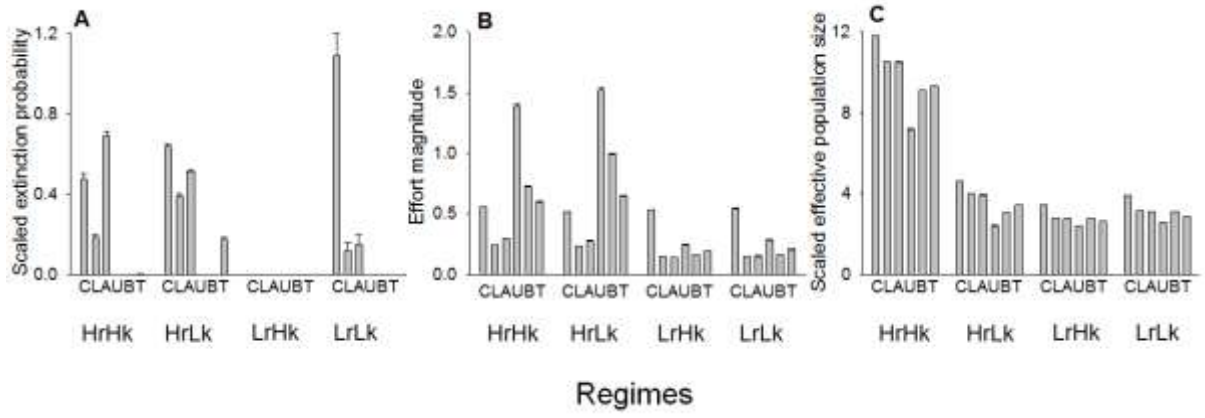

Figure 2.10. Comparison of the six methods for 50% reduction in Fluctuation Index. C = CP, L = LLC, A = ALC, U = ULC, B = BLC, T = TOC. (A) Average (± SEM) extinction probability. Note that there were no extinctions in the LrHk regime. The absence of a method within a regime indicates no extinctions. (B) Average (± SEM) effort magnitude corresponding to the six methods in four regimes. (C) Average (± SEM) effective population size corresponding to the six methods in four regimes. In panels A and C, each value has been scaled by the average value of the unperturbed population in that regime. In general, the methods that involve a culling step, i.e. ULC, BLC and TOC, are better at reducing extinction probability. However, they have higher effort magnitudes in the two Hr regimes. All methods were effective in increasing the effective population size compared to the controls. Some of the error bars are too small to be visible.

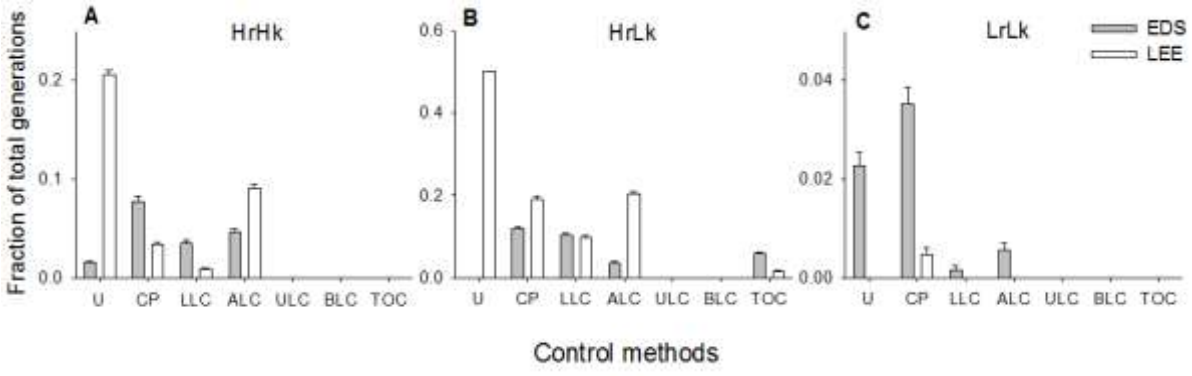

Figure 2.11. Lattice Effect Extinctions (LEE) Vs Extinctions due to Demographic Stochasticity (EDS) for 50% reduction in fluctuation index. Average (± SEM) fraction of times extinction occurred due to population size falling below 0.5 (LEE) or between 1 and 3 (EDS). (A) HrHk, (B) HrLk and (C) LrLk. No extinction occurs in LrHk regime and hence it has been omitted here. The extinctions were scored over 50 generations for 50 replicate runs for each method in each regime. Note the differences in the Y-axis scales in the three figures. For a similar reduction in fluctuation index, the relative frequencies of LEE and EDS varied between the control methods. Some of the error bars are too small to be visible.

Since culling enhances persistence when the population sizes are high, we expected the other two methods that also involve culling steps (i.e. BLC and TOC) to a degree, to be effective in terms of reducing extinction probabilities. Since the resolution in Fig 2.8-2.9 was too low for investigating this prediction, we explicitly studied the fraction of times the population sizes crashed low enough to cause concern in terms of extinction. Using all the replicate time series for each control method, we quantified the fraction of times extinction happened due to lattice effect (i.e. LEE) and demographic stochasticity (i.e. EDS). Even though the control magnitudes were set such that all methods caused an equal reduction in FI, the corresponding reductions in LEE and EDS were different, owing to how these methods changed the distribution of population sizes. ULC never allowed the population sizes to reach either the EDS or the LEE zone (Fig 2.11), which explains the complete lack of extinction in that control method (Fig 2.10A). Although, the upper ranges ($\geq 75^{th}$ percentiles) of BLC's post perturbation values were higher than those of ULC (Fig 2.8), they were not high enough to lead to EDS or LEE in the next generation (Fig 2.11) and therefore caused no extinctions (Fig 2.10A). The same stands true for TOC in general, except for low and very low frequencies of extinction in the HrLk and HrHk regimes respectively (Fig 2.10A). On the other hand, the three restocking control methods that did not have a component of directly reducing the peaks in population sizes (CP, LLC and ALC) had significant number of extinctions, particularly in

the two Hr regimes (Fig 2.10A). ALC and CP turned out to be the worst performers in terms of persistence in the HrHk and the HrLk regimes respectively. This observation was explained when we compared the EDS and LEE values in these two regimes (Fig 2.11A-B). The unperturbed populations in both regimes suffered primarily from LEE which both ALC and CP were able to partially ameliorate. However, CP was more effective in reducing LEE and suffered a higher fraction of EDS whereas the converse was true for ALC (Fig 2.11A-B). These observations were supported by the fact that ALC consistently had higher values for the 90^{th} and 95^{th} percentiles for the post-perturbation population sizes (Fig 2.8A, 2.8B) which means they are expected to hit lower values in the next generation more often. Interestingly, in the LrLk regime, CP actually induced LEEs where there were none in the unperturbed (Fig 2.11C) and ultimately increased the extinction probability. LLC was found to be the most effective in terms of reducing the extinction probability in all the regimes (Fig 2.10A) and primarily suffered from EDS (Fig 2.11A-C). The overall message from all these observations is that control methods interact with growth rate and carrying capacity to alter the distribution of population sizes, which in turn determines the relative frequencies of LEE and EDS, ultimately affecting the persistence stability of populations. Since in populations with high growth rates, extinctions can only happen following a crash from a peak, methods that control the upper ranges of population sizes are better in terms of controlling extinctions for a given level of fluctuation in population sizes. However, such methods have their own share of problems in terms of applicability.

3.3.2 50% reduction in FI: Effort Magnitude

All control methods investigated in this study involve restocking or culling of individuals. In practice, addition or removal would always incur some economic cost which can become a major factor in the choice of control methods. There are no simple ways of knowing how much the implementation of a method would cost as the figures would clearly be contingent on factors like the species under consideration, the technological know-how available etc. Therefore, following past studies (Hilker and Westerhoff, 2005; Sah et al., 2013), we considered the number of organisms added (or removed) to be a proxy of the economic cost involved. We assume here that all else being equal, the economic cost (and hence the undesirability of a method) is directly proportional to the number of organisms to be added or removed.

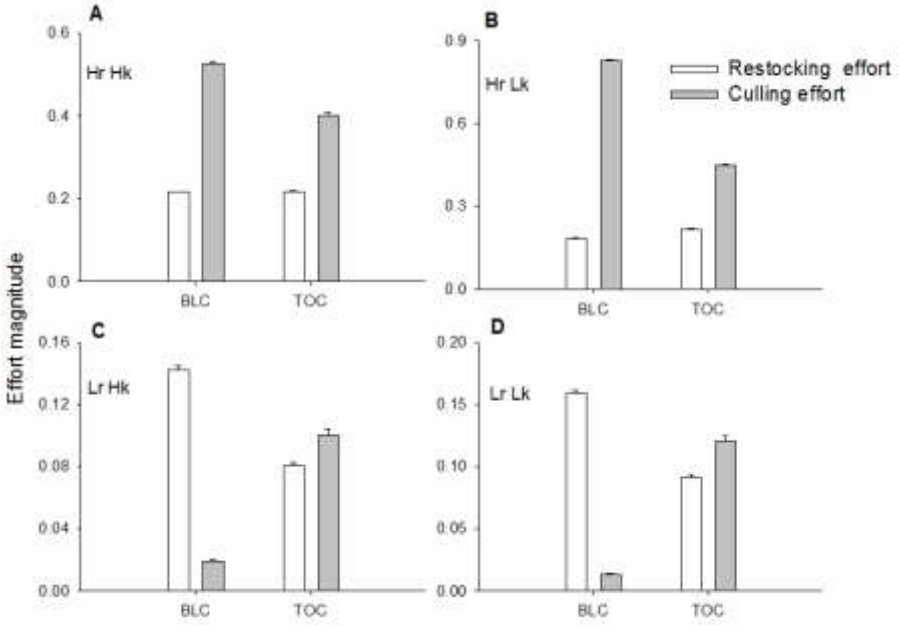

Figure 2.12. Restocking and culling efforts for BLC and TOC. Panel A, B, C and D represent the regimes HrHk, HrLk, LrHk and LrLk respectively.

In the two Hr regimes, the three methods that involved some amount of culling (ULC, BLC, TOC) required greater effort compared to the three that relied solely on restocking (CP, LLC, ALC) (Fig 2.10B). This is a direct consequence of the positive-skew in the distribution of the unperturbed populations under high intrinsic growth rate (Fig 2.9A-B). Ricker model of population growth ensures that whenever growth rate is high ($r > 2.0$), crashes bring the population size below the carrying capacity whereas increases in population sizes take them above *k*. Thus, in the absence of perturbation, for Hr, approximately 50% of the points of the return map are squeezed between 0 and *k* while the remaining 50% are spread over the interval *k* to approximately 5k (Fig 2.9A-B). Due to this long tail of the first return map of the Ricker model (May and Oster, 1976), even small magnitudes of restocking prevent the population from reaching relatively large intervals in terms of peak sizes in the next generation. On the other hand, a much wider interval of high population sizes need to be controlled, to restrict the magnitude of the crashes in the next generation. The net result of this is that restocking methods require lesser effort to reduce the fluctuation in population sizes, compared to methods that involve culling. This line of reasoning does not fully explain the working of control methods like BLC and TOC that involve both culling and restocking. However when we explicitly looked at the magnitudes of culling and restocking for these two methods it was found that under high *r*, more effort is expended in culling than in restocking (Fig 2.12A-B).

The above account is expected to hold only when the population size distribution is positively skewed. This explains why under low *r* (i.e. for LrLk and LrHk), where the distribution of population sizes is more symmetric (see unperturbed in Fig 2.9C-D), there was less difference in the effort magnitudes across the six methods (Fig 2.10B). This also highlights that the effort magnitude of a method depends primarily on the growth rate of the species. It should be noted here that, by definition, the effort values of a time series are scaled by the corresponding average population size (Section Methods: *Effort magnitude and Effective Population Size*). Thus, the actual values of the number of organisms to be added or removed will clearly depend on the carrying capacity. Moreover, from fig 2.12C-D, we note that for low *r*, there is more restocking for BLC whereas culling remains the dominant factor for TOC. This distinction can be important because these two processes have opposite effects on another crucial determinant of the extinction probability of a population, namely its genetic diversity.

3.3.3 50% reduction in FI: Effective Population Size (N_e)

A population which has lower genetic diversity can suffer from inbreeding-like deleterious effects and therefore have a greater risk of extinction (Newman and Pilson, 1997). In the present study, since all six methods increased the breeding size (i.e. post perturbation size, Fig 2.8) of the populations, the corresponding N_e values were enhanced under all four regimes (Fig 2.10C). Since ULC led to the lowest values for both the upper (95^{th} percentile) and the lower (5^{th} percentile) of population size distributions (Fig 2.8) it was relatively less effective in terms of N_e, particularly under HrHk (Fig 2.10C). For all the other methods, both the upper and the lower ranges played a role in determining the N_e and the knowledge of any one was not sufficient for prediction. In general, CP tended to lead to the highest N_e under all four regimes.

3.3.4 50% reduction in FI: Composite performance score

From our results it is clear that at the level of constancy stability investigated, no single method is unambiguously superior to the others. As is often the case in biology, the "best" method was context-dependent. However, such answers mean little for most practical purposes. Therefore we computed the composite performance score with equal weightage to extinction probability, effort magnitude and effective population size (Fig 2.13A). Note that for this score, lower values indicate better performance and vice versa. LLC was found to be

the optimal method under HrHk, HrLk and Lr Hk regimes, whereas BLC was the best performer under the LrLk regime (Fig 2.13A).

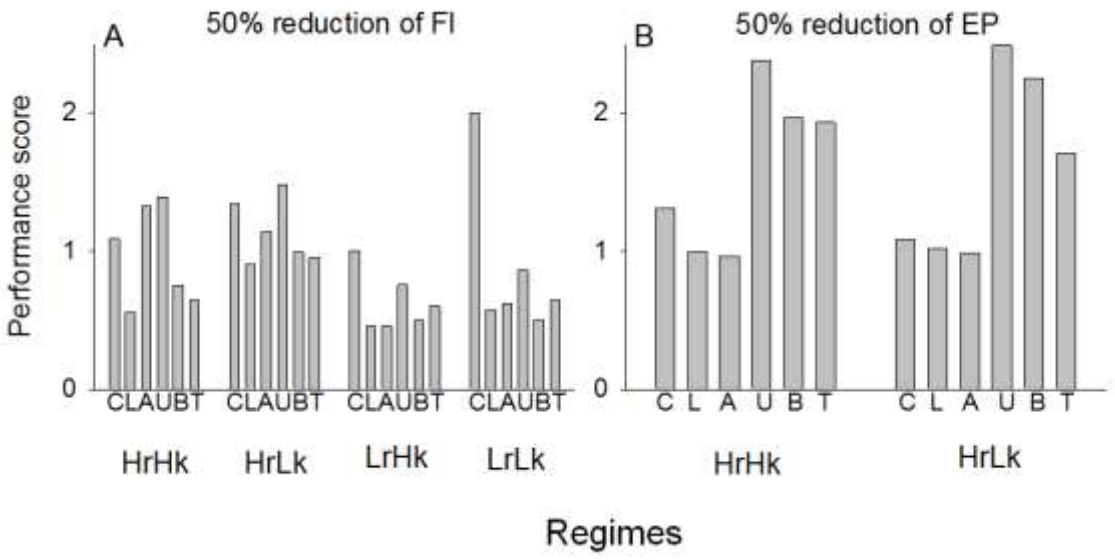

Figure 2.13. Composite performance score for comparison between the control methods. C = CP, L = LLC, A = ALC, U = ULC, B = BLC, T = TOC. A) For 50% reduction in FI. (B) For 50% reduction in extinction probability. Lower values indicate better performance. Although no method was clearly superior in all the regimes, in general, LLC performed the best under most circumstances and ALC was the second best.

3.4 50% reduction in extinction probability

The above discussion pertained to the correlates of attaining a 50% reduction in fluctuation index. However, for most practitioners of conservation, reducing the extinction probability of a population is perhaps a more pressing goal. Therefore, we repeated the entire analysis above in terms of 50% reduction in extinction probability. The exploratory analysis can be found in Figs 2.2- 2.7 whereas the persistence analogues of Figs 2.8-2.10 are Figs 2.14-2.16. It should be noted here that the two Lr regimes (LrHk and LrLk) suffered almost no extinctions and therefore were excluded from this part of the study.

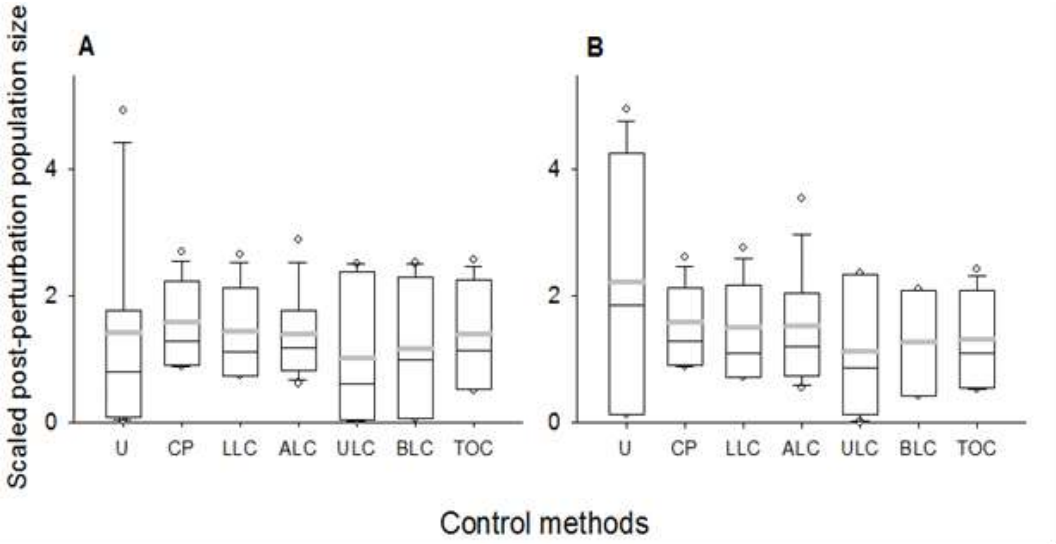

Figure **2.14. Box-plots of the post-perturbation population size distributions for 50% reduction in Extinction Probability**. A and B correspond to the HrHk and HrLk regimes respectively. Note that the unperturbed populations in LrHk and LrLk suffered negligible extinctions and hence they are excluded here. Thick grey lines = means, thin black lines in the box = medians. Lower and upper limits of the box represent 25th and 75th percentiles, lower and upper whiskers denote 10th and 90th percentiles while the lower and upper dots stand for 5th and 95th percentiles. U stands for the unperturbed population while the other abbreviations denote the six methods. All the values have been scaled by the corresponding carrying capacity (*k*) of the regime to facilitate comparison across regimes.

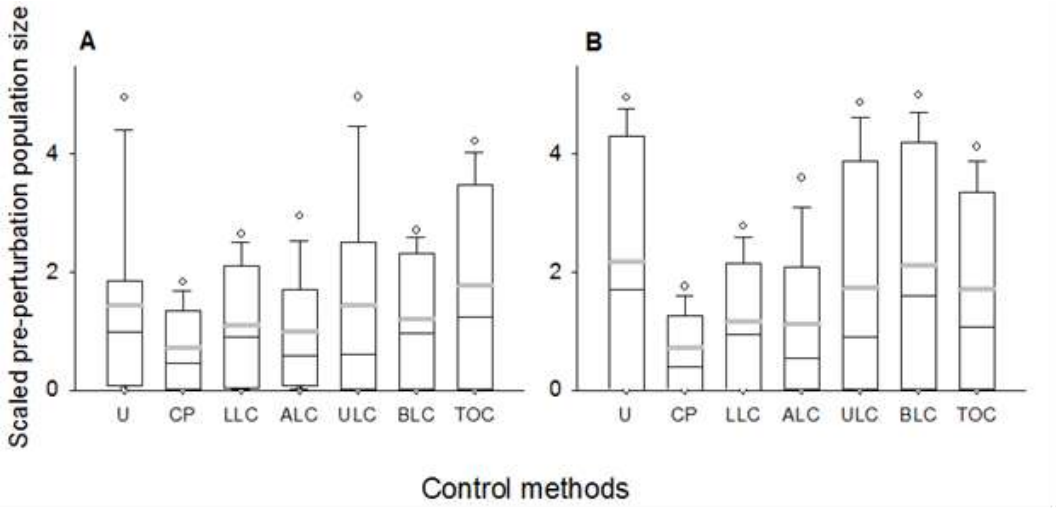

Figure 2.15. Box-plots of the pre-perturbation population size distributions for 50% reduction in Extinction Probability. A and B correspond to the HrHk and HrLk regimes respectively. Note that the unperturbed populations in LrHk and LrLk suffered negligible extinctions and hence they are excluded here. Thick grey lines = means, thin black lines in the box = medians. Lower and upper limits of the box represent 25th and 75th percentiles, lower and upper whiskers denote 10th and 90th percentiles while the lower and upper dots stand for 5th and 95th percentiles. U stands for the unperturbed population while the other abbreviations denote the six methods. All the values have been scaled by the corresponding carrying capacity (*k*) of the regime to facilitate comparison across regimes.

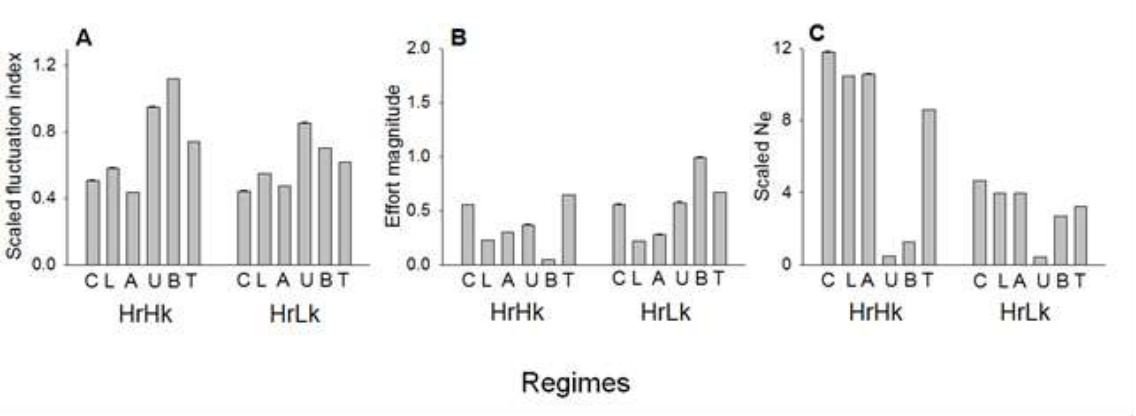

Figure 2.16. Comparison of the six methods for 50% reduction of extinction probability. Note that the unperturbed populations in LrHk and LrLk regimes suffered almost no extinctions and were excluded. (A) Average fluctuation index (± SE). (B) Average effort magnitude (± SE). (C) Average effective population size (± SE). In panels A and C, each value has been scaled by the average value of the unperturbed population in that regime.

We began by looking at the effects of reducing extinction probability by 50% on the corresponding FI. The three methods that did not have a culling step (i.e. CP, LLC and ALC) had lower FI compared to the three that included a culling step (i.e ULC, BLC and TOC) (Fig 2.16A). Interestingly, the former set of methods was actually worse off in reducing extinction probability for a given reduction in FI (*cf* Fig 2.10A and Fig 2.16A). This once again highlights the rather complex relationship between consistency and persistence, a fact that can be observed more directly by examining Fig 2.2-2.7.

The effort magnitude profiles were also very different particularly in the HrHk regime (*cf* Fig 2.10B and 2.16B). It can be immediately seen that it requires relatively lesser effort to reduce extinction by 50% than to achieve a similar reduction in FI. This is because the pre-images to prevent LEE or EDS are well above *k* and hence require relatively less culling effort, whereas a population needs to be more heavily controlled to obtain a similar magnitude of reduction in FI (Table 2.2). An interesting manifestation of this effect can be observed in the unnaturally low effort magnitude and N_e of BLC in the HrHk regime (Fig 2.16C). To explain this observation, we recall that for BLC, multiple combinations of the upper and the lower threshold led to a 50% reduction in extinction probability and therefore arbitrarily the combination that had the lowest effort magnitude was chosen (Table 2.2). In the lower-threshold-upper threshold parameter space, there is a small zone of relatively high upper thresholds and really small lower thresholds that showed a 50% reduction in extinction probability. Our arbitrarily set criteria picked a point in this zone of the parameter space. Since the extinctions were mitigated by preventing crashes in the pre-perturbation population

sizes, the very low value of the lower threshold did not matter in that context. However, since the N_e is computed on the post-perturbation population sizes, the low values of the lower threshold led to a very small increase in post-perturbation population size, which reflected in the very small N_e. The very small N_e of ULC in the HrHk regime arises due to a similar reason, except that in this case, there are no lower thresholds to begin with. This inability to enhance the N_e proved costly for ULC and BLC in terms of the composite performance score. Overall, ALC emerged as the primary method of choice for reducing extinction probabilities, with LLC and CP being second and third preferred methods respectively (Fig 2.13B).

3.5 Caveats

Although these comparisons were obtained from simulations that incorporated several biologically realistic features, from an application point of view, there are several other caveats that need to be considered. In this study, we gave equal weightage to culling and restocking for computing the effort which, depending on the species, need not always be the case. Thus, for example, if artificial breeding of a particular species is more expensive than killing them in wild, then the entire effort calculation needs to be suitably modified. Similarly, culling and restocking have very different effects on the standing genetic variation of a population. In this study, we compute N_e solely as a function of the population sizes. However, if the organisms that are used for restocking come from a different genetic stock, then for all control methods except ULC, the actual rate of loss of genetic variation would be less than what is indicated by the values of N_e reported here.

In the computation of the composite performance score, we gave equal weightage to fluctuation index / extinction probability, effort magnitude and effective population size, which might not be applicable under all scenarios. We also omitted factors like frequencies of external interventions, which might become crucial under certain scenarios (Franco and Hilker, 2013). Any changes in these relative weightages or inclusion of more parameters for comparison can possibly lead to different conclusions in terms of relative performances of the methods. For example, we did not take into account the cost of census of the population, which actually varies among the methods. CP needs no censuses for implementation, which perhaps explains its popularity among studies that explicitly consider more realistic frameworks (Gusset et al., 2009). LLC requires lower census efforts in peak years (i.e. till the point the threshold number of animals has been sighted) and greater census efforts in lean years (when the population sizes are low, larger fraction of the population will have to be

counted to decide whether the perturbations need to be implemented). The other four methods require complete census at all time intervals. It is perceivable that sometimes the cost of census might over-run the cost of implementation of the perturbations, which is evidently economically undesirable. Moreover, for many organisms like butterflies or fishes, a total count of the population might be impossible, and one would be forced to depend upon counts extrapolated from samples. Robustness of control methods towards such noisy implementations have been demonstrated for most of the methods referred here (Dattani et al., 2011; Hilker and Westerhoff, 2005; Sah et al., 2013; Solé et al., 1999) but was not included in the present study since it is relatively difficult to quantify. However, its importance in terms of usability of a control method cannot be overstated. Finally, this study is explicitly in the context of spatially unstructured, single-species populations whereas most natural populations are expected to exist as metapopulations in multi-species assemblages which might necessitate other kinds of control strategies (e.g. Hudson et al., 1998). Thus, a multitude of factors still need to be considered before adopting the various control methods investigated here, under field conditions. However, given that the consequences of controls going wrong can sometimes be catastrophic for ecosystems (e.g. Pyke, 2008), the kind of comparison that we attempt here will be a crucial part of translating theory into practice.

4. CONCLUSIONS

Several interesting observations emerged from this study. At least for the levels of stability investigated in this study, methods that have a culling step (ULC, BLC and TOC) are better at preventing extinctions although they are worse off in terms of reducing fluctuations in population sizes. The converse is true for methods that involve only restocking step (CP, LLC and ALC). The efficacy of control methods that incorporate both (like TOC and BLC) varies depending on whether restocking becomes the dominant force or culling. Of course, the efficacy of the methods also depend upon combination of growth rates and carrying capacities of the populations, which highlights that there is no alternative to gathering relevant biological information before the application of a control method (although see (Hilker and Westerhoff, 2007)). However, in the absence of detailed knowledge of the system, LLC (and to a lesser extent ALC), are the optimal methods to employ (Fig 2.13).

CHAPTER 3

Population stability through Upper Limiter Control (ULC) and Lower Limiter Control (LLC)

Highlights

- Empirical validation of two methods: Upper and Lower Limiter Control (ULC and LLC).
- ULC promotes constancy and persistence but has less effects on genetic stability.
- LLC enhances persistence and genetic stability but not constancy.
- Biologically realistic simulations match the data, indicating generalizability.

Adapted from: **Tung, S.**, Mishra, A., Dey, S. 2016. Stabilizing the dynamics of laboratory populations of Drosophila melanogaster through upper and lower limiter controls. Ecological Complexity 25, 18-25.

1. INTRODUCTION

Over the last two decades, several methods have been suggested in control theory (Chernousko et al., 2008) and theoretical nonlinear dynamics (Andrievskii and Fradkov, 2003, 2004; Schöll and Schuster, 2008) to stabilize unstable non-linear dynamical systems. Several of these methods have been proposed for system where the underlying dynamics are well-characterized and stability is achieved by perturbing system parameters in real time to attain desired behaviours like stable points or simple limit cycles (Garfinkel et al., 1992). Unfortunately, for even fairly simple biological populations, the exact equations underlying the dynamics are often unknown. Moreover, when available, the parameters of such equations (e.g. carrying capacity or intrinsic growth rate) can often only be estimated *a posteriori* through model-fitting and thus are not available for real time perturbations. Finally, due to the ubiquity of noise in biological systems, it is not only impossible to attain stable points or limit cycles in the strict mathematical sense, it also becomes very difficult to distinguish such behaviours from chaotic dynamics (although see Desharnais et al., 2001). Thus, a different class of control methods and observables are needed in the context of biological populations.

The choice of method also critically depends upon the desired goal of control. There are two major, typically mutually exclusive, motivations for stabilizing biological populations. The first is in the context of economically exploited species (e.g. fishes) where the aim is to maximize the yield over a long period of time and reduce the uncertainty of the yield (Lande et al., 1997). The second aim seeks to reduce the amplitude of fluctuation in sizes or increase the long term probability of persistence for populations (Gusset et al., 2009; Hilker and Westerhoff, 2007). Not surprisingly, stabilizing the yield of harvested populations has received far more theoretical and empirical attention (Milner-Gulland and Mace, 1998) than stabilizing threatened species. Part of the problem with the latter is that conservation efforts are typically directed towards charismatic species of mammals and birds. The dynamics of such species typically cannot be captured by the simple models that have been often used to investigate the various control methods (e.g. Dattani et al., 2011; Sah et al., 2013). However, it should be noted that a simple model like the Ricker map (Ricker, 1954) does provide fairly accurate descriptions of the dynamics of taxonomic groups including bacteria (Ponciano et al., 2005), fungi (Ives et al., 2004), ciliates (Fryxell et al., 2005), insects (Sheeba and Joshi, 1998) and fishes (Denney et al., 2002). Together, such taxa account for a huge fraction of the total biodiversity on earth, at least some part of which have already been recorded to be

extinct (Baillie and Butcher, 2012). Therefore, there is a need to study the methods that can stabilize the dynamics of such "non-charismatic" taxa.

A major hindrance in applying the insights gained from theoretical studies in controlling endangered populations is the fact that few of the proposed methods have been empirically validated even under laboratory conditions (however see Desharnais et al., 2001; Dey and Joshi, 2007; Sah et al., 2013), let alone in nature. Given that survivals of threatened species are at stake, it is understandable when practitioners of conservation are unwilling to try out untested methods in the field. On the other hand, new methods have to be validated somehow in order to assess their suitability for a given scenario. A reasonable way out of this impasse is to validate these methods under laboratory conditions. The success of a method to stabilize laboratory populations allows us to verify our understanding about how the method works. Unfortunately, it does not guarantee the method's success under field conditions but merely increases the confidence that can be placed on its success. On the other hand, the failure of a method under laboratory conditions would typically suggest lack of understanding regarding some crucial aspect of the biology of the system.

In this context, a well-investigated class of methods are the so called limiter control methods which seek to stabilize a population by implementing different kinds of thresholds in population sizes (Corron et al., 2000; Zhou, 2006). Extensive mathematical (Franco and Hilker, 2013, 2014), numerical (Sah and Dey, 2014; Sah et al., 2013) and empirical (Sah et al., 2013) studies suggest that at least for one method of this class – the so called Adaptive Limiter Control or ALC- the theoretical predictions match the empirical data rather well. In this study, we investigate the stabilizing properties of two other limiter control methods, namely upper limiter control (ULC) and lower limiter control (LLC) (Hilker and Westerhoff, 2005), using unstable laboratory populations of the common fruit-fly *Drosophila melanogaster*. For each of these control methods, we investigate two different arbitrarily chosen values of the controlling parameter. We chose these two methods over many such available culling / restocking schemes (e.g. Dattani et al., 2011; Liz and Franco, 2010) primarily because they have been extensively investigated theoretically and numerically (Hilker and Westerhoff, 2005, 2006; Tung et al., 2014). This means that a number of predictions already exist in the literature for verifying against our empirical data. Therefore, the main focus of this paper was on an intuitive understanding of how these two methods affect the dynamics.

Here we show that ULC reduces temporal fluctuations in population sizes, as well as the extinction probability of populations. However, it is unable to enhance the effective population size and has high effort magnitude. On the other hand, the efficacy of LLC in reducing the fluctuations in population sizes is equivocal. In spite of that, the method is able to cause significant reduction in extinction probability and increased effective population size. Most importantly, the effort magnitude required to stabilize the populations is much less compared to ULC. We provide biologically intuitive explanations of how these control methods stabilize the populations. We also experimentally verify several theoretical predictions from the literature and show that our empirical results agree well with biologically realistic simulations.

2. METHODS

2.1. Maintenance regime of the flies

In this study, we used individuals from a large (breeding size of ~2400) laboratory population of *Drosophila melanogaster* called DB_4. The detailed maintenance regime and ancestry of this population has been described elsewhere (Sah et al., 2013). From this population, we derived 30 single vial cultures, each of which represented an independent population. Each of these populations was initiated by placing exactly 10 eggs on 1.1 ml of banana-jaggery medium in a 30 ml plastic vial. The vials were placed in an incubator at 25°C under constant light conditions. Once eclosion started, the freshly emerged adults of a population were daily transferred to a corresponding adult-holding vial, containing approximately 6 ml of banana-jaggery medium. This process continued till the 18^{th} day after egg-collection, after which the egg-vials were discarded. The adult flies were then supplied with excess live yeast paste for three days to boost up their fecundity. On the 21^{st} day after egg-collection, the adults were counted and culling or restocking of flies was imposed as per the prescribed control regimes (see section 2.2). Since the dynamics of a sexually reproducing species is primarily governed by the number of females, culling or restocking was implemented only on the female flies (Dey and Joshi, 2006; Dey and Joshi, 2007). The adults were then allowed to oviposit in a vial containing 1.1 ml of medium for 24 hrs. After oviposition, the adults were rejected and the eggs formed the next generation. The experiment was run over 14 generations. Theoretical (Mueller, 1988) and empirical (Dey and Joshi, 2006; Mueller and Huynh, 1994; Sah et al., 2013) studies have shown that a combination of low levels of larval food (1.1 ml here) and excess live yeast paste destabilizes the populations by inducing large amplitude

oscillations in the time series. This nutritional regime thus allowed us to study the stabilizing effect of various control methods on populations whose dynamics were otherwise unstable.

2.2 Control methods

Upper Limiter Control (ULC) involves culling to a fixed threshold, i.e. the population size is not allowed to go beyond an upper value (Hilker and Westerhoff, 2005). Mathematically, this is written as $N_t' = min\ (N_t, U)$ where N_t and N_t' refer to the population sizes before and after the application of the control method, U is the pre-determined value of the upper threshold and $min(x,y)$ is the minimum operator. To impose ULC experimentally, we culled the number of females in a population to the arbitrarily set levels of 15 (U1) or 10 (U2). When the number of females in a population was less than the threshold, the population was left unperturbed. Note that for ULC, lower values of U represent more stringent control and therefore U2 is a stronger control than U1.

Lower Limiter Control (LLC) is achieved by restocking the population to a fixed number, i.e. the population size is never allowed to fall below a fixed limit (L). Mathematically, this is given as $N_t' = max\ (N_t, L)$ where L stands for the fixed lower threshold and $max(x, y)$ is the maximum operator. For experimental implementation, we chose two arbitrary lower thresholds of 4 (L1) and 10 (L2) females where L2 represents a stronger control than L1. Following an earlier protocol (Dey and Joshi, 2006), the flies were counted, the number was multiplied by half (i.e. assuming equal sex ratio) and rounded up to estimate the number of females in the population. If this number was greater than the pre-determined value of L (i.e. 4 or 10) then the population was left untouched, else the shortfall was made up by adding the required number of females from outside. Thus, we explicitly incorporated some degree of noise in terms of application of LLC (see section 4.2 for the rationale of the same).

We used 5 replicate single vial cultures each for U1, U2, L1 and L2. We also had two batches of unperturbed populations (designated C1 and C2), each consisting of 5 replicate populations. C1 served as the unperturbed experimental controls for the ULC treatments while C2 were the corresponding experimental controls for the LLC treatments. The pairing of C1 with ULC and C2 with LLC was done *a priori*, i.e. at the time of initiation of experiments, before the data analysis. An extra set of experimental controls ensures that the ULC and LLC experiments are completely independent of each other. It also allowed us to verify the reproducibility of the unperturbed dynamics.

In case of U1, U2, C1 and C2, an extinct population was rescued by adding 4 males and 4 female flies from outside (Dey and Joshi, 2006). No such intervention was needed for the L1 and L2 populations where the control method automatically ensured that the breeding population never went extinct. The flies used for rescue (as well as restocking) were maintained by allowing 4 males and 4 females (yeasted) to oviposit in each generation in vials containing ~5ml of larval medium. Thus, these flies experienced a lower level of larval crowding as compared to the actual populations which were maintained on 1.1 ml of medium. This was essential for logistic reasons, so as to ensure a steady supply of flies in the backups. However, this can be a potential problem in the context of these experiments, as flies raised under severe larval crowding are typically much smaller in size and have lower fecundity (Mueller and Joshi, 2000). Thus, the immigrant flies could potentially have different demographic parameters compared to the native flies of a population. However, in our experiments, this problem was ameliorated to some extent as we did not observe any major size differences between the treatment and the back-up flies (ST personal observation). This was perhaps because 4 yeasted females typically lay sufficient eggs to cause considerable crowding even with ~5ml of food which was evident from the degraded state of the medium during collection of adults. We used the time series of the post-perturbation (i.e. application of control method) population sizes for computing all indices of population stability except persistence (see below).

2.3. Constancy and persistence stability

Populations with larger fluctuations over time are deemed to have less 'constancy' stability and vice versa (Grimm and Wissel, 1997). We used the Fluctuation Index or FI (Dey and Joshi, 2006) to quantify the constancy stability of a given time series.

$$Fluctuation\ Index = \left(1 \Big/ \left(T \times \overline{N}\right)\right) \times \sum_{t=0}^{T-1} \left| N'_{t+1} - N'_t \right|$$

where N'_t is the breeding population size, $\overline{N}$ represents average population size and T denotes the length of the time series.

Persistence stability (Grimm and Wissel, 1997) of a population was scored as the extinction probability during the course of the experiment (= number of extinctions / length of the time series). Since one of the methods (LLC) involved restocking, persistence was scored using the pre-perturbation census size.

2.4. Average population size and effective population size:

Average population size ($\overline{N}$) was simply the arithmetic mean of the time series while effective population size (N_e) was quantified as the harmonic mean of the time series (Allendorf and Luikart, 2007).

$$N_e = T \Big/ \sum_{t=1}^{T} 1/N_t'$$

It should be noted that sex ratio can occasionally deviate from 1:1, which would make the actual effective size lower than what this formula would predict.

2.5. Effort magnitude:

Following previous studies (Hilker and Westerhoff, 2005; Sah et al., 2013), we assumed that the number of organisms to be removed or added for implementing a control method (i.e. the so called effort magnitude) is a proxy for the corresponding economic cost of implementation. Thus,

$$Effort\ magnitude = \left(1 \Big/ \left(T \times \overline{N}\right)\right) \times \sum_{t=1}^{T} \left|N_t - N_t'\right|$$

where N_t and N'_t represent the pre- and post-control population sizes respectively in the t^{th} generation, and $\overline{N}$ and T denote the average population size and length of the time series respectively. It should be noted here that effort magnitude is the actual number of individuals culled from or added to the population. Although the value of the *a priori* set threshold does have a bearing on the empirically observed effort magnitudes, the two are not the same quantity. Since the flies used for rescuing the extinct populations were counted as a part of effort, C1 and C2 have non-zero effort magnitudes.

2.6. Statistical analyses:

The data for ULC and LLC were analysed separately along with their matched unperturbed populations, i.e. C1 and C2 respectively. The fluctuation index, average population size, effective population size and effort magnitude data were subjected to separate one-way ANOVA (unperturbed population and the two levels of fixed thresholds being the fixed factors). In those cases, where a significant main effect was obtained, Tukey's HSD was employed for testing the significance of pair-wise differences among means. All ANOVA and associated comparisons were performed using STATISTICA® v5 (StatSoft. Inc., Tulsa,

Oklahoma). We also used the freeware Effect Size Generator (Devilly, 2004) to compute Cohen's *d* statistic (Cohen, 1988) as a measure of effect sizes for the pair-wise differences among the means. The effect sizes were interpreted as small, medium or large for $d < 0.5$, $0.5 < d < 0.8$, and $d > 0.8$.

2.7 Simulations

The dynamics of the populations were simulated using the Ricker model (Ricker, 1954). This model is given as $N_{t+1}=N_t*exp(r*(1-N_t/K))$, where *r*, *K* and N_t denote intrinsic growth rate, carrying capacity and population size at time *t* respectively. Upper and lower limiter control were imposed according to the following mathematical form (Tung et al., 2014):

Upper Limiter Control (ULC): $N'_t = min\ (N_t\ ,\ H)$

Lower Limiter Control (LLC): $N'_t = max(N_t\ ,\ h)$

where N_t *and* N'_t are the population sizes before and after perturbation in the t^{th} generation. $N_{t+1} = f(N'_t)$, where f stands for Ricker model. H and h are upper and lower threshold of ULC and LLC respectively. *min (x, y)* and *max (x, y)* are minimum and maximum operator respectively.

Parameter values: We fit the Ricker model to the unperturbed experimental time-series (C1 and C2) and obtained a mean *r* and *K* of 2.6 and 34 respectively. These values of *r* and *K* were then used to simulate the basic dynamics of unperturbed and controlled populations. Matching the experimental values, upper threshold for U1 and U2 were kept at 15 and 10, whereas lower threshold for L1 and L2 were 4 and 10 respectively.

Stochasticity and lattice effect: Noise can significantly influence the dynamics of perturbed populations (Dey and Joshi, 2007). Therefore we incorporated noise to both the parameters, *r* and *K*, in each iteration. For this, we picked the *r* and *K* values from uniform distributions of 2.6±0.5 and 34±15 respectively. Real organisms always come in integer numbers, and

incorporating this in simulations can affect the dynamics of the systems; a phenomenon termed as lattice effect (Domokos and Scheuring, 2004; Henson et al., 2001). We accounted for lattice effect by rounding off the number of organisms in each generation, as well as the values of the perturbations, to the nearest integer.

The magnitudes of perturbations were computed for each of the treatments of the control methods after assuming a 1:1 sex ratio. Thus, for example, when the lower limit of LLC was set to 4, the control method was implemented only when the population size fell below 8. Although for non-zero initial conditions, the Ricker model does not take zero-values and hence, can never show extinction, the same is not true for the integerized model. Therefore, we set reset rules similar to the experiment for our simulations. When the unperturbed populations and ULC treatments went extinct, the population size was reset to 8. No resets were necessary for the LLC treatments where the control method automatically ensured reset. Each simulation was run for 100 iterations and FI, average population size and effective population size were computed over the resulting time series. All figures (Fig. 3.4 and Fig. 3.9) represent means over 100 independent replicates for each treatment. We also repeated all simulations for 14 generations and 5 replicates (i.e. the same as our experimental conditions). We found no qualitative differences between the two cases, and chose to report the former set of simulations (i.e. 100 iterations and 100 replicates) here, as they represent the dynamics over a slightly longer timescale.

In short, the sequence of steps in the simulation is given as:

$$N'_{t-1} \rightarrow [\text{FUNC}] \rightarrow [\text{INT}] \rightarrow [\text{EXT}] \rightarrow N_t \rightarrow [\text{CTRL}] \rightarrow N'_t$$

where N'_t and N_t are the population sizes after and before application of control in the t^{th} generation, and FUNC, INT, EXT and CTRL represent the population recursion (here Ricker model), integerization, stochastic extinction and control (here ULC and LLC) functions respectively. The initial value for all simulations was set to 10, which was the same as the

starting population size in the experiments. All calculations, except extinction probability, were performed on the time series of the N'_t values.

3. RESULTS

3.1 Upper Limiter Control (ULC)

In the absence of any perturbation, the distribution of the population sizes was found to be positively skewed with a large difference between the mean and the median (Fig. 3.1a). However, in the presence of upper thresholds beyond which the population sizes were not allowed to venture, the distributions became more symmetric, as evidenced by the low values of skew and little difference between the means and the medians (Fig. 3.1b-c). This change in population distribution translated into a significant reduction in FI ($F_{2, 12}$ = 20.8, P = 0.0001). Post-hoc tests revealed that although U1 and U2 had similar FI, both were significantly more stable than the corresponding unperturbed populations with high effect sizes (Fig. 3.2a, Table 3.1). This is consistent with the fact that the population size distributions of U1 and U2 were almost identical to each other and greatly different from the unperturbed treatment (Fig. 3.1). Interestingly, although ULC involves culling above a threshold, the effective population sizes ($F_{2, 12}$ = 2.32, P = 0.14, Fig. 3.2b) and the average population sizes ($F_{2, 12}$ = 1.08, P = 0.37, Fig. 3.2c) of the unperturbed and the controlled populations did not differ significantly (see section 4.1 for discussion). In terms of effort magnitude (Fig. 3.2d), there was a significant main effect of treatment ($F_{2, 12}$ = 44.07, P = 0.000003). Tukey's HSD suggested that both U1 and U2 required significantly greater effort than the unperturbed populations and the corresponding effect sizes were large (Table 3.1). This is intuitive and confirms previous theoretical observations (Tung et al., 2014) that ULC requires a large amount of effort in terms of number of organisms to be removed from the controlled population. ULC also had an almost significant ($F_{2, 12}$ = 3.61, P = 0.06) effect in terms of reduction of extinction probability (Fig. 3.5a). The effect size of the reduction achieved by U1 and U2, compared to the unperturbed population, were found to be large (Table 3.1). Finally, comparing Fig. 3.1 and 3.2 with the corresponding simulations (Fig. 3.3 and 3.4) suggests that our simulations were able to capture the broad trends of the data remarkably well.

Table 3.1 Tukey *P* value and effect size for all pair wise combinations of C1, U1 and U2

ULC	Pairwise combinations	Tukey *P*	Effect size ± 95% CI
Fluctuation index	C1-U1	0.0007	3.66 ± 2.03
	C1-U2	0.0004	3.38 ± 1.93
	U1-U2	0.78	0.43 ± 1.25
Av. population size	C1-U1	*	0.95 ± 1.31
	C1-U2	*	0.85 ± 1.29
	U1-U2	*	0.17 ± 1.24
Effective population size	C1-U1	*	1.17 ± 1.34
	C1-U2	*	1.49 ± 1.4
	U1-U2	*	0.23 ± 1.24
Effort magnitude	C1-U1	0.0002	5.39 ± 2.67
	C1-U2	0.0002	6.00 ± 2.91
	U1-U2	0.08	1.25 ± 1.35
Extinction probability	C1-U1	0.21	1.4 ± 1.38
	C1-U2	0.05	1.8 ± 1.47
	U1-U2	0.7	0.48 ± 1.26

*Note: The post-hoc analysis was not performed, when the main effect was statistically insignificant.

Table 3.2 Tukey *P* value and effect size for all pair wise combinations of C2, L1 and L2

LLC	Pairwise combinations	Tukey *P*	Effect size ± 95% CI
Fluctuation index	C2-L1	0.11	1.43 ± 1.39
	C2-L2	0.17	1.14 ± 1.34
	L1-L2	0.004	2.85 ± 1.76
Av. population size	C2-L1	*	0.93 ± 1.31
	C2-L2	*	1.71 ± 1.45
	L1-L2	*	0.41 ± 1.25
Effective population size	C2-L1	0.17	1.43 ± 1.39
	C2-L2	0.0002	4.7 ± 2.4
	L1-L2	0.0003	4.26 ± 2.24
Effort magnitude	C2-L1	0.58	0.93 ± 1.31
	C2-L2	0.05	1.44 ± 1.39
	L1-L2	0.008	2.21 ± 1.57
Extinction probability	C2-L1	0.06	2.4 ± 1.63
	C2-L2	0.02	2.8 ± 1.74
	L1-L2	0.78	0.37 ± 1.25

*Note: The post-hoc analysis was not performed, when the main effect was statistically insignificant.

3.2 Lower Limiter Control (LLC)

A different set of five unperturbed populations (Fig. 3.6a) gave almost the same distributional properties as seen in Fig. 3.1a, thus attesting the reproducibility of the distribution for the unperturbed case. However, when the population size was not allowed to fall below a fixed threshold, the changes in the population size distributions (Fig. 3.6b-c) were less dramatic than those seen for ULC (Fig. 3.1b and Fig. 3.1c). The nature of the intervention assured that there were no individuals in the lowermost bins for the two levels of LLC (Fig. 3.6b-c). Although the skew values were somewhat reduced due to the perturbations, the distribution still had a sufficiently long tail indicating that the populations were capable of reaching very high sizes. As a result of this, although there was an overall significant effect of treatment in the ANOVA ($F_{2, 12}$ =8.75, P = 0.005), neither L1 nor L2 had significantly lower FI compared to the unperturbed treatment (Fig. 3.7a). Interestingly though, L1 had a significantly higher FI than L2 (Fig. 3.7a, Table 3.2), which is due to the fact that small values of LLC can actually increase the FI of populations (Tung et al., 2014). The effective population size of L2 was significantly higher ($F_{2, 12}$ = 39.86, P = 0.000005) than that of the unperturbed and L1 with high effect sizes (Fig. 3.7b, Table 3.2). This is intuitive as the effective population size is primarily affected by low values in a series and LLC ensures that the population size does not go below a lower threshold. Similarly, the observation that the effective population size of L2 is greater than that of L1 is trivially explained by the fact that L2 involved immigrating larger number of individuals than L1. However, in spite of adding individuals from outside, there was no significant difference ($F_{2, 12}$ = 2.8, P = 0.1) between the three treatments in terms of average population size (Fig. 3.7c). There was a significant effect in terms of effort magnitude ($F_{2, 12}$ = 7.32, P = 0.008) and the L2 treatment required significantly greater amount of effort (with large effect sizes of the difference) compared to both L1 and the unperturbed populations (Fig. 3.7d, Table 3.2). Overall, the empirical results suggest that the L1 level of LLC treatments were primarily ineffective in inducing constancy stability in the populations, while L2 was somewhat more effective (see discussion for possible explanations). However, in terms of persistence, LLC led to significant ($F_{2, 12}$ = 5.85, P = 0.02) reduction in extinction probability. The effect size of the reduction achieved by L1 and L2, compared to the unperturbed population, were found to be large (Table 3.2). Finally, as with ULC, our simulations were again able to reflect the broad trends of the empirical data (*cf* Fig. 3.6 and Fig. 3.7 with Fig. 3.8 and 3.9 respectively).

4. DISCUSSION

This study compares how the upper and lower limiter control methods affect the dynamics of biological populations. However, it is not a direct comparison of the efficiency of the two methods. This is because simulations (Tung et al., 2014) indicate that almost every control method is capable of inducing almost any level of constancy stability, depending on the value of the control parameter (i.e. *U* or *L*). As it is not possible to equate over the parameter values for two methods as different as ULC and LLC, it becomes meaningless to compare their efficiencies. A recent simulation study circumvented this problem by numerically figuring out the magnitude of perturbation needed by each control method to achieve an arbitrary pre-determined level of stability (Tung et al., 2014). The efficiencies of the control methods were then investigated at those parameter values by comparing the resultant effective population size, effort magnitude, etc. Clearly, such an exercise is logistically very difficult in the context of an experimental study, where one is severely constrained in terms of how many treatments can be investigated. Therefore, in this study, we limit ourselves to mechanistic understanding of how each control method functions and refrain from a direct comparison of their efficiencies in attaining stability. It should be noted here that although the efficiencies of ULC and BLC cannot be compared quantitatively, it is valid to make qualitative comparisons between these two methods, for the given parameter values, in terms of their effects on the dynamics.

4.1 Upper Limiter Control (ULC)

Population size distribution and FI: ULC affects the dynamics of fluctuating *Drosophila* population in two ways. Firstly, by definition, the breeding population size was not allowed to go beyond a pre-determined threshold. Secondly, reduction in the number of breeding adults in generation *t* decreased the number of eggs laid in the next generation (i.e. *t+1*), which in turn lowered the larval competition. As a result, there was an increase in the larval survivorship which reduced the occurrences of population crashes in generation *t+1*. This is demonstrated by the fact that application of ULC (Fig. 3.1b and 1c) reduced the frequency of the lower population sizes compared to the unperturbed case (Fig. 3.1a). Consequently, the distribution of population sizes became more symmetric, the median approached the mean and the skewness reduced. This result is the biological analogue of the truncation of the stock-recruitment curve demonstrated by previous numerical studies (Figure 3.10 in Hilker and Westerhoff, 2005) and provides a biological, mechanistic understanding of how ULC

enhances the constancy stability of populations (Fig. 3.2a). The reduction in population FI due to ULC is consistent with an earlier study on ciliates that had reported reduced variability in population size upon imposing an upper threshold (Fryxell et al., 2005).

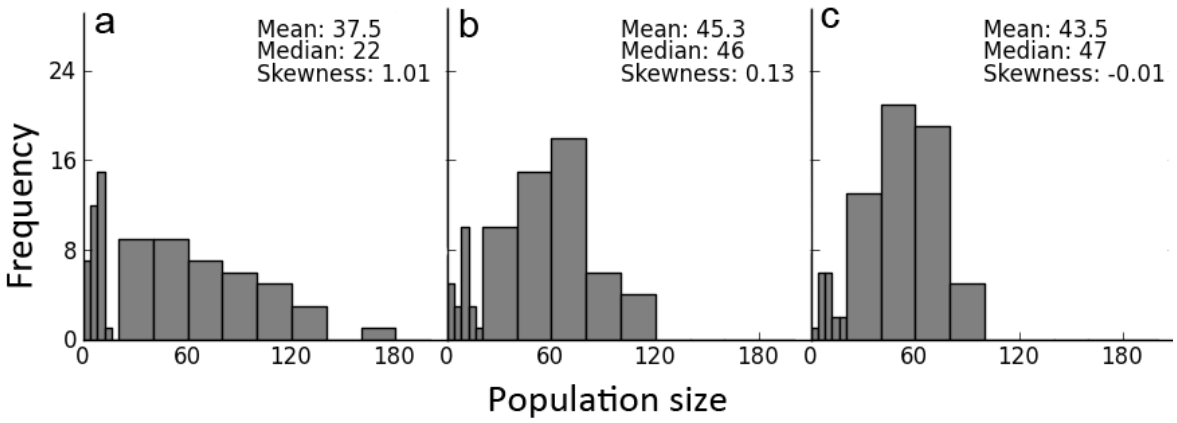

Fig. 3.1. Population size distributions for unperturbed and ULC-treated populations: (*a*) Unperturbed (C1), (*b*) U1 (upper threshold = 15), (*c*) U2 (upper threshold = 10). The bin size is 4 when the population size is in the range 0-20, and 20 otherwise. When the value of the upper threshold is lowered, thereby increasing control intensity, the frequency of the extreme values decreases and the overall distribution becomes more symmetric.

Effective population size: We also investigated the potential impact of ULC on the genetic stability of populations by quantifying the effective population size (N_e). N_e is defined as the corresponding size of an ideal population which loses heterozygosity at the same rate as the given population and is calculated here as the harmonic mean of the population sizes over time (Allendorf and Luikart, 2007). Since the rate of loss of heterozygosity has an inverse relationship with N_e, it follows that higher values of N_e are more desirable for enhanced genetic stability of populations. Also, as the harmonic mean gets affected more by the lower values in a time series, N_e is sensitive to population bottlenecks. Since ULC reduces population crashes, it was expected to enhance N_e. However, although both levels of ULC increased N_e compared to the unperturbed treatment (Fig. 3.2b), the difference was not statistically significant. Moreover, in spite of medium to large effect sizes of the differences between the means, the confidence intervals around the effect sizes were wide (Table 3.1). Together these suggest that there was wide variation in terms of the ability of ULC in enhancing N_e. This is primarily because although ULC reduces the frequency of population crashes, it cannot ameliorate it completely (Fig. 3.1b-c) and populations can still hit low values through demographic or environmental stochasticity. This observation is also

consistent with a recent study which found that ULC was the least effective among six control methods in terms of increasing N_e (Tung et al., 2014). To summarize, ULC is not a reliable method in terms of enhancing N_e.

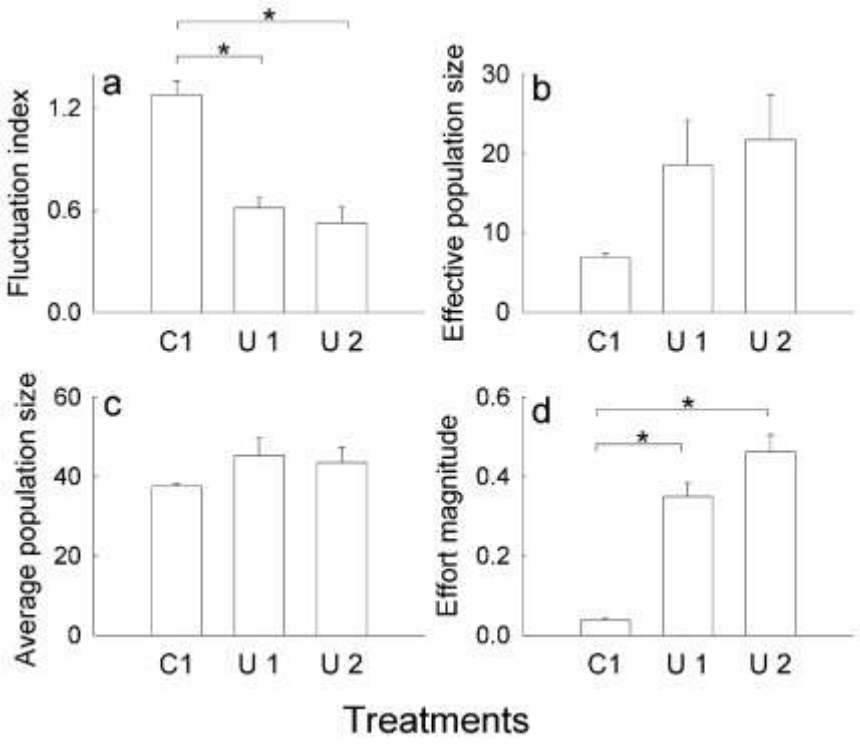

Fig. 3.2 Dynamics after applying ULC. C1 represents the unperturbed populations while U1 and U2 stand for ULC thresholds of 15 and 10 respectively. Each bar represents a mean over 5 replicate populations. Error bars denote standard error around the mean and * denotes $P < 0.05$. (*a*) Fluctuation index: Both the ULC treatments reduced population fluctuations significantly. Neither U1 nor U2 had a significant effect on (*b*) Effective population size and (*c*) Average population size. (*d*) Effort magnitude: Both U1 and U2 required significantly more external perturbation than C1. See text for possible explanations.

Average population size: ULC also failed to affect the average population size of the controlled populations (Fig. 3.2c). Although removal of individuals is expected to reduce the population size, control methods that involve culling can sometimes lead to an increase in average population size. This phenomenon has been called the paradox of limiter control (Hilker and Westerhoff, 2006) or the Hydra effect (Abrams, 2009) in the ecological literature. It happens because culling reduces the negative effects of density dependence on realized growth rate, thus off-setting the loss of numbers due to removal. However, when the stock-recruitment curve of a population shows a long flat tail, as can be inferred for our populations from Fig. 3.1a, then the hydra effect is not expected for a large range of ULC values. This is because for such populations, until and unless the ULC thresholds are fairly low, the increase

in the realized growth rate is not sufficient to cause an increase in the mean population size after compensating for the number of adults removed. This phenomenon has been observed in earlier theoretical studies (Hilker and Westerhoff, 2005) and was also captured in our simulations (Fig. 3.4c).

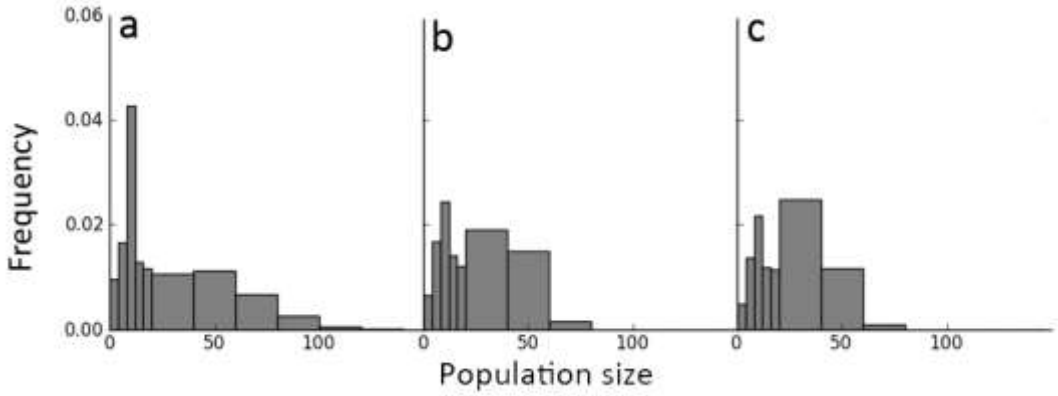

Fig. 3.3 Population size distributions of the simulated unperturbed and ULC-treated populations: **(***a*) Unperturbed (C1), (*b*) U1 (upper threshold=15), (*c*) U2 (upper threshold=10). When the population size is between 0-20, bin size is 4 and 20 otherwise.

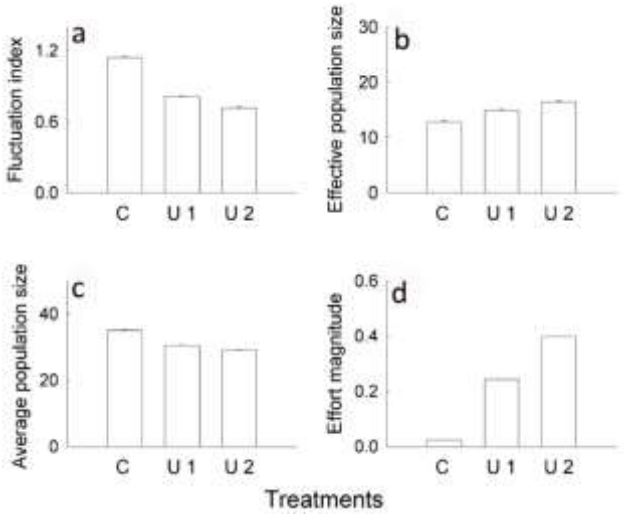

Fig. 3.4 Simulated dynamics after applying ULC. C1 represents the unperturbed populations while U1 and U2 stand for ULC thresholds of 15 and 10 respectively.Each bar represents a mean over 100 replicate populations. Error bars denote standard error around the mean. (*a*) Fluctuation index, (*b*) Effective population size, (*c*) Average population size and (*d*) Effort magnitude.

Effort magnitude: Both U1 and U2 required effort to the tune of 40-50% of the corresponding average population size (Fig. 3.2d). This is because although a fixed upper threshold restricts the number of breeding females, the high fecundity of the flies ensured a substantially higher-than-threshold population size in the next generation. Consequently, ULC had to be applied in most generations which translated into a high effort magnitude. This observation is consistent with a recent theoretical investigation which found that ULC entailed a relatively

high effort magnitude (Tung et al., 2014). Our results show that when cost of culling is high, the levels of ULC investigated here are likely to be prohibitively expensive. However, we note here that if harvesting the species in question has some economic benefits, then the cost of harvesting might be off-set by the income derived from it! Thus, like most issues in conservation, whether a given strategy is feasible or not, will be context dependent.

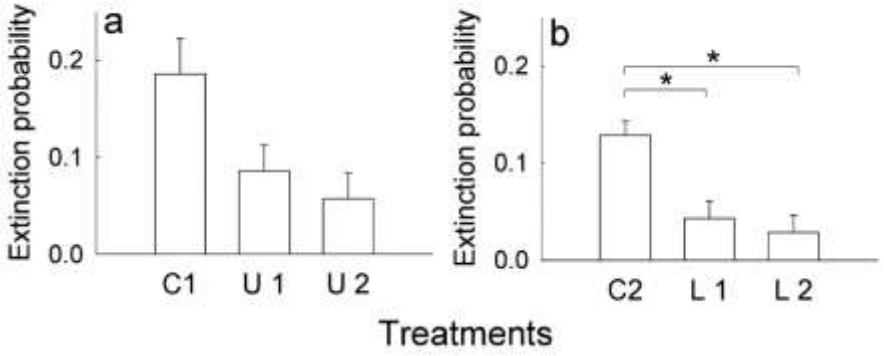

Fig. 3.5 Extinction probability after applying ULC and LLC. Each bar represents a mean over 5 replicate populations. Error bars denote standard error around the mean and * denotes $P < 0.05$. (*a*) ULC: C1, U1 and U2 represent the unperturbed populations, and ULC thresholds of 15 and 10 respectively. (*b*) LLC: C2, L1 and L2 stand for unperturbed populations, and LLC thresholds of 4 and 10 respectively. Both control methods were able to reduce the extinction probability, although the reduction was marginally insignificant for ULC (see text for possible explanation).

Persistence: Due to its tendency to diminish the frequency of population crashes, ULC reduced the extinction probability of the controlled populations, although the effect was statistically marginally insignificant (Fig. 3.5a). Similar observations have been made in the case of harvested populations where it is seen that the presence of some kind of upper threshold in population size is needed to optimize the yield and reduce the extinction probability under unpredictable environments (Lande et al., 1997).

To summarize, our results indicate that although ULC induces constancy and persistence stability, it is not very efficient in terms of enhancing the genetic stability of populations and is economically expensive.

4.2 Lower Limiter Control (LLC)

A numerical comparison of six control methods had concluded that LLC was the optimal control method under most (though not all) circumstances (Tung et al., 2014). Therefore, we investigated LLC under slightly more stringent conditions than ULC. Since under most real-life conditions, one is forced to operate on estimates of population sizes, rather than precise

counts, we explicitly incorporated some degree of imprecision in our implementation of LLC. For this, rather than using the exact counts of female numbers in the population, we followed a previous protocol (Dey and Joshi, 2006) and estimated the number of females under the assumption of 1:1 sex ratio. Since this assumption will evidently be not met in every generation, some amount of estimation error is built into the experimental procedure.

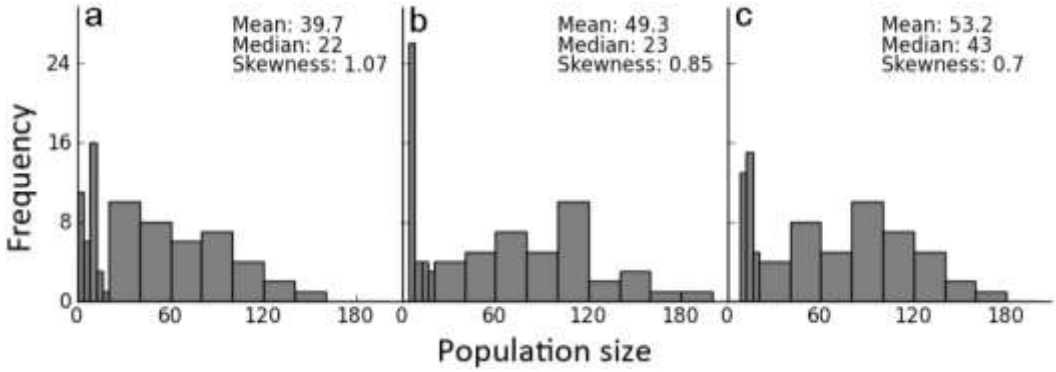

Fig. 3.6 Population size distributions of the unperturbed and LLC-treated populations: (*a*) Unperturbed (C2), (*b*) L1 (lower threshold=4), (*c*) L2 (lower threshold=10). When the population size is between 0-20, bin size is 4 and 20 otherwise. LLC treatments did not have a major effect on the population size distribution.

Population distribution and constancy stability: LLC directly prevents population sizes from going below a threshold without placing any restrictions on how high they can get. Consequently, although the population never hits the lower values in Fig. 3.6b-c, there is not much change in the long right hand tail, and the skew values reduce more slowly with increase in the threshold. Compared to ULC (Fig. 3.1b-c), the spread remains considerably wider. Not surprisingly therefore, the FI of neither L1 nor L2 were significantly different from the corresponding unperturbed population (Fig. 3.7a). This failure to reduce FI could indicate that LLC cannot enhance constancy in real populations. It could also be attributed to the fact that we incorporated some degree of imprecision in the application of LLC. Either way, our results indicate that a lot more empirical studies are needed to establish the efficacy of LLC in stabilizing biological populations. Interestingly, the FI of the L1 populations was larger than that of C2 populations but that of L2 was lower than both C2 and L1. This increase in FI for small values of restocking has been observed in numerical simulations of LLC (Tung et al., 2014) and other restocking methods like ALC (Franco and Hilker, 2013; Sah et al., 2013) and pinning (Tung et al., 2014) and it is tempting to wonder whether this is a generic property of all restocking methods. However, much more theoretical work would be needed to establish the reason for this observation.

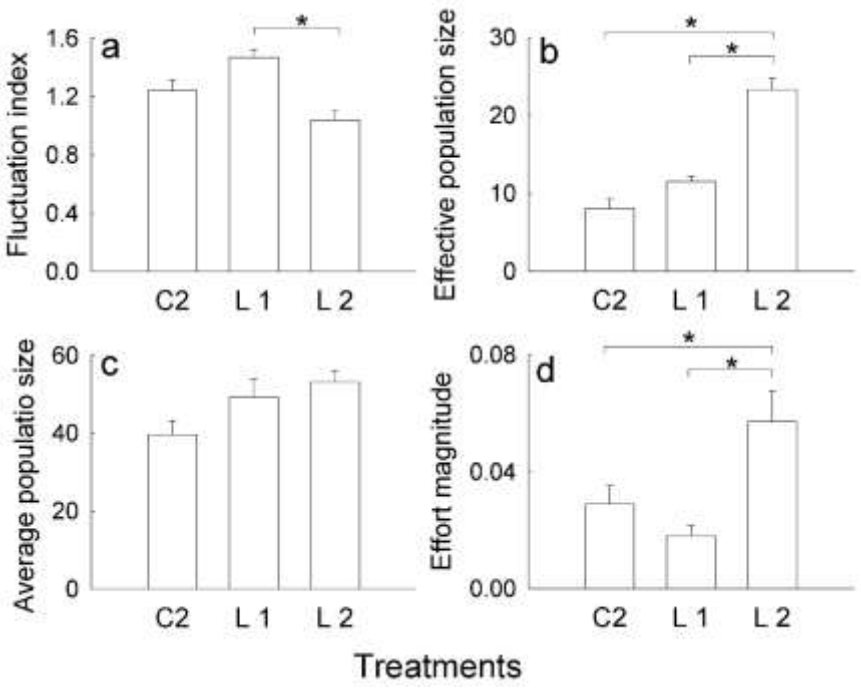

Fig. 3.7 Dynamics after applying LLC. C2 represents the unperturbed populations while L1 and L2 stand for LLC thresholds of 4 and 10 respectively. Each bar represents a mean over 5 replicate populations. Error bars denote standard error around the mean and * denotes $P < 0.05$. (*a*) Fluctuation index: Although there is a significant difference between L1 and L2, neither of them is significantly different from C2. L2 significantly increased (*b*) Effective population size but none of the LLC treatments affect (*c*) Average population size significantly. (*d*) Effort magnitude: L2 required significantly more external perturbation than both C2 and L1

Effective and average population size: Although LLC treatments could not enhance the constancy stability, L2 increased the N_e significantly (Fig. 3.7b). This is important as low N_e, and thereby enhanced chance of loss of genetic diversity, has been shown to have detrimental effect on population sustenance (Newman and Pilson, 1997). However, this attainment of higher N_e is trivial as restocking methods increases the harmonic mean by ensuring that the population size never goes below a lower threshold. More crucially, in spite of restocking, there was no statistically significant effect of LLC on the average population size (Fig. 3.7c) which contradicts earlier theoretical predictions (Hilker and Westerhoff, 2005). Interestingly though, the effect sizes of all pair-wise differences were large (Table 3.2) and Fig. 3.7c does reveal an increasing trend in average population size with increase in intensity of LLC. The lack of statistical significance is better explained by the presence of large amount of variation among replicates in the L1 and L2 treatment, which might be an artefact of the noise incorporated during census. Thus the effect of LLC on average population size merits further investigation.

Effort magnitude: The effort magnitude required for LLC is much, much less compared to that of ULC (*cf* Fig. 3.2d and 3.7d and note the difference in scale). Unfortunately, the two values are not directly comparable here, since they lead to different amounts of constancy stability. However, an earlier comparison of control methods have shown that for attaining comparable levels of stability, LLC typically requires the least amount of effort (Tung et al., 2014). This leads to the possibility that the performance of LLC can be probably improved significantly without incurring too much cost in terms of effort magnitude.

Persistence: Although LLC failed to enhance constancy, it had a significant effect in terms of persistence (Fig. 3.5b) which was almost comparable to that of ULC (*cf* 3.5a and 3.5b). *Prima facie*, this is counterintuitive as LLC does not involve culling and hence cannot reduce the frequency of population crashes to zero or very low numbers. This in turn means that it is not expected to reduce extinction probability which was scored before the imposition of perturbation. The discrepancy gets resolved when we recall that there are two ways in which a population can go extinct. The first is when the numbers in generation *t* crash to zero due to overpopulation in generation *t-1*. The second is when overpopulation in generation t-1 causes the numbers to become very low in generation t, which in turn leads to extinction in generation *t+1*. While LLC cannot ameliorate the first kind of extinction, it can reduce the probability of the second kind due to restocking by fecund individuals. This is the converse of ULC, which can only reduce the first kind of extinction but not the latter type. This leads to the prediction that the optimal method to promote persistence would depend on the kind of extinction suffered by the target population. For any population which, like our *Drosophila* cultures, suffer from both kinds of extinctions, a control method that incorporates both culling and restocking would be the most effective in terms of promoting persistence.

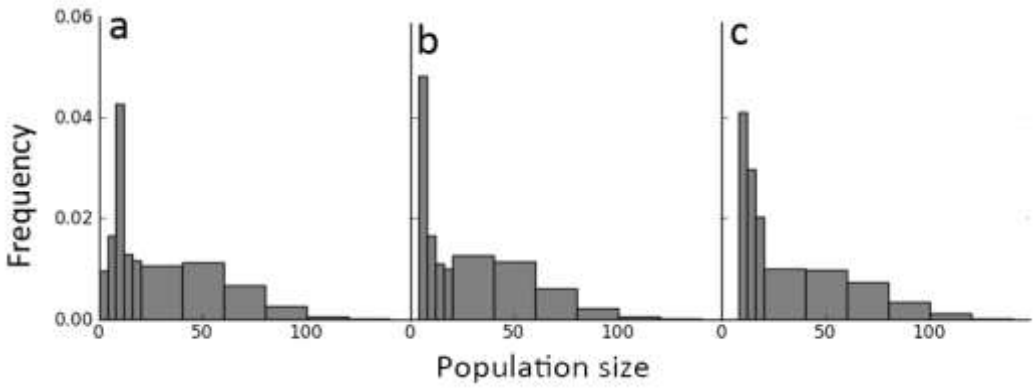

Fig. 3.8 Population size distributions of the simulated unperturbed and LLC-treated populations: (*a*) Unperturbed (C2), (*b*) L1 (lower threshold=4), (*c*) L2 (lower threshold=10). When the population size is between 0-20, bin size is 4 and 20 otherwise.

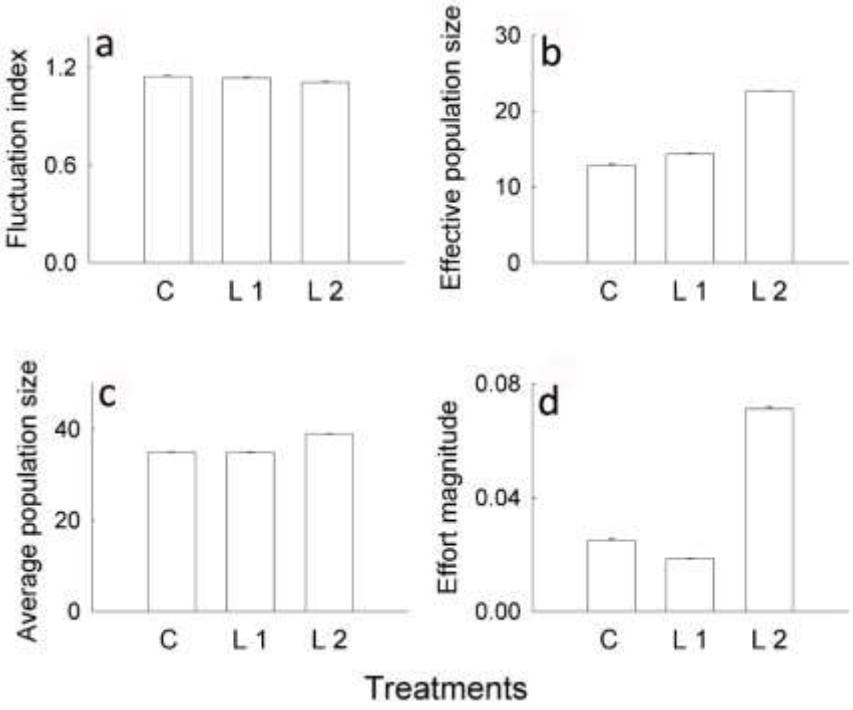

Fig. 3.9 Simulated dynamics after applying LLC. C2 represents the unperturbed populations while L1 and L2 stand for LLC thresholds of 4 and 10 respectively. Each bar represents a mean over 100 replicate populations. Error bars denote standard error around the mean. (*a*) Fluctuation index, (*b*) Effective population size, (*c*) Average population size and (*d*) Effort magnitude.

To summarize, the present experiments are inconclusive about the effects of LLC on constancy. However, it is clear that LLC boosts effective population size and persistence while requiring relatively less effort.

4.3 Caveats

Several caveats need to be considered while extrapolating our results to natural conditions. This study was conducted on *Drosophila* populations maintained under discrete generation cycles. This allowed us to test predictions emanating from discrete generation models like the Ricker map. The results of our study are therefore relevant for those species whose populations undergo non-overlapping generation cycles in nature. However, populations of several species in nature exhibit overlapping-generations which are sometimes age/stage structured. Such populations often have very different dynamics from those represented by simple maps (Caswell, 2001), which may or may not be controlled by methods like ULC or LLC.

In our study, we consider culling and restocking to be equivalent in terms of effort magnitude, which is clearly a simplifying assumption. Under many circumstances, killing individuals in a population might be less expensive than reintroduction from a source and vice versa. In all those cases, the effort magnitude needs to be suitably scaled to arrive at the actual economic cost. Unfortunately, this issue must be handled on a case-by-case basis, depending on the biology of the species and the available infrastructure, and it is impossible to incorporate those nuances in a general study like ours. Another fact not considered here is that the economic cost of census also might be very different for ULC and LLC. In the former case, the entire population needs to be counted while in the latter case, census effort is restricted to the point that the threshold number of organisms is cited. Furthermore, although we have calculated effective population size solely as a function of the census size, in reality, culling and restocking (and hence ULC and LLC) differ in how they affect the loss of genetic variation in a population. This is because if the individuals used in restocking belong to a different gene pool, then there would be relatively lower loss of genetic variation than what is expected based on the values of harmonic means. Simulation (Lacy, 1987), laboratory (Ball et al., 2000) and field (Vilà et al., 2003) studies suggest that even a small amount of immigration is sufficient to maintain / restore heterozygosity in populations. Thus, assuming the availability of a genetically diverse source population, LLC is a fundamentally superior method in terms of maintaining genetic diversity, which is consistent with our conclusions about genetic stability based on the harmonic mean of the population sizes. Finally, we have only examined the effects of ULC and LLC on spatially-unstructured populations of a given species, whereas most natural populations exist in spatially-structured communities of many species. Thus, several factors need to be taken into account before our experimental results can become usable in real-life scenarios. However, given that erroneous application of control methods can lead to ecological disasters (Pyke, 2008), studies such as ours can help in bridging the gap between theory and practical applications.

5. CONCLUSIONS

Our empirical results suggest that ULC is an efficient method in terms of reducing fluctuations as well as extinction probability. However, it does not increase the effective population size and needs fairly large magnitude perturbations. On the other hand, although the efficacy of LLC in reducing population fluctuations is equivocal, it increases effective

population size, reduces stochastic extinction probability and requires relatively less effort. Thus, the choice of methods under a given condition would depend upon the aspect of the dynamics that needs to be stabilized. We also investigated the generalizability of our empirical results. Our Ricker-based simulations did not contain any species-specific parameters, and yet were able to corroborate most of the empirical observations. This indicates that our experimental results are not due to some specific features of *Drosophila* biology, and hence, likely to be broadly applicable. It has been theoretically shown that populations that experience scramble competition and are randomly distributed over space, follow the Ricker dynamics (Brännström and Sumpter, 2005). Since large number of organisms belonging to diverse taxa fulfil these two conditions, our results are likely to be relevant in wide-ranging scenarios. However, there are several caveats to our results, and any extrapolation to real-life scenarios must be supplemented by relevant system-specific information.

CHAPTER 4

Population stability through Both Limiter Control (BLC) and Target Oriented Control (TOC)

Highlights

- First empirical validation of methods: BLC and TOC.
- Both methods stabilize populations and reduce extinction propensity simultaneously.
- Application of these methods also results in a higher effective population size.
- Non-Drosophila specific simulation results agree well with the data.

Adapted from: **Tung, S.**, Mishra, A., Dey, S. 2016. Simultaneous enhancement of multiple stability properties using two-parameter control methods in Drosophila melanogaster. Ecological Complexity 26, 128–136.

1. INTRODUCTION

Although several methods have been proposed for stabilizing biological populations over the last two decades (e.g. Dattani et al., 2011; Güémez and Matías, 1993; Hilker and Westerhoff, 2005; McCallum, 1992; Sah et al., 2013), few (if any) have been actually applied for conserving threatened populations. This gap between theory and application has multiple putative reasons. Firstly, in ecology, the term stability can refer to many concepts (Grimm and Wissel, 1997) and most theoretical studies typically restrict to any one of them. However, in any real world usage, the adopted control method must be able to simultaneously stabilize multiple aspects of the dynamics. Thus, for example, a method that reduces fluctuations in population sizes, but has relatively less impact on extinction probability, is of limited utility. Since different aspects of stability often do not correlate with each other (Dey and Joshi, 2013; Dey et al., 2008), choosing a method often becomes problematic.

To complicate matters further, most control methods proposed in the theoretical literature lack adequate empirical (i.e. ones that use real biological populations as opposed to computer simulations) verification. Some of the most well-known empirical studies on population control deal with methods that either require high levels of mathematical sophistication (e.g. Desharnais et al., 2001) or very detailed models of the system (Becks et al., 2005). The high degree of specificity of these studies can sometimes make it difficult to extend their insights into controlling other systems. Moreover, such studies (Becks et al., 2005; Desharnais et al., 2001) often deal with amelioration of chaos or characterization of the attractor, whereas the primary concern of most conservation biologists would be to prevent inbreeding or reduce extinction probability. Consequently, such empirically well-characterized control methods turn out to be of limited relevance for most real-world applications. As stated already, much of the proposed control methods have never been investigated using biological populations. Given that the survivals of species are at stake, the reluctance of the practitioners in adopting untested methods for controlling natural populations is well justified. The only way to bridge this gap between theory and practise is to empirically verify the control methods proposed in the literature under conditions that are as close to their conditions of intended use as possible. Clearly, methods that require relatively less system-specific information and are easier to implement (e.g. Gusset et al., 2009; McCallum, 1992), are likely to be more useful in this context. One such set of control methods is the so-called limiter class of methods.

Broadly speaking, the limiter methods do not allow the populations to go above or below (depending on the method) a pre-determined threshold through culling or restocking respectively. Recent empirical studies have shown that such methods can typically reduce either fluctuations in population sizes or extinction probability, but not both (Sah et al., 2013; Tung et al., 2016). This observation led to the conjecture that methods which involve both restocking and culling might prove to be more successful in simultaneous control of multiple aspects of stability. A well-studied method of this type is the Target-Oriented Control (TOC) (Braverman and Chan, 2014; Braverman and Franco, 2015; Dattani et al., 2011; Franco and Liz, 2013), which is a modification of the traditional proportional feedback method (Güémez and Matías, 1993; Liz, 2010). In TOC, the current population size is perturbed towards a predetermined 'target' by culling or restocking (Dattani et al., 2011; Franco and Liz, 2013). The magnitude of the perturbation is determined based on the difference between the pre-perturbation population size and the target value. Theoretical studies have shown that TOC globally stabilizes higher order difference equations (Braverman and Franco, 2015) and is particularly useful in those cases where the population size needs to be manipulated towards a pre-determined value (Dattani et al., 2011).

Another method that involves both culling and restocking is the recently proposed Both Limiter Control or BLC, which involves setting an upper and a lower threshold *a priori* (Tung et al., 2014). Each time the population size is outside the range set by these thresholds, appropriate culling or restocking is implemented to bring the size back to the upper or the lower threshold respectively. It has been shown numerically that BLC can protect populations from overcrowding and extinction risk due to demographic stochasticity (Tung et al., 2014). Further, both TOC (Fig 1 of Dattani et al., 2011) and BLC (Figure 4.1) can suppress the complex chaotic dynamics of a system to a stable point or limit cycles. However, till date, there has been no empirical investigation of how these two control methods affect the dynamics of real biological populations.

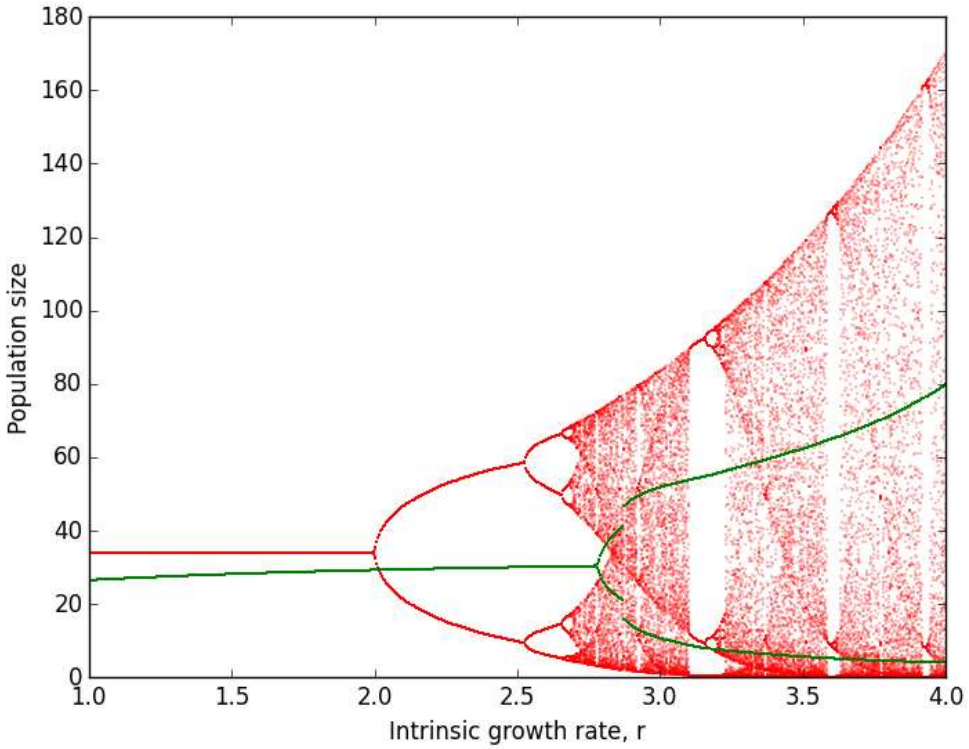

Figure 4.1. Effect of BLC on the long-term dynamics of a single, spatially unstructured population using Ricker model. Here N_0 =10 and K=34. The red scatter plot shows the classic period-doubling route to chaos exhibited by the Ricker model in the absence of any external perturbations. The green scatter plot indicates the same except here BLC method had been applied with upper and lower threshold on half of the population size being 10 and 4 respectively (same as the experiment here). Thus, in presence of BLC, the complex dynamics of the system gets suppressed and it largely becomes a two-point limit cycle with much reduced amplitude of population size fluctuations.

In this study, we investigate the effects of BLC and TOC in stabilizing the dynamics of spatially-unstructured laboratory populations of *Drosophila melanogaster*. Both these methods were found to be capable of inducing significant reduction in fluctuations in population sizes and extinction probability. Moreover, both methods also significantly increased the effective population sizes. However, the good performance of BLC and TOC came at the cost of a significantly large effort magnitude, which is likely to translate into relatively high economic expenditure. We also derive biologically intuitive understandings of how the control methods work by comparing the distribution of population sizes with and without control. Finally, we show that simulations using a biologically realistic, non-*Drosophila*-specific model, can capture most of the trends of our experimental results. This suggests that our observations are likely to be generalizable over a wide range of taxonomic groups.

2. METHODS

2.1 Experimental populations

In this study, we used 20 single vial populations of *Drosophila melanogaster* derived from a large outbred population, called DB_4. DB_4 population was maintained on a 21-day discrete generation cycle, as detailed elsewhere (Sheeba et al., 1998). Each of the 20 single vial populations used for this experiment were initiated by collecting 10 eggs on 1.1 ml of standard banana-jaggery medium and reared in an incubator at 25° C temperature under constant light. Once the adults started eclosing in a vial, they were transferred daily into corresponding adult-holding vials containing ~6ml of medium. The correspondence between an egg-collection vial and its adult-holding vial was strictly maintained. On the 18^{th} day from the day of egg collection, the flies were supplied with live yeast paste to boost fecundity. Three days later, the adults were counted under mild CO_2 anaesthesia and culling or restocking were implemented wherever necessary as per the protocol of the corresponding control method (see section 2.2). After this, the adults were put into fresh vials containing 1.1 ml food for oviposition. After 24 hours, the adults were discarded and the eggs formed the next generation. The experiment lasted for 14 generations.

2.2 Control methods and performance indices

Both Limiter Control (BLC): In BLC, the population size is not allowed to go beyond predetermined upper and lower threshold values (Tung et al., 2014). Mathematically, this can be represented as $N_t' = max(min(N_t, U), L)$, where N_t and N_t' are population sizes before and after the application of the control method, U and L are the pre-determined values of the upper and lower thresholds, and *max* and *min* denote the maximum and minimum operators. Here, we arbitrarily chose the upper and lower thresholds as 4 and 10 females respectively. Since the dynamics of sexually reproducing species are primarily driven by the number of females in the population, we restricted the implementation of the control to the females. In other words, when the number of females in a given generation was less than 4 or more than 10, BLC was applied by restocking to 4 females or culling to 10 females respectively.

Target Oriented Control (TOC): In TOC, the population size is nudged towards an *a priori* fixed target value (Dattani et al., 2011). It is a two-parameter control method which can be mathematically represented as $N_t' = N_t + c_d \times (T - N_t)$, where N_t and N_t' are population sizes before and after the application of TOC and T denotes the target population size. The

parameter c_d (arbitrarily set to 0.7 here) represents the fraction by which the difference between the target (set arbitrarily to 30) and current population size is restocked or culled. Thus, in our experiment, when the population size exceeds 30, 70% of the excess individuals are culled and when population size is below the target, 70% of the difference in number of individuals is added to the population.

Since earlier theoretical studies (Dattani et al., 2011; Tung et al., 2014) had suggested TOC to be a very effective control method, we decided to test it under somewhat more stringent conditions than BLC. For this, we incorporated some degree of imprecision in the implementation of the control method. Modifying the protocol of an earlier study (Dey and Joshi, 2006), in this study we estimated the number of females to be added or removed as *floor* $[0.5 \times c_d \times (T - N_t)]$, where *floor [x]* denotes the function leading to the largest integer not greater than x. This way of calculating the magnitude of the control assumes an equal sex ratio which will not be the case in every generation, thus introducing some noise in the implementation.

We used three measures of stability, namely constancy, persistence and effective population size. Constancy stability (Grimm and Wissel, 1997) of populations refers to the magnitude of temporal fluctuations in population sizes: population that have larger fluctuations have lesser constancy stability and vice versa. Persistence stability of a population is a measure of its probability of extinction (Grimm and Wissel, 1997) such that higher extinction probability denotes lower persistence stability and vice versa. Effective population size (N_e) is an indicator of how fast a population is expected to lose its genetic variation and thus, is a measure of its genetic stability (Hare et al., 2011). Following a previous study (Hilker and Westerhoff, 2005) we estimated the effort magnitude, which is a proxy for the cost of implementation of the control methods. This quantity computes the number of individuals externally added or removed per generation. Effort frequency was measured as the proportion of generations external perturbation was required. All performance indices (except extinction probability) were computed on the time series of the breeding population (i.e. after the implementation of restocking/culling). The mathematical expressions for all the indices are given in Table 4.1.

Table 4.1: Performance metrics and their mathematical expressions

Performance metric	Index	Formula	References for formula
Constancy stability	Fluctuation index (FI)	$FI = (1/(T \times \bar{N})) \times \sum_{t=0}^{T-1} \lvert N'_{t+1} - N'_t \rvert$	Dey & Joshi 2006
Persistence stability	Extinction probability (EP)	$EP = t_{ex}/T$	-
Genetic stability	Effective population size (N_e)	$N_e = T/\sum_{t=1}^{T} 1/N'_t$	Allendorf & Luikart 2007
Abundance	Average population size ($\bar{N}$)	$\overline{N} = \sum_{t=1}^{T} N'_t \Big/ T$	-
Economic Cost	Effort magnitude (EM)	$EM = (1/(T \times \bar{N})) \times \sum_{t=1}^{T} \lvert N_t - N'_t \rvert$	Hilker & Westerhoff 2005; Sah *et al.* 2013
Economic Cost	Effort frequency (EF)	$EP = t_{effort}/T$	-

*N_t and N'_t are the population sizes before and after external perturbation in the t^{th} generation respectively. t_{ex} and t_{effort} are total number of extinction events and the number of generations when external perturbation was required respectively. T denotes the length of the time series.

2.3 Unperturbed populations and experimental replicates

Apart from the BLC and TOC lines, we also had two sets of populations that were kept unperturbed, and maintained as mentioned in section 2.1. These two sets were called C1 and C2, and they served as unperturbed reference populations for BLC and TOC respectively. This pairing was done at the time of the experimental set up. The dynamics of the C1 and C2 populations have already been reported previously (Tung et al., 2016). When the C1 or C2 populations went extinct, they were reset by adding 4 males and 4 females from outside, which contributed to the effort magnitude (see below) of these populations. There were no needs for separate resets in the BLC and TOC lines, since the control methods automatically ensured that the extinct populations were rescued. All flies that were used for restocking were

maintained as mentioned in Appendix S1, except for the fact that they were provided with ~5 ml of larval food. Overall, there were four treatments (BLC, TOC, C1 and C2) in this study and each consisted of 5 replicate populations.

2.5 Statistical analyses

For statistical analyses, BLC and TOC were compared against the corresponding reference populations, C1 and C2, respectively. The data for the various indicators of stability along with the effort magnitude and frequency were subjected to separate one-way ANOVAs using STATISTICA®v5 (StatSoft. Inc., Tulsa, Oklahoma).We also computed the effect size of the difference between the means as Cohen's *d* (Cohen, 1988) using a freeware Effect Size Generator (Devilly, 2004). Following standard recommendations (Cohen, 1988), the value of effect size (*d*) was interpreted as large, medium and small when $d>0.8$, $0.8>d>0.5$ and $d<0.5$ respectively.

2.6 Simulations

In order to test the generalizability of our empirical results, we used the Ricker model (Ricker, 1954) for biologically-realistic simulations of the dynamics of the unperturbed populations. This model is given as $N_{t+1}=N_t*exp(r*(1-N_t/K))$, where *r, K* and N_t denote intrinsic growth rate, carrying capacity and population size at time *t* respectively. The two control methods, Both Limiter Control (BLC) and Target Oriented Control (TOC) were imposed according to the following mathematical form (Tung et al., 2014):

BLC: $N'_t = max\,[L, min\,[N_t, U]]$

TOC: $N'_t = max\,[0, c_d \times T + (1 - c_d) \times N_t)]$

where N_t and N'_t are the population sizes before and after perturbation in the t^{th} generation. $N_{t+1} = f(N'_t)$, where *f* stands for Ricker model. *U* and *L* are the upper and lower thresholds of BLC while T and c_d represent target and control intensity of TOC respectively. *min[]* and *max[]* stand for minimum and maximum operators.

<u>*Parameter values:*</u> We fit the Ricker model to the unperturbed experimental time-series (C1 and C2) and obtained a mean *r* and *K* of 2.6 and 34 respectively. These values of *r* and *K* were then used to simulate the dynamics of unperturbed and controlled populations. Matching the experimental values, upper (*U*) and lower (*L*) threshold for females in BLC

were kept at 10 and 4 whereas the target (T) and control intensity (c_d) for TOC were fixed at 30 and 0.7 respectively.

Stochasticity and lattice effect: Since noise can significantly influence the dynamics of perturbed populations (Dey and Joshi, 2007), we incorporated noise to both the parameters, *r* and *K*, in each iteration. Following an earlier study (Tung et al., 2016) we picked the *r* and *K* values from uniform distributions of 2.6 ±0.5 and 34±15 respectively. Real organisms always come in integer numbers (lattice effect, as described in Henson et al., 2001), a constraint that can potentially affect the dynamics of the systems. We incorporated the lattice effect in our simulations by rounding off the number of organisms as well as the values of the perturbations in each generation to the nearest integer less than the actual value. The Ricker model, when initiated with a non-zero value, does not take zero-values and hence can never show extinctions. However, the same is not true for the integerized model. Therefore, we set reset rules similar to the experiment for our simulations: when the unperturbed populations went extinct, the population size was reset to 8. No resets were necessary for the simulations incorporating BLC and TOC as both control methods involved restocking steps.

The magnitudes of external perturbation to be applied were computed by assuming a 1:1 sex ratio. Thus, for BLC, the control method was implemented only when half the population size fell below 4 or exceeded 10. For TOC, the amount of perturbation was estimated by subtracting the population size before perturbation (N_t) from the calculated post perturbation population size (N'_t) and then only half of the calculated perturbation value was actually realised in each generation.

To summarize, the sequence of steps in the simulation is given as:

$$N'_{t-1} \rightarrow [FUNC] \rightarrow [INT] \rightarrow [EXT] \rightarrow N_t \rightarrow [CTRL] \rightarrow N'_t$$

where N'_t and N_t are the population sizes after and before application of perturbation in the t^{th} generation, and *FUNC, INT, EXT* and *CTRL* represent the population recursion (here Ricker model), integerization, stochastic extinction and control (here BLC and TOC) functions respectively. The initial population size for all the simulations was set to 10, which was the same as the starting population size in the experiments. Each simulation was run for 100 iterations. From the resulting time series, fluctuation index, extinction probability, average population size, effective population size, effort magnitude and effort frequency were computed. All calculations, except extinction probability, were performed on the time series of the N'_t values. All figures (Fig. 4.7 and 4.8) represent means over 100 independent

replicates for each treatment and the error bars represents standard error around the means. This means that our simulations represent the dynamics of the populations over a longer time scale and larger number of replicates than what was performed in the experiments. This was important to study the long term behaviour of the populations and to ensure that our experimental results are not mere statistical artefacts. However, it is also important to ascertain whether we get back the same results if the simulations are repeated for the same number of replicates and number of generations as our experiments. Therefore, we also repeated all simulations for 14 generations and 5 replicates. Since there were no qualitative differences between the two cases, we chose to report the former set of simulations (i.e. 100 iterations and 100 replicates) here, as they represent the dynamics over a slightly longer timescale.

3. RESULTS

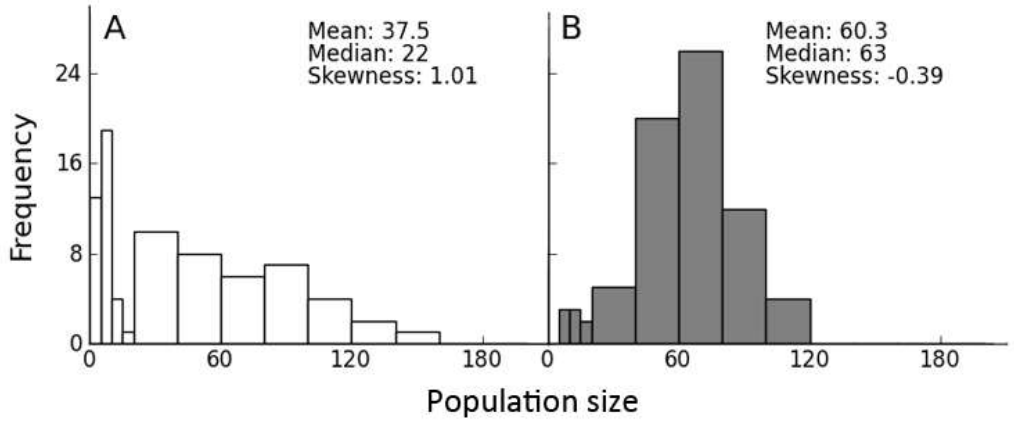

Figure 4.2. Population size distributions for unperturbed and BLC populations. A. Unperturbed (C1). **B.** BLC populations. Each distribution was plotted by pooling the data from all 5 populations over 14 generations. The bin size is 5 when the population size is in the range 0-20, and 20 otherwise. BLC made the distribution more symmetric and increased the measures of central tendencies (mean and median).

3.1 Both Limiter Control (BLC)

In the absence of control (*i.e.* for C1), population size distribution was observed to be positively skewed with a long right hand tail (Fig. 4.2A). When BLC was applied, the distribution became more symmetric with a higher mean and higher median population size (Fig. 4.2B). The absence of extreme values was reflected in terms of constancy and persistence. Compared to the C1 populations, the BLC populations had significantly reduced population fluctuation (Fig. 4.3A; $F_{1,8}$=56.83, p = 0.00007, d = 4.77) as well as extinction probability (Fig. 4.3B; $F_{1,8}$=26, p=0.0009, d= 3.23). Thus, both constancy and persistence

stability were significantly enhanced and so were effective population size (Fig. 4.3C; $F_{1,8}$=18.65, p = 0.003, d = 2.73) and average population size (Fig. 4.3D; $F_{1,8}$=23.21, p=0.001,d = 3.05). However, there was a steep cost to the attainment of this stability. BLC required significant effort magnitude (Fig. 4.3E, $F_{1,8}$=574.56, $p < 10^{-8}$,d= 15.16) and perturbations happened in almost every generation (Fig. 4.3F). Interestingly, although BLC involves both culling and restocking, here we found that culling events occurred more frequently than restocking ones (Fig. 4.4B) and are larger in magnitude (Fig. 4.4A). All the differences in means reported for BLC had large effect sizes (i.e. d > 0.8, see Table 4.2 for 95% CI around d).

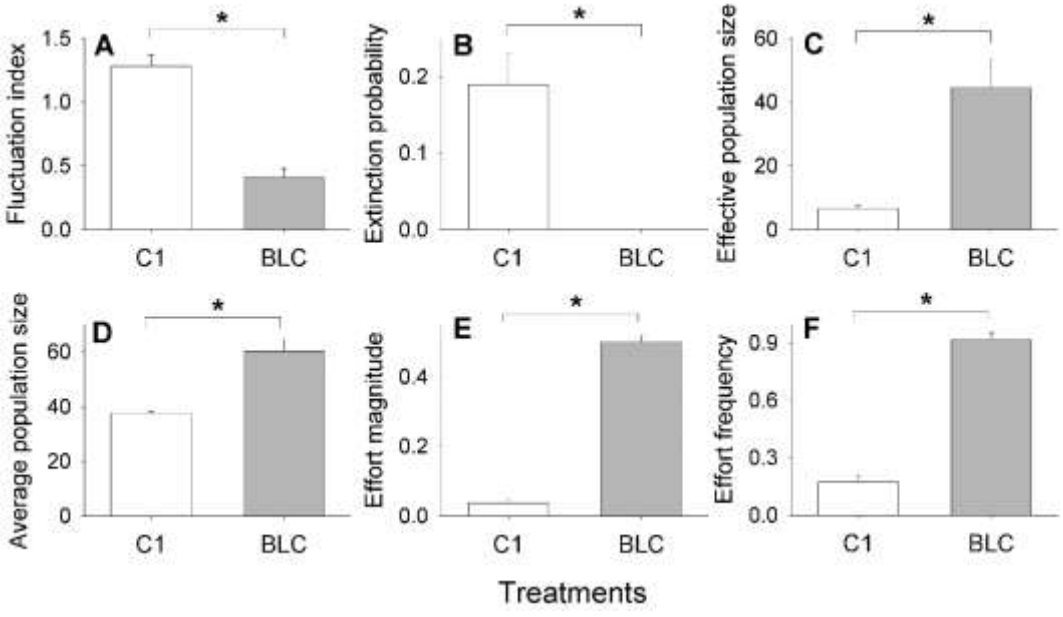

Figure 4.3. Empirical results for the effects of BLC. C1 represents corresponding unperturbed populations. Each bar represents a mean over 5 replicate populations. Error bars denote standard error around the mean and * denotes statistical significance (p< 0.05). BLC decreased **A. Fluctuation index** and **B. Extinction probability** significantly. It increased **C. Effective population size** and **D. Average population size** although at the cost of significantly high **E. Effort magnitude** and **F. Effort frequency**. See text for possible explanations.

3.2 Target Oriented Control (TOC)

The population size distribution of C2, like C1, had a long right hand tail (Fig. 4.5A). TOC prevented population sizes from taking extreme values, which made the distribution more symmetric with higher mean and median values (Fig. 4.5B). Thus, not surprisingly, TOC also increased both constancy and persistence stability by significantly decreasing fluctuation index (Fig. 4.6A; $F_{1,8}$= 31.16, p= 0.001, d= 3.5) and extinction probability (Fig. 4.6B;$F_{1,8}$= 32, p= 0.0005,d= 3.58) respectively. Interestingly, although TOC increased the effective population size of the controlled populations significantly (Fig. 4.6C; $F_{1,8}$= 30.7, p= 0.001, d= 3.5), it had no effect on the average population size (Fig. 4.6D; $F_{1,8}$=0.13, p=0.73, d= 0.23). Like BLC, the stability attained by TOC came at a high cost in terms of effort magnitude (Fig. 4.6E, $F_{1,8}$=

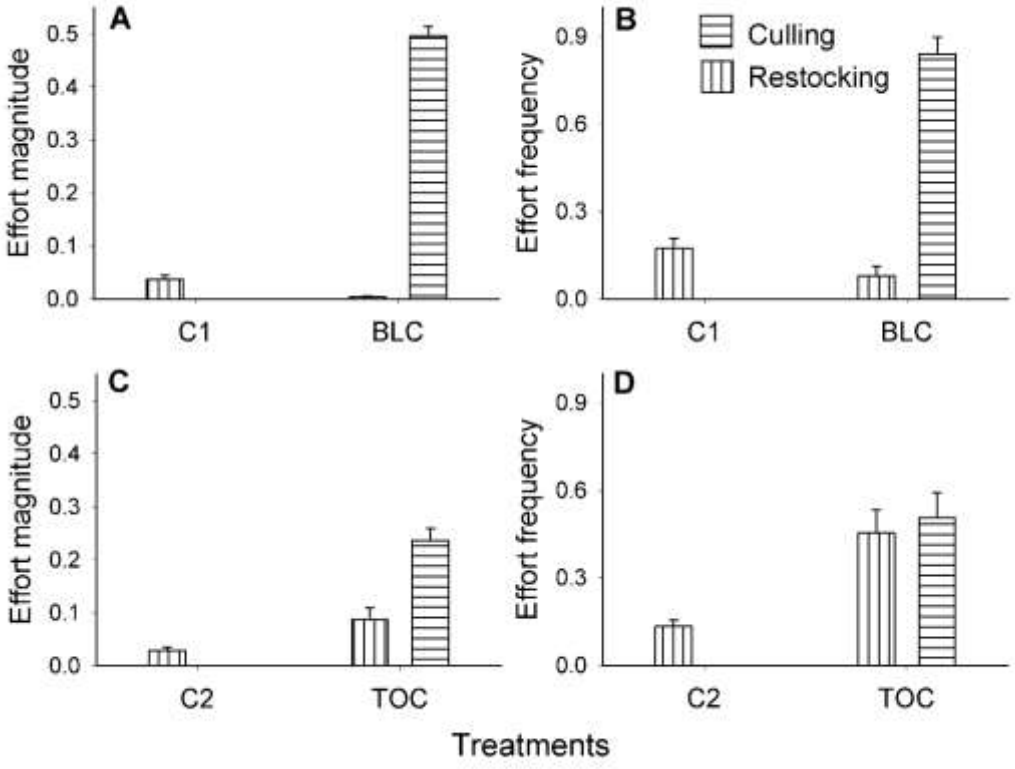

Figure 4.4. Magnitude and frequency of culling and restocking perturbation. Each bar represents a mean over 5 replicate populations and error bars denote standard error around the mean. C1 and C2 represent the unperturbed populations for BLC and TOC respectively. BLC mainly incurred culling perturbation w.r.t. both **A. Effort magnitude and B. Effort frequency**.TOC incurred more culling for. **C.Effort magnitude**, but similar amount of **D. Effort frequency** (see text for possible explanation and implications).

921.16, $p < 10^{-8}$, d= 19.2) and frequency (Fig. 4.6F, $F_{1,8}$= 591.4, $p < 10^{-8}$, d= 15.4). Unlike BLC though, in TOC, culling and restocking happened with almost similar frequency (Fig. 4.4D) and the effort magnitude for culling was only slightly greater than that for restocking (Fig. 4.4C). All the differences in means reported for TOC had large effect sizes (i.e. d> 0.8) except that for average population size, where it was found to be small (i.e. d< 0.5; see Table 4.3 for 95% CI around d).

Table 4.2. Summary statistics of the dynamics after applying BLC

BLC	Mean ±SE for C1	Mean ±SE for BLC	ANOVA F(1,8)	ANOVA p value	Effect size ± 95% CI	Inference
Fluctuation index	1.28± 0.09	0.41± 0.07	56.83	0.00007	4.77± 2.43	Large
Extinction probability	0.19± 0.04	0	26	0.0009	3.23± 1.88	Large
Effective population size	6.8± 0.67	44.6± 8.7	18.65	0.003	2.73± 1.72	Large
Average population size	37.5± 0.84	60.3± 4.68	23.21	0.001	3.05± 1.82	Large
Effort magnitude	0.04± 0.01	0.5± 0.02	574.56	$<10^{-8}$	15.16± 6.76	Large
Effort frequency	0.17± 0.03	0.92± 0.03	250.9	$<10^{-6}$	10.02± 4.6	Large

* C1 is unperturbed reference populations corresponding to BLC populations respectively. Cohen's d statistic (± 95% confidence interval) was considered as the measure for effect size and interpreted as small, medium and large for $0.2 < d < 0.5$, $0.5 < d < 0.8$ and $d > 0.8$ respectively.

Table 4.3. Summary statistics of the dynamics after applying TOC

TOC	Mean ±SE for C2	Mean ±SE for TOC	ANOVA F(1,8)	ANOVA p value	Effect size ± 95% CI	Inference
Fluctuation index	1.24± 0.08	0.69± 0.05	31.16	0.001	3.5± 1.98	Large
Extinction probability	0.13± 0.01	0.01± 0.01	32	0.0005	3.58± 2	Large
Effective population size	8.08± 1.33	25.4± 2.83	30.7	0.001	3.5± 1.97	Large
Average population size	39.7± 3.94	41.9± 4.6	0.13	0.73	0.23± 1.24	Small
Effort magnitude	0.03± 0.006	0.32± 0.007	921.16	$<10^{-8}$	19.2± 8.5	Large
Effort frequency	0.13± 0.02	0.96± 0.03	591.4	$<10^{-8}$	15.4± 6.9	Large

* C2 is unperturbed reference populations corresponding to TOC populations. Cohen's d statistic (± 95% confidence interval) was considered as the measure for effect size and interpreted as small, medium and large for $0.2 < d < 0.5$, $0.5 < d < 0.8$ and $d > 0.8$ respectively.

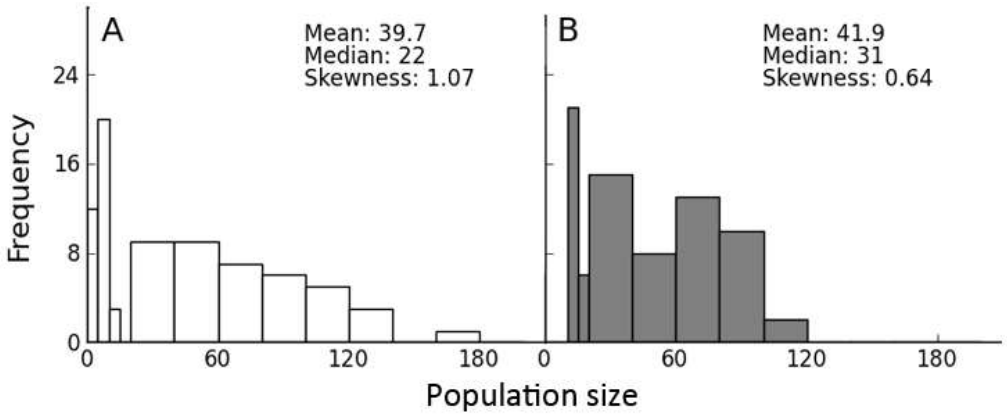

Figure 4.5. Population size distributions of the unperturbed and TOC populations. A. Unperturbed (C2). **B.** TOC populations. Each distribution was plotted by pooling the data from all 5 populations over 14 generations. The bin size is 5 when the population size is in the range 0-20, and 20 otherwise. TOC reduced the skewness and overall range of the population size distribution.

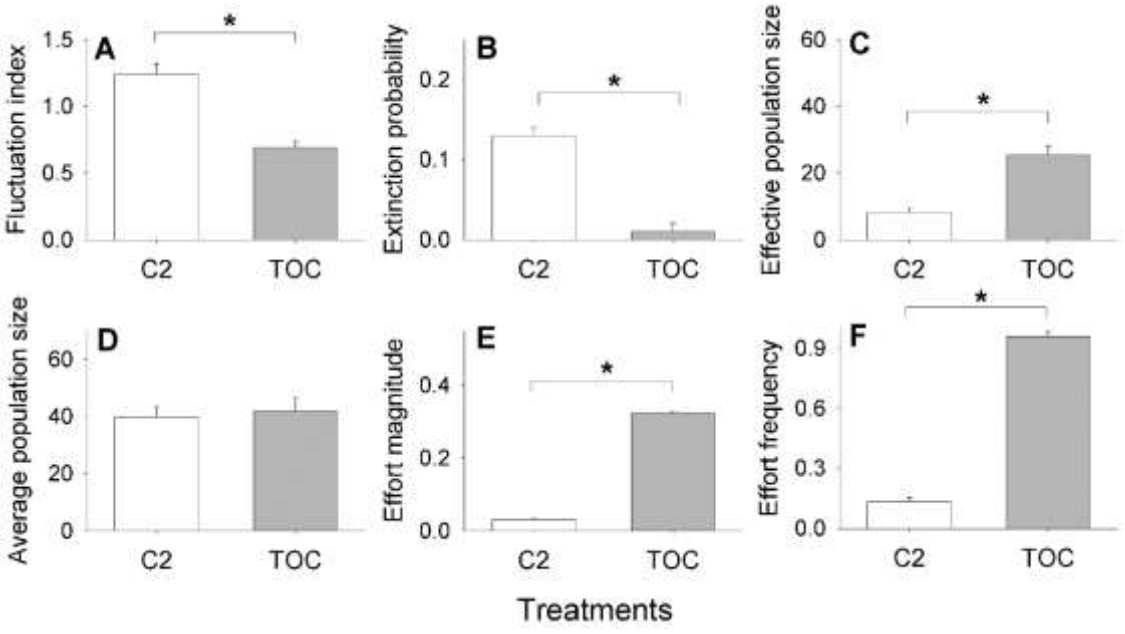

Figure 4.6. Empirical results for the effects of TOC. C2 represents the unperturbed populations. Each bar represents a mean over 5 replicate populations. Error bars denote standard error around the mean and * denotes $p < 0.05$. TOC decreased **A. Fluctuation index** and **B. Extinction probability** and increased **C. Effective population size** significantly, although it did not alter **D. Average population size.** TOC incurred significantly high **E. Effort magnitude** and **F. Effort frequency**. See text for possible explanations.

3.3 Simulations

For both BLC and TOC, the outputs of the simulations using Ricker model under biologically realistic conditions followed the same trends as the experimental results (*cf* Figs. 4.3 and 4.6 with Figs. 4.7 and 4.8 respectively). The sole exception to this observation was the average population size for BLC. In experimental populations, BLC had significantly higher average population size than the unperturbed populations, whereas in simulations they have similar values (*cf* Figs. 4.3D and 4.7D).

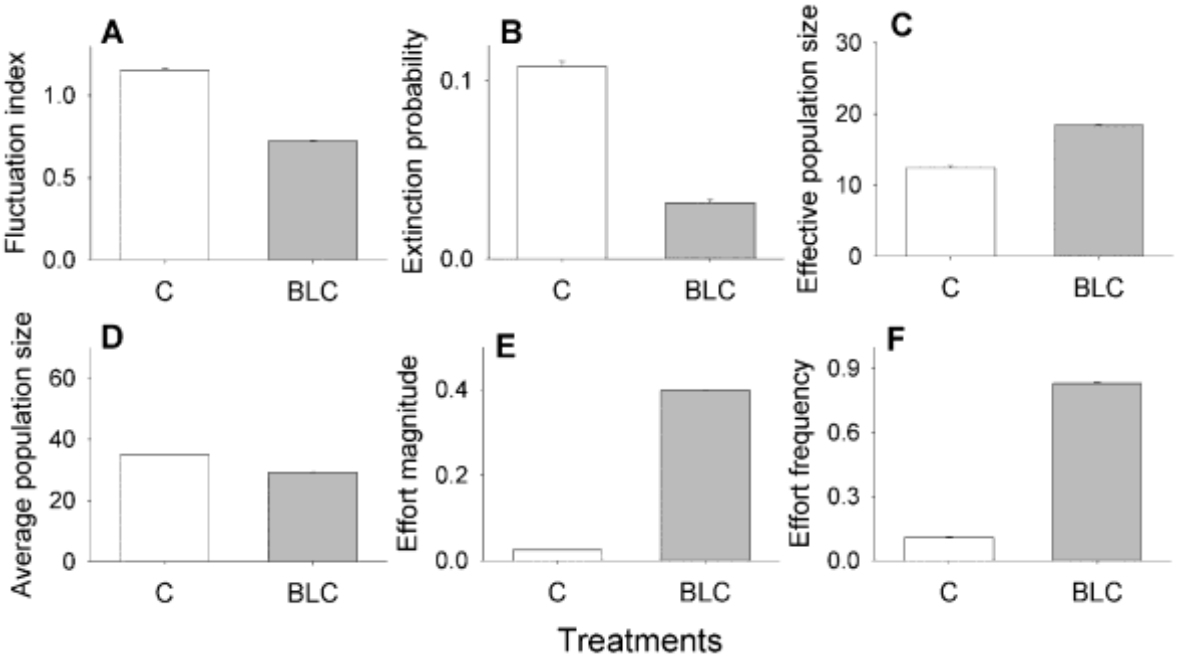

Figure 4.7. Simulation results for the effects of BLC. C represents unperturbed populations. Each bar represents a mean over 100 replicate populations and error bars denote standard error around the mean. **A. Fluctuation index**, **B. Extinction probability**, **C. Effective population size**, **D. Average population size**, **E. Effort magnitude** and **F. Effort frequency**.

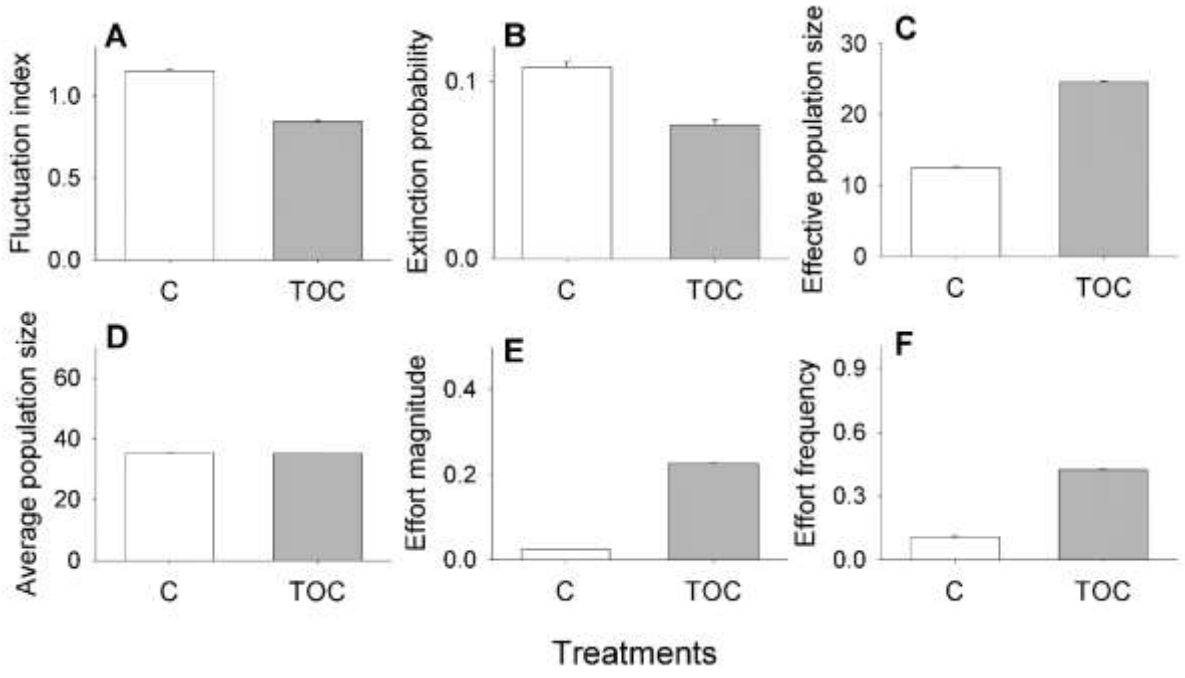

Figure 4.8. Simulation results for the effects of TOC. C represents unperturbed populations.Each bar represents a mean over 100 replicate populations and error bars denote standard error around the mean. **A. Fluctuation index**, **B. Extinction probability**, **C. Effective population size**, **D. Average population size**, **E. Effort magnitude** and **F. Effort frequency**.

4. DISCUSSION

Here we empirically studied the effects of two control methods in terms of inducing stability in unstable biological populations. However, it should be noted that this study does not concern itself with a quantitative comparison of the relative efficiency of these two methods and it is not possible for us to make statements like- "method 1 is 20% more efficient than method 2". This is because it has already been shown numerically that most control methods are capable of inducing any desired level of stability with the correct choice of the value of the control parameter (Tung et al., 2014). Thus, comparisons of relative efficiency are meaningful only when both methods are compared for similar values of a performance index, e.g. say a 50% enhancement of constancy as compared to the dynamics of the unperturbed populations (Tung et al., 2014). Clearly, in an experimental scenario, it is almost impossible to choose such parameter values *a priori* and therefore direct comparison of the efficiencies of the methods are improper. Hence, the goals of this study are to validate whether the methods can induce stability or not and to gain a biological understanding of the mechanisms by which these methods work. Thus, we prefer intuitive explanations based on how the

control methods affect the population size distributions, instead of mathematically rigorous theorems and lemmas on how stability is attained.

4.1 Both Limiter Control

Population size distribution and constancy: In the absence of any control, the distribution of population sizes had a long tail towards the right and about 49% of the values lay between 0-20 (Fig. 4.2A). As a result, the mean was much larger than the median which was also reflected as a high value for skewness (Fig. 4.2A). This kind of a distribution is a characteristic of populations undergoing high amplitude oscillations in sizes, which is known to be a feature of *Drosophila* populations subjected to a combination of low levels of larval nutrition and high levels of adult nutrition (Mueller, 1988; Mueller and Huynh, 1994). This is because high adult nutrition boosts the fecundity of the flies which allows them to overcome the negative effects of density-dependence on fecundity at high population densities in a given generation (say *t*). As a result, a large number of eggs are laid, which leads to an overcrowding in the larval population of the next generation (*t+1*). Now, if the amount of larval nutrition available is less, the larval mortality is greatly increased, which in turn causes a crash in the adult numbers in generation *t+1*. However, the high fecundity of the flies ensures an immediate recovery from this trough in adult population size in the next generation (*t+2*), and thus the high-amplitude cycles continue (Mueller and Joshi, 2000). Not surprisingly, the constancy stability of such dynamics is low (Mueller and Huynh, 1994; Mueller and Joshi, 2000; Sheeba and Joshi, 1998).

When BLC was applied, the distribution became more symmetric as the right hand tail was curtailed and only 10.6% of the values lay in the range of 0-20 (Fig. **4.2**B). Consequently, the mean came closer to the median and the value of the skewness was reduced (Fig. **4.2**B). There were two reasons for this. Firstly, by definition, BLC ensured culling of individuals when the population size was above the upper threshold. Secondly, the presence of an upper threshold prevented over-crowding in the adult stage. This reduced the number of eggs laid in the next generation, ensuring lower egg-to-adult mortality and thus reducing the amplitude of population crashes (Fig 4.9). This is the biological equivalent of truncating the stock-recruitment curve (Hilker and Westerhoff, 2005) and is known to reduce population size variability (Fryxell et al., 2005; Tung et al., 2016). Thus, not surprisingly, the fluctuation index of the BLC populations was found to be significantly lower than the corresponding unperturbed ones (Fig. **4.3**A). However, this enhanced constancy came at a cost.

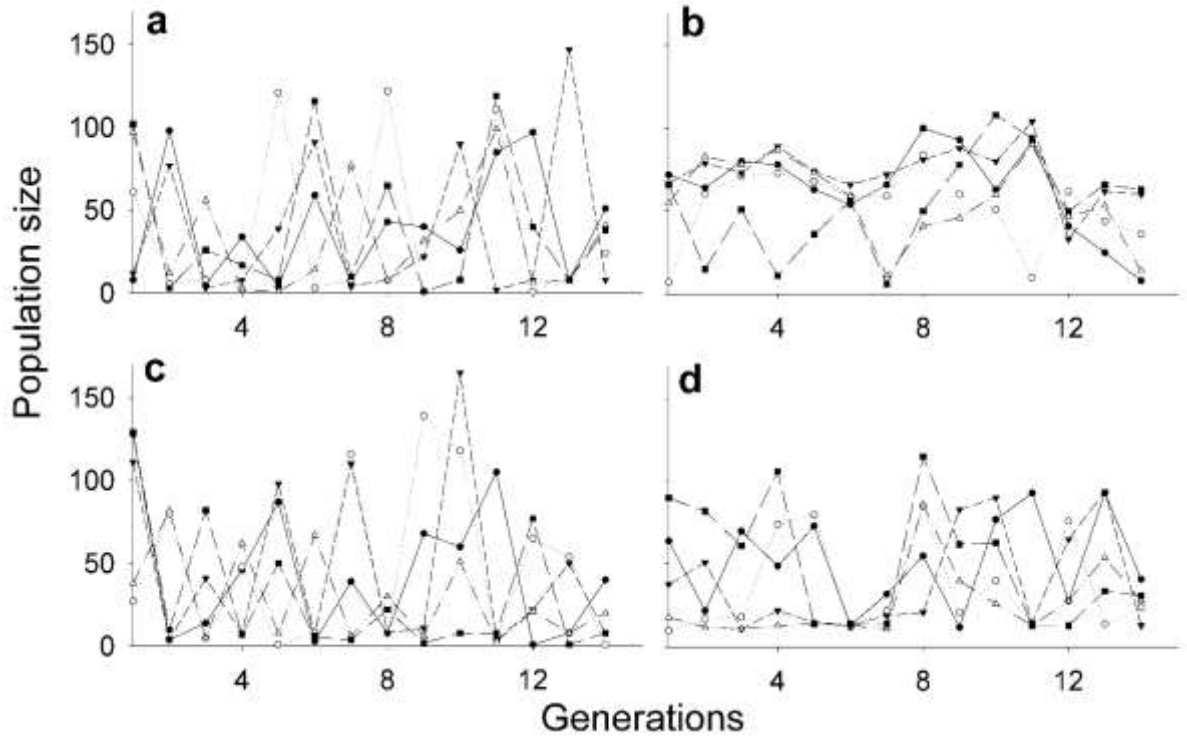

Figure 4.9. Empirical time-series of population sizes over generations. a, b, c and d represent the post-perturbation time-series for C1, BLC, C2 and TOC respectively. Five symbols represent the five independent replicate populations of each treatment.

Effort magnitude and frequency: Implementation of BLC required interventions in almost 90% of the generations (Fig. 4.3F) to the tune of ~30 individuals per generation (Fig. 4.3E). This implies that the effort required for BLC is likely to be prohibitively expensive for any real world application. Interestingly, although BLC provides for both culling and restocking, our data shows that ~99% of the total effort magnitude (Fig. 4.4A) and ~91% of the effort frequency (Fig. 4.4B) involved culling. This suggests that, at least for the parameter values used here, the culling part of BLC had a more significant impact on the dynamics. This is consistent with a recent empirical study (Tung et al., 2016) which shows that culling to a fixed upper threshold, *aka* Upper Limiter Control or ULC (Hilker and Westerhoff, 2005), is capable of significant reduction in fluctuation in population sizes by itself. Thus, it is tempting to think of the restocking part of BLC as superfluous in terms of affecting the stability properties of populations. However, this simple line of reasoning was found to be erroneous, when we examined other kinds of stability.

Persistence stability: An unperturbed *Drosophila* population can become extinct in two different ways. Firstly, as discussed above, large breeding population sizes in generation *t* can lead to extinction in the next generation (i.e. *t+1*), due to population crashes. Secondly, when the breeding population size is small, there is always a possibility of extinction in the next generation due to demographic stochasticity like all individuals being of the same sex or very low fecundity of the few available females etc (Lande, 1988). In other words, both large and small breeding population sizes can lead to extinctions in the next generation. Although culling reduces the frequency of population crashes, it does not completely ameliorate it and therefore is unlikely to be a sufficient condition to increase persistence. This line of reasoning is supported by recent experimental results that showed that although ULC could cause some reduction in extinction probability, the effect was not statistically significant (Tung et al., 2016). BLC ameliorates this problem by putting a threshold to the lower values that the population can attain, and thus was more successful in promoting persistence (Fig. 4.3B). In fact, no extinctions happened in any of the 5 replicates that were controlled using BLC, which is also consistent with past theoretical studies (Tung et al., 2014).

Effective and average population size: A fluctuating population tends to lose genetic variability whenever it experiences population crashes (Allendorf and Luikart, 2007). This can in turn lead to inbreeding-like effects, thus increasing the probability of extinction (Bijlsma et al., 2000). We quantified the effects of BLC on the genetic stability by estimating the effective population size (N_e) as the harmonic mean of the population time series which is sensitive to low population numbers. BLC was found to significantly increase the N_e by about 3 times (Fig. 4.3C). Interestingly, a previous study that employed an upper threshold of 10 individuals (*i.e.* the same as the upper threshold of our BLC), failed to find a significant increase in N_e (see U2 of Fig 2B in Tung et al., 2016). This was primarily because there was a large variation in terms of the change in N_e which is in turn consistent with the observation that culling reduces the frequency of crashes in *Drosophila* populations, but does not ameliorate them totally. As BLC also ensures that the lower population size never goes below a pre-determined threshold, it completely rules out population crashes, thus causing a significant increase in N_e.

Somewhat counter intuitively though, BLC also increased the average population size (Fig. 4.3D). Earlier theoretical studies have indicated that populations whose distributions have a long right tail (Fig. 4.2A) do not show an increase in average population size upon culling (Hilker and Westerhoff, 2005). This prediction has also been verified by a recent study which

employed the same level of culling as our current experiments (Tung et al., 2016). The only difference between the ULC treatment of the previous study (Tung et al., 2016) and our experiments is the restocking applied at very low magnitude (Fig. 4.4A) and frequency (Fig. 4.4B). However, this is insufficient to explain the observed increase in average population size as, unlike the harmonic mean, the arithmetic mean is not affected too much by low values in the population time series. Thus, it is not clear at this point as to why we observed an increase in the average population size in our experiments.

Simulations: Our simulations were able to capture all the trends of the empirical data (*cf* Fig. 4.3 and Fig. 4.7) except the one about average population size (Fig. 4.7D). Our simulation results predict no increase in average population size, which is consistent with earlier theoretical (Hilker and Westerhoff, 2005) and empirical (Tung et al., 2016) studies on the effects of culling. This result remained unaltered when the length of the time-series was increased up to 10000 generations, indicating that it is not due to any transient effect.

To summarize, at the level studied in this experiment, BLC enhanced all aspects of stability (constancy, persistence, effective population size) and average population size of unstable *Drosophila* population at the cost of very high effort.

4.2 Target Oriented Control (TOC)

Population size distribution and constancy stability: TOC is a two parameter control method in which a population is perturbed (i.e. culled or restocked) towards a pre-determined threshold (Dattani et al., 2011). For this experiment, the threshold was fixed at 30 and each generation, 70% of the difference between the threshold and the present population size was either culled or restocked when the population size was greater or lesser than 30 respectively. Consequently, TOC was also able to reduce the frequency of extreme values in the population size distribution (Fig. 4.5B). However, as can be seen from Fig 4.9, TOC was not very effective in ameliorating the extreme values in the distribution (i.e. population booms and crashes) which was reflected in the difference between the mean and the median and the high value of skewness for TOC (Fig. 4.5B).The presence of greater number of extreme values might also be a result of the intentionally induced experimental noise, which was incorporated to put TOC to a more stringent test. Nevertheless, TOC was able to cause a significant reduction in fluctuation index (Fig. 4.6A).

Effort magnitude and frequency: By its very design, application of TOC is expected to require culling or restocking except for the rare cases when population size would be exactly equal to the target value. Therefore, the effort frequency was found to be close to 1 (Fig. 4.6F). More crucially, there was a qualitative difference in terms of the pattern of the effort magnitude. Both culling and restocking were almost equally represented in the implementation of TOC in terms of magnitude (Fig. 4.4C) as well as frequency (Fig. 4.4D). This difference in the relative frequency of culling and restocking between the two methods can again be explained by the population size distributions. In BLC, culling to a fixed upper threshold (10 females in our experiment) reduces the frequency of population crashes and therefore, leads to few opportunities for restocking. This is reflected by the fact that there are few values towards the left in Fig. 4.2B. Hence, almost the entire effort in terms of both magnitude (Fig. 4.4A) and frequency (Fig. 4.4B) is devoted to culling. On the other hand, by definition, TOC requires restocking whenever the number of individuals is less than the target (= 30). Clearly, for the parameter values investigated in this study, TOC was not very effective in checking increases in population sizes (Fig. 4.5B) and hence experienced some amount of population crashes. Thus, it is reasonable to assume that it would be difficult for TOC to promote persistence.

Persistence stability, N_e and average population size: Although TOC significantly reduced the extinction probability of the populations (Fig. 4.6B), it was not able to ameliorate extinctions altogether. The level of TOC investigated in this study was sufficient to induce a significant increase in effective population size (Fig. 4.6C). As expected from previous numerical studies (Fig. 5B of Dattani et al., 2011) and our own simulations (Fig. 4.8D), we found no difference in the average population size of the TOC populations (Fig. 4.6D).

In short, our study found that TOC increased constancy, persistence and N_e of unstable *Drosophila* populations, but had no effect on average population size.

4.3 Generalizability

First principle derivations show that when organisms are randomly distributed over space and experience scramble competition, the population time series follows the Ricker dynamics (Brännström and Sumpter 2005). Since these two conditions are true for species from a large number of taxa, it is not surprising that the Ricker model has been shown to be a good descriptor of the dynamics of, *inter alia*, bacteria (Ponciano et al., 2005), fungi (Ives et al.,

2004), ciliates (Fryxell et al., 2005), insects (Sheeba and Joshi, 1998) and fishes (Denney et al., 2002). Together, these organisms represent an overwhelming majority of the biodiversity found on this planet, at least a fraction of which has already been recorded to be extinct (Baillie and Butcher, 2012). Our stochastic Ricker-based simulations were able to capture almost all the trends of the empirical data (*cf* Figs. 4.3 and 4.6 with Figs. 4.7 and 4.8 respectively), which indicates that these results are likely to be robust to noise and generalizable to other species.

4.4 Caveats and Conclusions

Although we demonstrate that BLC and TOC can increase constancy, persistence and genetic stability of real biological populations, a number of caveats should be considered before extrapolating the results to field populations. Our experiments and simulations were performed on spatially unstructured, single species populations. However, most natural populations exist in multi-species communities and inhabit patchy habitats connected via migration. The dynamics of the population in such cases are expected to be more complex (Hanski, 1999) and it is not possible to infer how much of our results would be applicable to those scenarios. Moreover, while calculating the effort of implementation, we have ascribed similar weightage to both culling and restocking. But this may not be the situation in the wild, where depending on the context, culling might be easier than restocking or vice versa. Additionally, during restocking, individuals coming from outside may harbour new genetic variation and thereby alter the genetic makeup of the native population. Thus the effects of culling and restocking on the standing genetic variation may be fundamentally different, something that is ignored when N_e is used as a proxy for estimating how fast the population loses its variation. Furthermore, both these methods demand good estimates of the population sizes which might be an economically expensive affair to begin with. Finally, one must never lose sight of the biology of the controlled species as improper control can be disastrous for the ecosystems (Pyke, 2008).

The primary insight of our study is that it is possible to attain multiple aspects of stability simultaneously, using two-parameter methods like BLC and TOC. Prior empirical studies (Dey and Joshi, 2013; Sah et al., 2013; Tung et al., 2016) on single-parameter methods have failed to attain this kind of simultaneous stability. This makes two-parameter methods more attractive in terms of management of unstable populations, in spite of their being a lot more labour-intensive.

CHAPTER 5

Understanding *Drosophila* dynamics through a stage-structured individual-based model

Highlights

- Construction of a stage-structured, individual-based model for *Drosophila* dynamics.
- The model is able to reproduce and explain several aspects of the dynamics of *Drosophila* populations, including those from two independent experiments.
- The effects of unequal sex-ratio and sex-specific culling on population dynamics are greatly influenced by fecundity.
- Interaction between juvenile food levels and adult fecundity determines efficiency of Sterile Insect Technique in controlling population sizes.

Adapted from: **Tung, S.**, Rajamani, M., Joshi, A., Dey, S. Understanding the dynamics of laboratory populations of *Drosophila melanogaster*: Long-term experiments meet individual-based modelling. bioRxiv (2017) 138446.

1. INTRODUCTION

The laboratory ecology of *Drosophila melanogaster* has been investigated for more than half a century, leading to considerable understanding of various density-dependent effects on the population dynamics of laboratory cultures of this species (reviewed by Mueller, 1985; Mueller and Joshi, 2000). Briefly, three density-dependent feedback loops — effects of larval crowding on larval survivorship and subsequent adult fecundity, and effects of adult crowding on adult fecundity — are thought to be the primary drivers of the dynamics of discrete generation cultures of *D. melanogaster* (reviewed in Mueller and Joshi, 2000). Several recursions incorporating one or more of these density-dependent feedback mechanisms have also been proposed to model the dynamics of laboratory cultures of *D. melanogaster* (Bakker, 1961; De Jong, 1976; Nunney, 1983; Prout and McChesney, 1985; Rodriguez, 1989). Mueller (Mueller, 1988) explicitly incorporated all three density-dependent feedback mechanisms into a single recursion as: $n_{t+1} = ½ \,.\, G(N_t).\, F(Vn_t).\, W(Vn_t).\, V.n_t$, where n_t and N_t represent the number of eggs and adults in generation t, respectively, V is the density-independent probability of larval viability, $W(Vn_t)$ and $F(Vn_t)$ are the functions representing the effects of larval density on larval survivorship and adult fecundity, respectively, and $G(N_t)$ is the function reflecting the effect of adult density on adult fecundity. This model remains the most detailed abstraction of *D. melanogaster* dynamics in the literature, and gave rise to several interesting predictions that were subsequently verified empirically (Mueller and Huynh, 1994; Mueller and Joshi, 2000; Sheeba and Joshi, 1998).

Despite this rich body of work, several aspects of *Drosophila* population dynamics in the laboratory still remain poorly understood. For example, although we know about the role of larval and adult nutrition in affecting population stability, it is still not clear if and how various life-history-related parameters, like egg hatchability and critical minimum size for pupation, interact with these nutritional regimes (although see Mueller and Bitner, 2015). Moreover, there is little theoretical or empirical understanding of how these nutritional regimes affect the various aspects of the population size distribution (mean, skewness and the position of the various quantiles) in *Drosophila*. There are two primary reasons for these lacunae. First, the absence of empirical datasets of sufficient length (although see Mueller et al., 2000) over multiple nutritional regimes precludes meaningful investigation of population size distributions. Second, most models of *Drosophila* dynamics are deterministic, which rules out a theoretical exploration of the population size distributions, except in the chaotic regime. This has limited the study of the dynamics of *Drosophila* populations to stability

properties (coefficient of variation, autocorrelations) and average population sizes, thus missing out on several interesting aspects of the dynamics with potentially large explanatory power.

In order to fill in some of these gaps in our understanding, we simulated *Drosophila* population dynamics using an individual-based model to generate time series data from four different nutritional regimes. These simulations were analogous to a previous 49-generation long laboratory experiment, where populations of *Drosophila melanogaster* were subjected to four different nutritional regimes. We then compared the experimental data with our simulation results to show that our model is able to capture various qualitative and quantitative aspects of *Drosophila* population dynamics in the laboratory. We then demonstrated the usefulness of our model for understanding the dynamics of laboratory populations of *D. melanogaster* in three ways. First, we used it to resolve a discrepancy between observations from an earlier study and our results. Second, we used it to generate clear predictions about how various life-history-related parameters affect the dynamics of the populations under the various nutritional regimes. Third, we used data from a previous experimental study to validate some of these predictions.

After establishing the bonafides of our model as a descriptor of *Drosophila* dynamics, we briefly compared its various features and predictions with those arising from models and experiments on other species. We then used our model to investigate various facets of a major question, namely the effects of unequal numbers of males and females on population dynamics. Simple models of population dynamics often assume that the entire dynamics can be understood in terms of the number of females in the population (May, 1976). Over the last few decades, the consequences of relaxing this assumption has been extensively investigated both theoretically and experimentally (reviewed in Rankin and Kokko, 2007). This line of study helps us understand the dynamics of those populations in which the two sexes face very different rates of mortality due to natural or anthropogenic reasons (Berec et al., 2016; Halvorsen et al., 2017; Perlman et al., 2015). Moreover, one of the highly successful pest eradication strategies, the Sterile Insect Technique (SIT), explicitly depends on altering the sex ratio of the population (Dyck et al., 2005). In nature, the amount of food available would obviously vary across space and time and this, in principle, can modulate the effects of sex ratio on the dynamics. Yet, the interaction of food and sex ratio in shaping the dynamics in general and stability in particular, has remained relatively poorly understood. Our simulations suggested that this interaction is best understood in conjunction with the density-independent

fecundity of the organisms. In a nutshell, the effects of changing the sex ratio on population stability and average size are dependent on certain aspects of the provided nutrition and not others. However, when it comes to SIT, the efficacy of the program depends more critically on the food provided over a large and biologically relevant parameter range.

2. MATERIALS AND METHODS

2.1 Laboratory ecology of *Drosophila melanogaster*:

In laboratory cultures of *D. melanogaster*, if the larval crowding is high, the mean amount of food available per larva is reduced. As a result, a large proportion of larvae are unable to attain the critical body mass needed for successful pupation, thus increasing larval mortality (Bakker, 1961). Since the body size of the adults depends mainly on the amount of resources gathered during the larval stage, the adults emerging out of crowded cultures are generally small in size (Marks, 1982) and exhibit low fecundity (Chiang and Hodson, 1950). Adult fecundity is also reduced with increasing density of adults in a culture and this is generally attributed to increased interference with egg laying (Pearl, 1932). Interestingly, this negative effect of adult density on fecundity can be ameliorated to a substantial degree by supplying the adults with excess amount of live yeast paste (Mueller and Huynh, 1994). Since survival and fecundity are the major factors affecting the growth rate of a population, it seems plausible that these three density-dependent feedback loops ― effects of larval crowding on larval survivorship and subsequent adult fecundity, and effects of adult crowding on adult fecundity ― play a major role in determining the dynamics and stability of *D. melanogaster* populations in the laboratory (Mueller and Joshi, 2000).

2.1 Experiment

The 49-generation experiment under four different nutritional regimes has been reported earlier (Dey, 2007). Here, we have used that data to for calibrating and validating my model. Since a basic understanding of the design of this experiment would aid the understanding of the simulations and subsequent analysis, the relevant experimental details are presented in Appendix I.

2.2 Statistical analyses

Distributional properties of the experimental time series of adult populations were assessed using the mean, median, 5th, 10th, 25th, 75th, 90th and 95th percentiles in box plots (Zar, 1999). The constancy stability (Grimm and Wissel, 1997) of the populations was measured as the fluctuation index (*FI*; Dey and Joshi, 2006) which is given as:

$$FI = \frac{1}{T \times \overline{N}} \times \sum_{t=0}^{t=T-1} \left| N_{t+1} - N_t \right|$$

where $\overline{N}$ is the mean population size over T generations and N_{t+1} and N_t are the population sizes at the $(t+1)^{th}$ and t^{th} generations, respectively. This formulation suggests that *FI* increases when the variance of the time series is increased, or the mean or the strength of autocorrelation of the time series is decreased.

In order to investigate the effects and interaction of larval and adult nutritional levels on constancy stability, the *FI* data were subjected to a two-way analysis of variance (ANOVA) with larval nutrition (fixed factor, two levels: Low and High) crossed with adult nutrition (fixed factor, two levels: Low and High). All statistical analyses were performed using STATISTICA® v5 (StatSoft. Inc., Tulsa, Oklahoma).

2.3 The model and simulations

2.3.1 *Model formulation*

The model can be divided into two modules: pre-adult and adult. For a given generation *t*, the pre-adult module takes the number of eggs and the total amount of larval food as input and computes the number of viable adults and the body size of each of those adults as an output. The output of the pre-adult module and the nature of the adult food available act as inputs for the adult module and the output is the total number of eggs produced that form the input for the pre-adult module in generation *t +1*. Thus, although our model produces the adult numbers in each generation, structurally it is an egg-to-egg recursion. This modelling strategy has been employed earlier (Mueller, 1988), and is preferred over an adult-to-adult recursion. This is because, due to density-dependent mortality, for a given amount of larval food, the relationship between adult numbers and the corresponding number of eggs from which they have arisen is single-humped (Chiang and Hodson, 1950). Consequently a given number of adults can arise from very different number of eggs (Prout and McChesney, 1985). Thus, for example, assuming say 10% mortality at low crowding, 10 eggs will always lead to ~9 adults.

However, if one sees 9 adults, this could have arisen from 10 eggs (assuming 10% mortality at low crowding) or 100 eggs (assuming say 91% mortality at high crowding). Thus, tracking the adult numbers is never sufficient for the purpose of modelling *Drosophila* dynamics (Prout and McChesney, 1985).

2.3.1.1 *Pre-adult module*:

Step 1: Obtaining number of larva (`numlarva`*) from the number of eggs (*`numegg`*):*

This module starts with a given number of eggs (`numegg`) and assumes that only a fixed fraction (*hatchability*) of them will hatch into larvae, due to density-independent mortality. Thus, the number of viable larvae is given by

$$\texttt{numlarva} = \textit{hatchability} \times \texttt{numegg} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where $0 \leq \textit{hatchability} \leq 1$. Reasons like intrinsic poor viability of the eggs, environmental stresses (say some toxins in the environment) or other ecological/random stochastic factors, can make *hatchability* < 1. However, under normal laboratory conditions, hatchability generally remains above 0.9 (Bakker, 1961). Therefore, we have taken *hatchability* = 0.98 and kept it same for all the simulations throughout the study (unless explicitly mentioned otherwise).

Note that here we consider *hatchability* as a density-independent parameter because there is no experimental evidence to suggest that hatchability is affected by egg density. However, *hatchability* can be easily made a function of egg or adult density, without affecting the other parts of the model.

Step 2: Obtaining the body size of each larva

In a *Drosophila* culture, the newly hatched larvae eat the larval food provided and grow in size and body mass. Although in the strict sense, size and mass are different quantities, for the sake of simplicity, we use them interchangeably to indicate biological growth. Due to among-individual variation in traits like larval feeding rate, food-to-biomass conversion efficiency etc., a distribution of larval body sizes ensues at the end of the larval growth period (Bakker, 1961). When the number of larvae in the food is increased, the amount of food available per larva, on an average, is reduced which, in turn, reduces the average body-size attained at the end of the larval stage (Chiang and Hodson, 1950; Miller and Thomas, 1958). Following a previous study (Bakker, 1961) we assumed the distribution of larval body sizes

at the end of feeding to be normal with a mean (mean_size) that was an increasing function of the total amount of larval food (*food*), but a decreasing function of the number of larvae (`numlarva`). Specifically,

$$\text{mean\_size} = x_1 \times (1\text{-}1/(x_2 + \exp(-x_3 \times \texttt{numlarva} + \textit{food}))) \quad \ldots\ldots\ldots(2)$$

where x_1, x_2 and x_3 are parameters (non-negative constants) and exp is the exponential function. The function is a logistic function of '*food* - x_3 × `numlarva`', which has an upper limit x_1 and lower limit $x1(1\text{-}1/x_2)$. Thus, theoretically, x_1 could be defined as the maximum possible average body size (theoretically attained when number of larvae is very small and

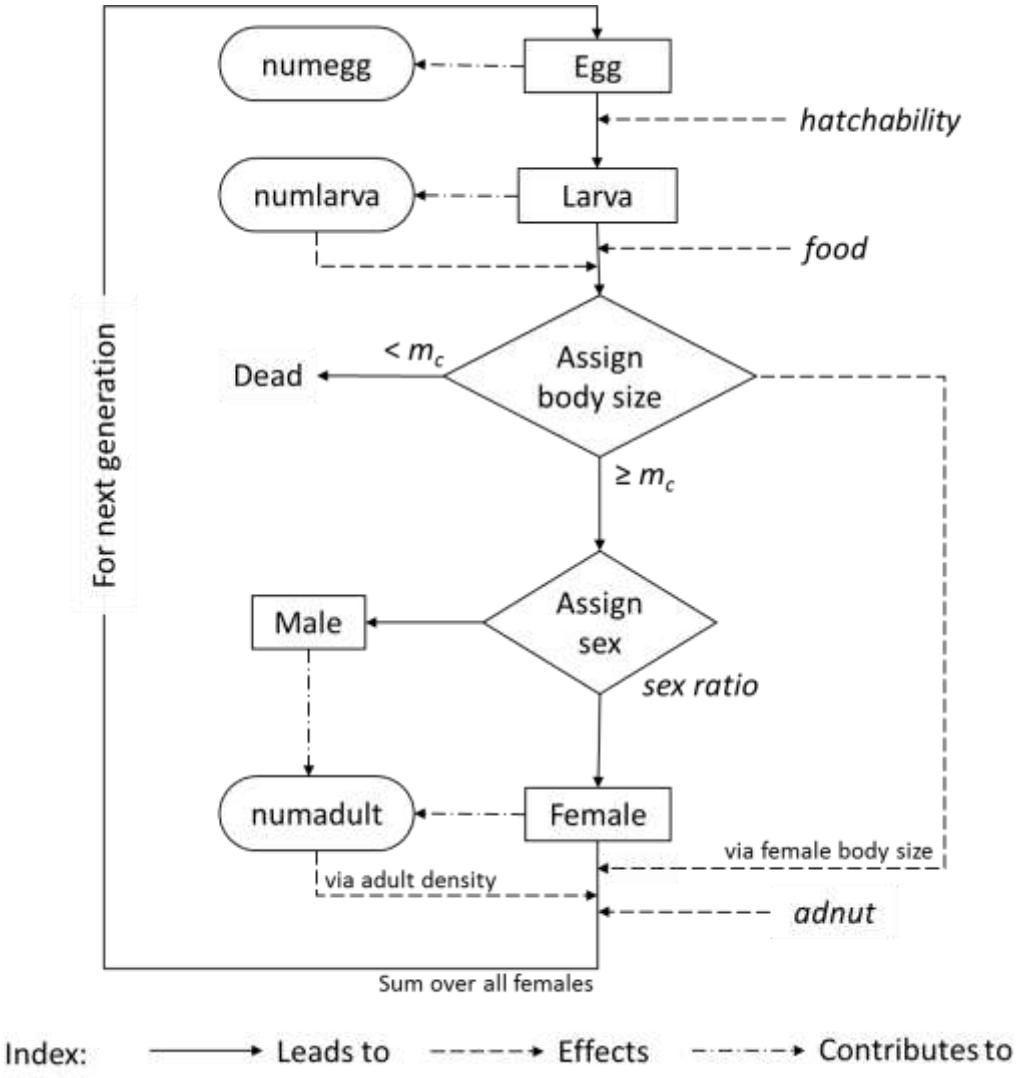

Figure 5.1. Schematic diagram of the model processes.

amount of food is close to infinite) and $1/x_2$ as the maximum fractional reduction in average body size (theoretically attained when the number of larvae is infinitely large and the amount of food is vanishingly small). We arbitrarily assigned $x_1 = 2.5$ and $x_2 = 1$. The parameter x_3 determines the rate of decline of mean_size with increasing the number of larvae for a given value of *food*. The values of x_3 and low-high values of *food* were calibrated systematically (see section 2.3.2) to match the observed empirical pattern of larval viability and body size for different egg densities with a fixed amount of larval food (Chiang and Hodson, 1950; Rodriguez, 1989). Note that here our intention was to adopt a function that can mimic the general qualitative features of the relationship between mean_size, *food* and `numlarva`. Equation 2 is just one function that fulfills this criterion and potentially many others would have also served our purpose (for example, logistic function of *food/*`numlarva` or variants thereof).

For the sake of simplicity, standard deviation (*sigma_size*) of the body-size distribution was kept as a density-independent constant (=0.45). Computationally, once `numlarva` is calculated from equation 1, each larva is assigned a body size value by drawing random numbers from a normal N(mean_size, $sigma\_size^2$) distribution. The absolute function (abs(x) = $-x$ if $x < 0$ else x) is used to avoid any negative value of body size.

Step 3: Larval body size assignment, critical mass cut off and adult body size

In order to complete metamorphosis and become an adult, *Drosophila* larvae, like those of many other holometabolous insect species (Davidowitz et al., 2003), need to attain a critical minimum size before pupation (Bakker, 1961; Robertson, 1963). To incorporate this phenomenon into our model, we considered a (deterministic) cut off, critical size (m_c), to be a density-independent constant (following Mueller, 1988) and compared the size of each larvae against it. All larvae whose body sizes were less than m_c were considered to have failed in becoming adults. The number of remaining larvae was considered to be the adult population size (`numadult`) of the current generation.

Empirical studies indicate a positive correlation between larval and adult body size in *Drosophila* (Bakker, 1961). Therefore, we considered adult body size to be a linear function of larval body size, i.e.

$$size\_adult_i = x_4 \times size\_larva_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

where $size\_adult_i$ and $size\_larva_i$ denote the body size of the i^{th} larva and the corresponding adult respectively ($size\_larva_i > m_c$) and x_4 is a parameter (= 1.0 for the sake of simplicity), which maps larval size to adult size. Note that x_4 is necessarily a positive quantity as it relates the body size of larvae to those of adults (both of which are positive). The nature of equation 3 ensures that heavier larvae lead to heavier adults, irrespective of the value of x_4.

Thus, the pre-adult module takes a life-history variable (`numegg`) as an input and gives two life-history related variables, number of adults (`numadult`) and the distribution of the adult body sizes ($size\_adult_i$), as output.

Recently, it has been discovered that *Drosophila* larvae can exhibit cannibalism under conditions of extreme food deprivation (Vijendravarma et al., 2013). However, we chose not to incorporate this phenomenon in the model since the extent of cannibalism among the larvae under the level of crowding found in our populations is still not known. More critically, there is no evidence till date to indicate that this is a density-dependent phenomenon. If we assume larval cannibalism to be density-independent, then this phenomenon can be easily incorporated into our model by multiplying `numadult` with another constant.

2.3.1.2 *Adult module*:

Step 1: Assigning a gender to each individual

The first task in this module is to assign a gender to each adult individual. In this study (unless explicitly mentioned otherwise), sex-ratio was considered to be independent of adult numbers and was always taken to be 1:1, so each adult was assigned to be female with probability 0.5 and male otherwise. However, due to the inherent stochasticity of the process, the realized sex ratio could deviate slightly from 1:1, which is biologically realistic, particularly in small populations of the kind that we are studying. *Drosophila* is a sexually dimorphic species with the females being significantly larger than the males. Therefore, ideally, only the heaviest individuals should have been designated as females. However, we ignore this complication in our model and assign sex irrespective of adult body size.

Step 2: Calculating the total number of eggs produced by all females

After the assignment of sex, fecundity of the females is computed based on their body size and current adult density. In many holometabolous insects, including *Drosophila*, fecundity or egg laying ability is positively correlated with the body size of the females (Honěk, 1993).

Prior studies have assumed that the fecundity of *Drosophila* females scale linearly on a ln-ln scale (Mueller, 1988), which implies that slight increases in body size values in the upper range can lead to large increases in fecundity. This is somewhat unrealistic as larger flies would also require to expend substantial amount of energy in body maintenance and therefore it is more likely that the rate of increase of fecundity would eventually slow down with increasing body size. This relationship could be modelled in many ways and we chose to use the logarithmic function to represent the effects of female body size on adult density-independent fecundity. Finally, live yeast paste is known to boost female fecundity irrespective of the density (Mueller and Huynh, 1994). This phenomenon, is incorporated by adding a density-independent constant (*adnut*) which denotes the fecundity boost due to yeast supplement to the adults (for not yeasted, *adnut* = 1; for yeasted, *adnut* >1). Taken together, the adult density-independent component of fecundity of the i^{th} female can be represented as:

$$\text{addens\_ind\_fec}_i = adnut \times x_5 \times \ln(x_6 + sen\_adsize \times \text{size\_adult}_i) \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where *sen_adsize* is the strength of relationship between female-fecundity and adult body size and x_5 and x_6 are scaling parameters. It should be noted here that in the above formulation, two of the constants (*adnut* and x_5) can easily be combined to create a single constant. However, we refrain from that in order to retain the ease of biological interpretation.

Another important factor that reduces per capita female fecundity in insects is adult density (Mueller, 1988; Rich, 1956). Following an earlier study (Mueller, 1988) we modelled this relationship as:

$$\text{addens\_eff} = (1/(1 + sen\_adden \times \texttt{numadult})) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where *sen_adden* is the sensitivity of female-fecundity to adult density. This hyperbolic function denotes negative feedback of adult density on adult fecundity, a relationship which is found not only in *Drosophila* (Mueller and Huynh, 1994; Rodriguez, 1989) but in many other species as well (Băncilă et al., 2016; Michael and Bundy, 1989). This negative feedback automatically makes population growth negatively density dependent and such negative feedback is known to be a necessary condition for population regulation (Turchin, 1999). The values of x_5, x_6, *sen_adsize* and *sen_adden* were determined by an extensive search over the parameter space in order to match the experimentally observed fecundity of a healthy female fly as well as to satisfy a previously observed pattern of female fecundity versus adult density

for both yeasted and un-yeasted conditions (Fig 2 in Mueller and Huynh, 1994; Fig 6.5 in Mueller and Joshi, 2000).

Combining equations 4 and 5, the fecundity of the i^{th}female is given as:

$$fec_i = addens\_ind\_fec_i \times addens\_eff \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

such that the number of eggs in the next generation,

$$\texttt{numegg}_{t+1} = \Sigma_i\, fec_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Thus, the adult module takes two life-history related parameters from the output of the pre-adult module and returns the number of eggs in the next generation as the output. This output then serves as the input for the pre-adult module for the next generation, and thus the iterations continue.

An earlier version of this model, and its correspondence with the experimental data, has been reported in the Master's Thesis of one of the authors (Tung, 2012).

2.3.2 Model calibration:

The calibration of the individual-based model was attained in three sequential steps.

2.3.2.1 *Step 1. Calibrating the pre-adult module*:

Previous studies have shown that the value of m_c is around half the maximum larval size that is physiologically attainable (reviewed in Prasad and Joshi, 2003). Since we had already fixed the maximum value of the mean larval size (i.e. *mean_size*) at 2.5, we started with an initial value of m_c = 1.25. Further, we arbitrarily started with x_3 = 0.01. We then simulated steps 1 and 2 combined (see section 2.3.1.1) to obtain the effects of `numegg` (range 1-1500) on larval viability and larval body size, for different amounts of *food* (range 0.5-6.0). The upper limits on the ranges of `numegg` and *food* roughly correspond respectively to the upper ranges in egg numbers typically seen and the amount of food (in mL) typically used in *Drosophila* vial populations in the laboratory (Dey et al., 2008). This set of simulations allowed us to narrow down the values of *food* to a range that roughly corresponded to the patterns and values of larval survivorship (Bakker, 1961; Mueller and Joshi, 2000; Rodriguez, 1989) and body size (Bakker, 1961; Chiang and Hodson, 1950; Rodriguez, 1989) in *Drosophila* laboratory populations. The correspondence between simulation results and prior experimental data were judged by visual pattern-matching as per standard methodological recommendations (Railsback and Grimm, 2011).

2.3.2.2 *Step 2. Calibrating the adult module*:

We next fine-tuned the parameters of the adult module, such that the fecundity of the female flies matched those of our flies (Dey et al., 2008). For the un-yeasted condition, we fixed *adnut* = 1, so that addens_ind_fec$_i$ in the LHS of eq 4 represented the adult density-independent fecundity of females. We arbitrarily fixed $x_6 = 2$ and simulated eq 4 across a range of values for *size_adult*. This range for *size_adult* was obtained from the larval module (after the calibration described above) for different values of `numegg`. Our aim was to obtain a value for x_5 and range for *sen_adsize* that that led to a matching of the empirically observed qualitative patterns of addens_ind_fec with increasing *size_adult* (Chiang and Hodson, 1950; Robertson, 1957; Rodriguez, 1989) (also see section 2.3.1.2 above). We then calibrated the *sen_adden* parameter in eq 5 by visually matching the simulation results to the existing empirical patterns of adult density-dependent female fecundity decline (Fig 2 in Mueller and Huynh, 1994, Fig 6.5 in Mueller and Joshi, 2000).

At this stage, we had ranges of parameter values based on prior studies that roughly matched various life-history patterns from different prior single-generation experiments. We now used these ranges to calibrate the model for the various population dynamics indices for our experimental dataset.

2.3.2.3 *Calibrating for our dataset:*

Using the ranges of the various parameter values obtained above, we simulated 49-generation long time series experiments to fine tune the parameter values for *food*, *adnut*, *sen_adden* and *sen_adsize*. For this, we crossed the ranges of these parameter values to obtain a total of 129,920 parameter combinations (i.e. 8 values of *food* × 58 *sen_adden* × 20 *sen_adsize* × 14 *adnut*) and for each combination, we ran 50 replicate simulations. From these 50 replicate time series for each parameter combination, we computed the means of the average population size, *FI*, coefficient of variation (CV), and first and second autocorrelation lags of the simulation output. We then obtained the corresponding indices for the eight replicates each of LL and HH regimes of the experimental time series. The match between the simulation and experimental data was judged by computing the absolute of the percentage deviation between the simulation mean and experimental mean for each index and summing them up over the five indices. This led to a set of parameter values for which the sum over all deviations were minimized separately for the LL and the HH regimes. We then simulated the

HH and LL time series regimes for these parameter values and did minor heuristic adjustments to obtain better matches (judged visually as per standard recommendations Railsback and Grimm, 2011) with the various indices of the dynamics of the experimental populations.

The above calibration process relied on fixing some parameters (e.g. x_1, x_2 etc.) arbitrarily as constants, some based on prior experimental results (e.g. m_c), and tuning the values of the other parameters to best match certain properties of the experimental data. Thus, the values of the best-fit parameters could be potentially very different if a different set of properties of the experimental data were chosen or the arbitrary constants were set to some other values. This is an inherent feature of pattern-oriented modelling. Since the main aim of this study was to vary these parameters and observe the corresponding effects on the dynamics, our calibration goal was not to arrive at impeccable parameter estimates but to derive a set of values that enabled our model to reasonably describe multiple facets of the empirical dynamics.

It should be noted here that once the values of *food* were obtained for the LL and HH regimes, we used the same values for the LH and HL regime. In other words, the LL and HH regime were equivalent to "training" datasets while the LH and HL regimes were equivalent to "prediction" datasets. This allowed us to avoid the issue of circularity in terms of parameterization and judging model performances. The final values of all the parameters used are presented in Table 5.1.

Table 5.1: List of parameters used in the model

Parameter	Description	Value
food	Amount of larval food present	1.76 (LL and LH) and 2.56 (HL and HH)
adnut	Quality of adult nutrition/fecundity booster	1 (LL and HL), 1.29 (HH) and 1.49 (LH)
hatchability	Egg-to-larval viability	0.98
m_c	Critical mass *i.e.* the minimum mass/size required to become a viable adult	1.1 (JB) and 1 (FEJ)
sen_adden	The coefficient of sensitivity of female-fecundity to adult density	0.17
sen_adsize	The coefficient of sensitivity of female-fecundity to adult size	1.7

sigma_size	Standard deviation of larval body size distribution	0.45
x_1	Parameter	2.5
x_2	Parameter	1
x_3	Parameter	0.009
x_4	Parameter	1
x_5	Parameter	85
x_6	Parameter	2

2.3.3 *Simulations:*

To investigate the population size distributions for each nutritional regime (LH, HH, HH or HL), we simulated eight replicate runs of the model with 49 generations in each replicate, to keep parity with the experimental data. However, none of our conclusions changed when the length of the time series was increased (see section 2.6 for discussion). Every simulation run started with 18 eggs. When there was extinction in any generation (i.e. `numadult` = 0), the time series was reset with four females with body size =2×m_c. Following previous studies (Sah et al., 2013; Tung et al., 2014), we also incorporated additional demographic stochasticity in the model by considering a 50% chance of extinction, whenever population size went below eight. If extinction occurred due to demographic stochasticity, the population was reset in the same way as mentioned above.

We also explored the effects of wide ranges of life-history related parameters (*hatchability*, m_c, *sen_adden*, *sen_adsize*) on population stability. For each value of a given parameter, we took an arithmetic mean of *FI* measured over 100 replicate time-series, each of which was 100-generations long. All other conditions of the simulations were identical to those in the previous paragraph.

Our empirical data revealed that the HL regime had a greater average population size and lower *FI* compared to the HH populations (see section 3.5 for details) whereas an earlier study (Mueller and Huynh, 1994) had shown that the population size of HH was greater than that of HL and the two regimes had similar constancy stability. In order to investigate this discrepancy between the two results, we simulated our model with five different values of larval food ranging from 3.0 to 7.0 in step size of 1.0. Each value of larval food level (*food*) was crossed with two values of *adnut* – 1.0 and 1.29- which represented the presence and

absence respectively of yeast for the adults. For each *food* × *adnut* combination, we simulated eight 49-generation long time-series, and obtained the corresponding population size distributions, and *FI* and egg-to-adult viability values. All other parameter values were identical to the earlier simulations (Table 5.1).

2.4 Comparisons with a previous model:

Our model is similar to a previous model of the population dynamics of *Drosophila melanogaster* (Mueller, 1988), with three major differences. First, the previous model was fully deterministic, while ours is stochastic and individual-based (for larvae and adults). This feature allowed us to study the various properties of the population size distributions and compare them with the experimental data, which would not have been possible with a deterministic model (except perhaps for chaotic dynamics). This also allowed us to account for certain biological features that can have a major impact on population dynamics. For example, allotting the sex of every individual using a uniform distribution allowed us to account for demographic stochasticity in the number of females, even though the expected sex ratio was 1:1. The previous model, being deterministic, assumed that a fixed fraction of the individuals in the population were females.

Second, we considered female fecundity to be a logarithmic function of female body size, whereas in the previous study, it was modelled as a power law. This implies that in the previous study, when body size was large, small differences in size translated into large differences in fecundity, which was not the case with our model.

The third difference relates to the way effects of adult density on adult fecundity was modelled. In the previous model, low nutrition for adults refers to a condition where a dilute solution of yeast is provided (Mueller and Huynh, 1994), as a result of which, yeast is not limiting at low densities and the fecundity of the flies is as high as the case when yeast is provided (see Fig 2 of Mueller and Huynh, 1994). For this reason, in the previous model, the effects of adult nutrition are conceptualized solely as a density-dependent reduction of fecundity, assuming that when densities are low, the fecundities are the same. However, in our study, low food for adults means no yeast at all and consequently, the density-independent fecundity of our non-yeasted flies is much lower. That is why, we model the effects of adding yeast using the parameter *adnut* (which determines how much the fecundity is boosted due to yeast supplement) and assume that the density-dependent reduction in fecundity is similar in magnitude for both yeasted and non-yeasted flies. The latter condition

is not a property of our model but gets imposed because we chose to use the same value of *sen_adden* for both LL and HH. Thus, this difference is simply a result of modelling strategies and the data used for model formulation and calibration.

Overall, as stated above, our model is more appropriately considered as a consequential extension of the earlier model (Mueller, 1988), rather than a completely new model.

2.5 Role of extinction in the present study:

Persistence, or the ability to resist extinction, is an important aspect of the dynamics of any population (Grimm and Wissel, 1997). Prior empirical studies have suggested that extinction plays a crucial role in determining the dynamics of laboratory populations of *Drosophila melanogaster* (Dey and Joshi, 2006; Dey and Joshi, 2007). However, most of these studies have been conducted on the LH regime, under extremely low levels of larval food which leads to high extinction frequencies. For example, Dey and Joshi (Dey and Joshi, 2006) provided ~ 1mL of larval food and observed a per generation extinction probability of ~0.37. However, the current empirical study used ~2mL of food in the low food regimes, which led to a per generation extinction frequency of 0.06 (± 0.0067 SEM) in LH and 0.03 (± 0.01 SEM) in LL populations. The two other regimes did not suffer any extinctions at all. Therefore, there was no way for us to compare the regimes in terms of extinctions, which is why we chose to neglect extinction in this study, including in the process of model calibration. Interestingly, even though we neglected extinction during model calibration, the observed values of extinction frequency from the simulations for parameter values mentioned in Table 5.1, were fairly close to the experimental values (LH: 0.069 ± 0.013 SEM; LL: 0.003 ± 0.003 SEM; HL and HH: 0). We note here that these values refer to the extinction probabilities under parameter values that were close to our experimental conditions (i.e. Figure 5.4). When we studied the effects of changing the parameter values, it is entirely possible that the amount of extinctions observed were very different. Therefore, in principle, for every figure showing the effects of changing a parameter on constancy stability (here, *FI*), a corresponding graph on persistence stability (in the form of extinction probabilities or some related measure) can also be investigated. Although important in its own right, such an investigation is clearly out of scope of this paper which focuses primarily on dynamics in terms of constancy stability.

2.6 No evidence of transients in our experiments and simulations:

Due to logistic constraints, most observed population dynamics time series tend to be short. However, it is well known that the transient behavior of population dynamics models can be very different from the equilibrium behaviors (Hastings, 2004; Hastings and Higgins, 1994). In this study, to keep parity with our experiments, we had limited the length of each simulated time series to 49. To investigate if the long-term behavior of these time series was any different from the short-term behavior, we simulated the dynamics in each regime for 1000 generations, and computed all the quantities represented in Figure 5.4 for the last 49 generations (Figure 5.2). Comparing generations 1-49 with generations 952-1000 revealed no major differences in either the population-size distributions or *FI*. This suggests that the transient dynamics in our model are almost indistinguishable from the longer-term dynamics.

Although their absence is re-assuring from a modelling perspective, transients are very much expected from a biological standpoint. This is because experimental evolution studies suggest that in *Drosophila melanogaster*, even 10-15 generations is often sufficient for noticeable divergence in life-history related traits that can affect the dynamics (for examples see Prasad and Joshi, 2003). Therefore, all else being equal, one would expect at least some of the stability determining parameters to evolve during the course of the experiment, which in turn is expected to lead to transient dynamics in a long time-series. Yet, we did not incorporate any evolution in our model, which meant that the various life-history parameters detailed in Table 5.1, remained constant in a particular simulation run. This was because it has been previously shown that at least over 45 generations, there are no observable changes in stability determining demographic parameters in laboratory populations of *D. melanogaster* (Mueller et al., 2000). Thus, we felt that it was safe to ignore changes in life-history related parameters in the context of our empirical data and, therefore, did not incorporate their evolution in our model. However, we note that the structure of our model is such that it can be very easily extended to incorporate the evolution of stability-determining parameters and the effects of such evolutionary change on population dynamics.

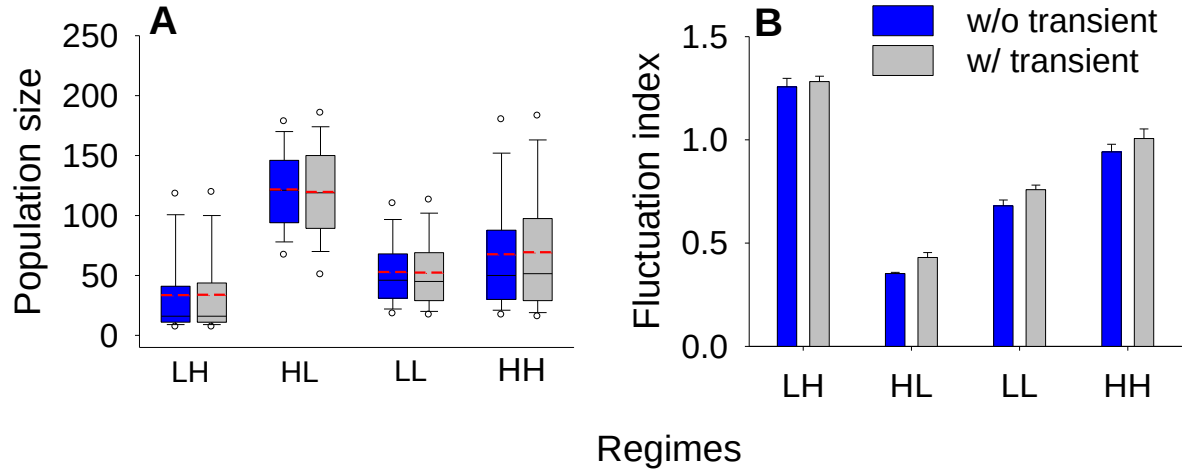

Figure 5.2. Population size distribution and constancy stability of short and long term dynamics. Blue boxes and bars represent the data for long term dynamics (generation 951-1000), where transients are excluded, whereas the grey shaded boxes and bars represents short term dynamics (generation 1-49). (**A**) Descriptive statistics of the population size distributions for long and short term dynamics in four regimes. Red dashed lines = means, thin black lines = medians, edges of the boxes=25th and 75th percentiles, whiskers=10th and 90th percentiles and the circles outside = 5th and 95th percentiles of the distributions. (**B**) Average (± SEM) fluctuation index of the population size distributions for long and short term dynamics in four regimes. In both panels, there are no systematic differences in population size distributions and constancy stability between the short- and long-term dynamics.

2.7 36-generation simulation and experiment

To validate one of the predictions arising from our model, we compared our model output with the dynamics of four *Drosophila* populations selected for faster development and early reproduction (henceforth called $FEJ_{1\text{-}4}$) for ~125 generations (Dey et al., 2008; Prasad et al., 2003). The $FEJ_{1\text{-}4}$ lines were derived from four ancestral populations ($JB_{1\text{-}4}$), which served as controls in that experiment. Incidentally, one of these JB populations is the ancestor for the 32 populations used in the present study. For each FEJ_i or JB_i ($i \in$ 1-4) population (represented by single vial cultures), there were four replicates each under HL and LH regimes. Thus, there were 16 FEJ populations and 16 JB populations, that experienced the LH regime and similarly 16+16 that experienced the HL regime. The maintenance details of this 36-generation long experiment are given elsewhere (Dey et al., 2008) and are similar to the experimental protocol of the present study.

The rationale and the details of the model recalibration are presented in the section 2.7.1. It should be noted here that the empirical *FI* values are those reported in Figure 5.8a of the earlier study (Dey et al., 2008) and are being re-plotted here only to facilitate comparison

with the simulation results. The population size distribution data from these experiments is being reported for the first time here.

2.7.1 Model Recalibration for the dynamics of the FEJ-JB populations:

To use the time-series data from an earlier experiment (Dey et al., 2008), we first re-calibrated our model by reducing the value of m_c of FEJs from 1.1 to 1.0. This is because it has been suggested that due to selection for faster pre-adult development, the FEJs had a lower value of m_c (Prasad et al., 2001). Moreover, to keep parity with the experimental data, we used 16 replicates each of FEJ and JB in both HL and LH nutritional regimes, and each replicate was simulated for 36 generations. Every other detail of the parameter values and the simulation were identical to those mentioned above. We then compared the population size distributions and *FI* values of the simulations against those observed from the empirical data

2.8 Simulating the dynamics of sex-biased populations

We investigated the dynamics and stability of populations under three scenarios where there could be unequal numbers of males and females, namely systematic distortion of sex ratio, sex-specific culling and the sterile insect technique (SIT). In all these cases, relevant alterations were made in the adult module while the pre-adult module was kept unchanged.

a) Distortion of sex ratio: To simulate sex ratio distortions, we varied the probability of a given adult to be a female in the adult module, while keeping the rest of the model unchanged. We studied the effects of these various sex ratios at two different levels of density-independent basal fecundity, namely high [($adnut \times x_5$) =1.49 × 85] and low [($adnut \times x_5$) = 1.49 × 17)]. For each sex ratio × fecundity combination, we also studied the effects of low and high levels of food to the juvenile stage (*food* = 1.76 and 2.56 respectively). For each sex ratio × fecundity × food combination, we ran 100 replicate time series, each of which was 100 generations long. The mean (±SEM) of average population size and fluctuation index over these 100 replicates was plotted.

b) Sex-specific culling: This was similar to the above case except that in every generation, after the gender was assigned to the adults, we explicitly removed a fixed percentage of males or females from the population. The effect of adult crowding on adult fecundity was computed after the culling.

c) Sterile insect technique (SIT): This is a popular pest control technique (Dyck et al., 2005), wherein large numbers of sterile males of the pest species are introduced into the population.

These sterile males compete with the fertile ones for potential mating partners, thereby bringing down the number of successful matings which can lead to offspring. The number of females that mate with fertile males is directly proportional to the ratio of number of fertile: sterile males and inversely proportional to the competitive ability of the sterile males (Knipling, 1955). For the sake of simplicity, we considered the proportion of females producing eggs for the next generation as a linear function of the fertile/sterile male ratio. Thus, proportion of egg laying females = min((`num_fertile`/(β×`num_sterile`)), 1), where `num_fertile` and `num_sterile` are the number of fertile and sterile males in the population, β is the competitive ability of the sterile males and min() is the minimum function. In this section, we studied the interaction of density-independent female fecundity with the amount of juvenile food available in determining the efficacy of SIT.

In our model, low values of *food* or high female fecundity can lead to extinctions due to larval overcrowding. This creates a problem in terms of judging the efficacy of introducing sterile males to cause extinction. Therefore, we first investigated the extinction probabilities over a large number of combinations of larval food (*food*, range = 0.5 - 3.0, step size = 0.2) and density-independent fecundity (i.e. *adnut* × x_5, range = 1 - 150, step size = 5) in the absence of any introduced sterile males. For each *food* × fecundity combination, we computed the probability of extinction within 10 generations over 10 replicates and plotted the average extinction probability (Figure 5.15). We then limited our explorations on the efficacy of SIT to only those *food* × fecundity combinations where the probability of an extinction event within the first 10 generations was less than 0.1. For each *food* × fecundity combination, we sought to compute the minimum number of sterile males that would lead to an extinction within 10 generations. Therefore, we increased the number of sterile males in step size of 1 and simulated 10 generation long time-series for each *food* × fecundity × sterile male number combination till there was an extinction. For a given *food* × fecundity combination, this step was repeated 100 times and the average number of sterile males that were needed to cause extinction was recorded.

3. RESULTS AND DISCUSSION

3.1 Experiments: Larval and adult nutritional regimes interact to shape the adult dynamics

There was a significant interaction between the larval and adult nutrition ($F_{1, 28}$= 17.92, p= 0.0002) suggesting that enhancing the fecundity of flies (through a supply of yeast) causes a much greater increase in *FI* when the amount of larval food is limiting (i.e. LL and LH) than when it not limiting (i.e. HL and HH) (Figure 5.3). This is because although both LL and LH experience substantial larval crowding, the greater fecundity of the LH flies leads to higher larval crowding even with moderate adult population sizes which, in turn, causes regular population crashes. On the other hand, even when there are population crashes, the greater fecundity of the LH flies ensures a high population size in the next generation. Together, these two effects ensure large amplitude oscillations in LH population sizes, and a substantially larger *FI* than the LLs **(**Tukey's HSD p = 0.00016**)**. On the other hand, although the fecundity of females in the HH populations is larger than those in the HLs, the non-limiting amount of larval food ensures that the population crashes are only marginally more severe in the former. This leads to a much lower (although statistically significant; Tukey's HSD p = 0.00017) increase in *FI* in the HH populations, compared to the HL populations (Figure 5.3).

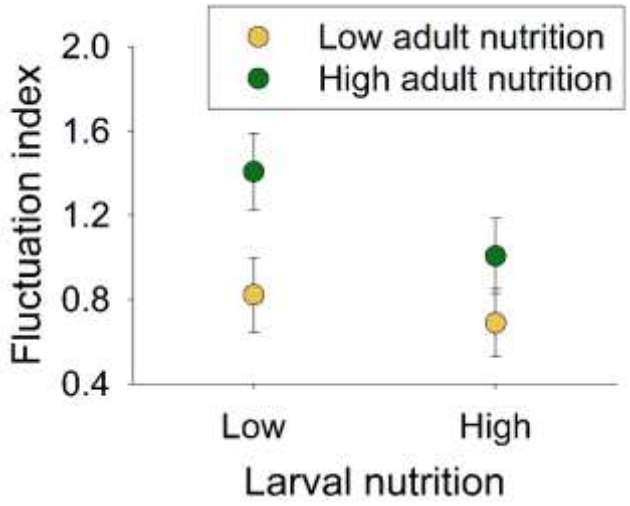

Figure 5.3. Effects of larval and adult nutrition on constancy stability. Interaction of larval and adult nutrition to determine constancy stability of population is statistically significant. High adult nutrition destabilizes population more when larval nutrition is low. Error bars = 95% CI.

3.2 Experiments: The differences in the dynamics of the populations are reflected in their population size distributions and *FI*

We began our investigation by examining the distribution of population size which is ultimately related to the temporal dynamics of populations (Figure 5.5). Both larval and adult nutritional levels were found to affect the population size distributions (Figure 5.4A, the

white boxes). Specifically, when larval food is low, population size distributions have lower values of mean, median, 25th percentiles and 75th percentiles, compared to when larval food is high (*cf* LH with HH and LL with HL in Figure 5.4A). Interestingly, irrespective of the level of larval nutrition, providing yeast to the adults reduced the population sizes (*cf* LH with LL and HH with HL in Figure 5.4A). Moreover, in the LH and HH regimes (Figure 5.4A), the population size distributions are much more skewed to the left (i.e. median < mean), which is indicative of crashes in population numbers from various medium to high population sizes (see also figure 4 in Dey and Joshi, 2013). All these observations are due to the fact that low levels of larval food or increased adult fecundity increase the larval crowding by reducing the per-capita food available to the larvae. Consequently, fewer larvae are able to acquire a body size greater than m_c, which reduces the egg-to-adult survivorship, and hence the adult population sizes. Interestingly, the mean and the median population sizes were very close for the HL populations, but not so for the other three (Figure 5.4A). This showed that the population size distributions of HL had little or no skew, while the other three regimes exhibited positive skewness. This implied that in spite of having a larger average population size compared to the other three regimes, the HL populations exhibited lower amplitude fluctuations relative to their own mean population size. Thus, not surprisingly, the HL populations were found to have the lowest *FI* (Figure 5.4B) amongst the four regimes.

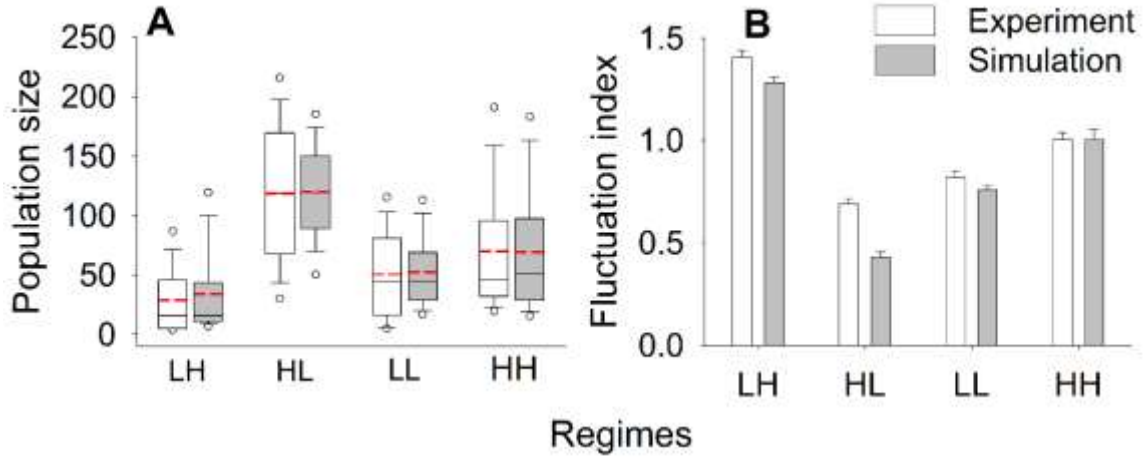

Figure 5.4. Population size distribution and constancy stability of experimental and simulated time-series. (**A**) Descriptive statistics of the population size distributions. Red dashed lines = means, thin black lines = medians, edges of the boxes=25th and 75th percentiles, whiskers=10th and 90th percentiles and the circles outside = 5th and 95th percentiles of the distributions. White boxes represent experimental data while grey shaded boxes are for simulated time-series. (**B**) Average (± SEM) *FI* of the experimental and simulated time-series in the four regimes. Both plots suggested a good agreement between the experiments and the simulations. The populations in the HL regime were the most stable with highest average population size while those in the LH regime were the least stable with lowest population size.

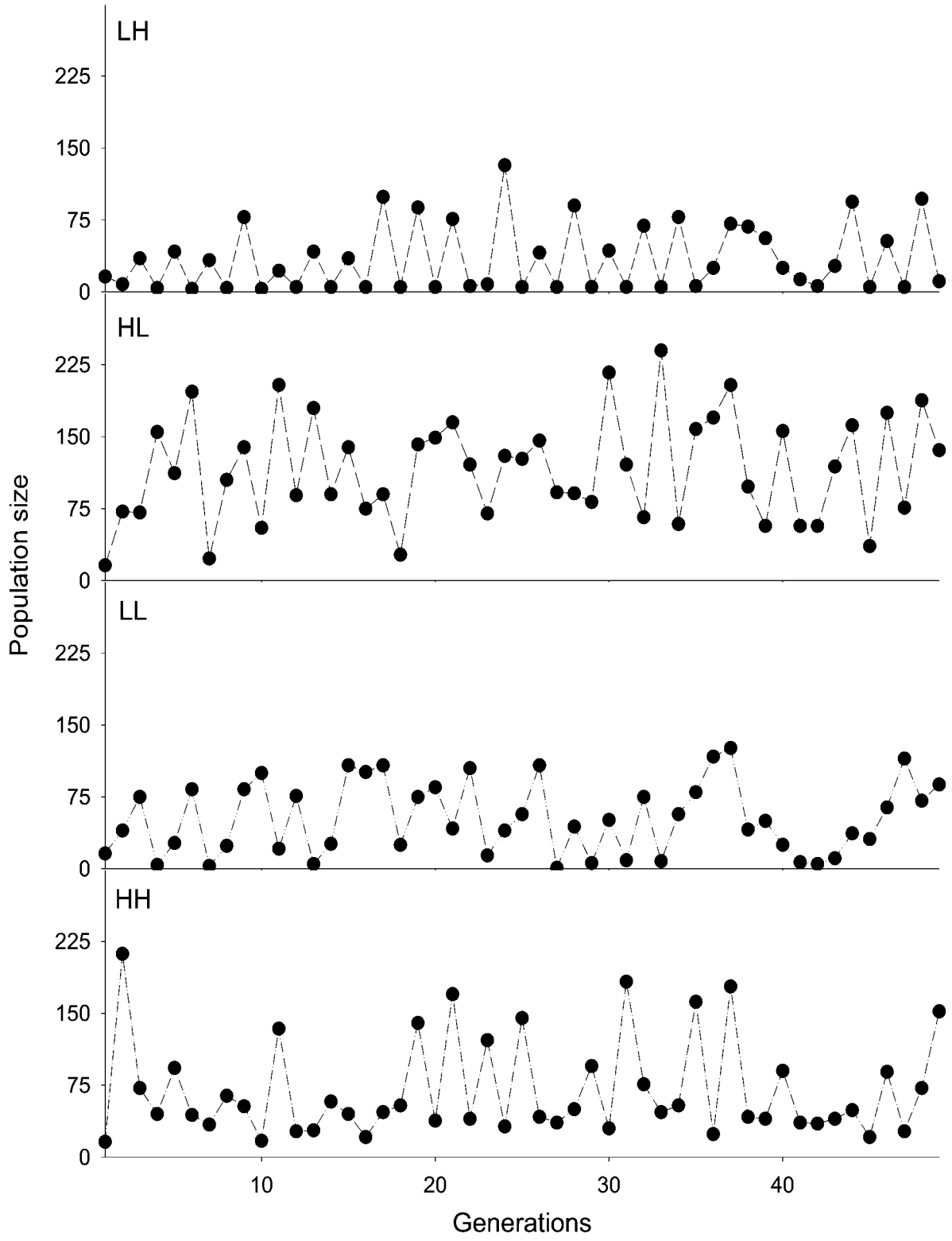

Figure 5.5. Representative empirical time-series of population size over 49-generations for LH, HL, LL and HH regimes respectively.

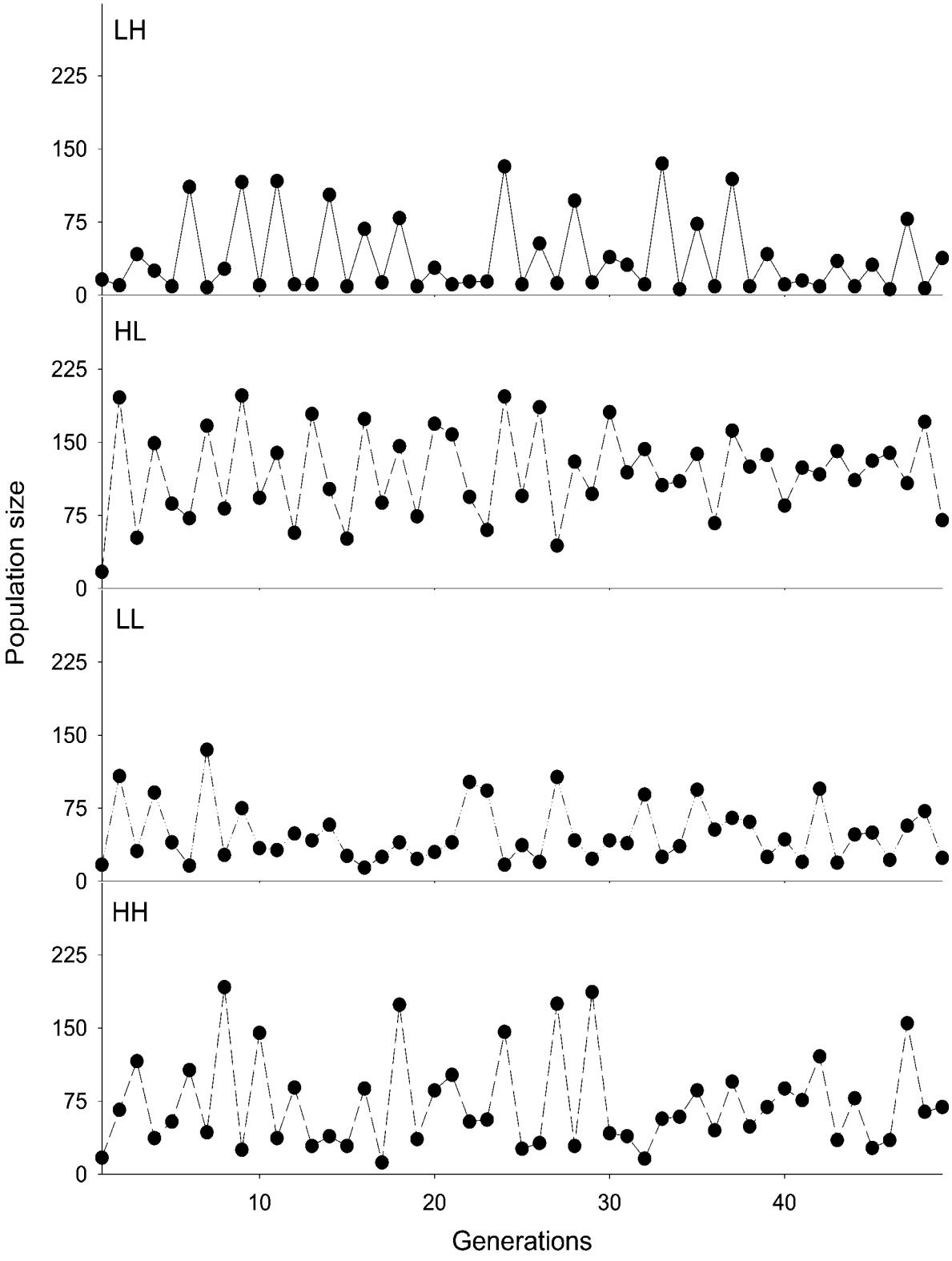

Figure 5.6. Representative simulation time-series of population size over 49-generations for LH, HL, LL and HH regimes respectively.

Post-hoc tests (Tukey's HSD) on *FI*s in the four nutritional regimes revealed all pair-wise differences to be significant with the rank order: LH > HH > LL > HL (Figure 5.4B, the white bars). Although these four regimes have never been studied together till date, subsets of them have been studied in all kinds of combinations. Thus, it has been shown that in terms of constancy stability LH< HL~HH (Mueller and Huynh, 1994), LH< HL and LH< LL (Dey and Joshi, 2013). Our results (Figure 5.4B) are in excellent agreement with all these studies except those of Mueller and Huynh (1994) who showed theoretically and empirically, that the constancy stability of HL and HH were not different. Moreover, Mueller and Huynh (Mueller and Huynh, 1994) also predicted the average population size of the HH regime to be much larger than that of the HL regime, which also did not match our observations (Figure 5.4A). We resolve this issue later in this study (section 3.5) using our individual-based model of *Drosophila* dynamics.

3.3 **Simulations: High level of correspondence between the experimental data and the model output**

The simulation results (grey bars) matched the various salient features of population size distribution (Figure 5.4A) and population stability (Figure 5.4B) in the empirical observations in all four nutritional regimes. To the best of our knowledge, there are no models of *Drosophila* dynamics whose predictions have been verified in this detail with experimental data. This is more a reflection on the paucity of good quality long time series data, rather than any shortcoming on the part of the modelers. In fact, in the context of dynamics of laboratory populations of *D. melanogaster*, our 49 generation data-set is perhaps the second-longest in the literature in terms of number of generations.

Although the model did an excellent job in capturing the various aspects of the experimental data (*cf* Figure 5.5 and 5.6; also Figure 5.7), these details (and therefore the parameter values that lead to them) are obviously experiment-specific and shall vary across studies. Therefore, the usefulness of our model is more in terms of the mechanistic understanding that it generates about how the dynamics is affected by the interaction of various life-history and environmental variables. That was our next object of investigation.

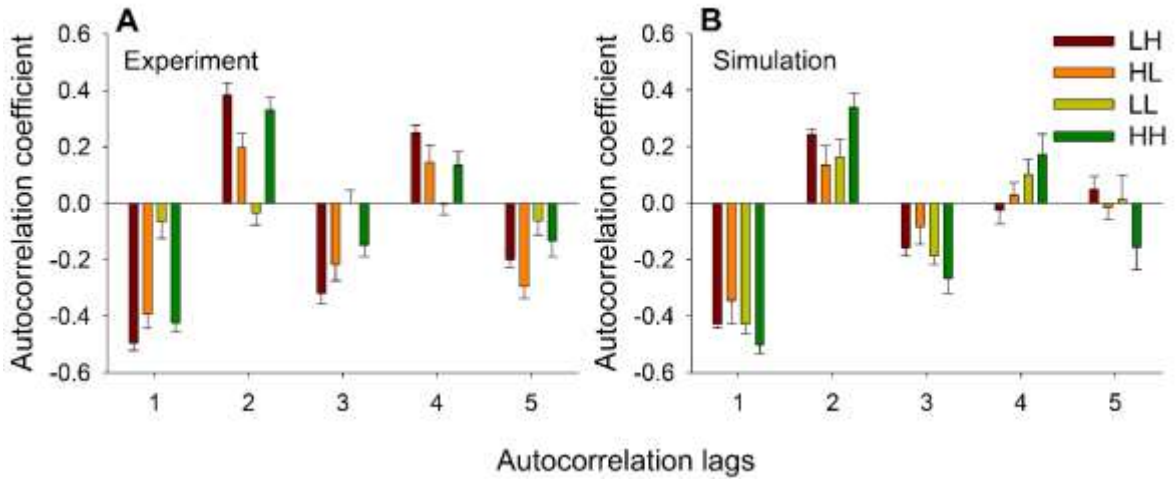

Figure 5.7. Autocorrelation coefficients of empirical and simulation time-series. For four regimes, we have computed first five autocorrelation lags, averaged over eight replicates for both (**A**) empirical and (**B**) simulation time-series. Error bars indicate SEM.

3.4 **Simulations: The effects of various life-history related traits on dynamics**

3.4.1 *Hatchability (hatchability) and critical size (m_c)*:

Our model predicted that with decreasing hatchability of the eggs up to moderate values, population *FI* decreases (i.e. constancy stability increases) in all four regimes (Figure 5.8A). This is because a reduced hatchability in generation *t* is conceptually equivalent to reduced fecundity in generation *t*-1, which is known to be a stabilizing factor (Mueller, 1988). As expected, the destabilizing effects of increasing hatchability are more pronounced where the larval crowding is already very high (LH) and are mildest where larval crowding is the lowest (i.e. HL). Interestingly when hatchability values are very low (~.35 or less), further reductions lead to a decline in the average population size without greatly affecting the variation in population sizes over time (Figure 5.9). Since the expression for *FI* includes the average population size in the denominator, this leads to a minor increase in the *FI* values for very low values of hatchability.

Like hatchability, increasing larval critical size (m_c) also has a negative effect on constancy stability (Figure 5.8B). This works in two ways. First, all else being equal, increasing m_c means that fewer larvae would be able to attain m_c, which would reduce larval survivorship. This is analogous to reducing survivorship through reduced larval food amount, which is a destabilizing factor. Secondly, increasing m_c means on an average, the surviving adults would have a greater body size, which would translate into larger fecundity and thus, destabilize the

dynamics. Conversely, decreasing m_c is always expected to stabilize the dynamics (Mueller, 1988): a prediction that we return to in section 3.6.

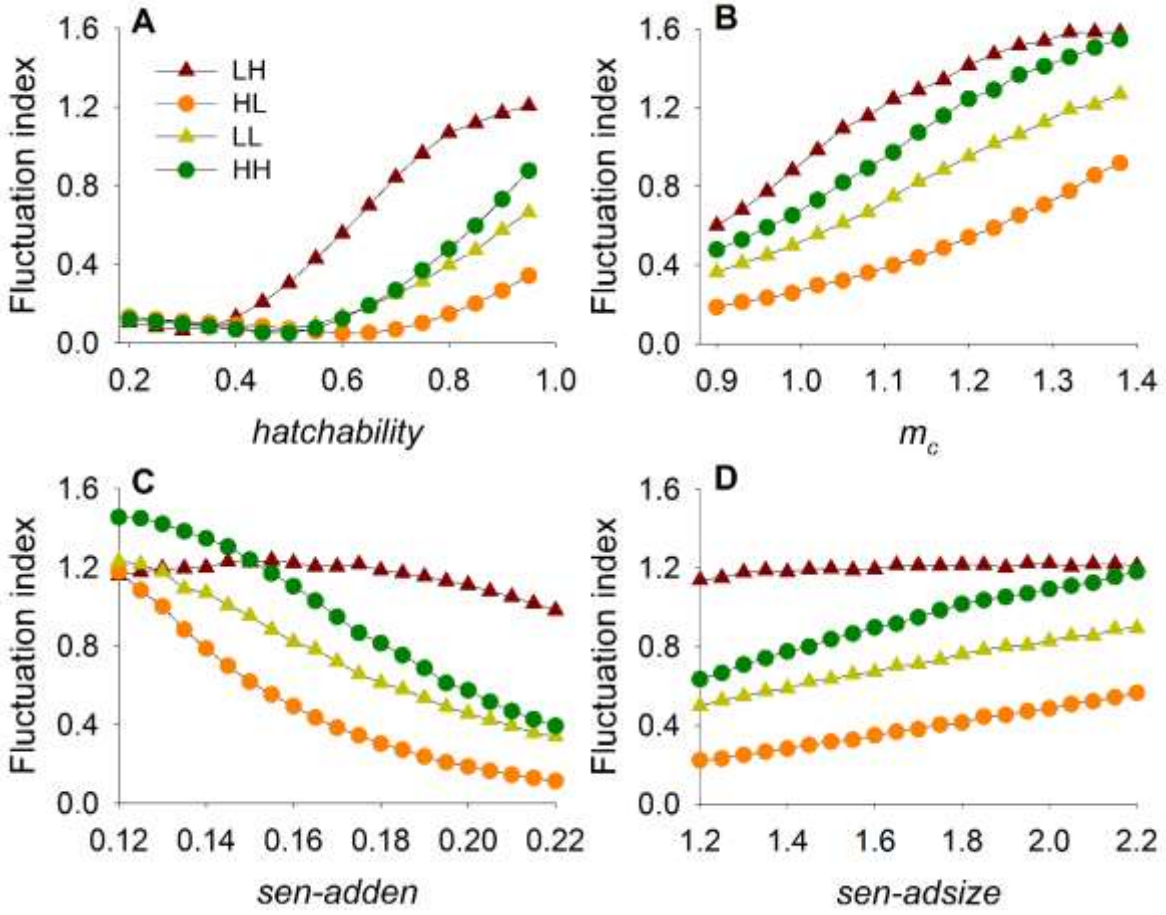

Figure 5.8. Effect of varying life-history related parameters of the model on constancy stability. Each point represents average (± SEM) fluctuation index of 100 replicates of 100-gen long simulated time series. Error bars are too small to be visible. (**A**) In all four regimes, as *hatchability* reduces, larval density also reduces and thus populations become more stable. (**B**) As critical size increases, the populations become more destabilized. (**C**) Increasing the sensitivity of female-fecundity to adult density (*sen_adden*) increases constancy stability in all regimes except LH. (**D**) Increasing the sensitivity of female-fecundity to adult body size (*sen_adsize*), reduces constancy stability in all regimes except LH. See section 3.4.2 for explanations for the anomalous behaviors in the LH regime.

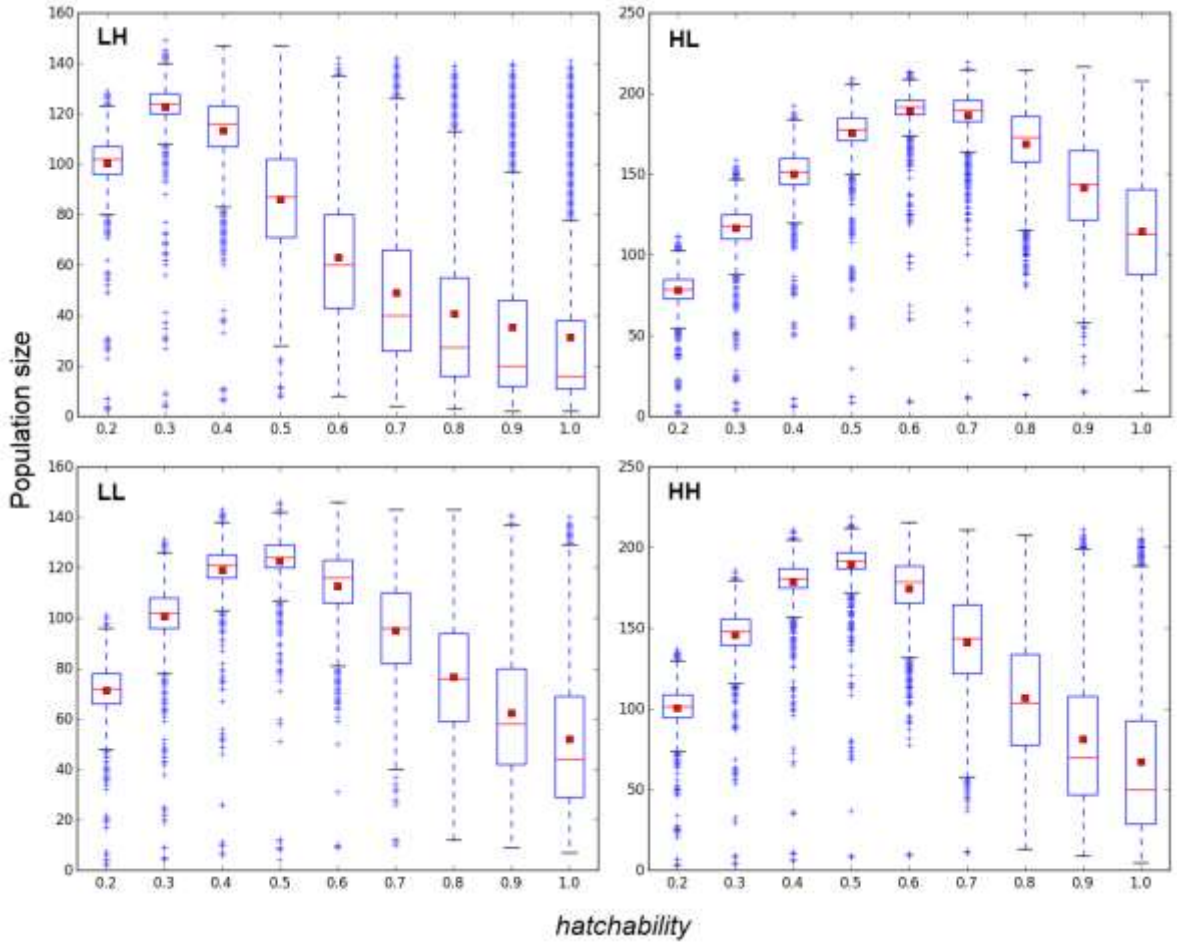

Figure 5.9. Effect of varying *hatchability* on population size distribution in four regimes. Red squares = means, thin red lines = medians, edges of the boxes=25th and 75th percentiles of the distributions.

3.4.2 *Sensitivity of female fecundity to adult density (sen_adden) and adult body size (sen_adsize)*:

Increasing adult density is known to negatively affect female fecundity in *Drosophila melanogaster* (Mueller and Huynh, 1994). In our model, *sen_adden* determines the strength of this effect, such that for same adult density, greater *sen_adden* results in reduced fecundity. This in turn enhances larval survivorship, by increasing the amount of food available per capita, which has a stabilizing effect on the dynamics. On the other hand, *sen_adsize* determines the strength of the positive correlation between body size and fecundity, such that increasing *sen_adsize* will increase fecundity, thereby reducing larval survivorship, ultimately leading to destabilized dynamics. In a nutshell, an increase in *sen_adden* and decrease in *sen_adsize* is expected to lead to a stabilization of the population dynamics. Our simulation results agreed with this prediction in all the regimes except LH (Figure 5.8C and 5.8D). In the LH regime, both *sen_adden* and *sen_adsize* seemed to have

little effect on *FI*, even though, reducing *sed-adden* and increasing *sen_adsize* caused the total egg number to go up (Figure 5.10A and 5.10C). The reason for this unintuitive behaviour was revealed when we investigated the effect of these two parameters on the egg-to-adult survivorship. Increasing *sen_adden* (Figure 5.10B), or decreasing *sen_adsize* (Figure 5.10D), hardly affected the egg-to-adult viability in the LH regime. This is because the reduced levels of larval food ensured that even with low fecundity, there was substantial larval crowding in this regime (as evidenced by the low levels of egg-to-adult viability) so that there was almost no effect of changing *sen_adden* or *sen_adsize* on larval mortality. As a result, the destabilizing effect of increasing fecundity was not seen in the LH populations. Another interesting observation from Fig 5.8C is that at low values (< 0.14) of *sen_adden*, HH has a much larger *FI* than LH, despite having similar levels of average egg number (Fig 5.10A). This is because for low values of *sen_adden*, the egg-to-adult viability of LH is lower than that of HH (Fig 5.10B), due to the fact that the amount of larval food for the former regime is much less than that of the latter. Consequently, greater number of adults can be supported by HH, which makes higher amplitude fluctuations in population sizes more likely, thereby increasing the values of *FI*.

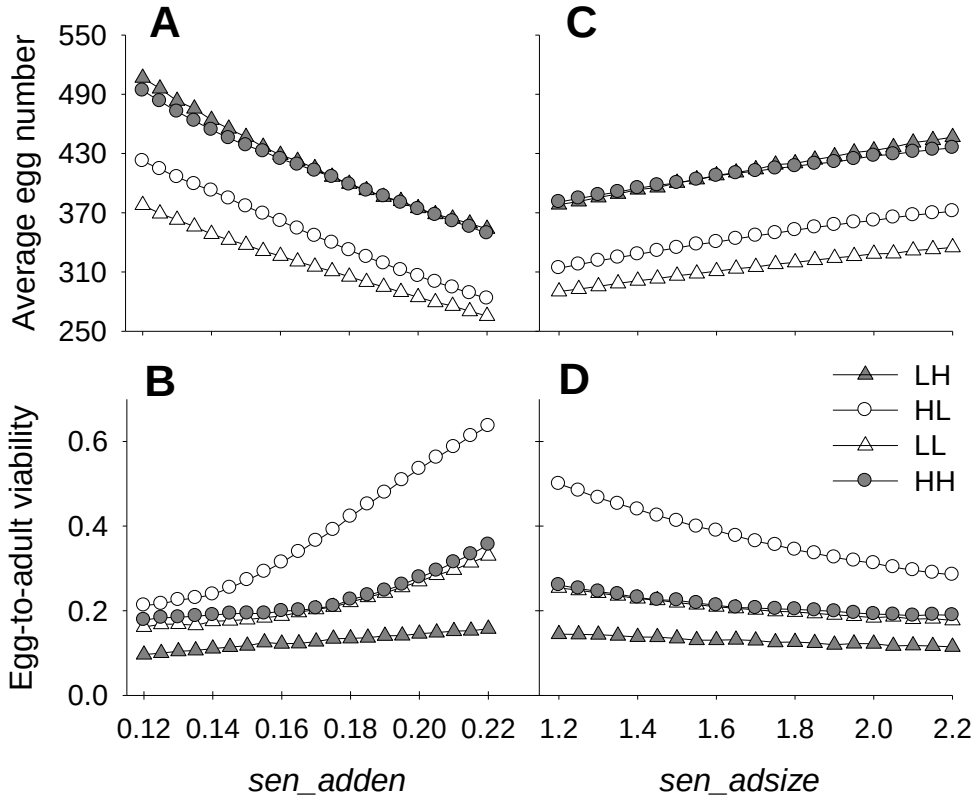

Figure 5.10. Effect of varying *sen_adden* and *sen_adsize* on the average egg number and egg-to-adult viability. Each point represents average (± SEM) fluctuation index of 100 replicates of 100-gen long simulated time series. Error bars are too small to be visible. Effects of sensitivity to

adult density (*sen_adden*) on **A.** Average egg number and **B.** Egg-to-adult viability. Effects of sensitivity to adult size (*sen_adsize*) on **C.** Average egg number and **D.** Egg-to-adult viability. Note that in B and D LH is the least affected by increases in the parameter values. See text (section 3.4.2) for explanation.

The primary insight from the above observations is that even in the highly simplified dynamics under laboratory conditions, the environment can interact with the life-history related traits of the organisms to lead to very counter-intuitive effects on the dynamics.

3.5 Simulations and Experiments: Population dynamics is shaped jointly by the quality and quantity of nutrition

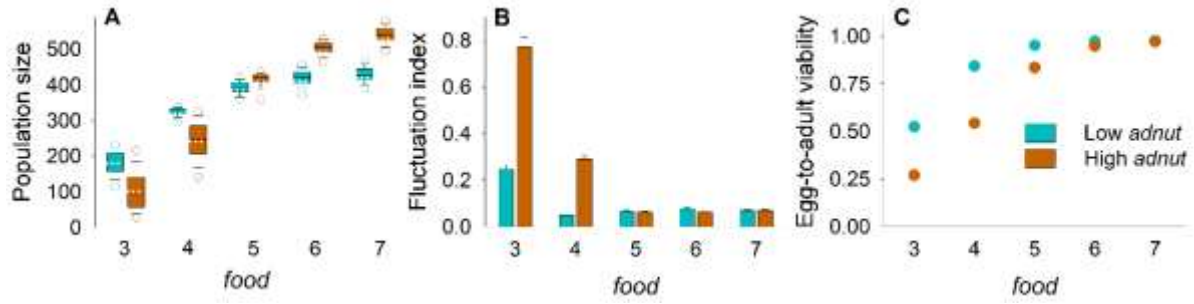

Figure 5.11. Simulations on effects of varying larval nutrition on population dynamics. (**A**) Population size distributions for the simulated time-series under low adult nutrition or *adnut* (cyan) and high *adnut* (orange) conditions for different levels of larval food amount (*food*). White dotted lines = means, thin black lines = medians and the circles outside = 5th and 95th percentiles of the distributions. The relative positions of the population size distribution of low *adnut* and high *adnut* regimes reverses as the larval food amount increases. (**B**) Average (± SEM) fluctuation index of the low *adnut* and high *adnut* regimes become comparable, when the level of larval food is high. (**C**) Although the low *adnut* regimes have greater average (± SEM) egg-to-adult viability than the high adult nutrition (*adnut*) regime for low values of larval food (*food*), the viabilities become comparable as *food* increases. Error bars are too small to be visible here.

As stated already, one of our empirical results did not match the observations of an earlier study (Mueller and Huynh, 1994). We found that HL populations had greater constancy stability and average size than the HH populations whereas Mueller and Huynh (Mueller and Huynh, 1994) reported that the HH populations had similar constancy stability but much greater average size than the HL populations. The primary difference between the two experiments was in terms of the amount of food given to the larvae. In the experiment of Mueller and Huynh (Mueller and Huynh, 1994), the HL and HH larva got 40 mL of food in a 250 mL bottle while in our experiment the corresponding larva got ~6 mL food in a 37 mL vial. Consequently, the adult population sizes in the HL and HH regime varied in the range of ~40-240 in our experiment, but ~ 400-1600 in the previous experiment.

To investigate whether the differences in the larval food amount could explain the observed discrepancies, we simulated the HH and HL regime for different levels of larval food, keeping all other parameters the same as in the earlier simulation. We found that as the level of larval food increased, the relationship between the population size distribution of HH and HL reversed (Figure 5.11A). Furthermore, with increasing value of larval food, the *FI* in the HH regime reduced and approached the value seen in the HL regime (Figure 5.11B). The underlying mechanisms behind these observations can be understood as follows.

Due to the availability of yeast paste to the adults in the HH regime, the per-capita fecundity of the females is very high. Consequently, when the amount of larval food is relatively less (as in our experiment) there is larval crowding which reduces the survivorship in the HH regime. Therefore, with increasing levels of food, the survivorship increases (as in Figure 5.11C), which is manifested as increased population size in the HH regime (Figure 5.11A, orange boxes). The HL populations also face some amount of larval crowding at lower levels of food. However, since they do not have increased fecundity at high adult population sizes (due to the absence of yeast), the increase in population size plateaus off at a much lower level of food (Figure 5.11A, cyan boxes).

In order to visualize the effects of increased food amount on constancy stability, we need to appreciate that reduced larval crowding has two opposing effects on the dynamics. First, it stabilizes the dynamics by increasing larval mortality. At the same time, it can destabilize the dynamics by increasing the body size of the females at eclosion. As the larval food level increases, both these factors come into play. However, as there are upper bounds to both survivorship (=1) and the body size of the flies (= the physiological limit of body size), beyond a certain amount of larval food, both these factors cease to play a major role, and the *FI* in both regimes become similar. This can be clearly seen in Figure 5.11B and explains why in the presence of large amount of larval food, HL and HH populations have similar constancy, as reported previously (Mueller and Huynh, 1994). When the amount of larval food is relatively small (while still being high, compared to LH or LL regimes), the destabilizing effect of reduced survivorship overpowers the stabilizing effect of diminished fecundity due to reduced body size. This is because there is a minimum value for the body size (= m_c), which automatically places a lower bound on the fecundity of the flies irrespective of the level of larval crowding. Since the HH populations experience greater larval crowding than the HL populations, they are expected to exhibit lower constancy, as seen in our experiments (Figure 5.4B, white bars) and simulations (Figure 5.4B, grey bars).

In the *Drosophila* population dynamics literature, labels like LH and HL have typically been used as qualitative descriptors to signify the levels of larval crowding (highly crowded *versus* un-crowded) and state of adult nutrition (yeasted *versus* un-yeasted). As described in the Introduction section, this categorization has broad explanatory power in terms of the nature of the dynamics: LH leads to high amplitude oscillations while HL leads to relatively stable dynamics. However, the above comparison between the HL and HH regimes from the two different studies shows that changing just one environmental parameter (here, the absolute quantity of otherwise 'high' larval food) can lead to a rich array of dynamics. This again highlights how the actual values of the environmental parameters interact with life-history related traits in determining population dynamics.

3.6 Simulations and Experiments: Reduction in m_c is one way for population stability to evolve

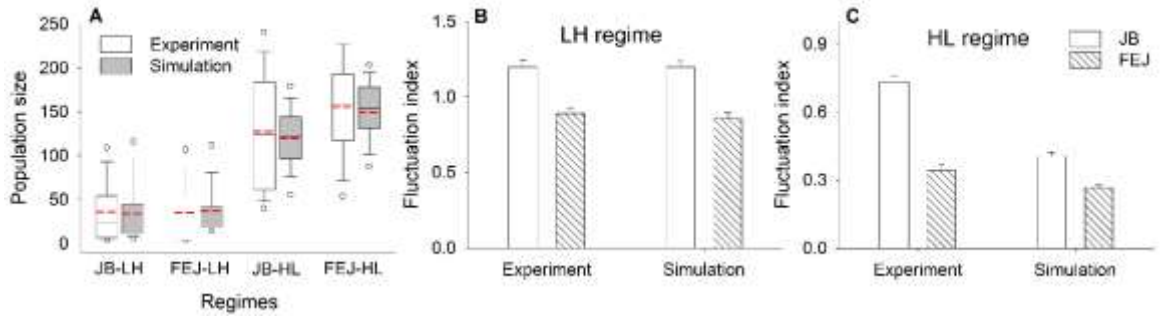

Figure 5.12. Validating model predictions on JB and FEJ populations. (**A**) Descriptive statistics of the population size distributions of experimental and simulated JB and FEJ populations. Red dashed lines = means, thin black lines = medians, edges of the boxes=25th and 75th percentiles, whiskers=10th and 90th percentiles and the circles outside = 5th and 95th percentiles of the distributions. White boxes represent experimental data while grey shaded boxes denote simulated time-series. Average (± SEM) *FI* of JB and FEJ populations corresponding to the experimental and simulated time-series under (**B**) LH and (**C**) HL regimes. Experimental data shows that in both the regimes, FEJs have lower *FI* than the JBs, as predicted by the model. Simulated FEJ populations capture well the empirical trends for population size distribution and constancy stability.

One of the predictions of our model is that decreasing m_c should lead to stabilization of the dynamics (Figure 5.8B and Section 3.3.1). This prediction is consistent with earlier theoretical studies (Mueller, 1988) and has been empirically validated using laboratory populations of *D. melanogaster* (Dey et al., 2008; Prasad et al., 2003). These earlier experiments used a population of flies (FEJs) that had evolved reduced m_c as a correlated response to selection for faster development and early reproduction. Consequently, they were found to have reduced *FI* compared to the corresponding ancestral control populations (JBs). In order to see whether our model was capable of recovering the other features of the

dynamics from the earlier experiment (Dey et al., 2008), we set a slightly lower value of m_c for the FEJs and kept all other parameters the same as in the previous simulations (Table 5.1). Our model was again able to capture the trends in the distributional properties (Figure 5.12A) and the *FI* values (Figure 5.12B and 5.12C) of JB and FEJ populations in both regimes. The empirical data in Figure 5.4 and Figure 5.12 are from two completely independent experiments done at different times. The fact that our model is able to predict the major features of the latter data-set based on parameterizations done for a subset of the former shows that our parameterization was robust. However, we again emphasize here that the main focus of this part of the study was to gather insights about how the various life-history and environmental parameters interact, and the excellent quantitative match between the data and the model is essentially a secondary, though greatly encouraging, result.

3.7 Comparison with experiments and models on other species

Since our model is based on fairly generic life-history features (see section 2.3.1), it is reasonable to assume that it would be a good predictor of the dynamics across a wide range of taxa. For example, in another dipteran, the pitcher-plant mosquito (*Wyeomyia smithii*) increased larval food amounts reduces larval mortality and increases adult fecundity (Istock et al., 1975). Furthermore, in the same species, adult fecundity is positively and negatively affected by increased levels of adult food and adult crowding respectively (Istock et al., 1975). Since these are exactly the processes that govern the dynamics of *Drosophila* laboratory populations, it can be safely predicted that the dynamics of *W. smithii*, under different nutritional levels, will be well captured by our model. Unfortunately, we could not locate any studies on the population dynamics of this species to verify this prediction.

Paucity of studies in the literature was not the problem with the widely-studied crustacean genus *Daphnia*. In this system, increasing food concentration increases size at maturity and fecundity (Boersma and Vijverberg, 1995; Rinke and Vijverberg, 2005), as well as the slope of the body length-clutch size relationship (Rinke and Vijverberg, 2005), which is equivalent to increasing the value of *sen_adsize* in our model. An IBM incorporating these relationships predicted increased amplitude of fluctuations in population sizes with increasing concentration of food (Vanoverbeke, 2008), which agrees well with experimental observations (McCauley et al., 1999) and our results (Figure 5.8D). Simulation studies on another crustacean (*Armadillidium vulgare*) showed that increased level of food also increases the population size (Rushton and Hassall, 1987), which is consistent with our

observations (Figure 5.11A). It should be noted here, though, that all these *Daphnia* experiments and models deal with continuous culture systems, as opposed to the discrete generation systems investigated in our study. Moreover, in these systems, the adults and the juveniles share the same food, whereas in our model, the effects of the larval food (determined by the parameter *food*) is different from the effects of the adult nutrition (modeled using *adnut*). In spite of these non-trivial differences, the primary mechanism by which food amounts affect the life history, and thereby the dynamics, remain alike.

Moving away from the arthropods, not surprisingly, leads to more serious departures from the insights gathered from our study, although some broad patterns are still discernible. For example, an IBM for the fish yellow perch (*Perca flavescens*) showed that increased food (forage fish) amount leads to larger body size, higher fecundity and lower adult abundance (Rose et al., 1999). However, in the same study, when the amount of food was increased for another fish, walleye (*Sander vitreus*), the model predicted larger body size, lower fecundity and higher adult abundance. This difference was partly due to the fact that the life-history parameters and the ecological food-bases of the two fishes were very different. Although the authors did not report the effects of these food manipulations on the population dynamics, it is clear that changing the levels of food can affect the population size, which appears to be a fairly robust observation in this context. In a comprehensive review covering 138 species, including reptiles, birds and small mammals, Boutin reported that adding food to the environment typically leads to 2-3 fold increase in population density but little or no change in the population dynamics (Boutin, 1990; although see Klenner and Krebs, 1991 and references therein). More importantly, litter or clutch sizes are hardly affected by food supply, except when there is severe food shortage due to natural reasons or severe overpopulation (Boutin, 1990).

There can be several reasons for the differences between the observations of these vertebrate studies and ours. First, there is no reason to expect that a model based on arthropod life history can accurately capture the population dynamics of vertebrates. Second, it is very difficult to estimate the parameter space for the vertebrate systems that is analogous to the parameter space used in our model. Third, all the vertebrate studies quoted here are on natural populations with multiple sources of food and other ecological interactions, at least some of which can interact with the effects of food manipulation in non-intuitive ways (e.g. Klenner and Krebs, 1991).

The above account suggests that there are substantial differences between how food levels affect the dynamics of insects / crustaceans on one hand and higher vertebrates on the other. Not surprisingly, our model is a better descriptor of the former. With that knowledge, we were in a position to go beyond laboratory populations of *D. melanogaster* and investigate the general consequences of various demographic processes for population dynamics. For the purpose of this study, we chose to investigate the effects of unequal numbers of males and females, and particularly its interaction with the amount of food available, on population size and stability.

3.8 Simulations: The effects of sex-ratio and sex-specific culling are greatly influenced by fecundity but not by levels of juvenile food

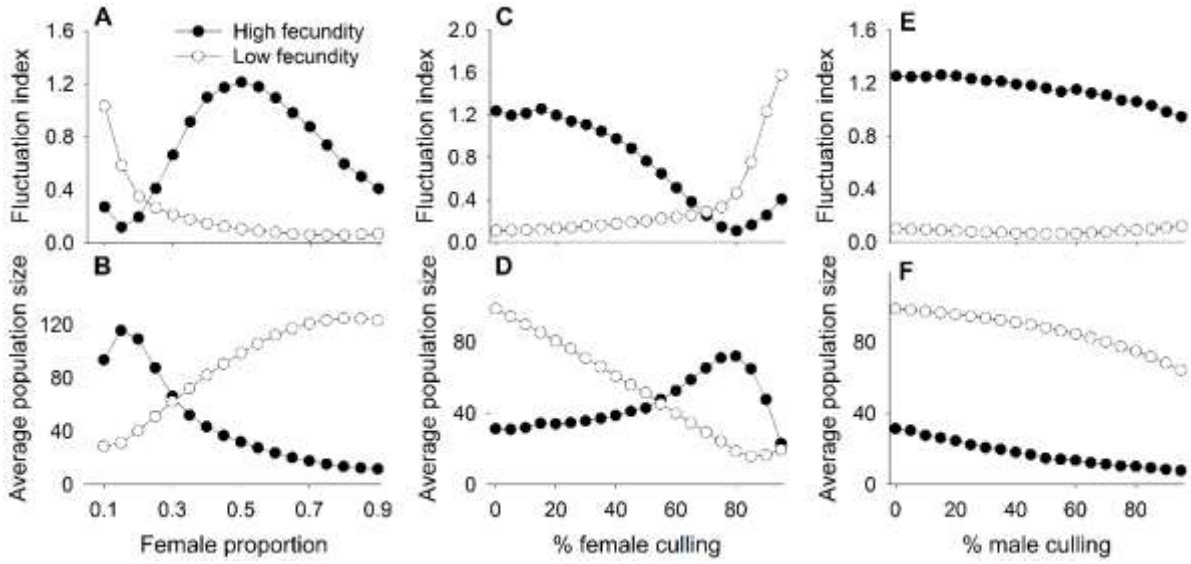

Figure 5.13. Effect of varying sex-ratio and sex-specific culling on population dynamics for low nutrient scenario. Effect of varying sex-ratio and sex-specific culling on population dynamics under low nutrient levels. **A)** Fluctuation index and **B)** Average population size as a function of the expected fraction of females in the population. **C)** Fluctuation index and **D)** Average population size as a function of the percentage of females culled. **E)** Fluctuation index and **F)** Average population size as a function of the percentage of males culled. Closed and open circles denote high and low levels of density-independent adult fecundity. See section 3.8 for explanations. Each point represents average (± SEM) fluctuation index or population size of 100 replicates of 100-gen long simulated time series. Error bars are too small to be visible.

Theoretical studies have typically neglected the effects of sex ratio on the stability of population dynamics (although see Johnson, 1994), an important lacuna that we address here. We found that when the baseline fecundity is high, the *FI* of the population is highest when the sex ratio is close to 1:1 and reduces when the sex ratio becomes more skewed (closed circles in Figure 5.13A). This is consistent with the observation that population growth rate typically maximizes either when both sexes are present in equal proportions or when the

proportion of females is slightly higher (Jenouvrier et al., 2010; Miller and Inouye, 2013). However, although equal or female-biased sex ratios are also expected to maximize the average population size (Rankin and Kokko, 2007; Schmickl and Karsai, 2010), our model showed that the maximum population size was reached when the proportion of females in the population was ~15%, i.e. when the population was male-biased (closed circles in Figure 5.13B). This general pattern was not altered irrespective of the amount of food given to the larvae (*cf* Figure 5.13 with Figure 5.14).

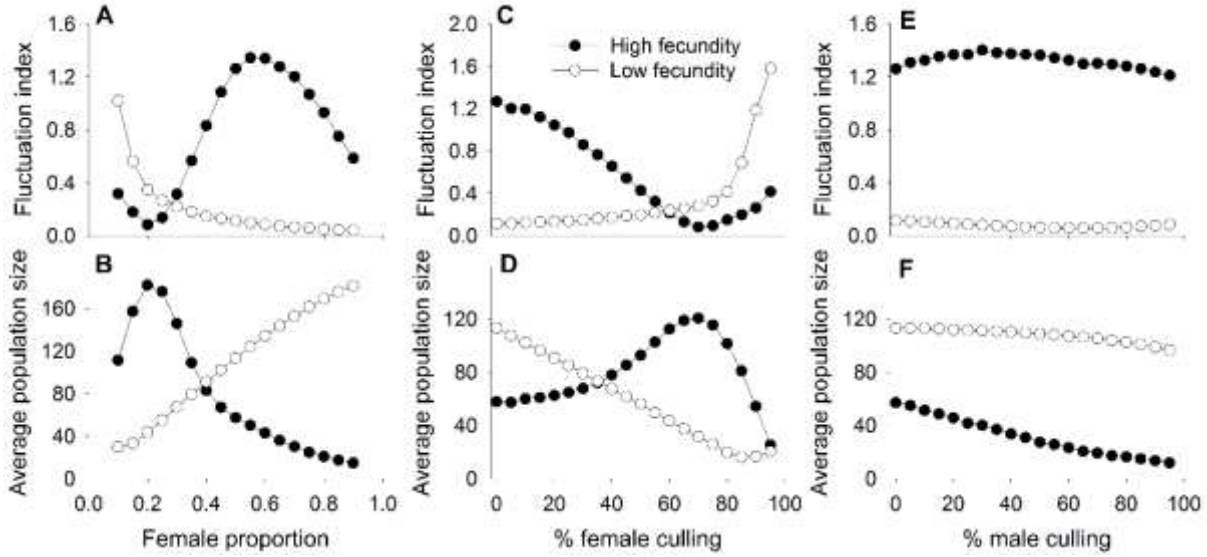

Figure 5.14. Effect of varying sex-ratio and sex-specific culling on population dynamics for high nutrient scenario. Fluctuation index and average population size were computed as the proportion of females (**A** and **B**), percentage of female culling (**C** and **D**) and male culling (**E** and **F**) increases in the population respectively. Each cases were investigated at two levels of density-independent fecundity. Here, each point represents average (± SEM) fluctuation index or population size of 100 replicates of 100-gen long simulated time series. Error bars are too small to be visible.

To explain this observed discrepancy, we note that many of the previous studies on effects of sex ratio assume the mating success of the females to be a function of the number of available males (Jenouvrier et al., 2010; Rankin and Kokko, 2007; Schmickl and Karsai, 2010). Although applicable for strictly monogamous species, this assumption need not always be true for insects like *D. melanogaster* where both males and females mate multiple times (although see Vahl et al., 2013), and a single mating can sustain high lifetime fecundity of a female, unless the proportion of males is extremely low. That is the reason our model assumed that all females contributed to the egg numbers of the next generation, irrespective of the number of males in the population (we relax this assumption in the next section). Consequently, male numbers can only have negative effects on the number of eggs in the next generation through the density-dependent reduction in fecundity. On the other hand,

each female in the population has a positive effect on the number of eggs in the next generation through its fecundity, and a negative effect through its contribution to the density-dependent reduction in fecundity (eq. 4). The sex ratio leading to the maximum population size is thus determined as an interaction between these two opposing effects. When female fecundity is high and not male-limited, this number is much lower than what is expected when these two conditions are not met. This explains why, in our model, the maximum population size is attained under male-biased conditions. When the number of females in the population is increased beyond this threshold, the number of eggs in the next generation increases, which, in turn, results in more intense larval crowding. Conceptually, this is similar to boosting the fecundity by supplying yeast to the adults which reduces the average population size (Figure 5.4A), as can be seen by comparing LL with LH and HL with HH in Figure 5.4A.

When the density-independent fecundity was low (open circles in Figure 5.13A and 5.13B) there was monotonic increase and decrease in average population size (Figure 5.13B) and *FI* (Figure 5.13A), respectively, with increasing proportion of females in the population. This trend was clearly different from the high fecundity case (*cf* open circles and closed circles in Figure 5.13A and Figure 5.13B), indicating that fecundity itself can alter the effects of sex ratio on population dynamics. To the best of our knowledge, this effect has previously not been reported in the literature and can be understood as follows. When the per capita female fecundity is low, the number of eggs in the next generation is reduced and the average size of the population is therefore limited more by the total number of eggs produced than the larval density-dependent mortality. Moreover, under such circumstances, the populations are also more vulnerable to demographic stochasticity, which leads to an increase in *FI*. Consequently, with increases in proportion of females, both the average size of the population and the population stability increases.

Sex-specific mortality or culling of the adult individuals: In a population, sexes often suffer unequal mortality rates. This can happen due to, *inter alia*, sex-biased predation (Boukal et al., 2008) or human preference for a given sex for commercial or other purposes. We modeled this phenomenon using our model by explicitly culling a fixed percentage of a sex in each generation and, not surprisingly, found that female (Figure 5.13C-D) and male (Figure 5.13E-F) culling have very different effects on the dynamics. The patterns observed in Figure 5.13C-F can be explained by noting that female culling increases the ratio of males in the population and vice versa, and the subsequent effects on *FI* and average population size can

be deduced based on Figure 5.13A-B. One interesting point to note is that when the female fecundity is high, culling of females can increase the average population size, a phenomenon that is termed as the Hydra effect in the population dynamics literature (Abrams, 2009). However, when female fecundity is low, increasing the fraction of females culled reduces population size, which is consistent with recent theoretical and empirical data on the beetle *Callosobruchus maculatus* (Snyder et al., 2014). Moreover, although changing the amount of juvenile food led to changes in the numerical values of the average population size and *FI*, the corresponding patterns remained unaltered (*cf* Figure 5.13C-F and Figure 5.14C-F), suggesting that the effects of changes in sex-ratio on population dynamics are not altered by juvenile nutrition.

To summarize, different amounts of juvenile food affects the interaction of sex ratio and population dynamics quantitatively but not qualitatively. However, in species like *Drosophila*, where adult fecundity can be increased by giving external food (equivalent to increasing *adnut*), adult food levels will play a role in determining the qualitative and quantitative effects of unequal numbers of sexes on the dynamics.

3.9 Simulations: Fecundity and nutrition levels interact to determine the efficacy of Sterile Insect Technique (SIT)

One of the major applications of skewed sex-ratios has been in the context of SIT, wherein a large number of sterile individuals (typically males) are released into an insect pest population (Klassen, 2005). These sterile individuals compete with the fertile individuals, thus reducing their reproductive fitness, which ultimately leads to effective suppression of population density or complete eradication of the pests (Knipling, 1955). This method has been successfully used to eradicate several pest species like melon fly, Tsetse fly, screwworm, Mexican fruit fly and West Indian fruit fly (see Dyck et al., 2005 for a comprehensive review). Not surprisingly, the consequences of releasing large number of sterile males have been extensively investigated in the theoretical population dynamics literature (reviewed in Barclay, 1980) primarily using simple non-linear models (e.g. Berryman et al., 1973). These studies have typically not incorporated the details of life-history or environmental variables like food amount on the efficacy of SIT. In fact, we could locate very few individual or agent-based models on SIT in the literature (Isidoro et al., 2009; Lin et al., 2015; Stone, 2013). Simple models predict that when the fecundity of females is high, introducing sterile males can increase the pest population size (Berryman et al., 1973),

whereas the present study shows that changes in larval food amount can affect the dynamics of populations. Therefore, we used our model to explore the interaction of the amount of food and fecundity of the pests on the expected number of individuals needed for a successful pest eradication using SIT.

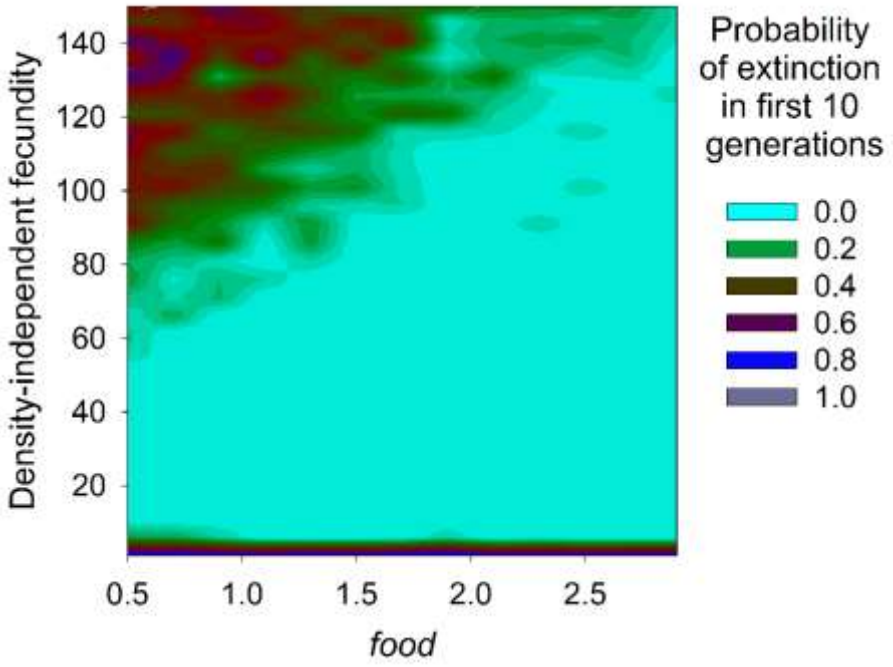

Figure 5.15. Probability of extinction within 10 generations in *food* × density-independent fecundity space. For each combination of food and fecundity values, we computed average probability of extinction within first 10 generations over 10 replicates and plotted according to the adjacent colour index.

Following prior studies (Knipling, 1959), we assumed that the number of females that get fertilized and lay eggs is a function of the ratio of fertile to sterile males in the population (see section 2.5). As noted earlier, neither our fly populations nor our model-derived time series underwent too many extinctions. However, for this set of simulations, we needed to consider parameter values of food and density-independent fecundity (i.e. $adnut \times x_5$ in equation 3) that were very different (greater as well as lesser) than what was applicable for our fly populations. Consequently, the extinction rates no longer remained negligible which in turn meant that sterile male induced extinctions needed to be distinguished from endogenous extinctions. Therefore, we first simulated the probability of extinction over 10 generations for various combinations of fecundity and food values (Figure 5.15). From this set, we used only those fecundity-food combinations which had less than 10% probability of going extinct without any perturbation to estimate the minimum number of sterile insects to be introduced that could lead to population extinction within 10 generations with a 90% probability (Figure 5.16).

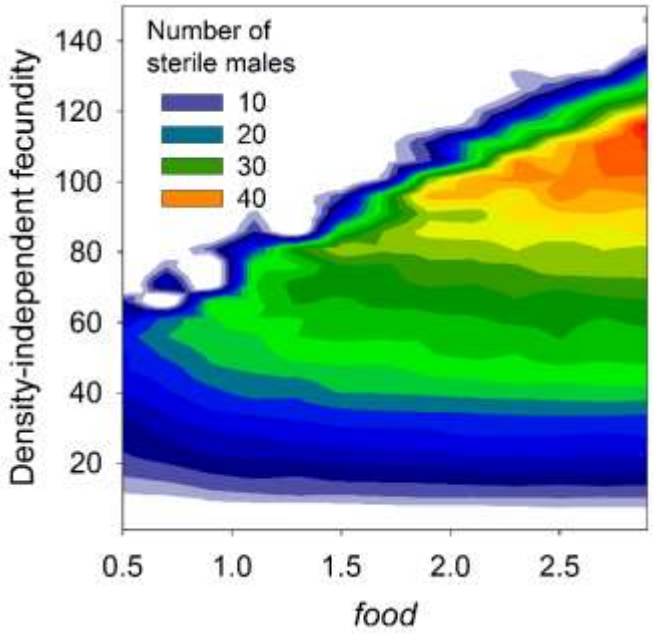

Figure 5.16. Effect of varying food and density-independent fecundity of the model on sterile male technique. For each combination of *food* and density-independent fecundity, this graph denotes the minimum number of sterile males needed to induce extinction within 10 generations with 90% certainty. Each point here is an average value obtained from 100 replicate simulations and plotted according to the adjacent colour index. Broadly, when fecundity is low, required number of sterile males does not depend on the amount of available food. But, for higher fecundity values, food and fecundity together determine the required number of sterile males needed for the desired level of extinction. For this analysis, we have considered only those food × fecundity combinations, where the basal probability of extinction is <10%.

Thus, the lower the number of males needed to induce extinction, the higher was the efficiency of SIT. We found that when density-independent fecundity is <~40, changing the amount of food had no effects on SIT efficiency. However, beyond this range, the efficiency of SIT reduced with the amount of food available, and the effect became more prominent as fecundity increased. The reason for this observation can be understood when we consider the effects of increasing the amounts of food for different fecundity levels. When fecundity is low, larval crowding is less and therefore, increasing the amount of food does not increase the population size. Consequently, the number of sterile males needed to bring extinction is also unaffected. However, when the fecundity is high, there is considerable larval crowding. Consequently, increasing the amount of food increases the average population size (as seen in Figure 5.11A) which in turn reduces the efficiency of SIT. Thus we find that the efficiency of SIT depends on an interaction between the amount of food available to the larva and its density independent fecundity (which can be altered by environmental factors). To the best of our knowledge, this interaction has not hitherto been reported. It should be noted here, that for our flies, the values of density independent fecundity (i.e. $adnut \times x_5$) considered were 85

and ~125 for un-yeasted and yeasted conditions respectively. Therefore, the observed interaction between food and fecundity happens in a parameter range for fecundity (>~40) which is very relevant biologically. Another point regarding these results is the order of magnitude of the population sizes. In nature, pest population sizes would typically be in orders of 10^5-10^7 whereas in our simulations, the population sizes were in orders of 10^1-10^2. This is because it is known that SIT is typically much more effective at low population sizes and therefore any application of SIT in the field is typically preceded by a chemical treatment that greatly reduces the population size (Klassen, 2005). Thus, although the population sizes in our simulations are smaller than what would be experienced in a real-world application of SIT, they are off by no more than 1-2 orders of magnitude. Nevertheless, we believe that our results are still relevant to SIT application since the purpose of our study was not to model the application of SIT to a particular real world population of a specific insect pest species, but rather to derive insights about how outcomes of sterile male release could possibly be affected by nutrition × fecundity interactions.

4. CONCLUSION

Mathematical modeling of the dynamics of laboratory populations has a long and venerable history (Kingsland, 1995; Mueller and Joshi, 2000) and has been extensively done for several model systems like *Tribolium* (Costantino et al., 1997), *Callosobruchus* (Tuda and Shimada, 2005), protists (Holyoak et al., 2000), mites (Benton and Beckerman, 2005) etc. Depending on the objectives of their investigation, these studies have employed different kinds of modeling tools, ranging from simple deterministic difference equations, to coupled differential equations and individual-based models (reviewed in Mueller and Joshi, 2000). The value of our study is first in the close correspondence between empirical observations and simulation results, and second in terms of the insights gained regarding the interaction of the environmental factors (larval and adult food level) with life-history related traits to determine population dynamics and stability. Our model was able to replicate several predictions about the dynamics of insects and crustaceans. Given that these two taxa represent about 70% of all animal species on earth (Zhang, 2011), one can be reasonably confident that the general insights derived from this study are broadly applicable.

CHAPTER 6

Simultaneous evolution of multiple dispersal components and kernel in laboratory populations of *Drosophila melanogaster*

Highlights

- Rapid, simultaneous evolution of dispersal propensity, ability and kernel in flies.
- Evolution of long-distance dispersers (LDDs) leading to 67% greater spatial extent.
- Context dependent sex-biased dispersal exists in *Drosophila melanogaster*.
- Male and female flies respond similarly to selection for greater dispersal.

Adapted from: **Tung, S.**, Mishra, A., Shreenidhi, P. M., Sadiq, M. A., Joshi, S., Sruti, V. S., Dey, S. 2017. Simultaneous evolution of multiple dispersal components and kernel. Oikos 127, 34–44.

INTRODUCTION

Climate change (reviewed in (Root et al., 2003) and various human activities (Vitousek et al., 1997) have led to massive habitat loss and fragmentation, which in turn have affected many natural populations all over the world. These effects include, *inter alia*, loss of biodiversity, increase in extinction probability, modified species interaction patterns within a community, decrease in the average length of the trophic chains and reduced reproductive success (reviewed in Fahrig, 2003). Dispersal is one of the ways by which organisms can cope with such stresses as it allows them to increase their survival probability by tracking favorable environmental conditions (Travis et al., 2013). As a result, evolution of dispersal and its consequences have been a major focus of research in evolutionary ecology for the last few decades (reviewed in Bowler and Benton, 2005; Clobert et al., 2012; Ronce, 2007). According to the classical theoretical literature, dispersal could evolve due to three primary reasons, namely, inbreeding avoidance (Charlesworth and Charlesworth, 1987), reduction of kin-competition (Gandon, 1999) and spatio-temporal environmental heterogeneity (McPeek and Holt, 1992). However, substantial bodies of empirical and theoretical work over the last few decades suggest that the picture may not be so simple after all (reviewed in Bonte et al., 2012).

In terms of movement, the phenomenon of dispersal is often subdivided into three stages, namely emigration from the natal habitat, inter-patch movement and immigration into the destination patch (Bowler and Benton, 2005). The environment experienced during each of these stages, and the corresponding behavioral and physiological attributes needed to tackle them, can be very different. Consequently, in terms of life-history, dispersal is actually a composite trait, made up of components like propensity (i.e. the fraction of dispersers leaving the current habitat) which is primarily related to emigration, and ability (i.e. the mean distance travelled) which is primarily related to inter-patch movement. Evidently, which component(s) of dispersal evolve(s) is contingent upon the nature of the selection pressure faced by each component, the costs associated with them, how these costs interact with each other and how they are countered by the organisms (Bonte et al., 2012). For example, in laboratory populations of *C. elegans*, dispersal propensity evolves when patch fitness is varied by externally imposed extinctions (Friedenberg, 2003). However, the same trait fails to evolve when patch fitness is altered by varying resource density (Friedenberg, 2003). Similarly, spatially correlated extinctions select for long distance dispersers in the spider mite (*Tetranychus urticae*) but randomly distributed local extinctions do not (Fronhofer et al.,

2014). To complicate matters further, although the various components of dispersal are related to each other, evolution of a given component does not necessarily make an organism better in terms of another component. For example, in spider mites, artificial selection can increase dispersal propensity (Yano and Takafuji, 2002) but not dispersal ability (Bitume et al., 2011). In the same organism, when selection is imposed in the form of spatially correlated extinctions, the frequency of long distance dispersers (LDDs) increases but dispersal propensity is reduced (Fronhofer et al., 2014).

Thus, for any organism under a given ecological scenario, a complete picture of dispersal evolution is possible only when all the dispersal components are simultaneously investigated. Unfortunately, most empirical studies typically focus on the evolution of any one of the several components of dispersal (Bitume et al., 2011; Friedenberg, 2003; Keil et al., 2001; Ogden, 1970; Tien et al., 2011; Yano and Takafuji, 2002; although see Fronhofer et al., 2014). This makes it somewhat difficult to envisage how the evolutionary responses to the individual components of dispersal ultimately come together to affect the distribution of dispersal distances of individuals, i.e. the dispersal kernel (Nathan et al., 2012).

The kernel is one of the most frequently used descriptors of the outcome of dispersal in the ecological and the evolutionary literature (reviewed in Nathan et al., 2012). Empirical studies suggest that dispersal kernels in natural populations are often "fat-tailed" (Clark et al., 2005; Van Houtan et al., 2007). This implies that many natural populations have a larger number of long-distance dispersers or LDDs, i.e. individuals who disperse far more than the mean dispersal distance of the population than what is expected by a Gaussian function. The presence of LDDs can impact several ecological phenomena like range advance (Phillips et al., 2008), effects of habitat fragmentation (Van Houtan et al., 2007), invasive potential (Kot et al., 1996) and disease spread (Rappole et al., 2006). Thus, evolution of the kernel in general, and the fraction of LDDs in particular, is a major topic of interest in the context of dispersal evolution (reviewed in Hovestadt et al., 2012). Unfortunately, although it is easy to conceptualize a dispersal kernel, it is not experimentally simple to measure it. Moreover, the observed dispersal kernel is a product of the phenotype of the organism and the environment through which dispersal is happening. Differentiating between these two effects is not always a straightforward task. Not surprisingly, therefore, although theoretically well-investigated (e.g. Phillips et al., 2008; Starrfelt and Kokko, 2010), we are aware of only one empirical study that has demonstrated kernel evolution (Fronhofer et al., 2014).

Another factor that can play a role in the evolution of dispersal in a population is sex. Sex-biased dispersal (SBD) is well-documented in the animal kingdom, particularly among birds and mammals (reviewed in Pusey, 1987), and also insects (Bennett et al., 2013; Lagisz et al., 2010). Although SBD is hypothesized to be primarily driven by the mating system of the species (Greenwood, 1980), recent studies have challenged this claim (Mabry et al., 2013; Trochet et al., 2016). One major complication here is that SBD has often been investigated in the context of any one component of dispersal like propensity or ability (Clutton-Brock and Lukas, 2012). However, the presence or absence of SBD for one dispersal component (say propensity) does not allow us to make any predictions about it in the context of another dispersal component. Moreover, potentially, the realization of SBD for a given dispersal component itself can be environment-dependent. To take a hypothetical example, one sex (for example, males) might show greater dispersal ability only under resource limitation and not when resources are plentiful. Clearly, this context-dependence of SBD has consequences for gene flow across the habitats and how that affects the standing variation (Prout, 1981). It can also potentially alter the evolutionary outcome depending on whether resources were available or not during the selection process. Thus, in order to obtain a more detailed picture of SBD in a given species, it is again critical to simultaneously investigate multiple dispersal components in different environments.

To address some of the issues discussed above, we subjected four large, replicate populations of *Drosophila melanogaster* to directional selection for increased dispersal. Our selection protocol mimicked increasing habitat- fragmentation over generations, with starvation and desiccation stress being the primary inducer of dispersal. The selected populations rapidly evolved to have significantly greater dispersal propensity and ability, irrespective of the presence or absence of starvation/desiccation stress. To the best of our knowledge, this is the first report of condition-dependent selection leading to the evolution of phenotype-dependent dispersal. We then describe the impact of these evolutionary changes on the shape of the dispersal kernel and how that affected the spatial extent. We also investigate whether there was sex-bias in different dispersal components and whether selection caused the two sexes to respond differently. Finally, we briefly discuss some of the eco-evolutionary implications of our results and why some of them do not match with previous observations in the literature.

METHODS

Ancestral populations: The experimental populations used in this study were derived from four independent large (breeding size of ~2400) laboratory populations of *Drosophila melanogaster* (DB_{1-4}) which in turn trace their ancestry to four outbred populations called JB_{1-4}. The detailed maintenance regime and ancestry of the JB_{1-4} populations has been described elsewhere (Sheeba et al., 1998). The maintenance regime of the DB_{1-4} populations are similar to the JB_{1-4}, except that the former set of flies are introduced into population cages on the 12th day after egg collection

From each DB_i population (where $i \in [1, 4]$), we derived two populations: VB_i (short for 'vagabond', subjected to selection for dispersal) and VBC_i (corresponding no-dispersal control). Thus VB and VBC populations that share a numerical subscript (e.g. say VB_1 and VBC_1) were related by ancestry (DB_1 in this case), and hence were always assayed together and treated as blocks in statistical analyses.

Maintenance regime of experimental populations: The adults of both VBs and VBCs were maintained in plexi-glass population cages (25 cm × 20 cm ×15 cm) at a high adult number (~2400 individuals) to avoid inbreeding. Following earlier protocols, both the larvae and the adults were maintained at 25°C and constant light conditions (Sheeba et al., 1998). The flies were made to oviposit on petri-plates containing banana-jaggery medium for 12-16 hours. After oviposition, we cut small strips of the medium, each containing ~60-80 eggs, and introduced them individually into 35 ml plastic vials that had ~6 ml of the same banana-jaggery medium. This ensured that the larvae were raised under low to moderate level of crowding, and there was no confounding effect of density-dependent selection (Joshi, 1997). The adults started emerging by the 8th-9th day after egg collection and on the 12th day, the VB populations underwent selection for dispersal (see below). Since at 25°C temperature, all normally developing adults eclose by 10th -11th day, our selection protocol ensured that there was no inadvertent selection for faster larval development (Prasad et al., 2001). After the imposition of selection, the flies were transferred to the population cages and immediately supplied with excess live yeast- paste to boost their fecundity. Around 40 hours after this, the flies were supplied with a fresh petri-plate containing banana-jaggery medium for oviposition. The eggs so collected formed the next generation and the egg-laying adults were discarded, ensuring that adults from two different generations never co-exist. Thus, both VBs and VBCs were maintained under 15-day discrete generation cycles. For each VB population,

we collected eggs in 80 vials (thus leading to approximately 4800 adults) while for VBCs, the corresponding number of vials was 40. This ensured that after selection (see next section), the breeding population of the VB populations was similar to that of the VBCs.

Selection protocol: The apparatus for selection for dispersal consisted of three components: a *source*, a *path* and a *destination*. The source was an empty transparent cylindrical plastic container of diameter 11 cm and height 16 cm with a funnel attached to one end (Fig. 6.1). The diameter of the broad end of the funnel matched that of the source, while the diameter of the exit to the stem was 1.8 cm. The path connecting the source with the destination consisted of a transparent plastic tube of inner diameter ~1 cm. The destination too was a cylindrical plastic container (diameter 11 cm and height 16 cm) and contained a supply of moisture in the form of a strip of wet cotton. The end of the path protruded ~10 cm inside the destination (Fig. 6.1). This protrusion helped in reducing the rate of backflow as, after getting out of the path, the flies typically spent most of their time on the walls or floors of the container, and hence mostly failed to locate this aperture. To make the overall setup compact, the path was coiled (in the horizontal plane). The length of the path was 2 m at the beginning of the selection, but was increased intermittently. By generation 33, when the last set of assays were performed, the path length had reached 10 m.

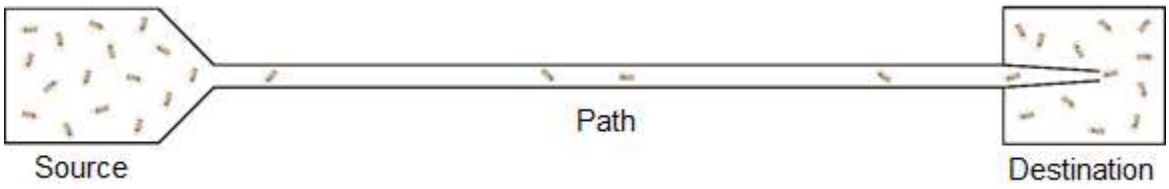

Figure 6.1. Schematic diagram of the selection and assay setup. The source and the destination are transparent plastic containers. The path is a transparent plastic tube. The path protrudes ~10 cm inside the destination; this protrusion considerably reduces backflow of the flies. Here, all the three parts-- the source, path and the destination are detachable. The tiny objects oriented randomly inside the setup denote the flies. The length of the path increased from 2m to 10m during the 33 generations of selection reported here.

In order to impose the selection, on the 12^{th} day after egg-collection, ~2400 adults (coming out of 40 vials) of a given VB_i population were placed in a source, which was then connected to the destination via the path. The entire setup was placed in a well-lit room maintained at 25 °C. Since the source had no moisture, the flies experienced desiccation. Pilot runs with the ancestral DB populations had shown that under these environmental conditions, a subset of the flies tended to move through the opening towards the destination. Pilot studies also

showed that very few flies dispersed in the presence of food in the source and therefore we decided to impose selection in the absence of food. The flies were allowed to disperse for six hours or until roughly 50% of the population reached the destination (whichever happened earlier). The arbitrary cut-off of six hours was chosen because assays in the lab had demonstrated that under desiccating conditions, there was almost no mortality during the first six hours (S. Tung personal observations). Only the flies that reached the destination were allowed to breed for the next generation. Since the imposed selection allowed ~50% of the flies to breed, there were two independent "source-path-destination" setups, with ~2400 flies in the source, for each VB_i population. Post-selection, the dispersed flies in the two destination containers for a given VB_i population were mixed and transferred to a population cage. They were then supplied with live-yeast paste and after ~40 hours, eggs were collected (as mentioned above). The VBCs were maintained similarly as the VBs except two major differences. Firstly, after transferring the flies into the source, the exit was blocked by a cotton plug and the flies were allowed to desiccate for 3 hours or till 25% of the VBs reached their destination (whichever was earlier). Following the protocol for the VB flies, the VBC flies were then supplied with a moist cotton plug for the remaining duration of VB dispersal. This controlled for the inadvertent desiccation experienced by the VB flies in the source and the path, as part of the selection protocol. It should be noted here that there was almost zero mortality in the VBC flies during this time, thus ensuring that the selection pressure for desiccation resistance was at best, mild. Secondly, all the flies in the VBC populations were allowed to breed, thus ensuring no selection for dispersal.

Assays:

All assays were performed after relaxing the selection on both VB and VBC populations for one generation. For this, the VB and VBC flies were transferred directly into the corresponding cages on the 12^{th} day after egg collection. The progeny of these flies, henceforth referred to as the relaxed populations, were used for the assays. This common-rearing ensured that influence of phenotypic plasticity or non-genetic parental effects were ameliorated. Additionally, to remove any extraneous influence due to larval crowding, egg density was kept to ~50 eggs on ~6mL food in each vial. Furthermore, as the assays for each of the four blocks required us to sex and count ~12,000 flies, it was not logistically possible to assay more than two blocks in a given generation. Therefore, each assay was conducted over two successive generations and it is the latter value which is reported in the paper (i.e. for the t^{th} generation assay, VB_1-VBC_1 and VB_2-VBC_2 were assayed in generation t-1 while

VB_3-VBC_3 and VB_4-VBC_4 were assayed in generation t). For example, for the 33rd generation assays, block 1 and 2 were assayed during the 32nd generation of selection, while block 3 and 4 were assayed during the 33rd generation and so on. This is not a problem in terms of our statistical analysis as block was explicitly recognized as a random factor in our ANOVA.

Dispersal assay in presence and absence of food: This assay was performed thrice- after 10, 20 and 33 generations of selection in order to assess the difference in dispersal propensity and ability between the VBs and the VBCs. The assay-setup was similar to the selection setup (see 'Selection protocol' above and Fig. 6.1) except for the length of the path. The path-length was 10 m for the assay performed after 10 generations of selection and for the rest it was 20 m. Furthermore, to obtain the distribution of the location of the files after dispersal, the path was divided into multiple detachable sections: 20 sections of length 0.5 m each for the first 10 m and followed by 10 sections of length 1 m each for the rest (1 m sections were not present when the path-length was only 10 m). The destination container (a 250 ml plastic bottle) did not contain food or water but had a long protrusion of the path into it, to reduce the backflow of flies. On the 12th day after egg collection, ~2000 adult flies were introduced into the source container and were allowed to disperse for 6 hours. During this interval, the entire setup was kept undisturbed under constant light and at a temperature of 25˚C. After the end of dispersal run, the setup was dismantled; the openings of the source, the destination, and each section of the path were secured carefully with cotton plugs, and labelled appropriately. The flies were then heat killed and the location (in terms of the distance from the source) and sex of each fly was recorded. For each VB_i and VBC_i population, there were three such replicate kernel setups.

We performed two kinds of kernel assays: a) with an empty source and b) in the presence of ~20 ml banana-jaggery medium in the source. The former set of assays was performed after 10 and 20 generations of selection while the latter set of assays happened after 33 generations of selection. In total, these assays involved segregating according to sex and scoring of ~140, 000 flies.

Dispersal components

a) Dispersal propensity

The proportion of total flies in the source that initiated dispersal was taken as the dispersal propensity (Friedenberg, 2003). Thus propensity = (Number of flies found outside the source/ Total number of flies).

b) Dispersal ability

The dispersal ability was computed only on the flies that left the source, based on the section of the path in which they were found after 6 hours. All flies found in a given section of the path were deemed to have travelled the distance between the source and the midpoint of the section. The destination container was considered as a part of the last path-section. Thus mathematically,

$$dispersal\ ability = \frac{\sum_{i=1}^{y} x_i n_i}{\sum_{i=1}^{y} n_i}$$

where, n_i is the number of flies found in the i^{th} path-section, x_i is the distance of the mid-point of this section from source and y is the total number of path-sections (here y = 30, see Section 'Dispersal kernel assay in presence and absence of food' for details). Since dispersal ability is measured only on the flies that came out of the source, the measure of propensity and ability were independent of each other.

Measures of dispersal kernel and spatial extent: Dispersal kernels of the VB/VBCs were characterized using the various percentiles of the distribution. Change in the mean distance travelled, in principle, can shift the kernel without changing its shape. We eliminated this effect by computing the percentiles after subtracting the mean distance travelled in a given kernel replicate from the distance travelled by each individual in the replicate. Thus for each replicate, mean-subtracted distance travelled by each individual (i.e. $x_i - \sum x_i / n$), where x_i is the distance travelled by i^{th} individual and n is the number of individuals that initiated dispersal in that replicate, was used for computing percentiles. To investigate shape, we calculated the higher moments of the dispersal kernel like standard deviation, skew and kurtosis (where the kurtosis of a normal distribution was taken to be zero).

To further characterize the kernel data obtained from the dispersal kernel assay in presence of food, were fitted with the negative exponential distribution $y=ae^{-bx}$, where x is the distance from the source, y is the frequency of individuals found at x, and a, b are the intercept and slope parameters respectively. For this we pooled the data of the three replicates for each of the four populations of VB and VBC, estimated the frequency for each distance, natural log-transformed all values and fitted the equation ln(y) = ln(a) – bx using linear regression. The estimated R^2 values (Table 6.1) ranged between 0.67 and 0.99 and the residuals showed no major trends. The value of population extent was estimated as b^{-1}. ln (a/ 0.01), i.e. the distance from the source beyond which 1% of the population is expected to disperse.

During the linear regression, we observed that one data point in the kernel of the VB_3 population seemed to be an outlier. Excluding this point from the kernel considerably improved the fit (R^2 = 0.26 became R^2 = 0.91) and the distribution of the residuals improved considerably. However, removing this outlier reduced the mean value of the spatial extent of VBs from 32.6 m to 28.01m. Incidentally, there were no changes in terms of the statistical significance in the Mann-Whitney U-tests for a, b or the spatial extent irrespective of whether the outlier is included or excluded. Therefore, in this study, we chose to report the value of population extent omitting the outlier. Note that this removal only makes our estimate of the spatial extent of VBs more conservative.

Statistical analyses: Since VB_i and VBC_j that shared a subscript (i.e. $i = j$) were related to each other by ancestry, they were analyzed together as a block. Data for dispersal propensity and dispersal distance were subjected to separate three-factor mixed-model ANOVA with selection (VB and VBC) and sex (male and female) as fixed factors and block (1-4) as a random factor. The propensity data, being fractions, were arcsine-square root transformed (Zar, 1999) before analysis. The standard deviation, skew, kurtosis, a, b and spatial extent data for each population were computed after pooling the data for the corresponding three measurement replicates. For these six quantities, we used separate Mann-Whitney U (MWU) tests to compare the VBs and the VBCs. The effect sizes (Cohen's d) for the differences between VBs and VBCs for these six quantities were estimated. Following standard recommendations (Cohen 1988), the value of effect size (d) was interpreted as large, medium and small when d>0.8, 0.8>d>0.5 and d<0.5 respectively. All statistical analyses were performed using STATISTICA® v5 (StatSoft. Inc., Tulsa, Oklahoma).

RESULTS

Rapid, simultaneous evolution of dispersal propensity and ability: After 10 generations of selection, the VB populations were found to have significantly greater dispersal propensity (Fig. 6.2a, $F_{1,3}$= 148.1, P= 0.001) and dispersal ability (Fig. 6.2b, $F_{1,3}$= 41.32, P= 0.008) compared to the VBCs. This suggests that compared to the controls, in the selected populations, a larger fraction of flies initiated dispersal and those dispersers travelled farther. It was interesting to note that only 10 generations of selection was sufficient to produce a significant divergence for these dispersal traits. We repeated the experiment after 20 generations of selection and the VB populations again had a significantly higher propensity (Fig. 6.2c, $F_{1,3}$= 22.68, P= 0.02) and ability (Fig. 6.2d, $F_{1,3}$= 68.8, P= 0.004) than the corresponding VBCs.

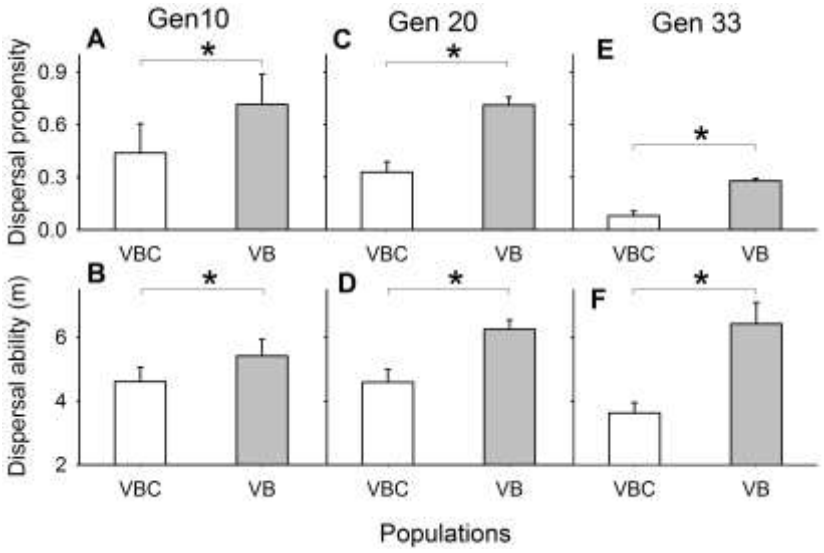

Figure 6.2. Evolution of dispersal propensity and ability. (**a, c and e**) Propensity refers to the fraction of the total population that disperses from the source. (**b, d and f**) Ability refers to the mean distance travelled by those individuals that come out of the source. The selected populations (VBs) had significantly greater propensity and ability compared to the controls (VBCs) in all the assays performed after 10 (**a, b**), 20 (**c, d**) and 33 (**e, f**) generations of selection. Food was present in the source container for the assay performed after 33 generations of selection (**e, f**). Each bar is a mean over four replicate populations each of which had three independent replicates. Error bars represent standard errors around the mean (SEM). * denotes $P < 0.05$ for the main effect of selection in the ANOVA.

Selected flies dispersed more even in the absence of stress: After 33 generations of selection, we again measured the dispersal traits of VBs and VBCs. The experimental set-up was identical to the previous assays mentioned above except each source now contained a supply of moisture and nutrition such that the flies were neither starved, nor desiccated. This

removed two of the major proximate reasons for dispersal present during the process of selection. However, even in the presence of food in the source, the VB populations were found to have significantly greater dispersal propensity than VBCs (Fig. 6.2e, $F_{1,3}$= 60.78, P= 0.004) and ability (Fig. 6.2f, $F_{1,3}$= 15.23, P= 0.03) compared to the VBCs.

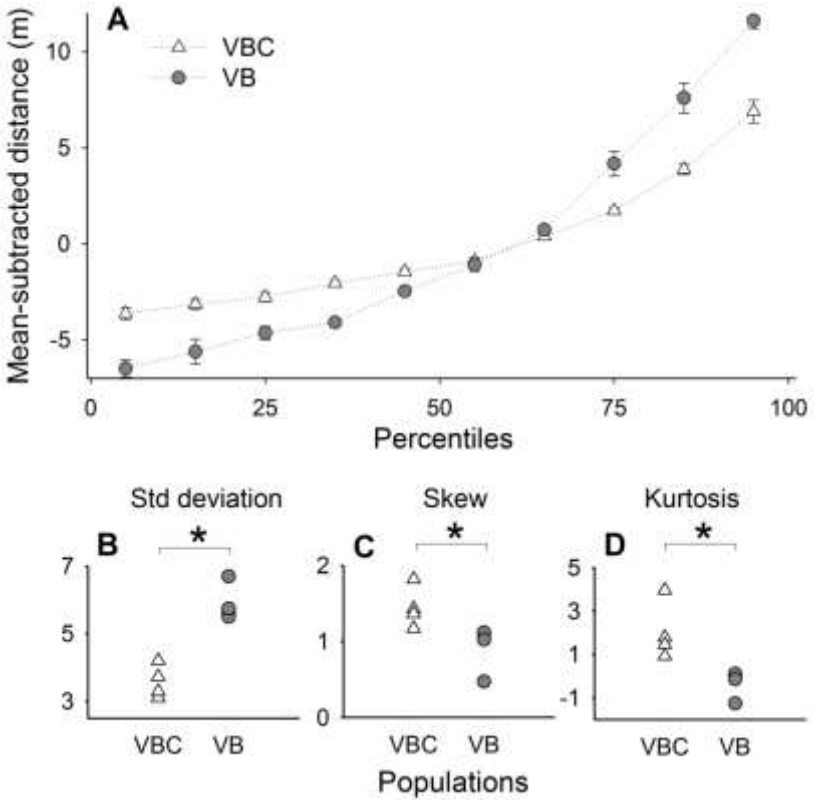

Figure 6.3. Evolution of location and shape parameters of dispersal kernel. (a) Overall dispersal kernel of VB and VBC populations. Each bar represents the average frequency of flies counted in the corresponding distance-bin for the four populations of each of VB and VBC. Error bars= standard error. (**b**) 5th to 95th percentile for the mean-subtracted kernels of VB and VBC populations. The error bars represent standard errors around the mean. In few cases the error bars are too small to be visible. Each percentile point represents data pooled over four VB or VBC populations each of which had three independent measurement replicates. (**c**) Standard deviation, (**d**) Skew and (**e**) Kurtosis of the dispersal kernels. Each point (triangle for VBC and circle for VB) represents data from one replicate population, pooled over three independent kernel measurements. Together these panels indicate that the dispersal kernels of VBs have become flatter and their tails have become fatter. * denotes P<0.05 for Mann-Whitney U-tests.

Taken together, the above results imply that multiple components of dispersal had rapidly and simultaneously evolved in the selected populations, and this difference was observable irrespective of the presence or absence of a proximate reason for them to disperse. We next investigated the implications of these changes in dispersal components, on the spatial distribution of the organisms, i.e. the dispersal kernel (Nathan et al., 2012).

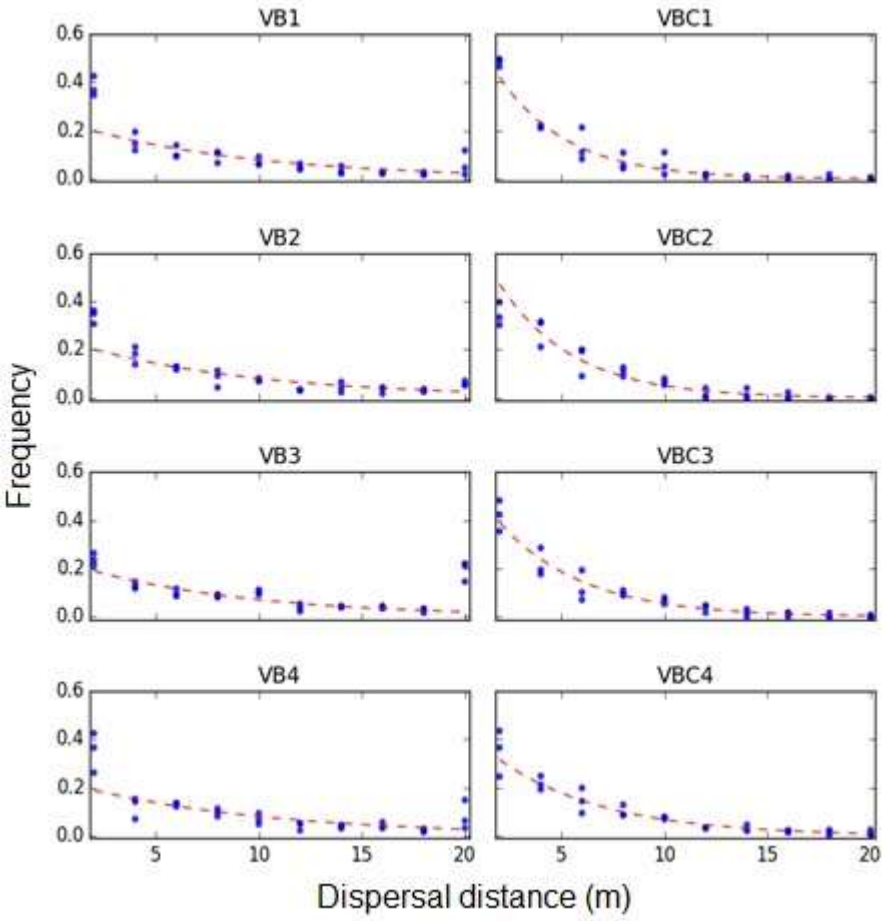

Figure 6.4. Fitted kernels of VB and VBC populations. In each panel, the frequency of individuals dispersed (scaled by total number of dispersed individuals) is plotted against the corresponding dispersal distances for each population. The three points at each dispersal distance correspond to the three measurement replicates of a population. The red dashed line is the negative exponential curve fitted to the pooled data of the corresponding population. Note, for VB3, the frequency value at dispersal distance 20 was considered as outliers and not considered during fitting.

Evolution of dispersal kernel and increased frequency of LDDs in the selected populations: There was a clear difference between the distributions of the dispersal distances of the VBs and the VBCs (Fig. 6.3a), suggesting that the VB kernel had evolved due to selection. All the higher percentiles (75 onwards) of the dispersal kernel of VBs were greater than the corresponding percentiles of VBCs (Fig. 6.3b) which indicates the presence of a greater number of Long-Distance-Dispersers (LDDs) in the selected populations. This also suggested that the overall kernel shape has changed, which was supported by the fact that VB populations had a significantly greater standard deviation (Fig. 6.3c, MWU= 0.0, P= 0.02, *d*= 4.45), lesser positive skew (Fig. 6.3d, MWU = 0.0, P= 0.02, *d*= 1.79) and more negative kurtosis (Fig. 6.3e, MWU= 0.0, P= 0.02, *d*= 2.23) compared to the VBCs. For all these shape

statistics, effect sizes of the differences between VB and VBC populations were large (i.e. $d >$ 0.8). When we fit a negative exponential distribution, ($y=ae^{-bx}$) to the data (Fig. 6.4), we found that the values of the intercept parameter a (Fig. 6.5a, MWU= 0.0, P= 0.02, d= 3.77) and the slope parameter b (Fig. 6.5b, MWU = 0.0, P= 0.02, d= 4.17) for the VB kernels were significantly lower than the VBCs (see Table 6.1 for R^2 values). This indicates a general flattening of the shape and fattening of the tail of the kernel in the selected populations. The mean spatial extent of the VBs and VBCs were found to be 28 m and 16.8 m respectively which implies an increase of 67% (Fig. 6.5c, MWU= 0.0, P= 0.02; d= 5.67). In other words, if we compare the top 1% of the dispersing individuals, VBs travel ~67% greater distance than VBCs. This implies an increase in the proportion of LDDs in the population (i.e. the fatness of the tail of the distribution). The fact that this increase was obtained after only 33 generations of selection suggests that evolvability of the kernel cannot be ignored in medium to long-range forecasts of phenomena like the rate of range expansion, disease spread and invasion speed.

Table 6.1. R^2 values of the fitted kernels

Populations	R^2
VB1	0.67
VB2	0.73
VB3	0.91
VB4	0.65
VBC1	0.97
VBC2	0.97
VBC3	0.99
VBC4	0.98

It should be noted here that the Mann-Whitney U test (MWUT) compares the ranks of the observations across two groups (Zar, 1999). This implies that for any number of tests, as long as the sample sizes and the relative ranks are the same, the U- and P-values will be identical. This is what is happening for the six MWUTs in Figs 6.4c-e and Fig 6.5. There are absolutely no overlaps between the VB and VBC values in any of these figures, as a result of which, in all the MWUTs, the ranks for one group is 1,2,3,4 and that for the other is 5, 6, 7, 8. Not surprisingly, all of them yield exactly the same values of U and P.

In short, our results indicate that even if we account for the increased mean, the shape of the dispersal kernels of the VBs had evolved to be substantially different from that of the VBCs.

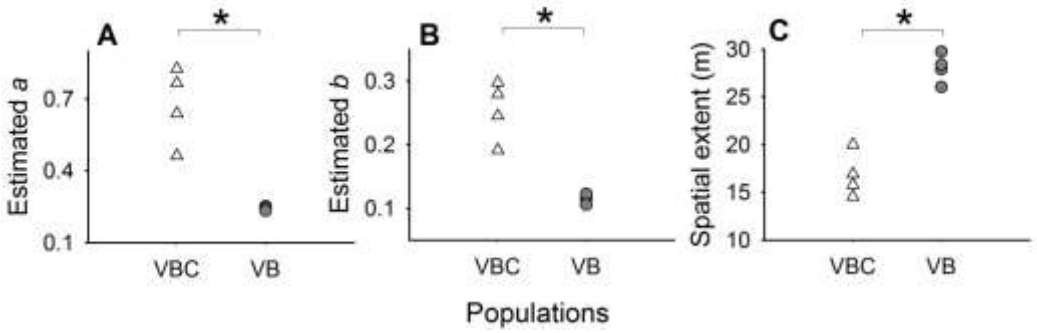

Figure 6.5. Evolution of the parameters of dispersal kernel and the spatial extent. Dispersal kernel parameters were estimated by fitting the negative exponential $y=ae^{-bx}$, where x is the distance from the source and y is the frequency of individuals found at x. Estimated values of (**a**) a and (**b**) b are significantly lower for VBs than VBCs. (**c**) Using the fitted curve, spatial extent of each of VB and VBC populations was computed by finding the distance from the source, beyond which 1% of the population is expected to reach. Spatial extents of VBs were greater than VBCs, indicating an increase in LDDs in the population. Each point (triangle for VBC and circle for VB) represents data from one replicate population, pooled over three independent kernel measurements. * denotes $P<0.05$ for Mann-Whitney U-tests.

***Drosophila* dispersal is sex-biased but both sexes respond similarly to selection:** In presence of stress (performed after 20 generations of selection), males had greater ability to disperse than females (Fig. 6.6b, $F_{1,3}= 16.28$, $P= 0.027$), although in terms of dispersal propensity both the sexes performed equally (Fig. 6.6a, $F_{1,3}= 3.47$, $P= 0.16$). Interestingly, when assayed in the absence of stress after 33 generations, the trend reversed. Here we found that females had significantly lower propensity than males (Fig. 6.6c, $F_{1,3}= 21.59$, $P= 0.019$) but the ability of both the sexes was not different from each other (Fig. 6.6d, $F_{1,3}= 2.23$, $P= 0.23$). Given the presence of sex-biased dispersal in ability and propensity (albeit in different environments) we continued to investigate whether individuals of both the sexes responded equally to selection for dispersal. But we did not find any significant treatment × sex interaction in presence of stress with respect to dispersal propensity (Fig. 6.7a, $F_{1,3}= 1.98$, $P= 0.25$) or ability (Fig. 6.7b, $F_{1,3}= 0.52$, $P= 0.52$). In the absence of stress too, the interaction term was not significant in case of either propensity (Fig. 6.7c, $F_{1,3}= 0.21$, $P= 0.68$) or ability (Fig. 6.7d, $F_{1,3}= 2.2$, $P= 0.24$).

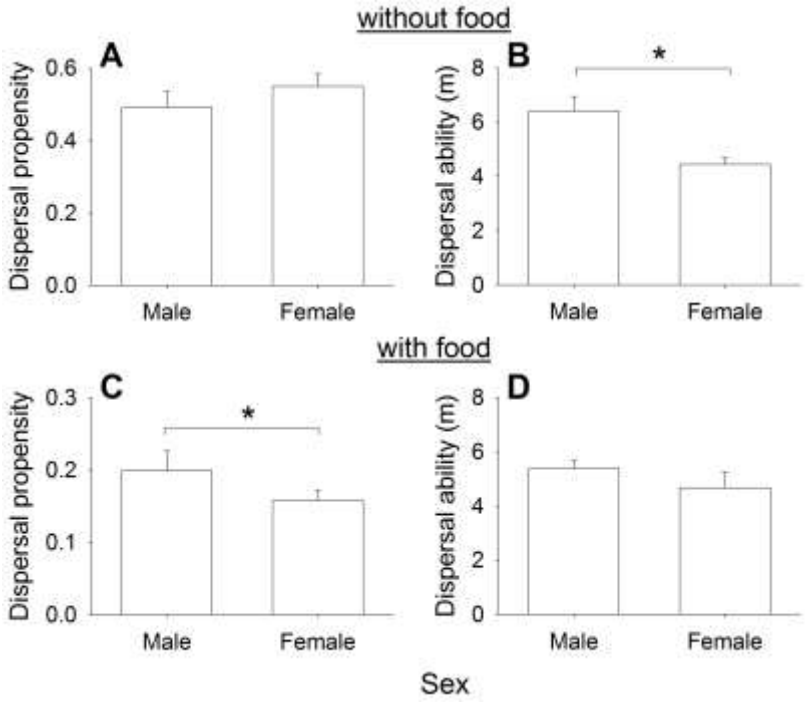

Figure 6.6. Sex-biased dispersal in the presence and absence of food. Dispersal (a) Propensity and (b) Ability in the absence of food in the source container. Dispersal (c) Propensity and (d) Ability in the presence of food in the source container. Males had significantly greater dispersal ability in the absence of food, but significantly higher propensity in the presence of food in the source. This shows that the expression of sex-biased dispersal can vary depending on which component is being measured and the environmental condition under which dispersal takes place. Error bars represent standard errors around the mean (SEM) and * denotes $P<0.05$ for the main effect of sex in the ANOVA.

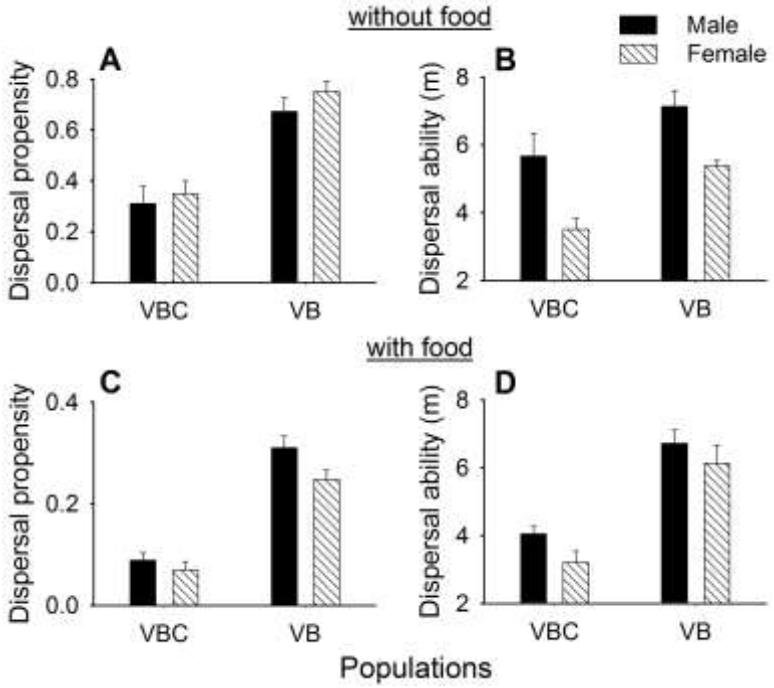

Figure 6.7. Selection × Sex interaction for dispersal propensity and ability in the presence and absence of food. Here none of the sex × selection interactions were statistically significant, thus we

could not conduct post-hoc tests for pairwise comparisons of each groups. However**,** dispersal propensity (**A**) and ability (**B**) of both the sexes of VB populations were greater than those in VBCs in absence of food in the source. Similarly, even in presence of food in the source, males and females of VB populations had higher dispersal propensity (**C**) and ability (**D**) than VBCs. This showed that both sexes in VB responded equivalently to the selection for dispersal. The error bars represent standard errors around the mean (SEM).

DISCUSSION

During dispersal, various physiological and behavioral attributes pertaining to the different dispersal stages (i.e. emigration, travel and arrival/settlement) (Bowler and Benton, 2005; Cote et al., 2010) interact with each other and the external environment. These interactions can lead to a variety of costs for the organism (Bonte et al., 2012). In order to evolve greater dispersal, organisms need to optimize over various physiological or genetic constraints to minimize the overall fitness cost of dispersal. For example, when the conditions are unfavorable and the cost of dispersal is less than the cost of staying back in the natal patch, enhanced dispersal propensity is likely to evolve (Friedenberg, 2003). However, when the cost of travelling or settlement is very high, dispersal propensity might fail to evolve, even if there is reduced fitness in the natal patch (Cheptou et al., 2008). As the number of factors and interactions that affect dispersal is very high, evolution of dispersal turns out to be a complex phenomenon. Thus, it becomes difficult to predict which components of dispersal would evolve and which would not, under the influence of various ecological circumstances.

Not surprisingly therefore, there is a large variation in the outcomes of experimental evolutionary studies on dispersal. For example, in spider-mites (*Tetranychus urticae*), it has been shown that dispersal propensity evolves when direct selection is imposed on dispersal rate (i.e. those who disperse early are selected) (Yano and Takafuji, 2002). Interestingly, in the same model system, propensity fails to evolve when selection is imposed directly on propensity (Tien et al., 2011) or on dispersal ability (Bitume et al., 2011). Another study with the same model organism (Fronhofer et al., 2014) showed that spatially-correlated extinctions favored the evolution of long-distance dispersers (LDDs) which is related to increased dispersal ability. However, in the same experiment, dispersal propensity did not evolve. This was because, positive spatial-correlation of extinction, in the absence of a significant increase in dispersal ability, substantially increases the cost of leaving the current habitat. A theoretical study on the evolution of passive dispersal of seeds on a fragmented landscape also suggests that spatial autocorrelation of nearby habitats can lead to the evolution of long-distance dispersal, but not propensity (Hovestadt et al., 2001). To summarize, all these

selection studies suggest that multiple components of dispersal (here propensity and ability) cannot evolve together.

Multiple dispersal components can evolve simultaneously under condition-dependent selection: In our study, only the first 50% of the adults that reached the destination were allowed to breed. Thus, there was a direct selection for dispersal propensity (i.e. the tendency to leave the source patch). Moreover, as the length of the path increased over generations, there was also a direct selection on dispersal ability (i.e. the ability to travel the required distance). Consequently, within 10 generations of selection, dispersal propensity (Fig. 6.2a) and ability (Fig. 6.2b) of the selected lines (VBs) was significantly greater than the controls (VBCs). We measured these two components of dispersal again after 20 generations of selection and reached an identical conclusion (Figs 6.2c and 6.2d). Both these assays were performed under conditions similar to the selection (i.e. no food in the source) which increases dispersal propensity of the flies (*cf* Fig. 6.2c and Fig. 6.2e), presumably due to starvation and desiccation stress. Thus, the dispersal, in this case was *condition-dependent* (*sensu* (Denno and Roderick, 1992; Matthysen, 2005), i.e. primarily driven by external cues.

The simultaneous evolution of dispersal propensity and ability in VBs was interesting because, in earlier studies, multiple components of dispersal had failed to evolve together (Bitume et al., 2011; Fronhofer et al., 2014; Tien et al., 2011; Yano and Takafuji, 2002). Our results also differ from theoretical (North et al., 2011) and field (Baguette et al., 2003; Cheptou et al., 2008; Schtickzelle et al., 2006) studies which predict that increased habitat fragmentation should have a negative effect on dispersal propensity. This apparent discrepancy is resolved when we observe that in some of these studies, the mortality during the travelling phase is so high that individuals with lower dispersal propensity have greater fitness even with habitat destruction (Baguette et al., 2003; Cheptou et al., 2008; although see Schtickzelle et al., 2006). In our study, since ~50% of the flies were able to reach the destination, the cost of dispersal was not prohibitively high. This allowed dispersal propensity to evolve, as predicted in some of the earlier theoretical studies (Heino and Hanski, 2001; Zheng et al., 2009).

Phenotype-dependent dispersal can evolve even under condition-dependent selection: After demonstrating the evolution of condition-dependent dispersal, we next investigated the evolution of phenotype-dependent dispersal. This refers to dispersal tendencies that are intrinsic to the organisms (Clobert et al., 2009), and thus independent of the dispersal cues.

For this set of assays, we placed a food plate (which also provided moisture) in the source. Thus, the flies in the source experienced no starvation or desiccation stress, which removed the two major proximate reasons for dispersal experienced during the process of selection. Even under these conditions, a significantly greater proportion of VBs dispersed (Fig. 6.2e) to a larger average distance (Fig. 6.2f) than the VBCs.

This result is important because all previous experimental evolution studies have both imposed and assayed for phenotype-dependent selection (Bitume et al., 2011; Fronhofer et al., 2014; Keil et al., 2001; Ogden, 1970; Tien et al., 2011; Yano and Takafuji, 2002). The evolution of phenotype-dependent dispersal in such studies is intuitive. However, our results suggest that phenotype-dependent dispersal can evolve rapidly as a result of selection for condition-dependent dispersal. The presence of such constitutive dispersers will evidently affect the dispersal-related properties of a population which in turn can affect a large number of community- and ecosystem- level processes including range expansion (Travis and Dytham, 2002), invasion (Kot et al., 1996; Shaw and Kokko, 2015), spread of diseases (Rappole et al., 2006) and community dynamics (Leibold et al., 2004). The other aspect of dispersal that would affect these processes is the dispersal kernel.

Evolution of dispersal kernel and LDDs in the selected populations: In principle, two populations can have very different dispersal kernels because of underlying evolved differences in the phenotype, or the environment, or both. Thus, in order to demonstrate the evolution of the kernel due to phenotypic evolution in the populations, it is important to control for the environment in which dispersal is assayed. We achieved this in our study by making the path through which the flies moved completely homogeneous and devoid of any potential environmental feature (like food or predators). More importantly, both the VBs and VBCs experienced the same environment during dispersal. Thus, all the differences observed between the kernels of these two populations were attributable to the underlying phenotypic differences. Evidently, we cannot claim this kernel to be the "natural kernel" of the flies as there are potentially infinite numbers of such "natural kernels" (one for every environmental state). However, this study showed that for a given environment, condition-dependent selection for dispersal can alter the location and shape of the kernel (Fig. 6.3 and Figs. 6.5a-b) and enhance the fraction of LDDs in the population (Fig. 6.5c), even when there is no proximate reason for dispersal. This is an interesting point because the shape of the dispersal kernel is often considered to be a static entity in much of the theoretical literature (Chapman et al., 2007; Krkošek et al., 2007). Our results are thus in line with more recent theoretical

(Phillips et al., 2008; Starrfelt and Kokko, 2010) and empirical studies (Fronhofer et al., 2014) that considered the possibility of evolving kernel shapes.

Our results also showed that the skew and kurtosis of the selected populations had reduced compared to the controls (Fig. 6.3d and Fig. 6.3e) which is consistent with observations on invasive cane toad populations in Australia and results of mathematical modelling on the same species (Phillips et al., 2008). For both studies, the change in the kernel shape parameters can be attributed to the increased frequency of LDDs in the population.

Sex-biased dispersal exists in *Drosophila*: Although there are many examples of sex-biased dispersal (SBD) among birds and mammals (reviewed in Pusey, 1987), relatively few cases of SBD have been reported among insects like butterflies (Bennett et al., 2013) and ground beetles (Lagisz et al., 2010). It has been shown that *Drosophila pachea* exhibits SBD while *D. nigrospiracula* and *D. mojavensis* do not (Markow and Castrezana, 2000). However, to the best of our knowledge, no prior study has looked at SBD in *Drosophila melanogaster*. Therefore, our first aim was to see whether SBD exists at all in this species.

When there was no food in the source, the males had a greater ability to disperse (Fig. 6.6b), but there was no sex-bias in dispersal propensity (Fig. 6.6a). Interestingly, the situation was reversed when there was food in the source, i.e. there was no difference in ability (Fig. 6.6d), but the males had greater dispersal propensity (Fig. 6.6c). These results highlight two major issues in studying SBD. First, the presence of SBD for any one component of dispersal is no guaranty for the presence of SBD for another dispersal component (Clutton-Brock and Lukas, 2012). Second, the fact that SBD for propensity and ability were seen in the absence and presence of food respectively, illustrates that the manifestation of SBD for a given dispersal component can be condition-dependent. Taken together, these observations suggest that across-study comparisons of SBD are not possible, until and unless they refer to the same dispersal component, under similar environmental conditions.

One potential complication with these experiments is that the dispersal assays in presence and absence of food were not conducted at the same time. Thus, in principle, it is possible that the differences between the results in presence and absence of food are due to the selection that happened during the intervening time. Although we failed to come up with any biological reasoning, we could not logically rule it out either. Note that our first observation (SBD for one component does not guaranty SBD for another) remains unaffected by this complication.

Both sexes respond similarly to selection for dispersal in *Drosophila*: Prior studies have shown that selection can lead to sex-specific effects on dispersal-related traits (Legrand et al., 2016). Therefore, our next major question was whether selection had made dispersal more sex-biased in the VB populations. This kind of a bias was expected because of an inherent asymmetry in our selection protocol: non-dispersing males could, in principle, pass their genes to the next generation by impregnating dispersing females, while the females had no such option in terms of their evolutionary contribution. This implied a potentially stronger selection pressure on the females for dispersal-related traits, which could lead to a sex × selection interaction for propensity or ability. In short, VB females were expected to diverge more from the VBC females than the VB males from the VBC males.

The sex × selection interaction was not statistically significant irrespective of the presence or absence of food (Fig. 6.7). Unfortunately, we could not conduct post-hoc tests for these differences, as the sex × selection effect was not significant in either ANOVA. However, it can be safely said that there was no evidence to support that the males had responded less to selection for either dispersal ability or propensity. There can be at least two potential (and mutually non-exclusive) reasons for this observation. First, in *Drosophila*, there is substantial evidence for last male precedence, i.e. when the females mate multiple times, the last male to mate sires more offspring (reviewed in Parker, 1970). Thus, the males that mated with the females after dispersal could have had much greater fitness than those that mated before dispersal. This could considerably increase the selection pressure on the males to disperse and explain why the males maintained their advantage in terms of propensity and ability in the VB populations. The other possibility is that dispersal traits are controlled by the same loci in both females and males in *D. melanogaster*, such that it is not possible for the sexes to respond differently to selection for dispersal. Interestingly, in terms of trends, the VB males always had greater dispersal propensity and ability than the VBC males (Fig. 6.7). This suggests that, irrespective of its relative magnitude with respect to the females, there was substantial positive selection pressure on VB males for dispersal components.

Caveats: In this study, we selected for ambulatory dispersal in fruit flies. It is well known that this is not the primary mechanism by which fruit flies disperse in nature (Dobzhansky, 1973) and we had no intention of examining that topic in a laboratory study. Our aim here was to investigate, given a mode of movement, how various aspects of dispersal interact and evolve. One can potentially argue that in our kernel assays the flies had not attained their equilibrium distribution of dispersal distances after six hours, which could potentially

invalidate our kernel measures. But, it should be noted that dispersal kernel has been defined as the "distribution of the post-dispersal locations relative to the source point" (Nathan et al., 2012). However, for constantly moving organisms like our fruit flies, it is impossible to define when dispersal has ended, particularly when they are still in the path. That is why we had to impose a temporal cut-off for the kernel assays, which is consistent with similar empirical studies in the literature (Bitume et al., 2011; Markow and Castrezana, 2000; Ogden, 1970). Thus, the problem (of organisms not settling down) is intrinsic to the study of the dispersal kernel of any actively moving organism that does not settle to make nests or occupy territories, and there is no way to obtain the equivalent of an equilibrium "distribution of the post-dispersal locations" for such animals. Moreover, we still believe that the kernel that we measured gives valuable information about dispersal evolution, for the following reasons. First, our study compared the dispersal of the VBs and the VBCs under identical conditions which means that all comparative statements about the various aspects of the kernel (Figs 6.3 and 6.5) are valid, irrespective of whether the kernels were static or not. Second, looking at the individual kernels (Fig 6.4) it is clear that there is relatively little variation across the three replicates for any given population (VB or VBC). The same is true for the various shape parameters across the four replicates of the VBs or the VBCs (Figs 6.3c-e and 6.5). This suggests that even if the populations have not attained their equilibrium distribution of dispersal distance, they are probably fairly close to it. This is not surprising as other experiments in our laboratory have shown that ~90% of the flies that leave the source in VBs and VBCs, do so within the first 90 minutes (Tung et al, manuscript under preparation). This implies that most of the flies spend ≥4.5 hours on the path, which is ~66% of the total dispersal time. To summarize, we believe that even if we cannot demonstrate that we have measured dispersal kernel at equilibrium, this is a problem inherent with most active dispersers, it does not change any of the conclusions of our study, and our measured kernels are probably very close to the equilibrium any way.

Implications of our results: There is a growing realization that multiple components must be investigated simultaneously to obtain a complete picture of dispersal evolution (Bonte et al., 2012). However, there is no theoretical or empirical expectation about the relationship between the various dispersal components, i.e. evolution of propensity does not let us predict anything about the evolution of ability and vice versa. Given this scenario, our result about the concurrent evolution of multiple dispersal components can be taken as a null model. In other words, whenever a particular component of dispersal is seen not to evolve, elucidating

the reasons for that can become the focus of an investigation. Furthermore, our study shows that under gradual directional selection of moderate intensity and in the absence of conflicting selection pressures, dispersal can evolve rapidly, and substantially. Such conditions are expected to be fairly common in nature, particularly in regions where climate changes or habitat degradations are gradual but steady. More critically, our results indicate that once evolved, these traits can express themselves even in absence of the proximal stresses (i.e. become phenotype-dependent). This could lead to organisms with intrinsically high rates of dispersal. On one hand, this could reduce the chances of local extinction (Brown and Kodric-Brown, 1977; Forney and Gilpin, 1989) and ensure greater gene flow among populations (Vilà et al., 2003). While on the other, this could increase the invasiveness of species (Kot et al., 1996; Neubert and Caswell, 2000), increase the rate of spread of diseases (Keeling et al., 2001) and induce instability in metapopulation dynamics through enhanced synchrony between neighboring subpopulations (Dey and Joshi, 2006). Figuring out the magnitude by which different dispersal components evolve and how that affect these ecological processes will be a major challenge not only for ecologists but also for ecological economists and conservation biologists (Buoro and Carlson, 2014).

CHAPTER 7

Evolution of dispersal syndrome and its corresponding metabolomic changes

Highlights

- Activity, aggression, and exploration evolved as correlated response to dispersal selection.
- Dispersal selected flies had similar body size, fecundity and longevity as the controls.
- Increased glucose, AMP and NAD levels suggest enhancement of cellular respiration in the selected flies.
- The selected lines had higher levels of neuropeptides related to aggression and exploration.

Adapted from: **Tung, S.**, Mishra, A., Gogna, N., Sadiq, M. A., Shreenidhi, P. M., Sruti, V. S., Dorai, K., Dey, S. 2017. Evolution of dispersal syndrome and its corresponding metabolomics changes. bioRxiv (2017) 178715.

1. INTRODUCTION

Dispersal affects several ecological and evolutionary processes (Clobert et al., 2012) and is one of the early responses of mobile organisms under environmental stresses. Faced with the spectre of global climate change and large-scale anthropogenic habitat destruction, fates of several natural populations depend, at least in part, on their dispersive abilities. Not surprisingly therefore, evolution of dispersal and its consequences on the organisms themselves have become major topics of investigation over the last two decades (reviewed in Ronce, 2007).

There are two somewhat different ways by which this issue has been investigated in the dispersal literature. The first approach involves comparing the behavioural or life-history attributes of dispersers with those of the non-dispersers in a given population (e.g. Ronce and Clobert, 2012). Such studies have led to the conclusion that very often (although not always) certain suites of behavioural and life-history traits are closely associated with dispersers (reviewed in Clobert et al., 2009). For example, in terms of behaviour, dispersers typically exhibit greater exploratory tendencies (Cote et al., 2010; Korsten et al., 2013) and are more aggressive (Duckworth and Badyaev, 2007), whereas in terms of life-history, the dispersers are often larger in size (Dingle et al., 1980) and have greater fecundity (Ebenhard, 1990). The primary motivation for investigating such suites (also called dispersal syndromes) is the assumption that associations between dispersal-related traits are expected to affect the genetic and demographic outcomes of dispersal (Clobert et al., 2009; Ronce and Clobert, 2012). While the assumption is fairly intuitive, the robustness of the dispersal syndrome is not, a fact that has been amply noted in the literature (Ronce and Clobert, 2012). This is primarily because dispersal is a complex process and what exactly evolves can be rather sensitive to differences in the nature of the selection pressure (Bonte et al., 2012). For example, in spider mites, selection applied through spatially correlated extinctions leads to an increase in the frequency of long distance dispersers (LDDs) even though the fraction of individuals dispersing (i.e. dispersal propensity) is reduced (Fronhofer et al., 2014). However, dispersal propensity does evolve when there is a direct selection on dispersal (Yano and Takafuji, 2002). Evidently, there is no reason to expect the same set of behaviour or life-history traits to have evolved in these two experiments, even though some attribute of dispersal had evolved in both cases.

The second approach to study the consequences of dispersal evolution is experimental evolution, which seeks to exercise greater control over the various selection pressures affecting dispersal, even at the cost of simplifying some of the ecological interactions. From a large number of studies in the last few years (Fronhofer et al., 2014; Matsumura and Miyatake, 2015; Yano and Takafuji, 2002), it is clear that under most laboratory conditions, dispersal can evolve reasonably fast. Surprisingly though, most laboratory experimental evolution studies report that life-history traits like longevity and fecundity do not evolve (Bitume et al., 2011; Li and Margolies, 1994) as a correlated response to selection for dispersal. Unfortunately, the experimental evolution studies have often not focused on the various behavioral attributes (although see Matsumura and Miyatake, 2015; Matsumura et al., 2016), which makes it difficult to compare the observations with those from association studies.

Another major question in this context is the underlying mechanism of dispersal evolution. Although a lot is known about the anatomical or physiological changes associated with dispersal phenotypes (reviewed in Zera and Brisson, 2012), we have relatively lesser understanding of what happens at the molecular level. Two genes that have been fairly conclusively shown to be related to dispersal in insects are the glycolytic enzyme Phosphoglucose isomerase (*Pgi*) in the Glanville fritillary butterflies (Niitepõld et al., 2009) and the cGMP-dependent protein kinase called foraging (*for*) gene in *Drosophila melanogaster* (Osborne et al., 1997). In *C. elegans*, three genes, namely G-protein coupled receptors *npr-1* (de Bono and Bargmann, 1998) and *tyra-3* (Bendesky et al., 2011) and *rol-1* (Friedenberg, 2003) have also been shown to have a connection with dispersal phenotypes. Although these are valuable insights, it is not always clear whether these genes would be the ones whose frequencies would change during dispersal evolution. More critically, given the complexity of dispersal, it is intuitive to assume that in any given species, dispersal evolution will probably involve changes in a fairly large number of genes and metabolic pathways. Therefore, a promising approach would be to look at the changes at the level of the metabolome and correlate that with the corresponding behavioural and life-history changes. Although this approach has been successfully used in the context of adaptation to stresses (Sørensen et al., 2017) or circadian profiles of metabolites (Gogna et al., 2015), to the best of our knowledge, it has never been attempted in the context of dispersal evolution.

Here we address some of these issues using four large, outbred populations of *Drosophila melanogaster* that have been selected for increased dispersal. These populations have evolved

significantly greater dispersal propensity and ability (i.e. distance covered by the dispersers) and a larger fraction of Long-Distance Dispersers (LDDs) in the population (Tung et al., 2017). In this study, we investigate three correlated behavioural traits (locomotor activity, exploration and aggression) and three life-history traits (body size, fecundity and life-span). We find that while all three behavioural traits have evolved significantly, none of the life-history traits had been altered due to selection for dispersal. We then investigate the metabolomic changes in the selected flies using non-targeted NMR spectroscopy and connect them with the corresponding behavioural and life-history changes.

2. MATERIALS AND METHODS

2.1 Ancestry and maintenance of the experimental populations

In this experiment, we used eight large (breeding size of ~2400) outbred laboratory populations of *Drosophila melanogaster*, four of which were subjected to selection for increased dispersal over 49 generations (called VB_{1-4}) and the other four populations served as corresponding controls (VBC_{1-4}). VB and VBC populations that share a numerical subscript (e.g. VB_1 and VBC_1) were related by ancestry (Tung et al., 2017), and hence were always assayed together and treated as blocks in statistical analyses.

Both VBs and VBCs were maintained on a 15-day discrete generation cycle at 25°C and constant light conditions. In each generation, ~60-80 eggs were collected in clear plastic vials containing ~6 mL of standard banana-jaggery food (following Sheeba et al., 1998). For each VB and VBC population, we collected eggs in 80 and 40 vials respectively. After 12 days from the day of egg collection, the adults were collected and subjected to the selection protocol (see section 2.3). Immediately after this, the adults of a given population were transferred to a plexi-glass cages (25 cm × 20 cm ×15 cm) and provided with yeast supplement along with standard banana-jaggery food. After ~40 hours, eggs were collected for the next generation. The adults were discarded after oviposition, thus ensuring that individuals of two successive generations never co-exist.

2.2 Selection procedure

The apparatus used for selection has three components- *a source, a path and a destination* (see Chapter 6 and Tung et al., 2017 for detailed description). In order to impose selection on VBs, the adults collected from 40 vials (~2400) were introduced into a *source* container. Thus, for each VB population, two *source* containers were used. Each *source* was then

connected to a *path* consisting of a plastic tube of inner diameter ~1 cm, which led to the *destination* container. The source and path did not contain any source of food or moisture, and the flies dispersed through the *path* into the *destination*, which contained a strip of moist cotton as a source of moisture. The process was continued until ~50% (visual estimation) of the population reached the *destination* or for six hours (whichever happened earlier). The arbitrary cut-off of six hours was chosen based on the result of a prior assay in the lab that under such condition, the flies do not die due to desiccation stress during the first six hours (S. Tung personal observations).

Similar to VBs, adults coming from the 40 vials of each VBC populations were maintained in separate *source* containers but they were not allowed to disperse. They were also provided with a moist cotton plug, after 25% of the VBs reached their destination or 3 hours (whichever was earlier).

At the end of this process, for VBCs, all the flies from respective source containers were transferred to the cages, thus ensuring that there was no selection for dispersal. For each VB population, only the flies which reached the *destination* in the two selection setups were transferred into a cage and allowed to breed for the next generation. Thus, the breeding population size of VBs and VBCs remained comparable in each generation.

Starting from 2 m, the length of the path was increased intermittently over generations in order to intensify the selection for grater dispersal ability. By generation 67, when the last set of assays were performed, the path length had reached 20 m.

2.3 Assays:

Prior to any assay, both VB and VBC populations were maintained under identical rearing conditions for one generation to ameliorate the influence of phenotypic plasticity or non-genetic parental effects. The progeny of these flies were used for the assays. Moreover, for all the assays, egg density was always maintained at ~50 eggs on ~6mL food in each vial to avoid any confounding effect of larval crowding on the life-history and behavioural traits measured.

2.3.1 Locomotor activity assay

After 49 generations of selection, locomotor activity of the selected and control lines were checked both in the presence and absence of food using *Drosophila* Activity Monitoring (DAM2) data collection system (Trikinetics Inc, Waltham, MA). The activity for a given fly was estimated as the average number of times the fly crossed the IR beam of *Drosophila* activity monitor per hour, while, continuous inactivity for five minutes or more was

considered as sleep/rest (Chiu et al., 2010; Hendricks et al., 2000). For each VB/VBC population, we measured the activity of only male flies as in females laying of eggs on the tubes could affect the accurate measurement of locomotor activity (Chiu et al., 2010). We performed this assay both in presence and absence of food. For the activity assay in the presence of food, on the 11^{th} day from the day of egg collection, between 1830 h -1930 h, single adult male flies were aspirated into glass activity tubes of 5 mm diameter, containing standard banana-jaggery food at one end. We preferred aspiration over CO_2 anaesthesia to avoid any lingering effects of anaesthetization on the activity of the flies (van Dijken et al., 1977). Details of the preparation of the tubes and the cleaning of the same can be found elsewhere (Chiu et al., 2010). The selected populations were always assayed along with their matched control populations (i.e. VB_1 was assayed with VBC_1 and so on) and there were 30-32 replicates for each population. Activity data were collected for 30 hours and were divided into two parts - i) first 6 hours and ii) next 24 hours. The first set captured the activity-rest pattern immediately after introduction of the flies in the tube, while the next set measured the steady state activity-rest pattern, after 6 hours of acclimatization, for a complete 24-hour cycle. For the entire duration of recording, the monitors containing the activity tubes were kept undisturbed inside an incubator maintained at 25 °C and constant light.

Locomotor activity assay in the absence of food was similar to the one mentioned above except that no food was provided and both ends of the activity tubes were secured with clean, dry cotton plugs. The setup for this assay was done on the 12^{th} day from the day of egg collection between 1200 h-1300 h which roughly corresponds to the time at which selection was imposed during the regular maintenance of VBs. Moreover, in the no-food case, locomotor activity was recorded only for 6 hours from the time of setup as, after this period, the flies become stressed and slowly start dying due to desiccation. 30-32 flies were assayed for each of the VB and VBC populations.

For each of these datasets, average number of activity counts per hour was calculated as a measure of the activity level of the flies and the fraction of time the flies did not show any activity count was computed as an indicator of the sleeping/resting duration. In other words, the activity level of a fly is the total number of activity counts while it was not resting/sleeping. Thus, mathematically, the activity level of a fly was independent of the fraction of time it spent in resting/sleeping. In order to assess the quality of rest/sleep, for the 24-hour dataset, we computed the average length of uninterrupted rest/sleep bout and duration of the longest rest/sleep bout for each of the flies.

2.3.2 Exploration assay

This assay was performed after 53 generations of selection. For each VB or VBC population, we used 10 replicate vials each containing around 50 eggs / 6ml of banana-jaggery food. The assay was performed on the 12th day after egg collection. The male flies were aspirated from the egg-collection vials and introduced into the experimental arena (modified from Soibam et al., 2012) where their activity was recorded using a video camera. The experimental arena was made of a clear polycarbonate petri dish lid with an inner diameter of 10 cm. A small hole was drilled into the centre of the lid to introduce flies into the setup. The lid was placed on top of a blank sheet of paper which contained the traces of two concentric circles. The outer circle was of the same diameter as the petri dish lid while the inner circle divided the total area of the lid into two zones: the outer containing one third of the total area while the inner one enclosing two-thirds of the area (Liu et al., 2007) of the lid. 32 replicates were assayed for each sex of all the VB and VBC populations. After the flies were introduced into the arena, they were given one minute to acclimatize to the new environment. They were then observed for the next 10 minutes and the number of times they entered the inner zone, considered as the number of exploratory trips, was recorded.

2.3.3 Male-male aggression assay

The aggression assay was performed after 52 generations of selection. For this assay, the flies were reared under low levels of larval crowding (~50 eggs in 6-8ml banana-jaggery food). Freshly eclosed males were collected and reared in social isolation (i.e. one male per vial) till the day of assay (following Yurkovic et al., 2006). We used 6 wells of a twelve-well culture plate (Corning®, NY, USA) as the assay apparatus, where each well served as the enclosure for one replicate of the aggression assay. A small plastic cup containing regular banana-jaggery medium was affixed at the centre of each well. A freshly decapitated female was stuck to the middle of the food cup using yeast paste. The food and the female served as defendable resources and potential reasons for conflict. Following an earlier protocol, VB and VBC males were colour-coated with daylight fluorescent pigments (DayGlo) for easy identification (Dickens and Brant, 2014). On the 12th day from egg collection, two males (one VB and one VBC) were introduced into the setup and their interaction was recorded for 45 minutes using a video camera. 30 such replicates were assayed for each of the four populations of VBs and the corresponding VBCs. Individual wells were visually isolated from each other using cotton to ensure no visual cues were being exchanged between replicates. Uniform lighting and constant temperature (25°C) were maintained.

The scoring for aggression was done after an initial five-minute acclimation period. For each of the replicates, the number of successful chase-aways from the food cup was recorded. A successful chase-away is defined as one in which one male completely chases the other male away from the top surface of the food cup (Yurkovic et al., 2006). Earlier studies have shown that in *Drosophila* a male that manages to complete three consecutive successful chase-aways usually manages to successfully chase away the other male in all future encounters (Yurkovic et al., 2006). Therefore, we used this criterion to identify the winner of each fight from all the blocks.

2.3.4 Body size assay

Dry body weight of the adults was measured as a proxy for body size after 49 generations of selection. For a given population, ~50 eggs were introduced into food vials containing ~6-8 mL of standard banana-jaggery medium. After 12 days from the day of egg collection, the adult flies were collected, sorted by sex, killed by flash freezing and stored at -80 °C till weighing. The flies were then dried at 60°C for 72 hours and (after thawing to room temperature) weighed to the nearest 0.1 mg in batches of 20 males or 20 females. Five batches of males and females were weighed for each of the four VB and VBC populations.

2.3.5 Female fecundity assay

Female fecundity of the selected (i.e., VB_{1-4}) and the control (i.e. VBC_{1-4}) populations were assayed after 53 generations of selection. Female fecundity was assayed both during early and late life. For early life fecundity, we used 15-day (post egg collection) old females, which is the same age at which the eggs are collected for the selection lines. Late life fecundity was measured on 33-day (post egg collection) old females. This is because, in *Drosophila*, it is known that during this time female fecundity reduces substantially due to aging but does not plateau out (Hanson and Ferris, 1929; Mueller et al., 2007). On the day of assay, flies were anaesthetized under mild CO_2 and one male and one female each were introduced into a 50 mL centrifuge tube containing a food cup. The tube had provision for aeration and the food in the food cup provided a surface for laying eggs. 40 such replicate setups were made for each VB_i and VBC_i (where $i \in$ 1-4) population. The setups were left undisturbed for 12 hours in a well-lit environment maintained at 25ºC and ambient humidity. At the end of 12 hours, the flies were discarded and the eggs laid on the food were counted under a stereo microscope. Since fecundity is largely determined by the body size of the females (Honěk, 1993) which is in turn critically dependent on larval density (Prout and McChesney, 1985), we maintained a

constant egg density (~50 eggs per vial containing ~6-8 mL of standard banana-jaggery food), while collecting eggs for generating the flies for this assay.

2.3.6 Longevity assay

Longevity assay was performed after 51 generations of selection in a constantly lit environment maintained at 25ºC. We initiated the assay by introducing 10 freshly eclosed, unmated individuals of the same sex into a food vial containing ~6 mL standard banana-jaggery food. 10 such replicate setups were prepared for males and females separately from each of the VB/VBC populations. Thus in total, we measured the life-span of 1600 flies in this assay. The alive flies were counted daily at a particular time (arbitrarily set at 1500 h) and every alternate day, they were transferred into fresh food vials, till the last individual died. Flies that escaped or died during transfers were not included in the analysis.

2.4 Metabolomic study using NMR spectroscopy

2.4.1 Sample preparation: After 67 generations of selection, NMR spectroscopy was performed on one block of selected-control populations (VB_4-VBC_4). For each VB/VBC population, we used 11 replicates, each of which comprised of 30 males. Samples were prepared following established protocols (Gogna et al., 2015). The flies were first flash-frozen in liquid nitrogen, transferred to pre-labelled microfuges containing 0.5 mL of 50% acetonitrile solution, homogenized using a battery run homogenizer and centrifuged at 10000 rpm for 10 min at 4ºC. The supernatant was then transferred to another set of pre-labelled microfuge tubes, lyophilized and stored at -80ºC, to be used for NMR experiments. Prior to the NMR experiments, the samples were rehydrated in 500 ml of 50 mM phosphate buffer prepared using D_2O (pH 7.4), containing 1 mg/ ml of 3-(trimethylsilyl)-propionic acid-D4, sodium salt (TMSP) as a chemical shift reference and transferred to 5mm NMR tubes.

2.4.2 NMR spectroscopy: NMR spectra were recorded on a Bruker Biospin 600 Avance-III spectrometer operating at a ^1H frequency of 600.13 MHz at 300 K using a 5 mm QXI probe. Gradient shimming was performed prior to signal acquisition to optimize magnetic field homogeneity. 1D ^1H NMR spectra were acquired using the water suppressed Car–Purcell–Meiboom– Gill (CPMG) spin-echo pulse sequence optimized with a spin -echo delay *t* of 300 ms and n= 400 and a total spin–spin relaxation delay (2n*t*) time of 240 ms to achieve attenuation of fast-relaxing broad signals from larger molecules. The proton spectra were collected with a 90-degree pulse width of 9.15 ms, a relaxation delay of 2 s, 16 scans, 16 K data points and a spectral width of 7211.54 Hz. Data were zero-filled by a factor of 2 and the

FIDs were multiplied by an exponential weighting function equivalent to a line broadening of 1 Hz prior to Fourier transformation. For resonance assignment and metabolite identification, two - dimensional NMR spectra were recorded, including $^{1}H–^{1}H$ correlation spectroscopy (COSY) and $^{1}H–\ ^{13}C$ heteronuclear and homonuclear single quantum coherence spectroscopy (HSQC, HMQC). 2D $^{1}H–^{13}C$ HMQC and HSQC spectra were obtained with a spectral width of 12 ppm and 200 ppm in the proton and carbon dimensions respectively, 1 K data points, 32 scans, 256 t1 increments and a recycle delay of 1.5 s. The COSY spectra were acquired with a spectral width of 12 ppm in both dimensions, 2 K data points, 32 scans and 128 t_1 increments. Metabolite fingerprinting for the *Drosophila* NMR spectra was done by checking identified metabolite peaks with standard NMR metabolite data deposited in databases such as MMCD (http://mmcd.nmrfam.wise.edu) and BMRB (http://www.bmrb.wise.edu). The NMR chemical shift assignments of several significant metabolites were further confirmed by recording the NMR spectra of pure compounds. For analysis of metabolites, single peak integrals for individual metabolites were chosen with minimal overlaps with peaks from other compounds.

2.4.3 Data Analysis: Multivariate statistical analysis was performed using SIMCA14.0 software (Umetrics, Umea, Sweden). Prior to analysis, all the spectra were converted into the ASCII format and imported into MATLAB for alignment using the Icoshift algorithm (Savorani et al., 2010). Spectral regions between 4.6 and 4.8 ppm were excluded from the analysis, to prevent errors due to any residual peak from the suppressed water signal. Data were normalized to the total area to compensate for possible differences in signal-to-noise ratios between spectra and to prevent separation due to variations in the amounts of sample. After importing the data into SIMCA, the data was Pareto-scaled and first analysed using the unsupervised pattern recognition method of principal component analysis (PCA), which helped to remove outliers, defined in the data as observations located outside the 95% confidence region of the Hotelling's T^2 ellipses in the PCA score plots. Such outliers were excluded from further analysis. PCA was followed by the supervised pattern recognition method of orthogonal projections to latent structure-discriminant analysis (OPLS-DA), which maximizes the class discrimination. The OPLS-DA scores and loadings plots were used to identify the metabolites responsible for separating VB and VBC flies. The quality of the model was described by R^2X and Q^2 values, explaining the variance explained (indicating goodness of fit) and variance predicted by the model (predictability) respectively. The significance test of the model was performed using CV- ANOVA (cross-validated ANOVA)

in the SIMCA software, where a *p* -value of 0.01 was considered to be statistically significant to validate the OPLS-DA model. Permutation analysis was also performed on the best model using 1000 permutation tests with a threshold *p*-value of 0.05 indicating that none of the results were better than the original one. t-tests coupled with Bonferroni corrections (to limit the family-wise error rate to 0.05) were performed to check for statistical significance of the differences in the metabolite levels between the VB and VBC flies.

The work described in sections 2.4.2 and 2.4.3 was performed by Navdeep Gogna and Dr. Kavita Dorai in the latter's lab at Indian Institute of Science Education and Research Mohali.

2.5 Statistical analyses

VB_i and VBC_j that shared a subscript (i.e. $i = j$) were assayed and analysed together as a block as they were related to each other by ancestry. For locomotor activity, the fraction of time spent resting/sleeping (arcsine-square root transformed), average sleep bout, length of the longest sleep bout and female fecundity data, we used two factor mixed-model ANOVA with selection (VB and VBC) as fixed factor crossed with block (1-4) as a random factor. Three-factor mixed-model ANOVA was performed for adult exploration, dry body weight and longevity data with selection (VB and VBC) and sex (male and female) as fixed factors and block (1-4) as a random factor, crossed with each other. For the aggression data, we used Mann-Whitney U tests to compare the performance of VB and the VBC males. The value of effect size (Cohen's *d*) was interpreted as large, medium and small when $d \geq 0.8$, $0.8 > d \geq 0.5$ and $d < 0.5$ respectively (Cohen, 1988). All the above statistical analyses were performed using STATISTICA® v5 (StatSoft. Inc., Tulsa, Oklahoma).

The NMR spectral data were analysed using standard procedures (see section 2.4). The spectral data were normalized to total area, Pareto scaled and subjected to Principal Component Analysis to identify and remove the outliers. This was followed by Orthogonal Projections to Latent Structure-Discriminant Analysis (OPLS-DA) to identify the metabolites responsible for separating VB and VBC flies. The significance test of the model was performed using CV- ANOVA (cross-validated ANOVA). Further, permutation analysis was performed on the best model using 1000 permutation tests with a threshold *P*-value of 0.05, which indicated that none of the results was better than the original one. The average level of the metabolites in the selected and control populations, were compared using Student's *t*-tests, followed by Bonferroni correction, thus restricting the family-wise error rate to <0.05.

3. RESULTS

3.1 VBs are restless but rest less

During the first 6 h after set-up, the VB populations had significantly greater locomotor activity irrespective of the absence (Fig. 7.1A, $F_{1, 3}$=60.3, 0.004) or presence (Fig. 7.1C, $F_{1,3}$= 423.3, *P*=0.0003) of food. Moreover, of the total duration of 6 h, the VBs spent significantly less time in rest/sleep, both in the absence (Fig. 7.1B, $F_{1, 3}$=50.4, *P*=0.006) and presence (Fig. 7.1D, $F_{1, 3}$=386.9, *P*=0.0003) of food. Interestingly though, when assayed in the presence of food over a duration of 24-h after the initial 6 h of acclimatization, although the difference in activity persisted (Fig. 7.1E, $F_{1, 3}$= 59.9, *P*= 0.004), the VBs spent similar amount of time in rest/sleep as the VBCs (Fig. 7.1F, $F_{1, 3}$= 5.47, *P*=0.1). The length of average sleep bouts (Fig. 7.2A, $F_{1, 3}$=2.2, *P*=0.23) and maximum sleep bouts (Fig. 7.2B, $F_{1, 3}$=4.7, *P*=0.12) of the VBs and the VBCs were also comparable.

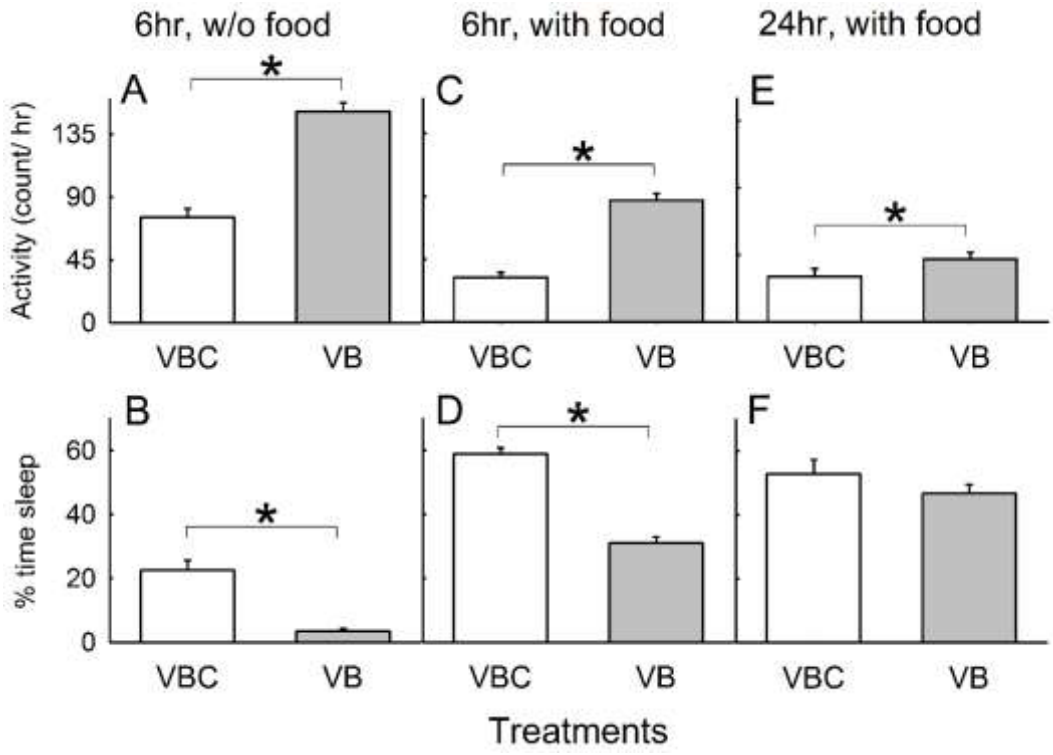

Figure 7.1. Locomotor activity-sleep profiles in the presence and absence of food. In the absence of food during the first 6 h after introduction **(A)** Locomotor activity of VBs was significantly greater than the VBCs although **(B)** duration of rest was significantly lower. In the presence of food during the first 6 h, similar results were obtained for **(C)** locomotor activity and **(D)** rest. After acclimatization for 6 h, over the next 24 h, the VBs had significantly greater (**E**) locomotor activity but similar levels of (**F**) sleep duration as the VBCs. The error bars represent standard errors around the mean (SEM) and * denotes *P*<0.05.

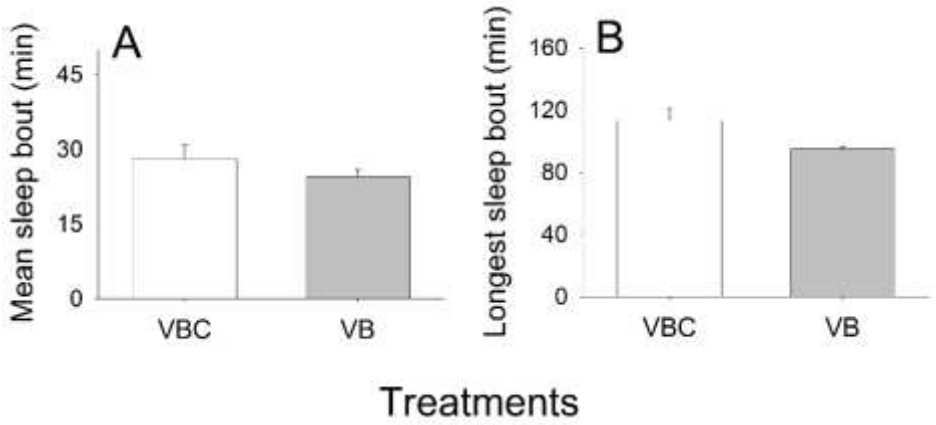

Figure 7.2. Average and maximum sleep bout over 24 hours post acclimatization. Over 24 hours in presence of food, (**A**) average sleep bout and (**B**) maximum sleep bout, are similar for both VBs and VBCs. The error bars represent standard errors around the mean (SEM) and * denotes $P<0.05$.

3.2 Selection for dispersal leads to greater exploratory behaviour and aggression

Selection regime had significant effects on adult exploratory behaviour, when the flies were introduced into a novel environment and the VB flies had significantly greater tendency to explore a novel area than the VBC flies (Fig. 7.3A, $F_{1, 3}=11.96$, $P=0.04$). Although we failed to find a selection × sex interaction ($F_{1, 3}=0.009$, $P=0.93$), for both sexes, the VB flies exhibited greater exploratory behaviour than the corresponding VBC flies (Fig. 7.4). Furthermore, we found significant effect of selection on male aggression (Mann-Whitney $U=0.0$, $P= 0.02$) in all the four blocks: VB males were found to be significantly more aggressive than the VBC males with large effect size ($d = 2.05$) in one-to-one fight for food and mate present in the arena (Fig. 7.3B).

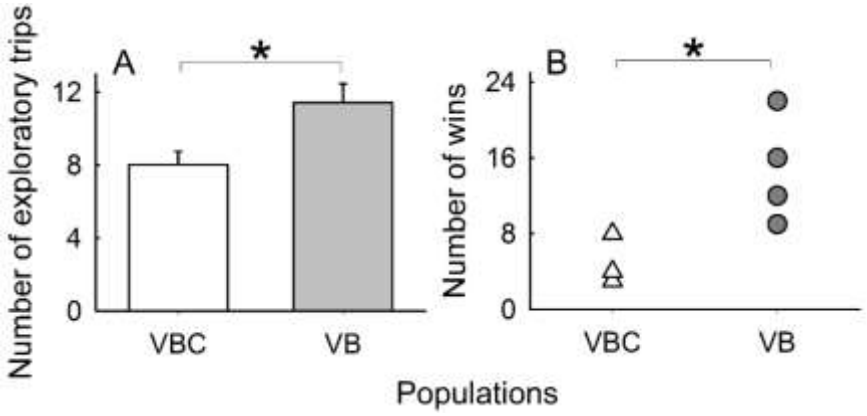

Figure 7.3. Exploration and aggression behavior of VBs and VBCs. (**A**) VB flies commenced significantly more number of exploratory trips than VBCs. The error bars represent standard errors around the mean (SEM). (**B**) VB males were more aggressive as they won significantly more number of fights against VBC males. Both these results were consistent across all the four blocks. * denotes $P<0.05$.

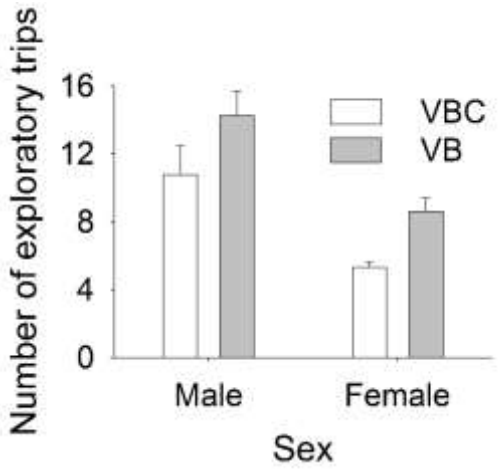

Figure 7.4. Exploratory behavior of males and females in VBs and VBCs. For both sexes, VBs performed more exploratory trips than VBCs. Error bars= SEM around the mean.

3.3 Selection for dispersal does not lead to changes in dry body weight, fecundity or longevity

The mean dry weight of VBs and VBCs were found to be comparable (Fig. 7.5A, $F_{1, 3}$= 0.76, *P*=0.45). Although *Drosophila* females are known to be heavier than males, we did not find any significant selection × sex interaction ($F_{1, 3}$= 2.1, *P*=0.24) in our study suggesting that selection did not affect the dry weight of the two sexes in VB and VBC flies differentially. There was no significant difference between the fecundity of the VB and VBC flies with respect to either early fecundity (Fig. 7.5B, $F_{1, 3}$= 0.25, *P*=0.65) or late fecundity (Fig. 7.5B, $F_{1, 3}$= 0.2, *P*= 0.68), indicating the absence of a trade-off between increased dispersal ability and reproductive output. We also did not find any trade-off between dispersal and longevity: the average life-span of VBs was found to be similar to that of the VBCs (Fig. 7.5C, $F_{1, 3}$= 4.9, *P*=0.11).

3.4 Selection for dispersal leads to changes in the metabolome profile

OPLS-DA scores plot (Fig. 7.6) documents the differences in the metabolite profile of the selected and control flies. Fig. 7.7 shows the colour coded coefficient loadings plot used to identify the metabolites responsible for differentiating both VB and VBC flies. The variance explained by the model (R^2X) was 0.968 and the variance predicted by the model (Q^2) was 0.953, showing that the model was effective and had a good predictive accuracy. The credibility and robust nature of the model were also confirmed by testing the statistical significance of the model with CV-ANOVA (p-value <0.01) and permutation test (p-value <0.05). The metabolites, which were significantly different between the selected and control flies in *t*-test followed by Bonferroni correction, are tabulated in Table 7.1.

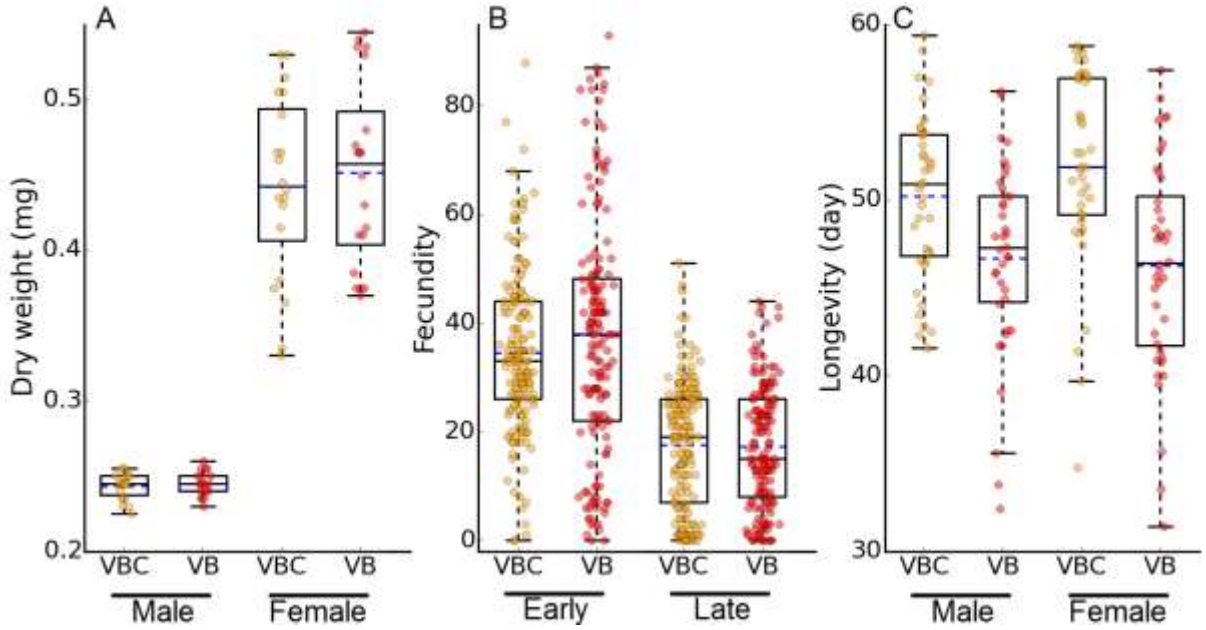

Figure 7.5. Life-history traits of VBs and VBCs. Cleveland-box plots show (**A**) dry body weight, (**B**) early-late fecundity and (**C**) longevity profiles of VB and VBC populations were not different from each other. The points represent the pooled data for all the replicates of VB and VBC populations with small random jitter along X-axis. The edges of the box denote 25th and 75th percentiles, while the black solid lines and blue broken lines represent the median and mean respectively.

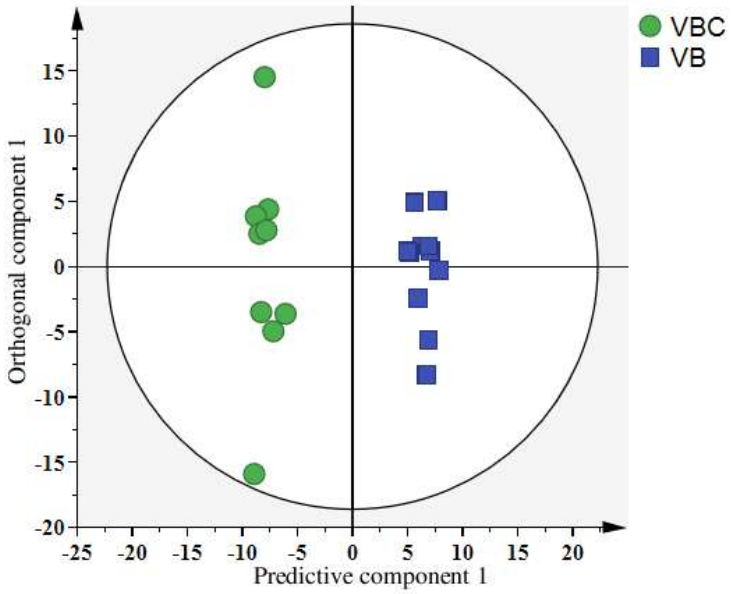

Figure 7.7. OPLS-DA score plot derived from 1D ^{1}H NMR spectra of VBs and VBCs.

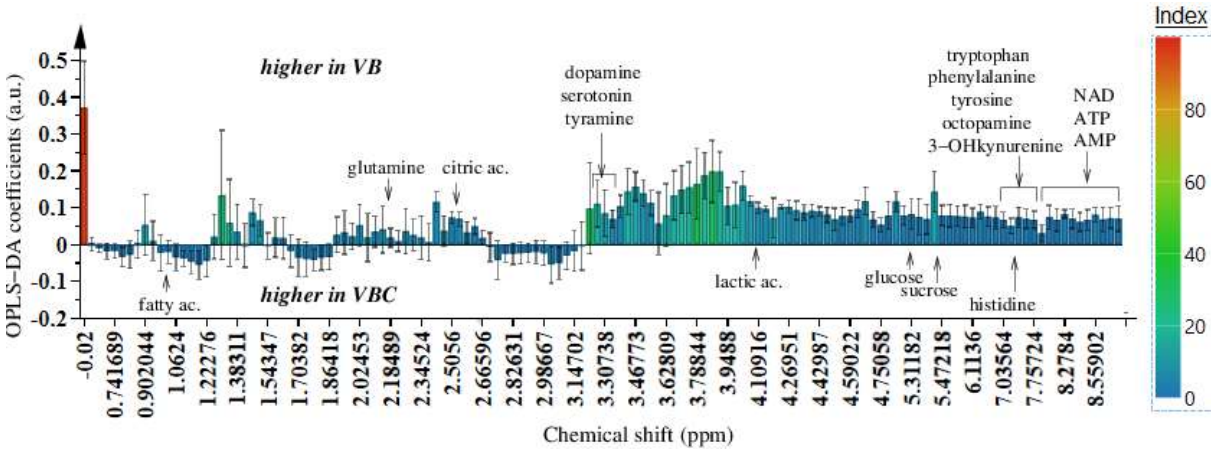

Figure 7.7. OPLS-DA loading plot obtained from the analysis of 1D ^{1}H NMR spectra of VBs and VBCs. Metabolites that are indicated above and below the baseline are present in higher quantity in VB and VBC flies respectively.

Table 7.1: List of metabolites altered during the course of selection for increased dispersal.

Metabolites	*p* value	Effect size (Cohen's *d*)	Fold change in VB (VB/VBC)
Tryptophan	1.15E-06*	3.77	3.27
AMP	6.93E-06*	3.16	2.63
3-HK	9.54E-07*	3.86	2.5
Octopamine	5.81E-08*	3.88	2.26
Phenylalanine	1.50E-06*	3.58	1.95
NAD	4.04E-08*	4.8	1.82
Tyrosine	6.19E-08*	3.96	1.73
Histidine	2.73E-05*	2.72	1.71
Glucose	2.41E-07*	4.18	1.65
ATP	1.06E-06*	3.69	1.57
Sucrose	2.15E-04*	2.39	1.48
Citric acid	1.57E-04*	2.15	1.36
Lactic acid	6.89E-05*	2.26	1.35
Glutamine	9.71E-04	1.94	1.27
Tyramine	5.57E-03	1.46	1.15
Serotonin	1.58E-02	1.16	1.15
Dopamine	1.63E-01	0.73	1.1
Fatty acid	2.14E-04*	2.49	0.77

Only those metabolites are shown that were either significantly different between the VBs and the VBCs or the fold change was >1. The *p*-values for a given metabolite were obtained from *t*-tests between the VBs and VBCs. * indicates that the differences were statistically significant even after Bonferroni correction at the 0.05 level. All effect size values are large (i.e. $d>0.8$), except for dopamine, where it is medium (i.e. $0.5<d<0.8$). Note that the AMP: ATP ratio for the VBs and VBCs were 0.43 and 0.26 respectively.

4. DISCUSSION

4.1 Selection for dispersal leads to similar patterns of activity but different patterns of rest in the short and long time-scale

During the first 6 h after introduction to a new environment (same duration for which the flies are allowed to disperse during selection), the selected populations had greater locomotor activity than the controls (Fig. 7.1A and 7.1C), and spent lesser time in rest (Fig. 7.1B and 7.1D). This observation is consistent with previous results (Hanski et al., 2006; Matsumura et al., 2016) and also with the fact that the VBs were under intense selection to reach a new environment within the first 6 h of introduction to the source (Tung et al., 2017). Consequently, maximizing the amount of activity and minimizing the resting period during that time would be of obvious advantage to the VB flies. More interestingly though, we found similar activity/rest patterns in the absence and presence of food (*cf* Figs 7.1A with 7.1C and 7.1B with 7.1D), which suggests that the increased activity is independent of starvation or desiccation cues. This is again consistent with earlier observations that the dispersal propensity and ability differences between VBs and VBCs were observed irrespective of the presence or absence of food (Tung et al., 2017). However, when we measured the activity of these flies over 24 h, after allowing a period of 6 h to acclimatize, although the VB males were found to be significantly more active than VBC males (Fig. 7.1E), the percentage of time spent resting was not different between these two lines (Fig. 7.1F). In other words, the VB flies rest lesser during the period that corresponds to the time when they face selection, but revert to normal levels of rest once that phase is over. During the latter period, the quality of the rest/sleep of the flies, as measured by the length of average bout of sleep (Fig. 7.2A) and maximum bout of sleep (Fig. 7.2B), during 24 h, was also found to be similar in VBs and VBCs. Thus, although the VBs are more active, it seems unlikely that they would face negative effects of rest-deprivation in the long run. To the best of our knowledge, this is the first demonstration that dispersers also modulate their rest-patterns temporally in way that could reduce the negative effects of rest-deprivation (Huber et al., 2004; Kayser et al., 2015). Increased activity of dispersers can positively correlate with another important behavioural trait, namely exploratory tendency (Cote et al., 2010) which is what we investigated next.

4.2 The evolution of dispersal led to simultaneous evolution of exploratory behaviour

Dispersers often have greater exploratory tendency (Cote et al., 2010; Korsten et al., 2013) which is thought to be beneficial for finding new habitats. In our selection protocol, there was no sensory cue in the path connecting the source to the destination. Therefore, only those flies

(of either sex) could disperse successfully that took the risk of getting into the path and then continuing along it, implying that exploratory tendency was under strong positive selection. Therefore, it was no surprise that the dispersal-selected VBs were more exploratory in nature than the VBCs (Fig. 7.3A), and the result was consistent across both sexes (Fig. 7.4). Elevated exploratory tendency can be important during range expansion as the individuals present at the range edges are more likely to experience environments different from their native and/or previously introduced habitats. For example, Kenyan house sparrows that were present at a range expansion front were found to be significantly more exploratory (Liebl and Martin, 2012). Interestingly, in many species, exploration is also found to be strongly related to invasion (Cote et al., 2010; Rehage et al., 2005; Russell et al., 2010), which involves conflict/confrontation with the native species. Consequently, aggression is another behavioural trait strongly correlated with exploration (Dingemanse and de Goede, 2004; Verbeek et al., 1996) and often closely related to personality-dependent dispersal (Cote et al., 2010). Thus we next investigated the effect of dispersal evolution on aggression.

4.3 Male-male aggression evolved as a correlated response to selection for dispersal

Aggression is an important trait that influences an individual's ability to retain resources and mates or gain new ones (O'Riain et al., 1996). Not surprisingly, several studies have reported a strong association between dispersal tendencies and aggression (Duckworth and Badyaev, 2007; Wahlström, 1994) which is consistent with our observations (Fig. 7.3B). However, while enhanced aggression might be a factor for the dispersal success in some of the natural populations (Duckworth and Badyaev, 2007), in our system, the dispersing flies have no obvious fitness advantage for being more aggressive, as they did not have to compete with any native individuals at the destination. Thus, in our experiment, aggression evolved as a correlated response of dispersal evolution, most likely due to changes at the biochemical/physiological levels.

4.4 Dispersal-selected lines have comparable body size as that of the controls

One life history trait that could potentially explain the increased levels of dispersal, locomotor activity and aggression in the VBs is adult body size. Bigger organisms are expected to have greater energy reserves and, in general, body size is positively correlated with dispersal (Dingle et al., 1980; Sutherland et al., 2000; although see Gu and Danthanarayana, 1992). Moreover, in *Drosophila melanogaster*, it is known that larger males win significantly more aggressive encounters compared to smaller males (Partridge and Farquhar, 1983). However, we failed to find a significant difference in body weight, a proxy

for body size, between VBs and VBCs (Fig. 7.5A) which suggests that the increased aggression and locomotor activity in VBs were not mere artefacts of differences in body sizes between the two populations. This result also highlights that how a trait evolves (here, no change in body size) due to a particular selection pressure may not always be inferred from existing trait-associations (here, the general observation that body size and dispersal are positively correlated).

In *Drosophila*, body size is generally considered to be a good proxy for the total amount of resources available to an organism. Our results suggest that the selected flies have similar levels of resources compared to the controls (Fig. 7.5A), but at the same time, display elevated levels of activity (Figs 7.1A, 7.1C and 7.1E). Given that the kind of nutrients available to both populations are the same, one way for the selected flies to manage this feat would be to alter the pattern of resource allocation among the various traits (van Noordwijk and de Jong, 1986). To investigate this possibility, we measured two crucial life-history traits, namely fecundity and longevity.

4.5 Selection for dispersal does not affect fecundity or longevity

The relationship between dispersal and fecundity has been somewhat controversial in the literature. On one hand, flight ability/ dispersal has been shown to be negatively correlated with fecundity in several insects including *Drosophila* (Roff, 1977), long-winged crickets (Roff and Fairbairn, 2007) and aphids (Dixon et al., 1993). This is thought to be due to energy limitation, as allocation of resources to the muscles reduces the availability of the same for reproductive functions. On the other hand, several investigators have reported a positive correlation between dispersal and fecundity (Hanski et al., 2006, reviewed in Rankin and Burchsted, 1992), which is possible if the dispersers are also the physically superior organisms of the population who abound in resources (Bonte and de la Peña, 2009). However, our results differed from both these expectations and there was no significant difference between the fecundity of the VB and the VBC populations either in early life or late life (Fig. 7.5B), which is consistent with an earlier dispersal evolution study on spotted-mites (Fronhofer et al., 2014). The lack of difference in fecundity between the VBs and the VBCs might be explained if we assume that the selected flies allocate a fixed amount of resources to reproductive functions and this amount has not been affected by selection for dispersal. While there are several theoretical studies in support of both assumptions (de Jong and van Noordwijk, 1992; Mezey and Houle, 2005), it is difficult to conclusively demonstrate

them using our data. Therefore, we shifted our attention to a crucial body maintenance trait, namely longevity.

In Glanville fritillary butterflies, higher dispersal and mobility correlate strongly with higher flight metabolic performance (Hanski et al., 2004). Since a strong negative correlation between life-span and metabolic rate has been reported across various taxa (reviewed in Rattan, 2008), dispersers are expected to have a shorter life-span, which was actually observed in a previous study on tropical butterflies (Tufto et al., 2012). However, in our study, we did not find any significant difference in longevity between the dispersal-selected and the control populations, a result which is consistent over all four populations and both the sexes (Fig. 7.5C). One reason for this might be the nutrient-rich laboratory conditions under which the flies were reared during selection, which ensured that resources were never limiting, and therefore did not lead to the expected trade-offs between dispersal ability and fecundity or longevity. If true, then this would suggest that the patterns of trait correlation that would evolve under selection for dispersal would depend closely on, *inter alia*, the resource availability, which would obviously vary greatly across populations under natural conditions. Thus, even for the same species, it might be difficult to predict the outcomes of dispersal evolution under various scenarios.

4.6 Selected flies have elevated levels of cellular respiration

There was a clear difference between the overall metabolite profiles of the VB and VBC flies (Fig. 7.6 and 7.7) and the levels of 14 metabolites were significantly different between these two populations (Table 7.1). Most notably, the glucose levels of VBs were significantly higher than the VBCs and glucose is the primary proximate source of energy in the cell through the process of cellular respiration. Moreover, the VBs had greater levels of citric acid, nicotinamide adenine dinucleotide (NAD) and adenosine monophosphate (AMP), all of which are critically associated with cellular respiration (Fig. 7.8). Finally, the VBs also had significantly greater amounts of lactic acid. It is known that when the demand for energy is more than what cellular respiration can generate (e.g. during intense muscular activity) glucose undergoes anaerobic oxidation via lactic acid fermentation to produce ATP. True to this observation, the ATP levels in VBs was significantly higher than the VBCs (Table 7.1). However, the AMP: ATP ratio, which is an indicator of levels of cellular energy crunch (Hardie and Hawley, 2001), is much higher for VBs. This suggests that, in spite of the greater

levels of ATP, the VBs are in a more energy-depleted state than the VBCs (Table 7.1). Taken together, these results suggest that the VBs have elevated levels of both aerobic and anaerobic cellular respiration and produces significantly more ATPs, which is consistent with the fact that they disperse to longer distances (Tung et al., 2017) and have greater locomotor activity (Figs 7.1A, 7.1C and 7.1E).

4.7 Selected flies have elevated levels of octopamine and precursors for other neurotransmitters

The VBs also had increased levels of four amino acids, namely histidine, phenylalanine, tyrosine and tryptophan (Table 7.1). The metabolic break-down products of all these amino acids form intermediates of citric acid cycle or their precursors and therefore play a role in energy production (Voet and Voet, 2011). More interestingly, phenylalanine and tyrosine act as precursors for octopamine and dopamine (Brandau and Axelrod, 1972), while tryptophan is a precursor for serotonin (Stone and Darlington, 2002). In *Drosophila*, increased levels of octopamine not only enhances aggression (Zhou et al., 2008) but also leads to greater activity (Yellman et al., 1997). This was consistent with the observation that octopamine levels had significantly increased in VBs (Table 7.1). Similarly, serotonin levels are also known to be positively correlated with activity (Yellman et al., 1997) and aggression (Dierick and Greenspan, 2007). Dopamine can elevate the activity level in flies (Yellman et al., 1997) although its relationship with aggression is not as straightforward as for the other molecules (Alekseyenko et al., 2010). Combining these evidences with the fact that the VBs are more active (Fig. 7.1A, 7.1C and 7.1E) and aggressive (Fig. 7.3B), strongly suggests that the levels of serotonin and dopamine have also increased in course of evolution for dispersal. We did detect an increase in the levels of both these neurotransmitters (Table 7.1), although the increase was not statistically significant after Bonferroni correction.

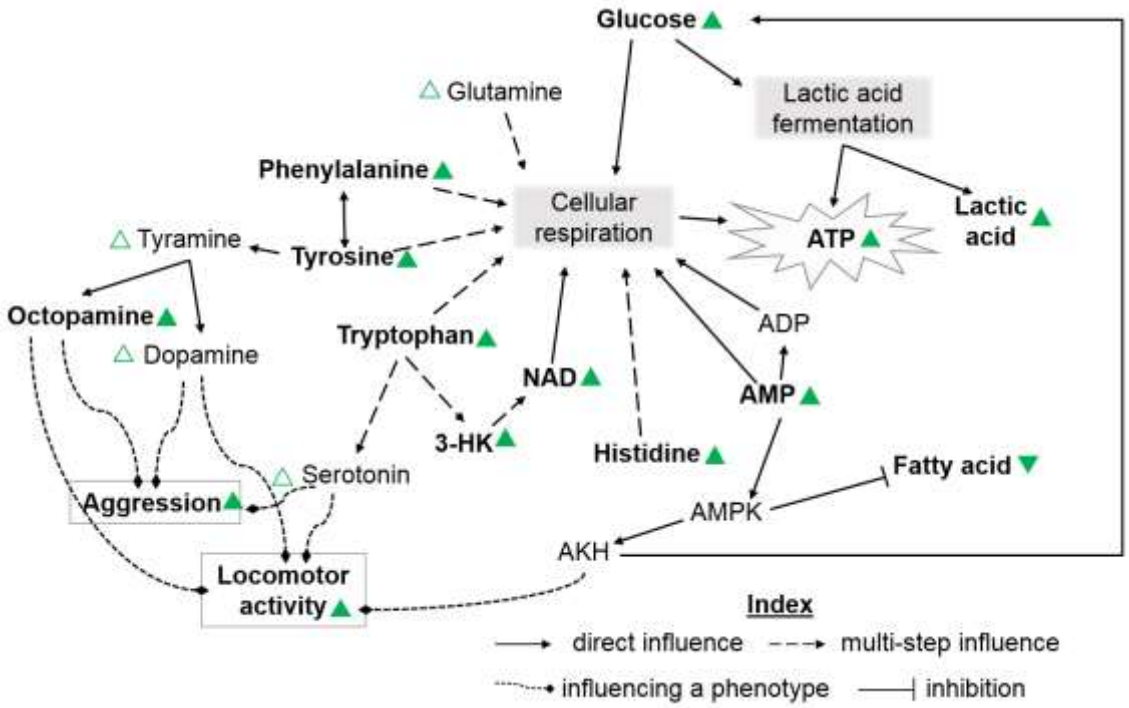

Figure 7.8. Alterations in metabolic pathways. Schematic diagram of the interactions between the metabolites that were found significantly changed in the VBs in the NMR data. Upright and inverted triangles adjacent to a metabolite denote whether its level had increased or decreased respectively. Filled and open triangles represent statistically significant and non-significant changes respectively (see Table 7.1 for values).

It should be noted here that apart from the three neurotransmitters discussed above, there are many more which can also potentially modulate fly behaviour. For example, it is known that increased levels of insulin (Belgacem and Martin, 2006; Luo et al., 2014) and tachykinin (Asahina et al., 2014) can either reduce aggression or activity or both. Unfortunately, although a comprehensive investigation of the changes in the levels of the various neurotransmitters in these flies would be of immense interest, it is also outside the scope of the present study.

4.8 Selected flies have reduced levels of fatty acids

The end-product of the main route of tryptophan metabolism is nicotinamide (Stone and Darlington, 2002), which subsequently produces NAD, a key element of cellular respiration (Khan et al., 2007). One of the main intermediates of the tryptophan-NAD pathway, 3-hydroxykynurenine (3-HK), is also found to be significantly higher in VBs, suggesting that the pathway has been enhanced in these flies. 3-HK is associated with free-radical generation and neural degeneration in flies (Savvateeva et al., 2000) which is consistent with the slightly

lower (although not significant) longevity of the VB flies (Fig. 7.5C). Histidine, another amino acid with elevated levels in the VBs, is known to be coregulated with AMP (Rébora et al., 2005). Abundant supply of AMP and depletion of ATP (Table 7.1) increases the AMP:ATP ratio, which in turn is expected to activate AMP-activated protein kinase (AMPK) (Hardie and Hawley, 2001). AMPK typically functions to facilitate the depletion of fat storage (Sinnett and Brenman, 2016) which is corroborated by the observation that VBs had significantly lower levels of fatty acid (Table 7.1). AMPK also controls the normal secretion of adipokinetic hormone (AKH) (Braco et al., 2012). AKH in turn stimulates locomotor activity and helps in maintaining a hyperglycaemic state in the body (Bharucha et al., 2008): two facts that are consistent with our observations on VBs.

Taken together, it is evident that the changes at the behavioural and life-history level are well correlated with the underlying metabolomic changes (Fig. 7.8).

5. CONCLUSIONS

Our study shows that in terms of relationship between dispersal and behavioural traits, there is excellent correspondence between the insights derived from association studies on field populations and experimental evolution studies. One reason for this might be that active dispersal is intimately related to locomotion which shares common control mechanisms with aggression and exploration via neurotransmitters like octopamine and serotonin. This automatically leads to the prediction that in passively-dispersing organisms, this trait-association is likely to breakdown. Incidentally, we also show that in terms of life-history traits, the correspondence between field and laboratory studies is poor. One reason for life-history traits not evolving in experimental evolution studies might be the fact that nutrition is typically non-limiting under laboratory conditions and therefore the organisms can increase expenditure in energy-intensive activities without paying major life-history costs. If true, then one can predict that artificial selection for dispersal under nutrient-limiting conditions would lead to a very different pattern of changes in life-history traits. Whether these changes would mimic the ones from field studies remains to be seen. Finally, our study gives the first glimpses of the metabolome-level changes that accompany dispersal evolution. This is best thought of as an over-view of the myriad changes that can occur when dispersal evolves and the complex ways by which they can affect the various traits of the organism. Establishing the robustness of these metabolic level changes (particularly under field conditions) and connecting them to the corresponding genes is going to be one of the next big challenges in dispersal ecology.

CHAPTER 8

Conclusions

Rapid changes in global climate coupled with wide-spread habitat degradation, pose a severe threat to biodiversity on earth. Much of these environmental changes are happening so rapidly that that a large number of species simply cannot evolve to adapt quickly enough. Some of these species can avoid extinction only with some help from us humans. The others can do so by dispersing to more favorable areas. In this thesis, I have examined some possible strategies for the former and the evolutionary consequences of the latter.

1. Theoretical and empirical investigations of control methods for stabilizing population dynamics

Although a number of methods have been proposed in the theoretical literature for stabilizing biological populations (Corron et al., 2000; Dattani et al., 2011; Hilker and Westerhoff, 2005, 2007; McCallum, 1992; Sah et al., 2013), they have rarely been implemented in practice. This is partly because these methods have often been proposed and investigated in the context of very different stability concepts and population dynamics models. Since there are no comparisons of their relative efficiencies under different ecological scenarios, it becomes difficult to choose a suitable method for any given case. More importantly, the majority of these methods had not been validated empirically, even under controlled laboratory conditions, let alone in natural populations. Thus, given that the survival of the threatened species is at risk, the reluctance of the practitioners of conservation and population management to try these methods under field conditions is understandable. I have tried to address both these issues in the first part of my thesis.

In Chapter 2 I have compared six popular population stability methods under a common theoretical platform. I show that although all the six methods can control population dynamics, their efficiencies vary in terms of the different aspects of stability (Grimm and Wissel, 1997). In other words, if a given control method performs well in terms of one aspect of stability (say constancy), that does not automatically guarantee its superior performance for another aspect of stability (say persistence). Unfortunately, these various aspects of stability are not entirely independent of each other. For example, if a population is undergoing high-amplitude oscillations, then it is very likely to face bottlenecks, which in turn will reduce the amount of genetic variation available, thus affecting its persistence. Therefore, in any real-life scenario, it is not always desirable to improve one kind of stability at the expense of another. Furthermore, practically speaking, it is not possible to ignore the economic aspects of any conservation measure. Therefore, while setting intervention goals, it

is imperative to think of a composite measure that is able to integrate these various concerns. Unfortunately, the current population dynamics literature lacks such a treatment. One of the primary messages from my theoretical (Chapter 2) and empirical (Chapter 3-4) results is that there is no simple solution to this problem. Methods that are good in terms of enhancing constancy can be bad in terms of getting persistence and vice versa. When it comes to two-parameter methods that can do both, the cost of implementation can be prohibitively expensive. I used a number of simplifying assumptions in reaching these conclusions (listed in section 3.5 of Chapter 2). For example, in my study, the performance score of the methods depends on the relative weightage given to different aspects of stability. But those relative weights can eventually vary based on the ecological or economic context of the population in question. Moreover, the calculation of costs of implementation of these methods can change based on the relative difficulty of culling vs restocking for different species. Consequently, the exact quantitative outcome of this study might alter for different species × environment combinations. However, there is no reason to expect that relaxing those assumptions would somehow lead one method to be superior to all others. Therefore, the search for usable methods for stabilizing natural populations continues.

2. Investigating the determinants of population dynamics and stability: A *Drosophila* model

The above conclusions were reached using the simple, but widely applicable Ricker model of population dynamics (Ricker, 1954). Although this choice of model increased the generalizability of my results, the simplicity of the Ricker function also prevented me from obtaining mechanistic insights about how the various determinants of the dynamics are affected by the control methods. For this, I needed a more detailed model of population dynamics that explicitly incorporated the details of the different density-dependent processes and individual life-history traits in governing population stability (Chapter 5). Therefore, I built an individual-based, stage-structured model of *Drosophila* dynamics which, nevertheless, included parameters that were common to the life-history of several holometabolus insects, and therefore carried some level of generality. After calibrating the parameters using data from laboratory populations of *Drosophila*, the model captured almost all the aspects of the dynamics observed in an experiment with real flies under four different nutritional regimes.

I then used this model to investigate how nutrition levels interact with demographic factors (like the presence of an unequal number of males and females in the population) and life-history traits (like fecundity and density-sensitivity) to affect population dynamics. These simulations showed that the effects of unequal sex-ratio and sex-specific culling are greatly influenced by fecundity but not by levels of juvenile nutrition. I also found that the efficiency of a widely-used pest control method (Sterile Insect Technique; see Dyck et al., 2005 for a comprehensive review) depends on a complex interaction between levels of juvenile nutrition and density-independent adult fecundity. Therefore, in the context of eradicating a pest species, accruing information related to the nutritional resource available for the juveniles and the fertility of the adult insects are important factors for efficient implementation of this technique.

There are several ways in which this individual-based model can be extended to answer various ecological and evolutionary questions. For example, incorporating heritable variation in the life-history parameters can allow one to study the influence of dynamic evolutionary changes on ecological dynamics (Pelletier et al., 2009; Schoener, 2011). By appropriately plugging in the various trait-correlations, this model can then be used to predict the outcomes of experimental evolution studies, particularly in the context of density-dependence (DeAngelis and Mooij, 2005). These predictions can then be compared with the observations from the rich body of literature available on this topic (Cameron et al., 2013; Kinnison and Hairston, 2007; Turcotte et al., 2011). Finally, this model can also be used to understand the mechanistic basis of how different control strategies affect population stability.

3. Evolution of dispersal components, kernel, syndrome and associated metabolomic changes: The *Drosophila* story

In the last part of my thesis, I have studied different aspects of dispersal evolution and its consequences on other life-history and behavioural traits. The process of dispersal consists of multiple stages like emigration, movement and immigration (Bowler and Benton, 2005). Consequently, the trait of dispersal consists of components related to each of these stages, like dispersal propensity (*i.e.* the proportion of individuals dispersed), dispersal ability (*i.e.* the average distance travelled by the dispersed individuals) and settlement efficiency (i.e. what fraction of the dispersers who reach a new patch are able to settle and reproduce). Evidently, in order to obtain a complete picture of evolution of dispersal as a trait, it is important to investigate the evolution of these individual components of dispersal (Bonte et

al., 2012), as well as their interaction. However, in practice, the relationship between the various dispersal components remains less investigated and understood.

I used a series of experiments on *Drosophila melanogaster* populations, which were artificially selected for higher dispersal to report the first empirical demonstration of the simultaneous evolution of multiple components of dispersal. This is noteworthy because none of the previous studies on dispersal evolution found that multiple dispersal components can evolve together. This happened because, unlike some of these earlier studies (Bitume et al., 2011; Friedenberg, 2003; Keil et al., 2001; Ogden, 1970; Tien et al., 2011; Yano and Takafuji, 2002; Fronhofer et al., 2014), there was moderate positive selection pressure for both propensity and ability in my selection protocol (Chapter 6). Consequently, we were able to show, that in principle, it is possible to have multiple dispersal components evolve together. Thus, the results of this study can be taken as a null model, such that whenever a particular component of dispersal is seen not to evolve, elucidating the reasons for that can become one of the goals of the investigation.

In my selection protocol, there was gradual directional selection of moderate intensity and the absence of conflicting selection pressures across the various dispersal stages. Such conditions are expected to be common in nature, particularly in regions where climate changes or habitat degradations are gradual but steady. This suggests that dispersal is likely to be under positive selection in many parts of the world. More importantly, I found that once evolved, the enhanced dispersal propensity and ability persists even in the absence of the proximal stresses (i.e. become phenotype-dependent). Finally, I show that selection for dispersal leads to the evolution of three traits related to the invasive potential of a species, namely enhanced activity, exploration and aggression (Chapter 7). This is consistent with results obtained from single-generation association studies on natural populations (Cote et al., 2010; Duckworth and Badyaev, 2007; Hanski et al., 2006; Korsten et al., 2013). Taken together, all these results are perhaps cause for some alarm. It is obviously difficult to conclude that selection for dispersal under various natural conditions would lead to similar correlated responses as I found in my study under laboratory conditions. However, even if a small fraction of the natural populations show similar evolutionary outcomes as those in this study, the number of invasive species in the nature will likely increase. This is consistent with the empirical finding that invasive species are often found in disturbed habitats (Lee and Gelembiuk, 2008), which presumably also have a positive selection for dispersal. Note that for invasive species to arise this way, it would not be necessarily needed for a non-native species to be

introduced and then “invade” into a new area. An increase in aggression, explorative tendencies and locomotor abilities can potentially turn even native species into invaders. This can in turn destabilize existing ecosystems. Thus, the potential increase in invasiveness of species due to selection for dispersal needs to be carefully investigated in natural populations.

Although there was close correspondence between my results and those obtained from single-generation association studies in terms of behavioural traits like aggression, the same was not true for the life-history traits (Chapter 7). The dispersal selected lines had similar body weight, fecundity and longevity as the controls, suggesting the absence any major life-history trade-off. One potential reason for this discrepancy might be the fact that nutrition is typically non-limiting under laboratory conditions. Therefore, in such scenarios, the organisms can afford to increase expenditure in energy-intensive processes like dispersal and locomotor activity without paying any major costs with respect to other life-history traits. Following this hypothesis, one can predict that artificial selection for dispersal under nutrient-limiting conditions might lead to a very different pattern of evolution in life-history traits. Whether these changes would be similar to those observed in field studies, remains to be seen.

The observations from the phenotypic assays led to predictions about putative mechanisms that were confirmed through untargeted metabolomic fingerprinting using NMR spectroscopy (Chapter 7). The selected flies evolved greater amounts of glucose, AMP and NAD, suggesting elevated levels of cellular respiration. At the same time, levels of neurotransmitters, such as octopamine, serotonin and dopamine, which are related to aggression and exploration, had increased. Thus, the experiments presented here give the first glimpses of the metabolome-level changes that accompany dispersal evolution. This is best thought of as an overview of the myriad changes that can occur when dispersal evolves and the complex ways by which they can affect the various traits of the organism. Establishing the robustness of these metabolic level changes (particularly under field conditions), and connecting them to the corresponding genes, is going to be one of the next big challenges in dispersal ecology. Moreover, many of these metabolites are known to be closely associated with learning (Dudai et al., 1987; Hammer and Menzel, 1998), metabolic syndromes (Roeder, 2005), stress (Hammen, 2005) and depression (Ries et al., 2017; Weiss et al., 1981). It will be interesting to investigate if there is any collateral effect of dispersal evolution on these important physiological conditions.

Finally, the rapid evolution of multiple components of dispersal and the kernel, expressed even in the absence of stress, without any perceivable life-history trade-off indicates that dispersal evolution cannot be ignored while investigating a large number of eco-evolutionary processes like range expansion, disease spread, evolution of invasive species and stability of spatially structured populations.

To summarize, the results of my theoretical studies and *Drosophila*-based investigations have led to several interesting insights, predictions and possible avenues for further work. I finish this thesis with the hope that these will be useful not only for ecologists in general and dispersal biologists in particular, but also for various practitioners of ecosystem management.

APPENDIX I

Description of the 49-generation time-series experiment

In order to facilitate the understanding of the simulations and subsequent analysis present in Chapter 5, here I briefly describe the basic design of the 49-generation time-series experiment. The complete details of this experiment have been reported elsewhere (Dey, 2007).

The experiment was comprised of thirty-two populations of *D. melanogaster*, each represented by a single vial (9 cm h × 2.4 cm dia.) culture. These populations were derived from a long standing, large outbred population (JB_1), maintained on a 21-day discrete generation cycle. Details of the ancestry and maintenance protocol of the JB populations can be found elsewhere (Sheeba et al., 1998), and are not germane to this study. These 32 populations were randomly allotted to one of four nutritional regimes, such that there were eight populations per regime. Following established norms (Mueller and Huynh, 1994; Mueller et al., 2000) these regimes were called HH, HL, LH, LL — where the first letter indicates the quantity of larval food and the second letter represents the status of adult nutrition. In case of larval food, H and L denoted ~6 mL and ~2 mL of banana-jaggery medium per vial, respectively, whereas in the case of adult nutrition, H and L referred, respectively, to the presence and absence of live yeast paste supplement to banana-jaggery medium. Thus, for example, HL denotes a nutritional regime comprising of ~6 mL medium per vial for the larvae, but no live yeast paste supplement for the adults, and so on.

Each population was initiated (generation 0) with eight male and eight female flies, and from this point onwards (except for extinction) there was no direct control on the number of adults in a vial. After oviposition in the vial for 24 hours (day 0), the adults were counted and discarded and the eggs formed the next generation. Once the adults started eclosing in these vials, they were transferred to adult collection vials every day with a change of medium every alternate day. Strict vial-to-vial correspondence was maintained between the egg vials and their corresponding adult collection vials. The process of adult collection continued until 18 days after day 0, after which the flies were conditioned for three days in the presence / absence of live yeast paste. The live yeast paste is known to boost the fecundity of the females (Chippindale et al., 1993) and reduce the effect of adult density on adult fecundity (Mueller and Huynh, 1994). On day 21 after egg collection, the adults were transferred to

fresh food vials containing ~2 mL or ~6 mL of banana-jaggery medium for a duration of 24 hours. After this, the adults were counted and discarded, while the eggs laid during this period formed the next generation. If there were no adults in a population, then an extinction event was recorded and the population was rescued by allowing four female flies from the ancestral JB_1 population to lay eggs for 24 hours. All extinction were recorded in the adult time series as 4 individuals (i.e. the number of rescuing females).

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LIST OF PUBLICATIONS, AND MANUSCRIPTS UNDER PREPARATION

Published manuscripts

i. **Tung, S.**, Mishra, A., Shreenidhi, P. M., Sadiq, M. A., Joshi, S., Sruti, V. S., Dey, S. 2018. Simultaneous evolution of multiple dispersal components and kernel. Oikos 127, 34–44.
ii. **Tung, S.**, Mishra, A., Dey, S. 2016. Simultaneous enhancement of multiple stability properties using two-parameter control methods in Drosophila melanogaster. Ecological Complexity 26, 128–136.
iii. **Tung, S.**, Mishra, A., Dey, S. 2016. Stabilizing the dynamics of laboratory populations of Drosophila melanogaster through upper and lower limiter controls. Ecological Complexity 25, 18-25.
iv. **Tung, S.**, Mishra, A. and Dey, S. 2014. A comparison of six methods for stabilizing population dynamics. Journal of Theoretical Biology 356, 163-173.
v. Mishra, A., **Tung, S.**, Shree Sruti, V. R., Sadiq, M. A., Srivathsa, S., and Dey, S. Pre-dispersal context and presence of opposite sex modulate density dependence and sex bias of dispersal. Oikos (accepted).

Manuscripts under review

i. Mishra, A.*, **Tung, S.***, Shreenidhi, P. M., Sadiq, M. A., Sruti, V. S., Chakraborty, P. P., and Dey, S. Sex differences in dispersal syndromes are modulated by environment and evolution (2018) *Equal contribution.
ii. **Tung, S.**, Rajamani, M., Joshi, A., Dey, S. Complex interaction of resource availability, life-history and demography determines the dynamics and stability of stage-structured populations. bioRxiv (2018) 138446.
iii. **Tung, S.**, Mishra, A., Gogna, N., Sadiq, M. A., Shreenidhi, P. M., Sruti, V. S., Dorai, K., Dey, S. 2017. Evolution of dispersal syndrome and its corresponding metabolomics changes. bioRxiv (2017) 178715.

Manuscripts under preparation

i. **Tung, S.**, Mishra, A., Shreenidhi, P. M., Sadiq, M. A., Sruti, V. S., Dey, S. Evolution of larval and adult life-history traits as a correlated response to selection for increased dispersal in *Drosophila melanogaster*.
ii. **Tung, S.**, Mishra, A., Shreenidhi, P. M., Sadiq, M. A., Sruti, V. S., Dey, S. Selection for condition-dependent dispersal leads to the evolution of phenotypic-dependent dispersal in *Drosophila*.
iii. **Tung, S.**, Mishra, A., Sruti, V. S., Sadiq, M. A., Shreenidhi, P. M., Dey, S. Evolution of dispersal in *Drosophila*: the story of two sexes.