University of Kentucky
UKnowledge

Theses and Dissertations--Biology | Biology

2012

THE EFFECTS OF SPATIAL CONFIGURATION OF POPULATIONS ON THE MAINTENANCE OF THE SEXES IN A CLONAL ORGANISM

Christopher Stieha
University of Kentucky, stieha@hotmail.com

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation
Stieha, Christopher, "THE EFFECTS OF SPATIAL CONFIGURATION OF POPULATIONS ON THE MAINTENANCE OF THE SEXES IN A CLONAL ORGANISM" (2012). *Theses and Dissertations--Biology*. 4.
https://uknowledge.uky.edu/biology_etds/4

This Doctoral Dissertation is brought to you for free and open access by the Biology at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Biology by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained and attached hereto needed written permission statements(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine).

I hereby grant to The University of Kentucky and its agents the non-exclusive license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless a preapproved embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's dissertation including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Christopher Stieha, Student

Dr. Philip H. Crowley, Major Professor

Dr. Brain C. Rymond, Director of Graduate Studies

THE EFFECTS OF SPATIAL CONFIGURATION OF POPULATIONS ON THE MAINTENANCE OF THE SEXES IN A CLONAL ORGANISM

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
Department of Biology
at the University of Kentucky

By

Christopher Stieha

Lexington, Kentucky

Co-Directors: Dr. Philip H. Crowley, Professor of Biology

and Dr. D. Nicholas McLetchie, Associate Professor of Biology

Lexington, Kentucky

2012

Copyright ©Christopher Stieha 2012

ABSTRACT OF DISSERTATION

THE EFFECTS OF SPATIAL CONFIGURATION OF POPULATIONS ON THE MAINTENANCE OF THE SEXES IN A CLONAL ORGANISM

Despite the two-fold advantage to asexual reproduction and its prevalence in a variety of organisms, sexual reproduction is prevalent across all taxa. The maintenance of two sexes is required to ensure genetic diversity and to prevent "evolutionary dead ends," especially in clonal organisms. Many mechanisms have been proposed for the maintenance of two sexes, ranging from environmental variation and stochasticity, parasites and predators, and mutation rates. Spatial configuration, the size and location of populations with respect to other populations, can allow two competitors to coexist when one would normally be lost. This is especially important when the two competitors are the two sexes. In the clonal organism *Marchantia inflexa*, I determined that spatial configuration of populations can directly influence the maintenance of both sexes in a population and in an aggregate of populations (a metapopulation) using a combination of theoretical models and field studies. Based on field studies, population size has a significant influence on whether a subpopulation will contain both sexes, with populations smaller than $1m^2$ being more likely to contain only one sex while populations greater than $1m^2$ are more likely to contain both sexes. Based on mathematical models, the spatial arrangement of subpopulations within a metapopulation can greatly influence whether a metapopulation maintains both sexes as well as whether the metapopulation persists once one sex has been lost. Field data suggest that distance to nearest neighbor, a measurement of spatial arrangement, influences the maintenance of the sexes within subpopulations, but could affect maintenance differently depending on the metapopulation identity. In some metapopulations, both sexes are maintained when the nearest neighbor is close, while in other streams, one sex is lost when the nearest neighbor is close. When mathematical models are used to explicitly simulate natural metapopulations, the mathematical model predicts the observed sex ratios in one metapopulation, predicts the observed bias in another metapopulation, and fails to predicted observed values in two other metapopulations. Understanding spatial configuration helps us understand the maintenance and loss of sex, but other factors, such as environmental differences, may be required to accurately predict which sex will be lost.

KEYWORDS: clonal organisms, maintenance of the sexes, *Marchantia inflexa*, metapopulation dynamics, spatial configuration

Christopher Stieha

May 14, 2012

THE EFFECTS OF SPATIAL CONFIGURATION OF POPULATIONS ON THE MAINTENANCE OF THE SEXES IN A CLONAL ORGANISM

By

Christopher Stieha

Philip H. Crowley, PhD
Co-Director of Dissertation
D. Nicholas McLetchie, PhD
Co-Director of Dissertation

Brain C. Rymond, PhD
Director of Graduate Studies

May 14, 2012

ACKNOWLEDGEMENTS

My dissertation, the research, and experiences contained within this document would not have been possible without the support and help of many people. I thank my family, John and Vicki Stieha and Chris and Dusty Needham who supported me emotionally and logistically through this process while often times not completely understanding why I was traveling to foreign countries or what I was doing with computers. Very important in this process is the family I had with me in Lexington who also provided emotional and logistical support while ensuring that I took enough walks. I couldn't imagine this process (or my life!) without Sara Cilles and the furballs: Bailey, Timber, Fozzie, Gobi, Raspberry, and Prairie. I thank them for being supportive during my whole graduate career and especially patient during the home stretch.

My academic family has also been instrumental in this process, ranging from the undergraduates in the McLetchie Lab, my fellow cohorts (both my Master's cohort and my Doctorate cohort), lab members in the McLetchie and Crowley Labs, other faculty in the department, Dr. Gisela García-Ramos, my outside examiner Dr. Liang Liang and my graduate committee: Dr. Scott Gleeson, Dr. Daehyun Kim, Dr. Nicholas McLetchie, and Dr. Phil Crowley. My co-advisors Dr. Nicholas McLetchie and Dr. Phil Crowley have been wonderful in providing guidance, discussion, confusion, and humor throughout this process while allowing me to be me and putting up with me being me. I also thank my two teaching advisors Dr. John Seabolt and Dr. Jim Krupa for giving me the instruction, support, and freedom to become the teacher I am.

My time spent in Trinidad was made homelier with the support of the McLetchie family, specifically Darryl, Andrea, and Daryann McLetchie, who allowed me to live near them and harass them with questions and cultural problems, and Mike and Doreen Oatham at the University of West Indies, who incorporated me into their lives and took me to see some cool parts of Trinidad. Alex Flecker, Mike Marshall, Steven Thomas, and other members of the FIBR group contributed scientific and cultural

discussion, while allowing me to piggyback projects. Ronnie and Linda Hernandez provided a constant helpful presence at the Simla research station throughout all my visits. I also thank the Wildlife Section of the Forestry Division of The Republic of Trinidad and Tobago for collection permits and the Water and Sewage Authority in Trinidad for access to research sites.

The office staff in the Department of Biology was wonderful and helped answer my many questions, whether it involved graduate school questions (thanks Bev Taulbee!) or money and ordering questions (thanks Cheryl Edwards!).

Funding was greatly appreciated for the international component of this project and I am grateful for the grants, fellowships, and support I have received from many programs: The Graduate School of the University of Kentucky, The Biology Department of the University of Kentucky, Gertrude Flora Ribble Fund, US National Science Foundation (DEB 9974086-DNM [PI] and PHC [co-PI]), the US Department of State, and the Institute of International Education, Fulbright Program 2006-2007.

I also thank my collaborators: Amy Bowman, Jessica Brzyski, Phil Crowley, Gisela Garcia-Ramos, Daehyun Kim, Nicholas McLetchie, Aurea Middleton, Joseph Stieha, and Skylar Trott.

I am sure there are people I have missed; I apologize for that and thank you for helping.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vii

LIST OF TABLES x

LIST OF FIGURES xi

CHAPTER ONE 1
Introduction 1

CHAPTER TWO 7
The Evolution of Sexual Reproduction: Were Males Expensive? 7
The Two-Fold Cost of Sex 7
The Two-Fold Cost of Sex? 8
Environmental Stochasticity 9
Parasites and Red Queen Dynamics 12
Mutations 15
What can maintain two sexes or mating types? 17
Future Research Directions 19
1. Determine the population level variation in frequency of mating types/sexes 20
2. Determine the cause of variation in sex ratios or mating type frequencies at the subpopulation level and metapopulation level 21
3. Set up experimental populations and explicitly test mechanisms 22
Pitfalls 22

CHAPTER THREE 24
Production, Dispersal, and Survival of Asexual Propagules and Their Contributions to Population Maintenance in a Clonal Organism 24
Introduction 24
Model Organism 26
Methods 27
Production of Gemmae 27
Intrapatch Dispersal 28
Interpatch Dispersal 30
Gemmae Survival under Water 32
Gemmae Survival after Desiccation 33
Results 34
Production of Gemmae 34
Intrapatch Dispersal 35
Interpatch Dispersal 35
Gemmae Survival under Water 35
Gemmae Survival after Desiccation 36
Discussion 36

CHAPTER FOUR 53
Maintenance of the sexes: a field test of the effects of population size and distance to nearest neighbor in a clonal plant 53
Introduction 53
Model Organism 56

Methods 57
Survey 57
Predicting One-Sex versus Two-Sex Subpopulations 58
Predicting Subpopulation Sex Ratios (Sex Bias Index) 59
Results 60
Predicting One versus Two Sex Subpopulations 60
Predicting Subpopulation Sex Ratio (Sex Bias Index) 61
Nearest Neighbor with Both Sexes 62
Discussion 63
Effects of Size 63
Effects of Distance to Nearest Neighbor 64
Maintenance of Competitors 66

CHAPTER FIVE 84
Maintenance of the Sexes and Persistence of a Clonal Organism in Spatially Complex Metapopulations 84
Introduction 84
Model Organism 86
Model 88
Subpopulation Model 88
Metapopulation Model 90
Spatial Model 93
Analysis 95
Results 96
Spatial Configuration – Island Model 96
Spatial Configuration – Single Linear System 96
Spatial Configuration – Linear system, Double 97
Spatial Configuration - Linear system, Triplets 98
Spatial Configuration – Grid Model 98
Spatial Configuration – Randomized Grid Model 99
Discussion 100

CHAPTER SIX 114
Predicting the Maintenance of the Sexes in a Clonal Organism using Mathematical Models 114
Introduction 114
Model Organism 116
Methods 118
Quantifying Natural Metapopulations 118
Model 119
Subpopulation Model 119
Metapopulation Model 121
Spatial Configuration Model 123
Analysis 124
Results 127
Overall Metapopulation Dynamics 127
Subpopulation Dynamics 127
Eastern Turure River 127
Quare River 128
Western Turure downstream 129
Western Turure upstream 130

Predicted Disturbance Regimes 131
Eastern Turure River 132
Quare River 132
Western Turure River downstream 132
Western Turure River upstream 133
Discussion 133
Overall Metapopulation Dynamics 133
Subpopulation Dynamics 135

CHAPTER SEVEN 152
Conclusion and Future Directions 152
Overall Conclusions 152
Use of genetic information 153
Application of spatial statistics 153
Dispersal capabilities of sexual propagules 154
Long term subpopulation and metapopulation dynamics 155
Enhancement of mathematical model 155

APPENDIX 1 157
Spatial Analysis of Proportion of Males 157
Methods 157
Results and Discussion 159

REFERENCES 165

VITA 173

LIST OF TABLES

Table 3.1. Gemmae Production Sequential Comparisons 41
Table 3.2. Coefficients of GLM for Gemmae Desiccation Experimentation 42
Table 4.1. Deviance table for the effects on whether the subpopulation has lost a sex. 69
Table 4.2. Deviance table for the effects on the Sex Bias Index (SBI)..... 71
Table 4.3. Coefficients for the final models when nearest neighbor is closest neighboring subpopulation. 73
Table 4.4. Coefficients for the final model when nearest neighbor must contain both males and females. 75
Table 5.1. Equations describing the population dynamics of male and females stages of M. inflexa. 104
Table 5.2. Parameter values of the default model used to predict sex ratio and sex bias index. 105
Table 6.1. Equations representing the life cycle of *Marchantia inflexa*. 138
Table 6.2. Parameter values of the default model used to predict sex ratio and sex bias index. 139

LIST OF FIGURES

Figure 2.1. Growth Differences under Various Reproductive Strategies 23
Figure 3.1 Conceptual Diagram of Dispersal 43
Figure 3.2. Production of Gemmae 44
Figure 3.3. Intrapatch movement speed of gemmae versus rainfall rate. 45
Figure 3.4a. Spatial configuration of populations found along Bird's Mill creek, female-only. 46
Figure 3.4b. Relationship between mean gemmae dispersal and proportion of metapopulation occupied for the female-only Bird's Mill Creek 47
Figure 3.5a. Spatial configuration of populations found along Honey Creek, male-only. 48
Figure 3.5b. Relationship between mean gemmae dispersal and proportion of metapopulation occupied for the male-only Honey Creek. 49
Figure 3.6. Proportion of individuals surviving submersion. 50
Figure 3.7a. Proportion of individuals germinating after desiccation, males. 51
Figure 3.7b. Proportion of individuals germinating after desiccation, females. 52
Figure 4.1. Spatial Configuration of the Four Metapopulations. 1a) Location and size of subpopulations along 4 streams 77
Figure4.2. Relationship between sex ratio as proportion of males and Sex Bias Index (SBI 78
Figure 4.3. Loss of One Sex versus Subpopulation Size. 79
Figure 4.4. The Effects of Spatial Configuration on Sex Bias Index. 81
Figure 4.5. Comparison of model predictions and observed data. 82
Figure 4.6. The Interaction of Subpopulation Size and Distance to Nearest Neighbor on Sex Bias Index (SBI). 83
Figure 5.1. Diagram of subpopulation arrangements. 106
Figure 5.2. Dispersal capabilities of sexual and asexual propagules. 107
Figure 5.3. Transition diagram of the model. 108
Figure 5.4. Effects of spatial configuration and dispersal on the maintenance of the sexes and persistence of single-sex metapopulations. 110
Figure 5.5. Sex ratio of metapopulations containing both sexes 112
Figure 5.6. Effect of randomization of patch location on the maintenance of the sexes and persistence of single sex metapopulations. 113
Figure 6.1. Transition diagram of equations 140
Figure 6.2. Comparison between the predicted and observed metapopulation sex ratios. 141
Figure 6.3. Comparison between the observed and predicted distribution of subpopulation sex ratios. 142
Figure 6.4. Observed versus predicted values for the Eastern Turure Metapopulation, meter. 144
Figure 6.5. Observed versus predicted values for the Eastern Turure Metapopulation, patch. 145
Figure 6.6. Observed versus predicted values for the Quare Metapopulation 146
Figure 6.7. Observed versus predicted values for the Western Turure Downstream Metapopulation, meter. 147
Figure 6.8. Observed versus predicted values for the Western Turure Downstream Metapopulation, patch 148

Figure 6.9. Observed versus predicted values for the Western Turure Downstream Metapopulation, meter 149
Figure 6.10. Observed versus predicted values for the Western Turure Downstream Metapopulation, patch. 150
Figure 6.11. Predicted disturbance regime to produce the observed proportion of males..... 151

CHAPTER ONE

Introduction

The evolution of sex has garnered much attention as researchers attempt to explain the persistence of sexual reproduction. Research focuses on what mechanisms make genetic recombination beneficial and prevent an asexual population from invading a sexual population, despite the two-fold cost of sex. The two-fold cost of sex is the cost that sexual females occur either through 1) production of males or 2) genome dilution compared to asexual females that 1) produce only females and 2) pass on all their genes to their offspring. Spatial configuration, defined as size of populations and location of populations with respect to other populations, can allow two competitors to coexist when one would normally be competitively excluded. In many organisms across a range of taxa, males and females compete intensely to the point of competitive exclusion and are both capable of sexual and asexual reproduction. In these taxa, the maintenance of the sexes may be based on the spatial configuration of populations.

Chapter Two considers the question: considering the evolution of sex, was there a two-fold cost? The evolution of sexual reproduction is thought to have occurred when isogamous gametes were present and when mating types all had the capabilities of asexual reproduction, which reduces the "cost of males" as males can also asexually reproduce. The question then becomes: what mechanisms maintain multiple mating types or both sexes in a system so that the potential for sexual reproduction still persists? I review three proposed mechanisms for the evolution of sex (environmental stochasticity, Red Queen dynamics, and mutation rates) and determine if these mechanisms can account for the maintenance of multiple mating types or sexes. I then discuss the types of organisms that should be used to best study the evolution of sexual reproduction and research avenues that should be pursued.

In the dissertation I do not specifically relate my research to the evolution of sex. I focus on the maintenance of two sexes, although the mechanism could be the same for both the evolution and

maintenance of sex. *Marchantia inflexa* is a clonal organism where both males and females can sexually reproduce as well as asexually reproduce. There is observed variation in the proportion of males at both the population level and the metapopulation level (an aggregation of subpopulations). The focus of my research is to understand the sex ratio variation and determine why some populations maintain both sexes while other populations lose one sex from a metapopulation perspecitve. I use laboratory experiments, field experiments, field surveys, and mathematical models to understand and quantify the effects of spatial configuration (size and location of subpopulations) on the maintenance of the sexes.

In the metapopulation framework, dispersal to other populations and new habitat is vital for the long-term persistence of a species. Understanding dispersal requires knowledge of emigration away from a focal source, dispersal through the landscape, and immigration into a new population or habitat. In many systems, the migrants are the products of sexual reproduction. In clonal organisms, asexual propagules are assumed to contribute to local population growth and not disperse out of the source population. In single-sex clonal organisms, however, the persistence of the species is dependent upon dispersal of asexual propagules. While the significance of asexual reproduction in population persistence via local population growth is well documented, the significance of asexual reproduction in population persistence via interpopulation dispersal is limited and understudied.

In Chapter Three, I combine laboratory experiments, field studies, and mathematical models to quantify the production, dispersal, immigration, and survival of water-dispersed asexual propagules of *M. inflexa*. Males and females differ in their overall rate of asexual propagule production. These propagules are capable of moving great distances even during light rain, which suggests they can leave the source population. Upon reaching the new habitat, gemmae survival and germination are high if the asexual propagules stay moist. Desiccation appears to affect survival and germination, with males being affected more negatively than females. I offer a diverse array of evidence that asexual propagules have the potential to affect population and metapopulation dynamics. The capabilities of asexual propagules

are important to understand for the maintenance of both sexes, the persistence of single -sex metapopulations and species, and the invasibility of clonal organisms.

Chapter Four focuses on the mechanisms that maintain both sexes within a subpopulation and therefore enable sexual reproduction. Sexual reproductionimportant in almost all organisms, requires spatial-temporal overlap of the sexes. However, competition between the sexes in clonal organisms can produce single sex populations unable to sexually reproduce. Many clonal organisms contain single-sex and two-sex populations, but the long term persistence of both sexes and their mechanisms are not well understood. Analogous with competing species, spatial configuration (size and location of a subpopulation with respect to other subpopulations) could prevent exclusion of one sex at the subpopulation or the metapopulation level. Mathematical models predict 1) subpopulations with larger carrying capacities require longer times before resources are limiting and competitive exclusion of one sex occurs and 2) the interaction of subpopulations through dispersal allow sexes to be maintained.

I use logistic regression to field-test the effects of subpopulation size (described as area) and distance to nearest neighbor on the maintenance of the sexes within subpopulations of four metapopulations of *M. inflexa*. When subpopulations are classified as single-sex or two-sex, area is the only significant explanatory variable. Subpopulations smaller than 1 m^2 are more likely to contain only one sex, while subpopulations greater than 1 m^2 are more likely to contain both sexes. Because single-sex subpopulations could contain only females or only males, I compute the Sex Bias Index (SBI), the deviation from an equal sex ratio. Both area and distance to nearest neighbors are significant. As area increases, SBI decreases; larger subpopulations have a more equal sex ratio. The effects of distance to nearest neighbor depends on the metapopulation, suggesting dispersal differences among metapopulations. This chapter shows that the spatial configuration of the metapopulation is another mechanism influencing the maintenance of the sexes in patchy systems.

The spatial arrangement of subpopulations within the metapopulation (not just nearest

neighbor) can affect the population dynamics and persistence of the subpopulations. Understanding the effects of neighboring populations on the dynamics of a focal subpopulation is required to predict persistence. Distance between subpopulations affects the probability that migrants can disperse between the subpopulations. Subpopulations close to one another may act synchronously, while subpopulations separated by a larger distance may act asynchronously. Synchrony can affect persistence of intense competitors; synchronous subpopulations will lose a competitor, while asynchronous subpopulations will maintain both competitors. When competitors are the two sexes, competition may undermine sexual reproduction. In Chapter Five, I use a mathematical model of competition between the sexes in *M. inflexa* to determine the relationship between subpopulation spread and the spatial arrangement of the subpopulations on the maintenance of both sexes within a metapopulation and on the persistence of metapopulations once one sex is lost.

Marchantia inflexa has two forms of dispersing propagules: 1) short distance, water-dispersed asexual propagules (the focus of Chapter Three) and 2) long distance, wind-dispersed sexual propagules. When subpopulations are close to one another, asexual propagules drive population dynamics and cause one sex to be lost, but the metapopulation persists as a single-sex metapopulation. When competition for space is intense and the subpopulations are far apart, subpopulations lose one sex, leading to single-sex metapopulations that go extinct due to lack of any propagules capable of recolonizing empty habitat. In between these two extremes, metapopulations persist with both sexes maintained at the metapopulation level. Spatial arrangement, whether island, linear, or grid, changes the threshold distance when one sex is lost and the metapopulation persists. These are idealized scenarios and their implications help clarify the effects of the spatial arrangement of subpopulations within a metapopulation on the maintenance of competitors. The model also suggests subpopulation arrangements that allow for the persistence of single sex metapopulations, which can explain the persistence of asexual species and inform the conservation of clonal organisms.

Conservation focuses on preserving a series of habitats or documenting habitat fragmentation and predicting species persistence. Mathematical models based on the spatial configuration of subpopulations within a metapopulation are used to predict persistence of the species. Although field and empirical data are used to inform the model, models are rarely compared to field data. In Chapter Six, I extend the current mathematical model from Chapter Five to predict the maintenance of both sexes in four spatially complex, naturally occurring metapopulations. To test the conceptual understanding of the *Marchantia* system, I use the model to predict sex ratios and deviation from a 1:1 (male:female) sex ratio of the metapopulations and the subpopulations within individual metapopulations. I then compare the predicted values to the observed values. With respect to the well-studied metapopulation along the Quare River, the observed and predicted values at the metapopulation level are similar. When I use the model to predict metapopulation dynamics along the other streams, the predictions are either qualitatively similar (a bias is predicted) or do not match the observed patterns. By including subpopulation carrying capacity explicitly in the model, the observed values are more often contained within the range of predicted values. The mathematical model matches observed values in the metapopulation containing many subpopulations at various stages, but does not work well for metapopulations with few subpopulations or for predicting the values of individual subpopulations. These results suggest that the current mathematical model is better at predicting averages than extremes, and future work should incorporate environmental heterogeneity and spatial-temporal autocorrelation of subpopulations.

This dissertation research addresses effects of dispersal of sexual and asexual propagules, including the influence of spatial configuration of subpopulations within a metapopulation, on metapopulation dynamics, the maintenance of the sexes, and the persistence of single sex metapopulations. In my final chapter, Chapter Seven, I suggest future avenues of research on these issues.

Copyright ©Christopher Stieha 2012

CHAPTER TWO

The Evolution of Sexual Reproduction: Were Males Expensive?

The evolution of sex has garnered much attention (Williams 1975; Maynard Smith 1978; Bell 1982; Kondrashov 1993; West *et al.* 1999; Lehtonen et al. 2012). The question still remains: Given the "two-fold cost of sex" compared to asexual reproduction (Maynard Smith 1978), why does sex exist and persist? I argue, however, that the question of the persistence of sexual reproduction in the presence of the two-fold cost of sex is the wrong question to ask. To understand the evolution of sexual reproduction, the question should be: In the absence of a cost of sexual reproduction, what mechanisms would allow multiple genotypes to coexist to allow sexual reproduction to occur?

The Two-Fold Cost of Sex

The two-fold cost of sex has two proposed mechanisms: 1) production of males (Maynard Smith 1978) or 2) genome dilution (Williams 1975; Bell 1982). Females produce larger gametes and are the drivers of population growth, while males produce smaller gametes that contribute genetic material but do not contribute mass for the survival of the gametes. Assuming that females can produce two offspring, an asexually reproducing female produces two female offspring (Figure 2.1a), while a sexually reproducing female produces one female and one male offspring (Figure 2.1b). In the next generation, the two female offspring produced via asexual reproduction can produce a total of four offspring, while the one female offspring produced via sexual reproduction must mate with a male and will produce one female and one male (Maynard Smith 1978). Genome dilution argues that asexually reproducing individuals pass on all of their genome to their offspring while sexually reproducing individuals pass on half of their genome to their offspring (Williams 1975; Bell 1982), but the validity of this mechanism for the twofold cost of sex is questioned because only the genes responsible for sexual or asexual reproduction (not the whole genome) and their potential to be passed to offspring is important (see

argument in Lehtonen et al. 2012). Despite the two-fold cost, sexual organisms and sexual reproduction are prevalent (Maynard Smith 1978), using males as a mechanism for outcrossing.

The Two-Fold Cost of Sex?

Organisms with isogamous (same sized) gametes and multiple mating types do not have the two-fold cost of sex and could gain the benefits of sexual reproduction and recombination (such as consolidation of beneficial alleles, production of novel genotypes) while still maintaining the capabilities of asexual reproduction (Maynard Smith 1974). However, most research on the evolution of sexual reproduction occurs on two-mating-type systems with anisogamous gametes (males and females), looking more at the maintenance or prevalence of sexual reproduction. To better understand the evolution of sexual reproduction, we need systems where asexual and sexual reproduction can occur across all mating types – e.g. when both males and females are able to asexually reproduce (Figure 2.1c). Although the capability of asexual reproduction does not completely negate the cost of sex, it does minimize the cost of producing males (Figure 2.1c). When using systems with a reduced cost of producing males because of asexual reproduction, studying the maintenance of both sexes (or multiple mating types) would be very similar to studying the evolution of sex itself.

Examining systems where both males and females (or mating types) asexually reproduce increases our understanding of the evolution of sexual reproduction and the maintenance of sexual reproduction in the presence of asexual competitors. Various mating types are known that are capable of both sexual and asexual reproduction, such as the plus and minus mating types of bacteria (Lederberg *et al.* 1952; Hayes 1953), fungus (Nelson 1996; Coppin *et al.* 1997; reviewed in Kronstad and Staben 1997) and algae (Smith and Regnery 1950; Wreede and Klinger 1988), sex I and sex II (or E and O for even and odd) in paramecium (Sonneborn 1937), and the multiple mating types of other protists (Elliott and Hayes 1953). Isogamy is unstable (Matsuda and Abrams 1999), leading to dimorphic gametes between the mating types and giving rise to anisogamy and the sexes – with both sexes still capable of

asexual reproduction. Males in many taxa asexually reproduce (animals: Bell 1982 for a review; Hand and Uhlinger 1992; McGovern 2002; plants reviewed in Eckert 2002; algae reviewed in Wreede and Klinger 1988).

I reevaluate the current literature on the evolution of sexual reproduction with a focus on asexual reproduction in multiple mating types. I focus on three main hypotheses for the evolution of sex: 1) environmental stochasticity, 2) Red Queen dynamics and parasite effects, and 3) accrual of mutations. In many cases, the literature focuses only on systems where one sex can asexually reproduce. In those cases, I review the conclusions and determine how they would change if both sexes could asexually reproduce. I then propose new avenues of research and new research programs to increase our understanding of the evolution of sex.

Environmental Stochasticity

Environmental stochasticity is random change in the environment across time and space. Environments that do not change in space or time are homogeneous, suggesting that they can be dominated by a single well adapted genotype. The world is not a single uniform landscape consistent throughout time, but a shifting mosaic of abiotic factors, such as temperature, rainfall, nutrients, and light – and biotic factors such as abundances of predators, competitors, mutualists, and conspecifics. The variability in the environment contributes to the maintenance of males and sexual reproduction (Otto 2009 but see Maynard Smith 1978).

The tangled bank hypothesis describes the world as a spatially heterogeneous collection of niches (Bell 1982; Sigmund 1993). Asexually reproducing individuals survive in niches to which they are best adapted. Their offspring, being exact copies, are also adapted to these niches. Competition for resources in the niche then occurs between parents and offspring. If the offspring disperse, they will only do well in areas with a niche similar to the parent's niche and will be outcompeted in niches where an adapted population already exists. In order to be able to occupy new niches, asexual offspring

require multiple independent mutations. On the other hand, sexual reproduction combines genes to produce novel genotypes that may be better adapted to the parents' niche or, when dispersed, are capable of utilizing surrounding niches that are not like the parents' niche. Because sexually reproducing populations are more likely to produce offspring that can occupy new niches and have greater fitness than asexually reproducing populations, spatial environmental heterogeneity should allow sexual populations to outcompete and prevent invasion by asexual populations (Weisman hypothesis; Bell 1982). But sexual reproduction also disrupts beneficial gene combinations (Daly 1978; Xu 2004), reducing the fitness of the sexually reproducing individual and potentially allowing invasion and competitive exclusion by asexual populations (Holmström and Jensen 2004).

In the small brown morph of the ostracod *Candonocypris novaezelandiae*, asexual populations are found in environmentally homogeneous artificial lakes. These individuals have been observed to invade the environmentally heterogeneous Lake Purrumbete and increase in abundance relative to the sexual population (Chaplin 1993). Colonizers could reproduce sexually or asexually, although asexual reproducers are found in harsher and more disturbed areas compared to their sexually reproducing relatives (reviewed in Tomiuk and Köhler 2007). Many common colonizers asexually reproduce (such as *Pilosella officinarum*; Chapman *et al.* 2000), suggesting that environmental variation alone is not enough to maintain sexual reproduction (Maynard Smith 1978). Also, a large proportion of the environment must be refuge for sexual reproducers to outcompete asexual reproducers (Lomnicki 2001).

Environments vary through time as well as space. For example, a habitat can be warm and dry one season followed by cold and wet the next season. If there is no environmental variation or if the environmental variation occurs at regular intervals through time, the asexual population best adapted to the homogeneous environment or the frequency of environmental change will dominate (Maynard Smith 1978). Sexual populations are expected to dominate only in areas with temporal environmental variation where the correlation between abiotic factors changes (Maynard Smith 1978). Sexual

reproduction breaks apart gene complexes beneficial to previous environments to produce novel genotypes capable of surviving the new environments (Maynard Smith 1978; Otto 2009). In the nematode *C. elegans*, temporal environmental heterogeneity (starvation events) appears to maintain males (Morran, Cappy, *et al.* 2009) and therefore sexual reproduction.

But the characteristics of the products of sexual reproduction could be more important than the increase in genetic diversity. In the aphid *Rhopalosiphum padi*, females can either produce offspring asexually or produce male and female offspring capable of sexual reproduction (Halkett *et al.* 2006). Asexually reproduced offspring are unable to survive cold winters, but the eggs produced via sexual reproduction can. When winter begins around the same time each year (low environmental heterogeneity), populations invest more in asexual reproduction and only invest in sexual reproduction at the very end of the season (Halkett *et al.* 2004, 2005). On the other hand, environments with variation in the start date of winter contain populations with a mix of sexual and asexual reproductive strategies (Halkett *et al.* 2004, 2005) and contain males and females throughout the growing season (Papura *et al.* 2003; Halkett *et al.* 2006). Daphnia follow a similar cycle, where asexual reproduction occurs in benign conditions, but the products of sexual reproduction can survive harsh desiccation events (Deng 1996). If the main mode of dispersal (either through time or space) is via sexually produced offspring, sexual reproduction could have developed and be enforced by a gene attempting to remove itself from a poor environment (Otto 2009).

In organisms where both males and females can asexually reproduce, niche partitioning may drive the maintenance of mating types. As a benefit, sexual reproduction would produce novel genotypes able to use novel environments. On the other hand, purely sexually reproducing populations require a niche refuge for persistence (Pound *et al.* 2002) and cannot invade the niche of a well-adapted asexually reproducing population (Peck *et al.* 1998). Niche partitioning of a heterogeneous environment can produce spatial segregation of the mating types or sexes, a frequently observed phenomenon

(Bierzychudek and Eckhart 1988). Segregation would allow for the persistence of multiple mating types or sexes and the possibility of sexual reproduction. Strong frequency dependent selection on genotype can cause asexually reproducing genotypes to cycle, where one genotype becomes abundant and then is inhibited, allowing another genotype to increase in frequency (Peck 1993). If the various genotypes are various mating types, both mating types can be maintained and sexual reproduction can still occur.

The extreme situation where sexual reproduction produces a protected object (egg, diaspore, seed) that is capable of surviving a temporary harsh environment to wait for a better environment is a perfect example of a mechanism for the maintenance of sexual reproduction (as asexual populations that do not produce this object die), but it does not explain the evolution of sexual reproduction or the association of sex with the resting stage. Spatial variation and temporal fluctuations in environmental variation could drive the evolution of sex because populations capable of producing novel genotypes could utilize the novel environments produced by the variation, while purely asexual populations could not.

Environmental stochasticity allows the maintenance of multiple mating types. Therefore, environmental stochasticity is a potential mechanism for the evolution of sex.

Parasites and Red Queen Dynamics

Red Queen dynamics describe a situation where a population must constantly change (such as develop new defensive strategies, modify life history strategies, etc.) to maintain the same reproductive output as before (Van Valen 1973). Often, Red Queen dynamics are driven by biotic interactions, where all interacting populations can evolve. Predators can force prey populations to evolve (Yoshida et al. 2003), while evolving themselves. Parasites evolve to be close to their optimum fitness, while their hosts evolve to reduce their virulence (Ebert and Hamilton 1996). Parasites often have a faster generational time and are in constant battle with a host's immune system, hastening parasite evolution. The longer generation time of the host slows its adaptation to the parasite and emphasizes immune defense (Ebert

and Hamilton 1996, Jokela *et al.* 2009). The mode of reproduction influences the host population's response rate.

Only when the parasite transmission rate was greater than 0.8 and the parasite reduced the host's fitness to almost zero did sexually reproducing populations resist invasion by an asexual reproducer (Howard and Lively 1994). Under most parameter values, asexual and sexual reproducers coexisted, or asexually reproducing populations dominated (Howard and Lively 1994). Coexistence was less frequent (Howard and Lively 1998). When sexually reproducing populations dominated, the asexually reproducing population of a single clone was outcompeted, much like pathogens destroying a monoculture (Ebert and Hamilton 1996). The limited range of values where sexual populations outcompeted asexual populations suggests that parasites are not the driving force behind the maintenance of sex. Tobler and Schlupp (2005) found no relationship between parasite load and mode of reproduction in female mollies. Asexual and sexual populations of mollies differed in parasite load but not in a consistent manner. If parasites were driving sexual reproduction and the maintenance of the sexes, a positive relationship between outcrossing rates or the frequency of males in the population and the parasite load is expected. Across a range of plant species, the outcrossing rate increases as the number of pathogens increases (Busch *et al.* 2004). In the New Zealand snail *Potamopyrgus antipodarum*, male frequency is positively correlated with parasites but not with temporal or spatial heterogeneity (Lively 1987). Pathogens increased the outcrossing rate in populations of *C. elegans.* In the presence of the pathogen, sexually reproducing populations increased fitness while asexually reproducing populations did not (Morran, Parmenter, and Phillips 2009).

The driving mechanism for parasite infection is genotype matching between host and parasite. The parasite that can infect the most abundant host genotype has the greatest fitness. A host population consisting of a single genotype will quickly be overrun with parasites (Ebert and Hamilton 1996; Howard and Lively, 1994, 1998). However, asexual populations can consist of multiple genotypes

(Jokela et al. 2009). In this case, parasites that can attack the most common host genotype will have the highest fitness. The fitness of the parasitized genotype decreases, which allows other genotypes to increase in number. Parasites then shift from the previous genotype to one of the more abundant genotypes (Jokela *et al.* 2009). In the snail *P. antipodarum*, clones in high abundance in 1994 were rare in 2001. Resistance tests to both allopatric and sympatric parasites suggest a lag for parasites to effectively infect hosts and cause a clonal population decline (Jokela *et al.* 2009).

Once sexual reproduction has developed, Red Queen dynamics can help maintain sexual reproduction. If we assume that multiple clones can be different sexes, the evolution of sex is similar to the maintenance of multiple clones of the snail *P. antipodarum*. If we assume there is a single male clone and a single female clone and the population can only asexually reproduce, we would expect the frequency of males to fluctuate. The parasite would affect one sex initially and reduce that sex's offspring output. The other sex would increase in frequency, only to become the new target of the parasite and experience a reduction in offspring output. The cycle would repeat. The asexually reproducing population could go extinct if the male and female were identical with respect to the immunity genes.

The combination of sexual and asexual reproduction would not break this cycle. Sexual reproduction would produce novel genotypes. One genotype would increase through asexual reproduction to become the new target of the parasite. Assuming that the asexual population consists of multiple genotypes, the production of novel genotypes is not required to maintain a population under pressure from a parasite.

In the case of parasites, Red Queen dynamics can potentially maintain multiple genotypes. The production of novel genotypes through sexual reproduction may not be required, but this could depend on whether the parasite has multiple genotypes or is capable of sexually reproduction. Depending on some assumptions, Red Queen dynamics could be a mechanism for the evolution of sex.

Mutations

Mutation accumulation of beneficial and deleterious alleles has been a major hypothesis for the evolution and maintenance of sexual reproduction (Hurst and Peck 1996). In an asexual population, mutations are transferred directly from parent to offspring and will be transferred from parent to offspring for generations. In sexual populations, mutations from one parent are only inherited, on average, by half of the offspring and may not be expressed in diploid species.

Deleterious Mutations

If the mutations are deleterious, asexual reproduction cannot purge the mutations, and deleterious mutations slowly accumulate within the offspring. For every new deleterious mutation, Muller's ratchet clicks one notch closer to mutational meltdown (Muller 1932; Lynch and Gabriel 1990). However, sexual reproduction offers the possibilities that deleterious mutations 1) will not be transferred to the offspring or 2) will be combined into a single individual with a reduced fitness and its death will remove the alleles from the population. Two deleterious mutations per genome per generation is expected to maintain sexual reproduction (Howard 1994). When mutation rates were experimentally manipulated in the nematode *Caenorhabditis elegans*, populations with high imposed mutation rates (about 1.2 deleterious mutations per genome per generation) had a greater proportion of males than populations with low imposed mutation rates (Cutter 2005). Asexually reproducing populations showed a significant decrease in fitness from the high mutation rates (Morran, Parmenter, et al. 2009). Populations did not go extinct, but male frequencies declined during the experiment (Cutter 2005), suggesting that 1) only a small number of males are required for the benefits of sexual reproduction, 2) the persistence of males (and therefore sexual reproduction) was temporary or 3) the populations had become adapted to the mutagen. In another experiment, populations exposed to multiple mutagens maintained twice as many males as the control treatment (Figure 1 in Manoel et al. 2007).

Deleterious mutation rates are not assumed to be static and can evolve. Sexually reproducing populations evolved a two-fold higher mutation rate than asexually reproducing populations (Sloan and Panjeti 2009). The presence of a higher mutation rate coupled with epistasis (where the effects of mutations on fitness do not increase linearly based on the number of mutations but increase multiplicatively) could inhibit an asexually reproducing population from invading a sexually reproducing population. The mutational load of the sexually reproducing population would be half the mutational load of the asexually reproducing population (Kimura and Maruyama 1966). In order to see a difference between sexually and asexually reproducing populations, a population must first evolve the higher mutation rate, which helps explain the maintenance of sexual reproduction, once sexual reproduction has evolved, but does not explain the evolution of sexual reproduction.

Beneficial Mutations

Although rarer, mutations can also be beneficial (García-Dorado 1997; Bataillon 2000). If mutations are beneficial, asexual reproduction produces offspring that will have the beneficial allele, while sexual reproduction produces offspring where only half the individuals will contain the beneficial allele. In an asexually reproducing population, beneficial mutations increase the number of offspring from a single parent and are present in all the offspring of that parent but do not spread across lineages (Peck 1993). Frequency-dependent selection at a separate locus from the beneficial mutations can inhibit the invasion of a sexually reproducing population by an asexual one. Because asexual populations contain identical individuals, frequency dependent selection quickly negates the benefit of the beneficial mutations. Sexual reproducers are not constrained (Peck 1993).

Beneficial and deleterious mutations occur simultaneously. In sexually reproducing populations, beneficial mutations become established at a rate about twice the fitness advantage of the beneficial mutation, independent of deleterious alleles (Table 1 in Peck 1994). On the other hand, the probability

of a beneficial mutation becoming established in an asexually reproducing population depends on the deleterious mutation rate and the effect size of both deleterious and beneficial mutations (Peck 1994).

When we focus only on mutation rates, what would we expect if both sexes could sexually as well as asexually reproduce? If deleterious mutations rates are low (below 1.5 mutations per genome per generation), asexual reproduction will dominate (Kimura and Maruyama 1966; Howard 1994), whether the asexual population is only males or only females. If the asexual population contains both males and females, one sex will be lost through competitive exclusion, as only the genotype with the highest fitness persists. If deleterious mutation rates are high (greater than 2 deleterious mutations per genome per generation), both males and females will persist due to the benefit of outcrossing (Kimura and Maruyama 1966; Howard 1994). Populations that only reproduce asexually will be at a serious disadvantage. If between 10% to 25% of the offspring are produced by sexual reproduction, the population does not experience disadvantages due to deleterious alleles and gains the advantages of beneficial alleles (Peck 1994). Asexual reproduction will also keep beneficial mutations together. The mixing of genes and the creation of variability through sexual reproduction can disrupt the accumulation of beneficial mutations and the genetic background in which they developed, slowing adaptation (Holmström and Jensen 2004). In this case, a population where both males and females can asexually reproduce will have a higher fitness than a population of sexual reproducers.

All arguments assume that a high mutation rate evolved initially, which is unlikely in an asexually reproducing population (Sloan and Panjeti 2009). Without the evolution of a high mutation rate, mutation rate does not appear to be a mechanism for the evolution of sex.

What can maintain two sexes or mating types?

The three major mechanisms that are hypothesized to have led to the evolution of sexual reproduction are all thought to help maintain outcrossing in a population and prevent an asexually reproducing population from invading a sexually reproducing population. “Maintain” is the operative

word. With respect to the evolution of sexual reproduction, only environmental stochasticity and Red Queen dynamics create a scenario where multiple mating types (and multiple sexes) could develop and persist where asexual populations of a single genotype resist invasion by asexual populations of another genotype.

Conceptually, the evolution of sexual reproduction is the maintenance of competitors that also mate with each other. Research that investigates the maintenance of competitors can also be used to develop predictions for the maintenance of multiple mating types and sexes. As with competition between two species, competition between asexual populations and sexual populations has four outcomes:asexual reproducers win, sexual reproducers win, both types coexist, or the winner depends on initial conditions (Doncaster *et al.* 2000; Pound *et al.* 2002). When intra-population competition is greater than interpopulation competition, asexual and sexual populations coexist (Doncaster *et al.* 2000). When coexistence is not possible, the population that persists is the population that uses the resource most efficiently (Case and Taper 1986), often the well adapted asexual reproducer as opposed to the sexually reproducing population that may have broken up beneficial gene complexes. Extending basic competition theory across clones, as long as intraclonal competition is greater than interclonal competition, clones should coexist. With the strength of sibling competition, especially in the presence of genetically identical siblings (Williams 1975), this should be the case assuming that each clone is better adapted than all others in its niche.

Populations do not exist in isolation but in an aggregate of populations forming a metapopulation (Hanski 1999). Having multiple populations loosely connected via dispersal can drastically affect population dynamics and allow sexual reproducers to stave off invasion by asexual populations (Peck *et al.* 1999). In systems where males and females compete for resources and both sexes can asexually reproduce, competitive exclusion is predicted to occur in single isolated populations (McLetchie et al. 2002; Crowley et al. 2005). When a collection of environmentally homogeneous

populations are loosely connected via dispersal, persistence of both males and females occurs at the metapopulation level, although individual subpopulations within the metapopulation may lose a sex (García-Ramos et al. 2007). Spatial structure also allows persistence of multiple asexually reproducing competitors (Kerr et al. 2002). Spatial structure may have made the persistence of multiple genotypes likely, and then the multiple genotypes benefited from sexual reproduction.

Future Research Directions

Our understanding of the evolution of sexual reproduction will be helped immensely by a conceptual shift away from focusing on the maintenance of sexually reproducing populations that have been invaded by asexually reproducing populations. When we consider that multiple matings types (both males and females) could initially asexually reproduce, this almost eliminates the proposed two-fold benefit required for the evolution of sexual reproduction. Instead we should be asking what prevents one mating type (or sex) from dominating a system when both mating types (or sexes) can asexually reproduce. I discussed some of the currently proposed mechanisms for the evolution of sexual reproduction and determined whether these hypotheses would still be applicable when both sexes asexually reproduce. Due to a reduction in the two-fold cost of sex, the evolution of sexual reproduction could occur much more easily than previously thought. The problem becomes maintaining both mating types or sexes so that sexual reproduction has the potential to occur. In this new framework, environmental stochasticty and Red Queen dynamics appear to be mechanisms for the evolution of sex, while mutation accumulation appears to not affect the evolution of sex.

Basic research into the evolution of sexual reproduction requires 1) organisms with mating types or sexes where all individuals asexually reproduce, 2) variation in mating type frequency among populations, and 3) correlation between some abiotic or biotic factor and mating type frequency. To study the evolution of sex, I propose these research foci.

1. Determine the population level variation in frequency of mating types/sexes

Despite being present in taxa ranging from single celled organisms to multicellular organisms, organisms where both males and females can asexually reproduce and the population variation appears to be understudied. By variation, I mean proportion of various genotypes and proportion of various mating types/sexes. For example, ten different natural isolates of *C. elegans* from across the world had a range of male frequencies from 1% to 35% of the population (Figure 3 in Anderson *et al.* 2010), although observing males in nature is rare. Despite the knowledge of the various mating types of *Paramecium*, I could not find any information on the frequency of those mating types or even the frequency of multiple mating types in the same populations. Genetic techniques would allow us to quantify variation at the genetic level, explicitly determine mating type or sex, and estimate levels of outcrossing.

Many single celled organisms participate in sexual reproduction, and Xu (2004) argues that no ancient asexually reproducing microorganism has been found. About 15,000 of the almost 70,000 identified fungal species need their sex cycle identified (Hawkesworth *et al.* 1995 as reported in Xu 2004), suggesting that rare instances of sexual reproduction mitigate the effects of long bouts of asexual reproduction. Variation in frequencies of sexual and asexual reproduction within fungal species is known, including strains that only sexually reproduce (Xu 1995 and Yan *et al.* 2002 both as reported in Xu 2004). In many examples, both mating types can asexually reproduce and coexist; the loss of one mating type is rare (Xu 2004).

Many species across a range of taxa have separate sexes (dioecious or gonochoristic) that asexually reproduce (see Bell 1982 for animals; Hand and Uhlinger 1992; McGovern 2002; plants reviewed in Eckert 2002;). These organisms may be better for investigating the evolution of sexual reproduction because asexual reproduction has been a key part of their life cycle for a large part of evolutionary history. Many of the currently studied organisms are asexual species that evolved from sexual species and carry constraints associated with their evolutionary history (Engelstädter 2008).

These systems are good for the study of the maintenance of sexual reproduction and offer insight into the evolution of sexual reproduction, but they are not suitable for the study of the evolution of sexual reproduction itself. It is important to consider the costs of sexual reproduction in these systems (Lehtonen et al. 2012). For example, with no parental care or female-only parental care, asexually reproduced offspring have a two-fold advantage, but male parental care alleviates the cost of sexual reproduction (Dawson 1995).

2. Determine the cause of variation in sex ratios or mating type frequencies at the subpopulation level and metapopulation level

Quantifying differences in sex ratio across populations and then determining the mechanisms that produce the differences is essential to understand the evolution and maintenance of sexual reproduction. Research on the New Zealand snail (Lively 1987) and Amazon mollies (Tobler and Schlupp 2005) are prime examples of the technique to determine the maintenance of males in a population. But greater variation and more extreme sex ratios exist (Longton and Schuster 1983) where populations can contain only females, only males, or both sexes. Due to competitive exclusion, I would expect all populations to contain only one sex or one mating type or one genotype. By understanding what drives one sex extinct in some of these populations while both sexes are maintained in other populations, researchers may gain insights into the mechanisms for the evolution of sexual reproduction.

The study of sex ratios has been productive (Hardy 2002). Frequency dependence alone is enough to maintain both sexes and allow sexual reproduction to persist (Seger and Stubblefield 2002). But is frequency dependence acting through sexual reproduction enough to maintain both sexes when both can asexually reproduce? The answer is probably no, unless there is some requirement for sexual reproduction and genetic recombination.

3. Set up experimental populations and explicitly test mechanisms

When natural populations are used to determine underlying mechanisms unmeasured explanatory variables can often introduce variation and obscure the results. Only by setting up laboratory experiments that explicitly test the various mechanisms, can we understand the driving force (or forces) leading to the evolution and maintenance of sexual reproduction. The work on *C. elegans* determined the effects of mutation rate (Manoel et al. 2007; Morran, Parmenter, et al. 2009), environmental variation (Morran, Cappy, et al. 2009), and Red Queen dynamics (Morran, Parmenter, and Phillips 2009) on the proportion of males, which is directly related to outcrossing and sexual reproduction. More experiments like these are needed, such as Gause's experiments (1932) on *Paramecium* competition, but with multiple mating types and potential reasons to sexually reproduce.

Pitfalls

Even though I have explicitly outlined new directions for the study of the evolution of sex, there are pitfalls to be avoided. Many organisms carry constraints due to phylogeny (Engelstädter 2008), which can influence dynamics. Careful consideration is required in choosing a study organism such that constraints are minimized (such as the cost required to transition from meiosis back to mitosis) to avoid the conclusion that a constraint is the mechanism for the evolution of sexual reproduction. The mechanism for the evolution of sexual reproduction may not be the mechanism that currently maintains sexual reproduction. For example, life history differences between mating types and sexes could influence the maintenance of sex but not the evolution of sex, especially differences associated with anisogamy. Males may require one life history strategy due to the production of small gametes, while females require a different life history strategy to support large gametes. Also, a single driving force may not be the mechanism for the evolution of sex and the maintenance of the sexes (West *et al.* 1999).

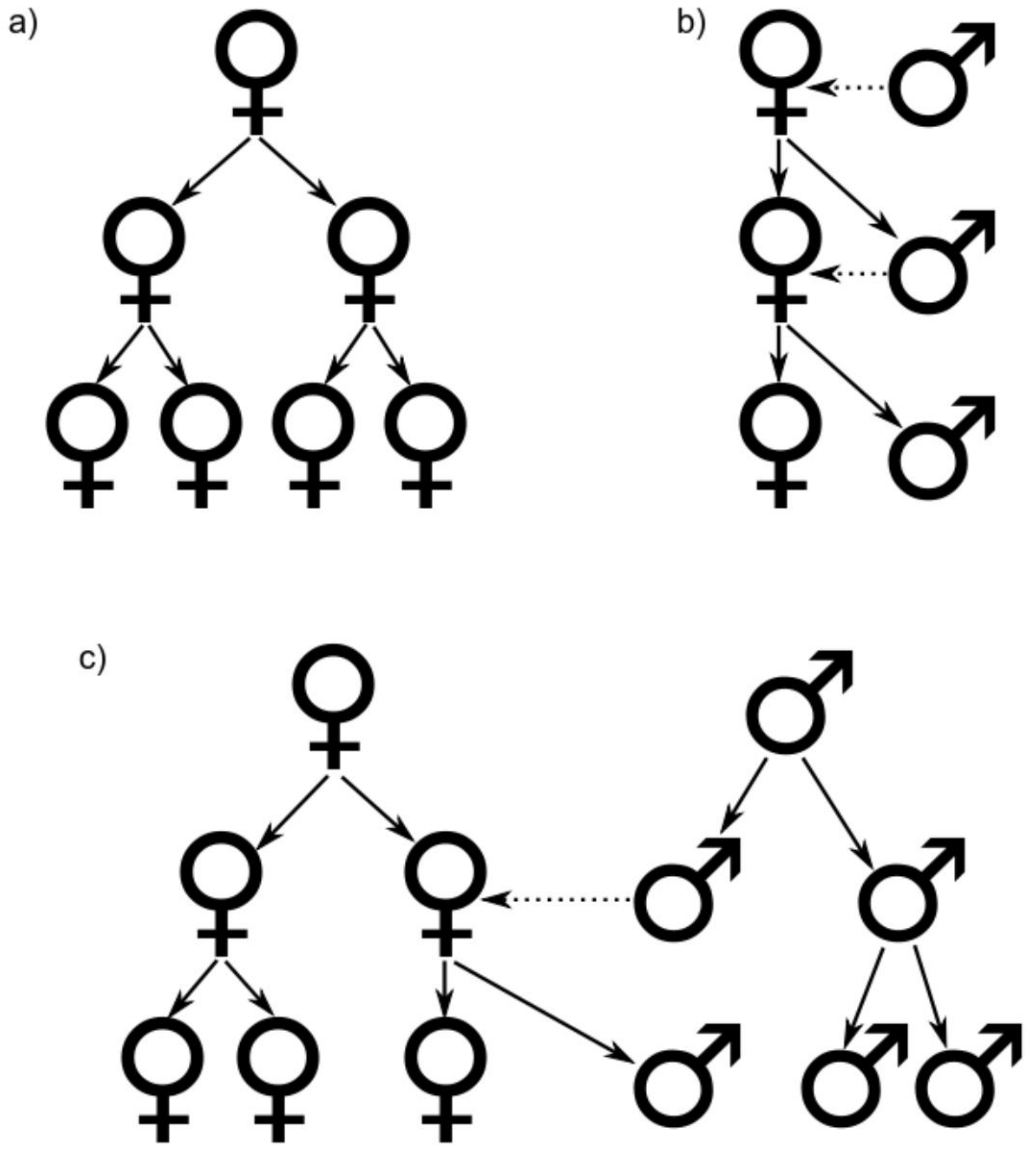


Figure 2.1. Growth Differences under Various Reproductive Strategies
Pictorially, the difference between the population growth rates becomes apparent when a) a female can only asexually reproduce, b) males and females can only sexually reproduce, c) both males and females can asexually and sexually reproduce. Figure c assumes that individuals can either do one or the other, not both simultaneously, but relaxing that assumption would not qualitatively change the results – population growth rate of c would still be higher than b. A key note is that to visualize the effects on population growth, one needs to start with a population of the same size. For example, compare two females that both asexually reproduce to a male and female that must sexually reproduce.

Copyright ©Christopher Stieha 2012

CHAPTER THREE

Production, Dispersal, and Survival of Asexual Propagules and Their Contributions to Population Maintenance in a Clonal Organism

Introduction

Dispersal of individuals between populations is required for the persistence of populations and species in the common type of landscape where populations form an aggregated network (metapopulation; Hanski 1999). Grasping the effects of dispersal on populations and metapopulations requires understanding the three stages of dispersal: emigration, dispersal, and immigration (Ims and Yoccoz 1997; Baguette and Van Dyck 2007). In organisms with dispersing propagules as opposed to dispersing individuals, understanding dispersal requires knowledge of propagule production capabilities (Longton 1992), the dispersal capabilities of the propagules (Cousens et al. 2008), and the survival of the propagules (During 2006; Cousens et al. 2008; Figure 3.1).

In many organisms, sexual propagules are the main or only form of dispersal (Longton and Schuster 1983; Ashton and Mitchell 1989; Hansson et al. 1992; Starfinger and Stocklin 1996; Laaka-Lindberg et al. 2003). But organisms from many taxa ranging from bacteria to fungi, plants, and animals produce asexual propagules or asexual individuals capable of dispersal (algae in Wreede and Klinger 1988; plants reviewed in Eckert 2002; animals in Bell 1982, Avise 2008). Asexual propagules have the potential to alter population dynamics of the source population (Longton and Schuster 1983) and of other populations via dispersal (Cousens et al. 2008), but their dispersal capabilities are not well studied (Laaka-Lindberg et al. 2003). In the organisms that have been well studied, asexual propagules vary in their contribution to dispersal between populations, contributing negligible and unobservable amounts for some species (Richmond and Hunter 1990; Mizuki and Takahashi 2009; Wintzer et al. 2011) and being the main form of interpopulation dispersal for other species, such as species with two separate sexes (dioecious or gonochoristic organisms) that have lost one sex (parthenogenetic populations as in Browne 1980, Avise 2008; single-sex populations of dioecious clonal plants in Longton and Schuster

1983; Longton 1992). For example, coral dispersal can be via sexual or asexual propagules, with major differences in the dispersal capabilities of asexual propagules based on whether the propagules are fragments or clonal planulae (Richmond and Hunter 1990). Asexual and sexual propagules of the soft coral *Alcyonium rudyi* travel similar distances, but asexual propagules drive local population dynamics, while sexual propagules are expected to be the main form of recruitment and colonization, given the high genetic diversity among populations (McFadden 1997).

In many systems, sexual propagules are thought to contribute equally to the number of individuals of both sexes. On the other hand, asexual propagules are exact replicas of the parent capable of greatly affecting the sex ratios at the population and metapopulation level, particularly when sex-determination is genetic. In extreme cases, one sex can be lost from a population, metapopulation, or species. The interpopulation dispersal of asexual propagules is vital for the persistence of single sex populations and metapopulations of species with separate genetically determined sexes (Longton and Schuster 1983; Longton 1992). Despite the fact asexually reproducing populations are classified as "evolutionary dead ends" (Maynard Smith 1978), single sex populations, metapopulations, and species have persisted for long periods of time exclusively via asexual reproduction (Longton and Schuster 1983; Judson and Normark 1996). By focusing on instances of single sex metapopulations, one can better understand the dispersal capabilities of asexual propagules independent of the dispersal of sexual propagules.

Understanding dispersal requires the study of multiple processes, ranging from production of the propagules, dispersal of the propagules both within (intrapopulation dispersal) and between populations (interpopulation dispersal), and the survival of the propagules after dispersal (Figure 3.1). I use a novel combination of laboratory experiments, field experiments, and mathematical models to understand the dispersal of asexual propagules of the clonal plant *Marchantia inflexa*. I will quantify the production of asexual propagules using a laboratory experiment, determine intrapopulation dispersal

with a field experiment, estimate interpopulation dispersal using a mathematical model, and determine the survival of propagules with laboratory experiments.

Model Organism

Marchantia inflexa Nees & Mont. is a New World liverwort found along streams from northern Venezuela to the southern United States as far north as Tennessee (Bischler 1984, Schuster 1992). Plants are found on rocks within the stream or along the banks of the stream in discrete areas separated by water or obvious breaks, producing quantifiable populations. Males and females are separate individuals determined by sex chromosomes (Bischler 1986). Streams containing only one sex are found in the southern United States, while most streams in the Caribbean contain both sexes (Bischler 1984; Fuselier and McLetchie 2004). Asexual dispersal can occur by two mechanisms: 1) vegetative material or 2) distinct asexual propagules. As with many clonal plants, vegetative material can become dislodged and float downstream. The main form of asexual dispersal, however, is through asexual propagules known as gemmae. Gemmae are produced in specialized structures known as gemmae cups which function as splash cups as described in Brodie (1951). Gemmae are about 0.2 mm in diameter and dispersed by water. Sexual propagules known as spores are about 28 µm in size (Schuster 1992) and are wind dispersed.

Given the differences in size and dispersal medium, spores are thought to travel farther than gemmae and be the main mechanism for colonization events. In other bryophytes, the asexual propagules are thought stay inside the patch (Kimmerer 1991). Given the possibility that single sex metapopulations are the products of post-Pleistocene unisexual colonization events of suitable habitat due to the changing environment (Longton and Schuster 1983), gemmae must be able to disperse out of the population and colonize suitable habitat along the stream. These single sex populations are ideal natural systems in which to study interpatch dispersal using metapopulation theory. In this study, I look at the production, dispersal, and survival of the asexual propagules of the liverwort *M. inflexa* using a

combination of laboratory experiments, field studies, and theoretical models. In the Discussion, I will relate these results to other taxa known to form metapopulations in nature.

Methods

Production of Gemmae

The production capabilities and differences in production between the sexes directly determine the number and sex of asexual propagules contributing to local population dynamics and available for dispersal to other populations. Twenty males and twenty females were raised from spores produced through controlled crosses involving two males and two females. The initial parents were collected along Quare River, Trinidad, West Indies. Gemmae were taken from the spore-started plants to be used as the individuals within the experiment. All plants were grown in a greenhouse at the University of Kentucky, Lexington, KY. Initially, three gemmae were planted in each well of a 50 well plug tray filled with steam-sterilized soil on December 17 and 18, 2001. After eight weeks, all plugs were weeded to leave only one individual.

Plants were watched for the development of gemmae cups, which occurred in February, March, and April, 2002. After a gemmae cup formed, extractions of the gemmae occurred once per week for nine weeks. Initially, 0.1 µL of distilled water was pipetted into the cup. The gemmae and water were extracted from the cup using a 1000 µL pippete. The larger pippete provided the necessary force needed to remove the gemmae-water solution from the cup. This solution was combined in an Eppendorph 1.5 ml tube with 500 µL of 5% Tween 20 solution to disperse the gemmae in the solution. Each cup was extracted twice per sampling event. Gemmae were counted using a grid-lined culture plate under a dissecting scope. Over the course of the experiment, up to three cups were extracted for each plant. From a total of 19 female individuals (1 died) and 20 male individuals, 14 unique female genotypes and 15 unique male genotypes were used in the experiment. Due to the repeated nature of the

measurements, if no gemmae were counted at a specific extraction time, the remaining extraction times were removed from the analysis.

Data were analyzed with a mixed-effects generalized linear model with a Poisson error distribution and log link function with extraction time as a repeated measure. Fixed effects were the sex of the individual, the extraction time, and the interaction between sex and extraction time. Random effects were cup within individual within genotype. The lmer function from the lme4 library (Bates et al. 2011) for R version 2.10.1 (R Development Core Team 2009) was used for analysis. To determine the effects of sex and extraction time on the number of gemmae counted, I developed the model sequentially, comparing models that initially started with only the random factors, then sex, then sex and extraction time, and finally sex, extraction time, and the interaction. Models were compared using a χ^2 test to determine if the term significantly increased the explanatory power of the model compared to a model without the term (Crawley 2007).

Intrapatch Dispersal

Asexual propagules are usually thought to stay within the source population. I first quantify how quickly asexual propagules move within a population to determine if the movement is sufficient for emigration from the source population. A 1.23m by 1.23m flat platform built out of four porcelain tiles was constructed at the Simla Research Station, Trinidad, West Indies. Cinder blocks elevated the platform off the ground and created a negligible slope (0º to 5°). Slopes of the substrate with current populations averaged 42º (Stieha, unpublished data). The porcelain tiles were covered with capillary mat (to prevent water from pooling) and three layers of white cotton (to make the gemmae easily observable). Trials occurred at least twenty minutes apart on the 27th and 28th of August, 2007, and the 10th through the 14th of September, 2007. Extraneous material and gemmae were removed by washing the platform and removing excess water between trials. The capillary mat and cotton were kept moist for trials.

Trials consisted of placing ten gemmae collected from plants at Quare River, Trinidad, West Indies in a two-centimeter circle on Whatman 70mm filter papers. Gemmae were subjected to natural rain for up to five minutes. The experiment was stopped after 30s from the time the first gemmae moved outside of the two-centimeter circle to increase the probability of finding the moved gemmae. For each gemma, I measured distance the gemma moved from the center of the filter paper. Gemmae remaining inside the initial 2cm circle were given a distance of 0cm. Gemmae that remained on the filter paper after the experiment, but were outside of the initial circle were given a distance value of 3.5cm. A circle with a radius of 45cm centered on the filter paper was drawn on the platform. The 45cm circle was exhaustively searched for all gemmae. Any gemmae not found within that area were assumed to have left the study area and given a distance value of 45cm. The average distance traveled by all gemmae was computed and divided by the duration of the experiment, giving a gemmae velocity in cm per minute.

The amount of rain received during the trial was measured using a HOBO tipping bucket rain gauge with a 0.2 mm sensitivity and a HOBO event data logger (Onset Computer Corporation). The tipping bucket mechanisms allowed me to determine how often 0.2 mm of rain had fallen. Time of the trial and time on the event data logger could be combined to determine the number of tips per trial. Tipping of the bucket in the rain gauge did not occur simultaneously with the beginning or end of a trial. To account for this, tipping points up to two minutes before and after the trial were used as discrete beginning and end points to determine the number of tips that had occurred. The time between the beginning tipping event and the final tipping event was determined. To calculate the rate of rainfall, the amount of rain was divided by the length of time between the first and last tipping event. Even though the rate of rainfall was computed over a longer duration than the experiment, the procedure produced a rainfall rate from the point the tipping bucket was empty to when it was empty again. Rainfall events ranged from 0 mm per minute, which consisted of no rainfall and rainfall so slight that not enough was

collected to fire the tipping bucket mechanism, to 3mm per minute, which was enough rainfall to completely soak a person in a few seconds.

Results were analyzed using linear regression in the statistical program R version 2.10.1 (R Core Development Team 2009). Because most rain events had a rate of less than 1 mm per minute, two lines were fitted to the data: one containing all values collected during all trials and one containing only values less than 1 mm per minute.

Interpatch Dispersal

Single-sex metapopulations can only persist via dispersal of asexual propagules among populations and from source populations to new areas that can be colonized. Using single-sex metapopulations allows me to study dispersal of asexual propagules without the confounding factor of sexual propagule dispersal that could occur in systems with both sexes. Mathematical techniques can be used to estimate interpatch dispersal without extensive, costly, and time consuming field experiments and mark-recapture procedures (Moilanen 1999, 2004).

Two single sex metapopulations (Fuselier and McLetchie 2004) were surveyed in Oklahoma, United States : 1) the all-female metapopulation at Bird's Mill Creek, Oklahoma (Figure 3.4a; 17 populations on 10 June 2006, lat/long: 34.615/-96.634) and 2) the all-male metapopulation at Honey Creek, Oklahoma (Figure 3.5a; 23 populations on 9 June 2006, lat/long: 34.447/-97.133). Surveying consisted of taking measurements of the size of each population, computed by measuring the length of the patch along the stream and then a width perpendicular to the stream at the largest point in the patch. Distances and degrees from North were collected between adjacent populations. The distances and degrees were used to compute relative spatial locations and distances among all populations in the metapopulation using a script written in MATLAB version 7.12.0.635 (2011). Distances were hand corrected to give distances along the stream between populations.

Using the distances between populations and the size of the populations (assumed to be at carrying capacity), I extended current mathematical models of *Marchantia inflexa* (McLetchie et al. 2002; García-Ramos et al. 2007; Stieha, unpublished manuscript) to be spatially explicit with respect to distances between patches (that can support populations), and I incorporate known carrying capacities. The mathematical model simulates the *M. inflexa* life cycle with seven coupled ordinary differential equations: four for the female and three for the male (McLetchie et al 2002; García-Ramos et al. 2007). The life history stages for each sex include a non-reproductive (vegetative growth) stage, an asexually reproductive stage, and a sexually reproductive stage. Males have only one sexually reproductive stage, while females have two, unfertilized and fertilized (McLetchie et al 2002; García-Ramos et al. 2007). In my model, dispersal of asexual propagules is described by the probability density function $y = \alpha e^{-\alpha d}$, where d is the distance and α is the dispersal capability of the gemmae. I then varied the dispersal capabilities of asexual propagules and determined the average proportion of patches that were occupied given the dispersal of gemmae. For dispersal capability α, $1/\alpha$ is the mean dispersal distance of the propagules. I incorporated two different dispersal functions: 1) a 1-dimensional dispersal kernel (see García-Ramos et al. 2007) and 2) a 2-dimensional dispersal kernel (see Stieha, unpublished). A 1-dimensional dispersal function focuses on the linear aspect of the stream, while a 2-dimensional dispersal function includes the increase in area as the distance from the source patch is increased. I predict the actual dispersal function falls between these two extremes.

I also looked at the effect patch size (carrying capacity) had on the persistence of single-sex metapopulations. I assumed that all carrying capacities matched the population sizes measured in the field. I also ran simulations assuming all patches had a carrying capacity of 1 m^2.

All populations within a metapopulation were initialized with 20 individuals of the sex found in the stream. For Bird's Mill Creek, I used only females. For Honey Creek, I used only males. Fifty simulations were run for each value of the dispersal parameter. Simulations were run for 500 years; I averaged the

proportion of patches occupied for each time step (*dt* = 0.1 month) over the last 400 years to exclude transient effects. Subpopulations experienced disturbance, where 20% of the individuals were removed from the population, every five months, on average (McLetchie et al. 2002; García-Ramos et al. 2007). Subpopulations also experienced extinction, where all individuals within a subpopulation are removed, once every 40 years, on average (García-Ramos et al. 2007). I assumed that extinction probability was independent of patch size. For a detailed description of the mathematical model, see McLetchie et al. (2002), García-Ramos et al. (2007), and Stieha (unpublished). I was interested in the proportion of patches that contained individuals during the simulations. All simulations were run in MATLAB R2011a (2011).

Gemmae Survival under Water

Because gemmae are dispersed by water, I measure the survivability of gemmae under prolonged submergence. On July 10, 2006, male and female gemmae were collected from stock plants grown in a common greenhouse at the University of Kentucky, Lexington, KY. From these collections, 336 male gemmae and 336 female gemmae were subsampled and placed individually in a 10x75mm test tube filled with a 25% Hoagland's solution (Hoagland and Arnon 1950) and randomly assigned to one of seven treatments: no time submerged, one week submerged, 18 days submerged, four weeks submerged, eight weeks submerged, 12 weeks submerged, and extra. During the experiment, test tubes containing algal growth were replaced with test tubes from the extra treatment. Test tubes were gently agitated to cause gemmae to sink to the bottom of the test tube and placed in a single container in a growth chamber with a light regime of (22ºC/18ºC 12/12 day/night). After one week, many gemmae required manual force to make them sink. Treatment times were computed from this date. To test survival, gemmae were removed from the test tubes at the end treatment time and planted in 96 well plates with soil and placed in a growth chamber (22ºC/18ºC 14/10 day/night) for two weeks to monitor germination. At two weeks, gemmae were classified as either alive (germinated) or dead.

I analyzed the results with a generalized linear model with a logit link function in R version 2.10.1 (R Development Core Team 2009). Initial analysis revealed underdispersion (residual deviation 5.65 for 8 degrees of freedom). Therefore, I used a quasi-binomial error distribution. Analysis was weighted for sample size. Model reduction (Crawley 2007) was used to determine whether the sex, the time spent under water, or the interaction has significant effects of survival.

Gemmae Survival after Desiccation

Even though gemmae are dispersed via water and land along the edges of the stream, they may land on a substrate that quickly dries out. To understand the effect of desiccation on gemmae survival, I tested gemma survival and germination after various lengths of drying time under controlled conditions. Three 96-well plates (Falcon 353911) were filled with steamed soil. The plates were allowed to sit in the open air for 1 week to dry. Male gemmae were collected from stock plants and placed in containers with water and thoroughly mixed together. Female gemmae were collected similarly. All gemmae were collected and planted on the March 20th, 2012 at the University of Kentucky, Lexington, KY. Gemmae (144 male and 144 female) were randomly allocated to individual wells. Each well contained only a single gemma. To get the gemmae into the well, the well was moistened with a small drop of water. This drop appeared to dry within 2 to 3 hours at laboratory conditions (21°C and 54% humidity, temperature and humidity sensor RHT03, MaxDetect Technology Co., Ltd.). All three trays were left out over night to make sure all excess moisture had evaporated. After that initial period, well plates were lidded and placed in a growth chamber (22ºC/18ºC 14/10 day/night). All gemmae were randomly allocated to one of 5 treatments: 0 days dry, 1 day dry, 2 days dry, 3 days dry, 4 days dry. The 0 days dry treatment consisted of gemmae that were consistently grown on moist soil throughout the experiment, even though the overnight drying period. Gemmae in the 1 day dry treatment were allowed to be dry from the time they were planted until 24 hours later, at which point they were on constantly moist soil. Other treatments followed a similar protocol. Placing the gemmae in water and putting them on soil that was

moist mirrors the interpatch dispersal of gemmae during which rainfall splashes the gemmae out of the cup (Brodie 1951; Equihua 1987), and they enter the stream to be carried away until they land on a moist substrate that slowly dries over time.

During all waterings, gemmae were observed. After the final treatment had received water, all plants were kept moist for 17 days from the start of the experiment (when gemmae were initially dispersed). After 17 days, I checked whether the gemma had attached itself to the substrate, could still float, or had died. I determined whether the gemmae were still alive or not by viewing them under the microscope. When planted, the gemma is green and round with a flared ring. Death is assumed if the gemma is brown. If the gemma was alive, I evaluated its attachment to the substrate by slowly filling the well with water. If the gemma was attached, the water would pool around the it; otherwise the gemmae would immediately float. Two weeks is more than enough time for gemmae to germinate and begin growing (C. Stieha and N. McLetchie, personal observation). Results were analyzed using a generalized linear model with a log link function and quasipossion error distribution due to underdispersion (10.05 residual deviance on 18 degrees of freedom; Wilson and Hardy 2002; Crawley 2007). Days dry, sex, and status (dead, floating, attached) were included as explanatory variables. The number of gemmae was my dependent variable. Analysis was performed in R version 2.10.1 (R Development Core Team 2009).

Results

Production of Gemmae

Gemmae production within cups peaked at week four (males 150.5±11.6(Mean±SE); females 132.9±11.0(Mean±SE); Figure 3.2). Male production of gemmae appears to increase until week four, at which point production decreases and then stops around week 9. On the other hand, female production appears stable for the first three weeks, followed by a sharp increase at week four and a slow decline as production decreases. Sequential addition of terms to a model initially containing only the random

factors (Table 3.1) shows that sex by itself does not significantly explain gemmae production (df=1, χ^2 = 0.62, p = 0.43), but the age of the gemmae cup (extraction time: df = 8, χ^2 = 24275, p < 0.001) and the interaction between age of cup and sex (df = 8, χ^2 = 226.97, p < 0.001) do.

Intrapatch Dispersal

The rate of rainfall was a good predictor of gemmae speed ($F_{1,17}$ = 195.5, p < 0.001, adjusted R^2 = 0.915). The speed of gemmae movement (*y*, cm of gemmae movement per minute) was positively related to the strength of the rainfall (*x*, mm of rainfall per minute; y = -1.35(±1.40 cm min^{-1}) + 25.27(±1.81 cm mm^{-1}) x, β(±SE)). The removal of the heaviest rainfall event did not change the results ($F_{1,16}$ = 16.34, p < 0.001; y = -0.097(±1.833 cm min^{-1}) + 20.332(±5.031 cm mm^{-1})x, β(±SE)). Figure 3.3 shows the best fit lines for both scenarios.

Interpatch Dispersal

Dispersal capabilities of gemmae greatly affected the proportion of occupied patches in my simulations (Figure 3.4b and 3.5b). For Bird's Mill Creek, a mean dispersal distance of 0.1 m or less led to an extinct metapopulation, where no individuals were found in any patches after 500 years (Figure 3.4b). From 0.1m to 1m, the proportion of patches occupied increased and a mean dispersal distance of greater than 1 ensured that almost all patches were occupied. For simulations of Honey Creek, very few scenarios led to the extinct of metapopulations (only short mean dispersal distances and actual patch sizes; Figure 3.5b). A mean dispersal distance greater than 2m was required for persistence of the single sex metapopulations. For both metapopulations, whether I assumed patches had a carrying capacity of $1m^2$ or had the measured carrying capacity, the results did not change.

Gemmae Survival under Water

Across trials gemmae survival ranged from 85.4% to 97.9% survival (Figure 3.6). To determine the significance of sex, the time spent submerged, and the interaction of the terms, I employed model

reduction (Crawley 2007). There was no significant difference in gemmae survival given the sex of the gemmae ($F_{1,8}$ = 0.577, p = 0.465), the amount of time the gemmae spent submerged ($F_{1,9}$ = 0.086, p = 0.776) or the interaction between time submerged and sex of the gemmae ($F_{1,8}$ = 4.327, p = 0.071).

Gemmae Survival after Desiccation

Gemmae attachment ranged from a low of 34.5% for male gemmae that had experienced four days without water (Figure 3.7a) to a high of 82.1% for female gemmae that experienced zero days without water (Figure 3.7b). Using model reduction (Crawley 2007), I determined that the interaction between sex, status (dead, floating, attached), and the number of days without water was not significant ($\Delta deviance$ = 0.1590, $F_{2,18}$ =0.1801, p = 0.8367). The interaction between sex and number of days dry was also not significant ($\Delta deviance$ = 0.2065, $F_{1,20}$ =0.1801, p = 0.4875), which is expected given the balanced design of the experiment. The interaction of sex and status was significant ($\Delta deviance$ = 7.6616, $F_{2,21}$ =9.6276, p = 0.0011). Males have more dead gemmae, fewer floating gemmae, and fewer attaching gemmae than females (Table 3.2). The interaction of status and number of days without water was also significant ($\Delta deviance$ = 19.088, $F_{2,21}$ =23.986, p <0.0001). As the number of days without water increased, the number of gemmae that have germinated and attached themselves to the substrate decreased and the number of gemmae that floated increased (Table 3.2).

Discussion

I focused on the production, dispersal, and survival of asexual propagules to get an integrative perspective on the capabilities of asexual propagules. Using field, laboratory, and modeling approaches, I found that asexual propagules have the potential to greatly affect population dynamics of the source population and neighboring populations.

On average, a male cup produces 529 gemmae during its lifetime, while a female cup produces 492 gemmae. Overall, males make more cups than females (10 versus 7, Figure 3 in McLetchie and

Puterbaugh 2000). The combination of these results suggests that males (females) can produce 5290 (3444) copies of themselves in only 18 weeks! This high level of production is found in other species as well (Laaka-Lindberg 1999).

Depending on the rate of rainfall, these gemmae can easily move up to 20 cm per minute of rainfall. Traps set up on along edge of populations of *Marchantia inflexa* caught many gemmae leaving the populations and entering the streams after small rainfall amounts (Stieha, unpublished data). Including a slight slope (even as little as 6º, the lower bound of the 95% confidence interval for slopes observed in nature, Stieha, unpublished data) increases the intrapopulation dispersal rate of the gemmae, with many leaving the source population after a slight rain (Stieha, unpublished data). Intrapopulation dispersal of gemmae from splash cups has been shown to be large, ranging from greater than half a meter (Brodie 1951) to 1.2 meters (Equihua 1987)!

Previously, the importance of asexual reproduction in interpatch dispersal and metapopulation dynamics has been ignored (Hansson et al. 1992; Laaka-Lindberg et. al. 2003) either due to the nature of asexual reproduction (large vegetative pieces Johansson and Nilsson 1993; larger asexual propagules Ronsheim 1994) or data suggesting small dispersal scales (Kimmerer 1991). Recently, there has been a call to better quantify the dispersal capabilities of asexual propagules (Laaka-Lindberg et al. 2003), and experiments have suggested that asexual propagules can and do disperse out of the focal population (Johanssen and Nilsson 1993; Rudolphi 2009; Pohjamo et al. 2006). Sometimes dispersal is accomplished by means of vectors, such as invertebrates (Kimmerer and Young 1995; Rudolphi 2009) or vertebrates (Parsons et al. 2007) to travel long distances, but in some instances, abiotic factors can disperse propagules long distances (Equihua 1987; Johanssen and Nilsson 1993; Walser 2004).

In some cases, the dispersal capability of asexual propagules is very similar to the dispersal capability of the sexual propagules (Ronsheim 1994; Pohjamo et al. 2003). For *Allium vineale*, the mean dispersal distance of asexual bulbils and sexual seeds from scapes of the same height is the same, although the

maximum distances differ (1.3m versus 0.95m; Ronsheim 1994). In other cases, asexual propagules are thought to disperse farther along the river than sexually produced seeds due to differences in floating abilities (Johannssen and Nilsson 1993). In lichens, molecular techniques have been used to determine that clones can be found up to 230m from one another (Walser 2004). I show that asexual propagules must travel far (a mean of 2 meters for Bird's Mill Creek and a mean >5m for Honey Creek) for the persistence of unisexual metapopulations.

Once the asexual propagules disperse outside of the focal population, they persist and are able to colonize and initiate a new population, as long as they remain moist. Even though desiccation affected germination rate, mortality rate was not increased (data not shown). In the research station, gemmae would turn brown (suggesting death) after only a couple of hours on unmoistened filter paper (Stieha, personal observation). Often the asexual propagules survive better than the sexual counterparts (Kimmerer 1991), but asexual propagules can suffer high mortality (Johansson and Nilsson 1993). With the number of asexual propagules produced and the difference in male versus female production, it is difficult to envision a scenario where asexual propagules do not affect metapopulation dynamics and the ratio of males-to-females in the individual population as well as in the metapopulation. The dispersal of asexual propagules can affect sex ratios at the population level and metapopulation level. The biased sex ratios produced by asexual propagules could affect population growth (Bisang et al 2004; Rydgren et al. 2010; Miller and Inouye 2011).

Dispersal of asexual propagules could change the optimal allocation of resources to sexual reproduction and asexual reproduction. When asexual propagules only contribute to local fitness (and not metapopulation/global fitness) and strategies are optimized based on the fitness of the individual in a metapopulation framework, there is no difference in the optimal allocation strategy when competition is lottery (allows utilization of unoccupied resources) versus overgrowth (competition for currently used resources; Crowley and McLetchie 2002). If asexual reproduction were to contribute to an individual's

fitness at the metapopulation level, I predict the optimal allocation strategy would change the overall results of Crowley and McLetchie (2002). The optimal allocation strategy could be influenced by the type of competition, where overgrowth competition inherently limits the production of asexual propagules and benefits an individual within a population (high local population fitness), but limits the individual's ability to disperse to other populations (low metapopulation fitness). On the other hand, lottery competition will favor the production of asexual propagules, which would increase the local population fitness and the metapopulation fitness. I would expect that single-sex metapopulations would contain individuals that invested more in asexual reproduction, therefore increasing interpopulation dispersal and the likelihood of colonizations. Colonizations are vital for the persistence of any metapopulation. But recent research shows that individuals in single-sex metapopulations of *M. inflexa* actually invest more into vegetative growth and less in gemmae production than their counterparts found in two-sex metapopulations (Fuselier 2008).

Dispersal capabilities are greatly affected by the environment through which the propagule must travel (Wiens 1997). Given the stream-based dispersal mechanism of asexual propagules of *M. inflexa*, pools, riffles, and the meanderings of the stream could influence dispersal between populations and the location of new populations. The effects of the environment through which the propagule must disperse may be evident in the mean dispersal difference between Honey Creek and Bird's Mill Creek. Although the locations of pools and ripples were not collected, comparison of the spatial configuration of populations along both streams suggests that streams were relatively straight (Figures 3.4a and 3.5a). In the aquatic plant *Ranunculus lingua*, curved parts of the stream captured more asexual propagules (rhizomes) than did straight parts but there was no difference between pools and rapids (Johansson and Nilsson 1993). The dispersal difference may be due to water flow differences between the two streams.

Contrary to previous assumptions, gemmae have the capability of emigrating from the source population and influencing population and metapopulation dynamics. Understanding the capabilities of

asexual propagules is vital for population dynamics in clonal organisms. Ignoring asexual propagules could lead erroneous predictions of the sex ratio for both populations and metapopulations, leading to errors in predicting the loss of one sex and estimating genetic diversity. By discounting dispersal of asexual propagules in invasive organisms, the rate of invasion could be underestimated, with the best example being dandelions (Lyman and Ellstrand 1984), although the dispersal of other species is also enhanced by asexual propagules (such as Amat et al. 2005)

Table 3.1. Gemmae Production Sequential Comparisons

	df	χ^2	*p*
Sex	1	0.62	0.43
Time	8	24275	<0.0001
Sex*Time	8	226.97	<0.0001

Table 3.2. Coefficients of GLM for Gemmae Desiccation Experimentation. The change is with respect to the number of females that are dead when the gemma does not experience desiccation (Intercept).

	Coefficient (±Std Error)	*t* value	*p* value
Intercept	0.623(±0.245)	2.547	0.019
Sex(males)	0.774(±0.220)	3.512	0.002
Status(floating)	0.701(±0.299)	2.345	0.029
Status(germinating)	2.552(±0.260)	9.804	<0.0001
Time Spent Dry	0.119(±0.073)	1.632	0.118
Sex(males) by Status(floating)	-0.619(±0.263)	-2.357	0.028
Sex(males) by Status(germinating)	-0.988(±0.241)	-4.102	0.0005
Time Spent Dry by Status(floating)	0.166(±0.090)	1.839	0.080
Time Spent Dry by Status(geminating)	-0.254(±0.081)	-3.141	0.005

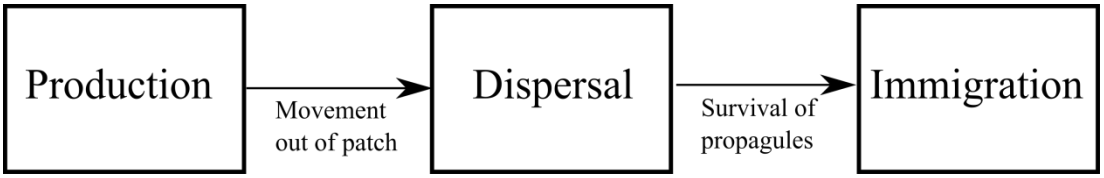


Figure 3.1 Conceptual Diagram of Dispersal. The standard framework focuses on emigration-dispersal-immigration (Ims and Yoccoz 1997; Baguette and Van Dyck 2007). In plants, the propagules are the dispersers as opposed to the individual. First, these propagules must be produced. A fraction of these propagules emigrate from the source population and have the potential to disperse between populations (Dispersal). Of these dispersers, only propagules that find habitable area and survive the initial colonization can be described as immigrants.

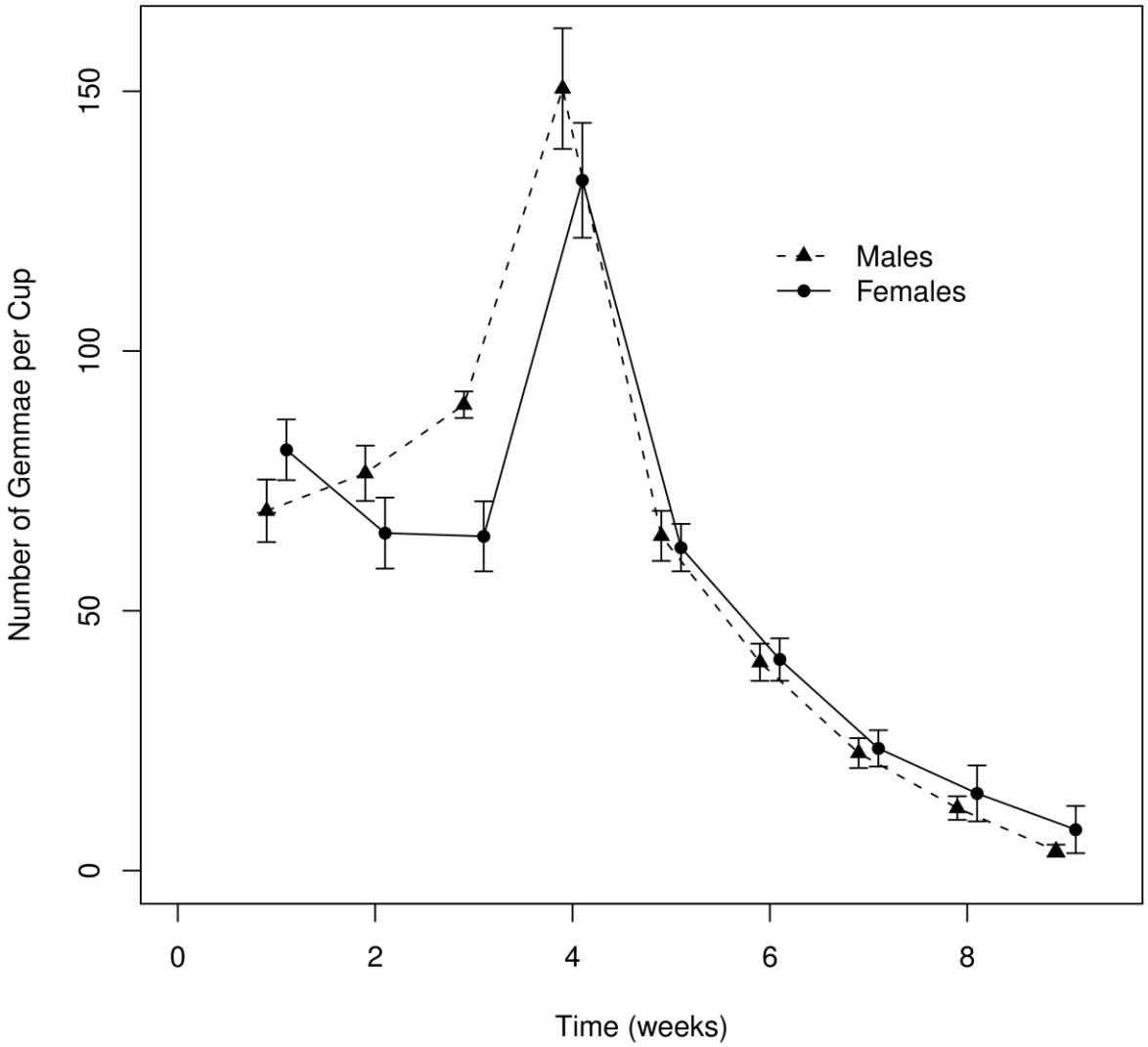


Figure 3.2. Production of Gemmae. Lines for the sexes are staggered to show the details of each line. Bars represent standard error. All measurements are collected based on the age of the cup, not the age of the plant.

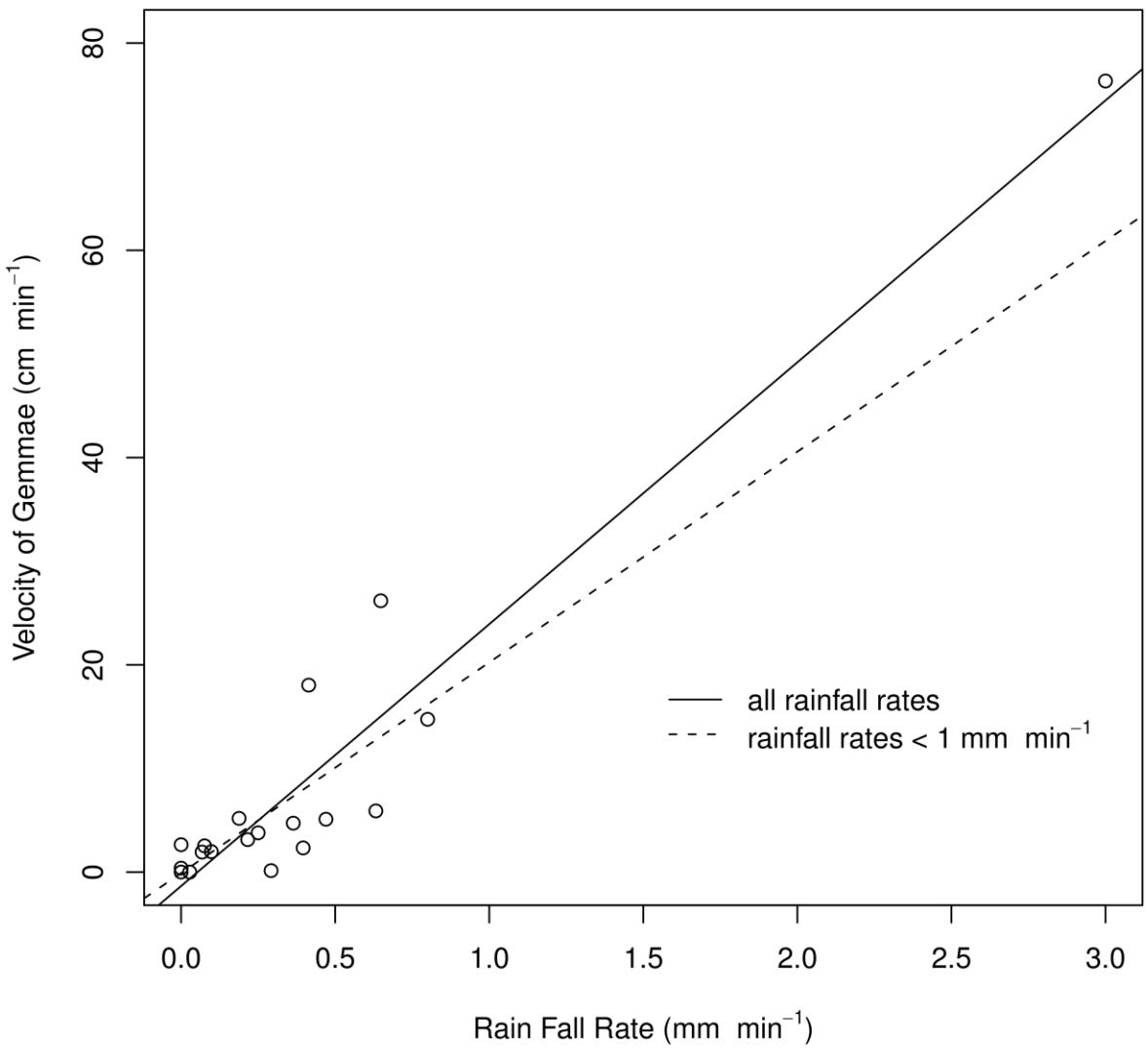


Figure 3.3. Intrapatch movement speed of gemmae versus rainfall rate. Conceptually, a rainfall rate of 0 would be no rain. Given the sensitivity of the tipping bucket, very light rains were often not recorded, but movement of the gemmae still occurred. Light rainfall may not have enough force to eject the gemmae from the cup, requiring further experimentation.

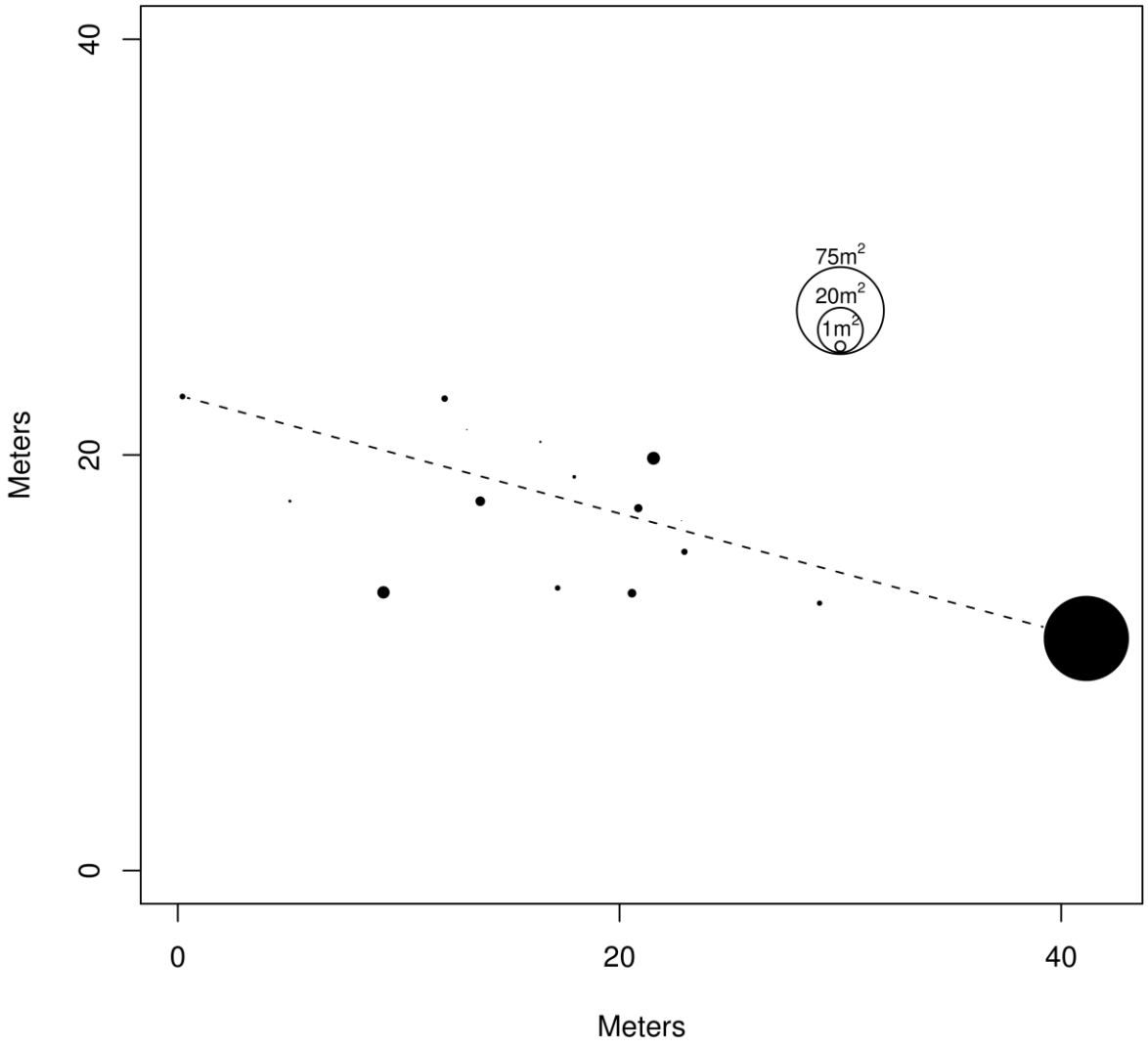


Figure 3.4a. Spatial configuration of populations found along Bird's Mill creek, female-only. One large population dominates the upstream segment of the stream. Other populations were quantified downstream but removed from analysis due to proximity to a recent construction area. Key indicates relative patch size.

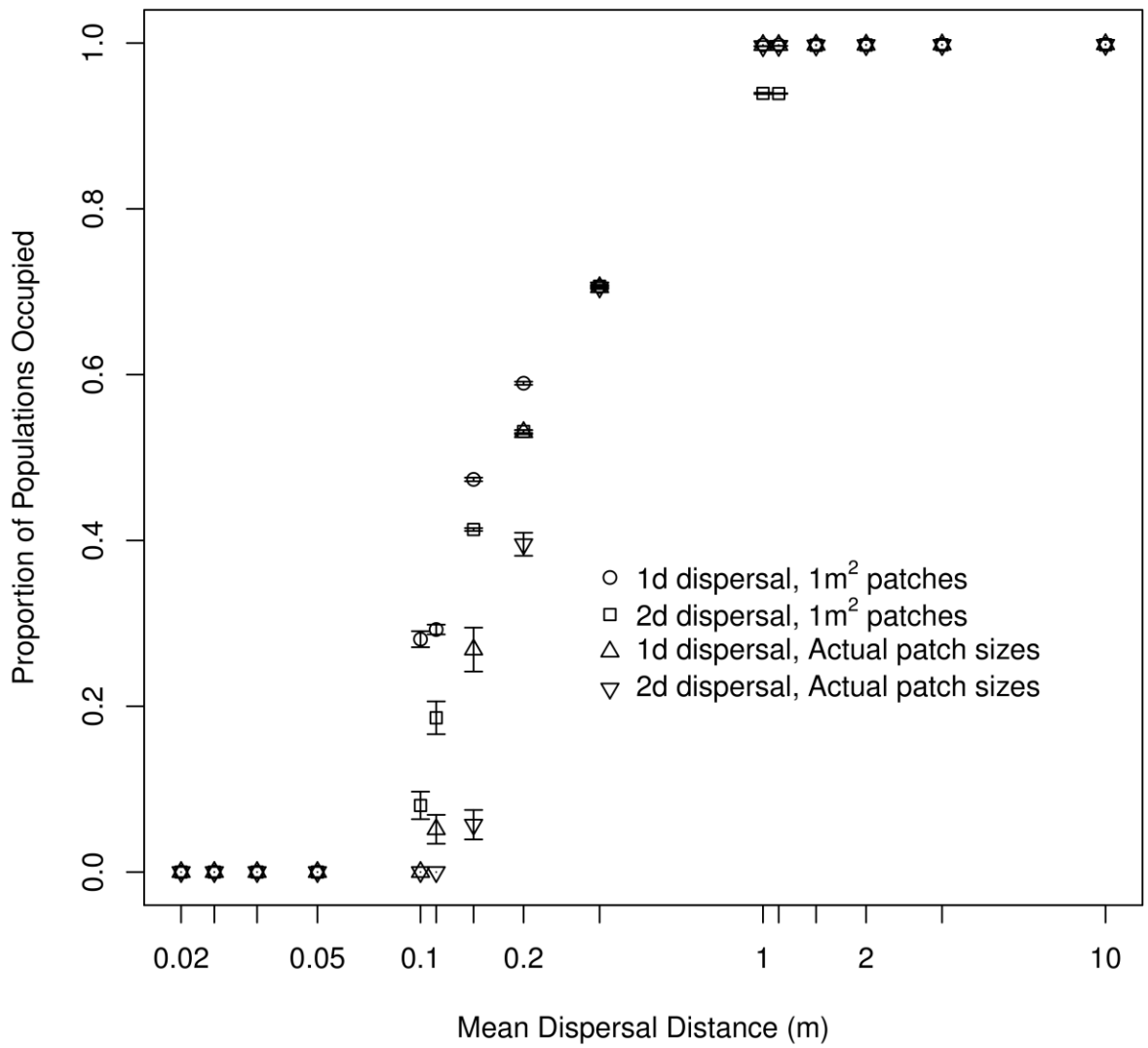


Figure 3.4b. Relationship between mean gemmae dispersal and proportion of metapopulation occupied for the female-only Bird's Mill Creek. Any α (decay parameter in the exponential dispersal function) with a mean above 1m predicts a metapopulation with all possible patches occupied. Mean distances less than 0.1m led to metapopulation extinction. Using the actual carrying capacity (patch size) or assuming all carrying capacities were $1m^2$ appeared to have little effect.

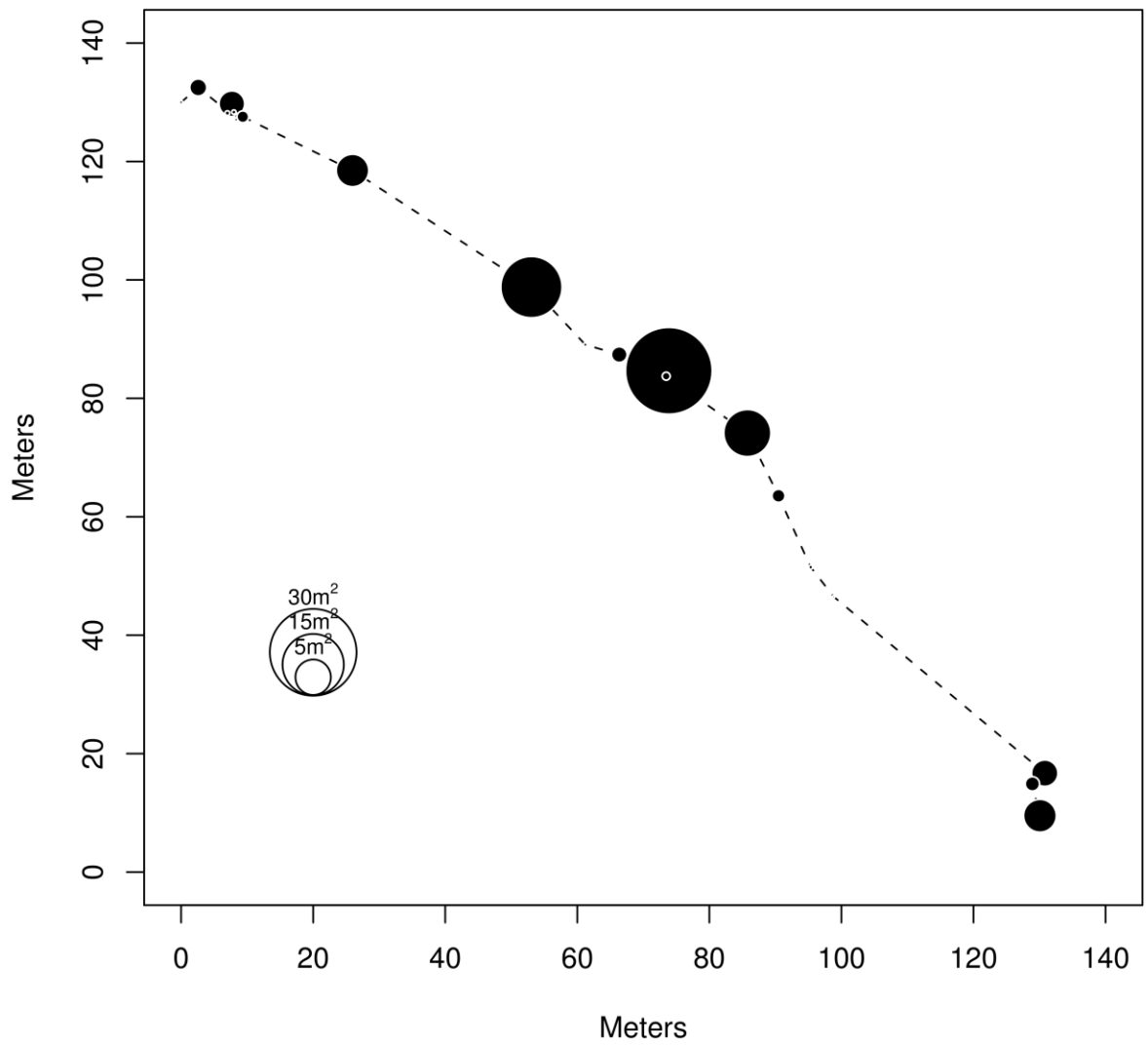


Figure 3.5a. Spatial configuration of populations found along Honey Creek, male-only. Both Honey Creek and Bird's Mill Creek have very large or aggregates of larger populations towards the upstream end of the metapopulation. For example, the three populations at the upstream segment were found on a cement retaining wall. These could be persistent source populations while the other populations are sinks (periphery populations) and ephemeral.

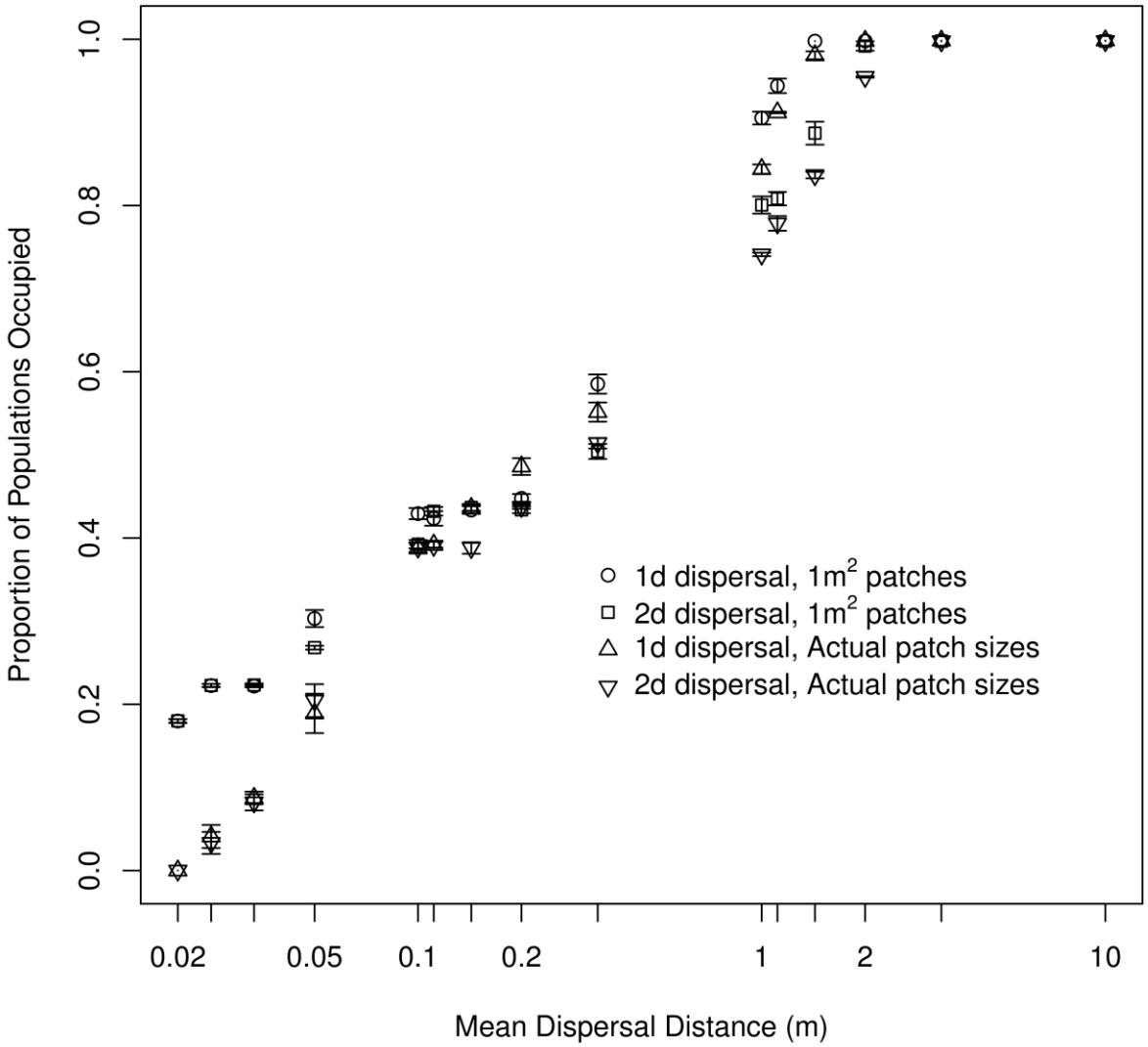


Figure 3.5b. Relationship between mean gemmae dispersal and proportion of metapopulation occupied for the male-only Honey Creek. No mean dispersal distances led to metapopulation extinctions, but low dispersal capabilities did decrease the number of patches occupied. For Honey Creek, a mean dispersal distance greater than 2m is required for 100% occupancy of the patches.

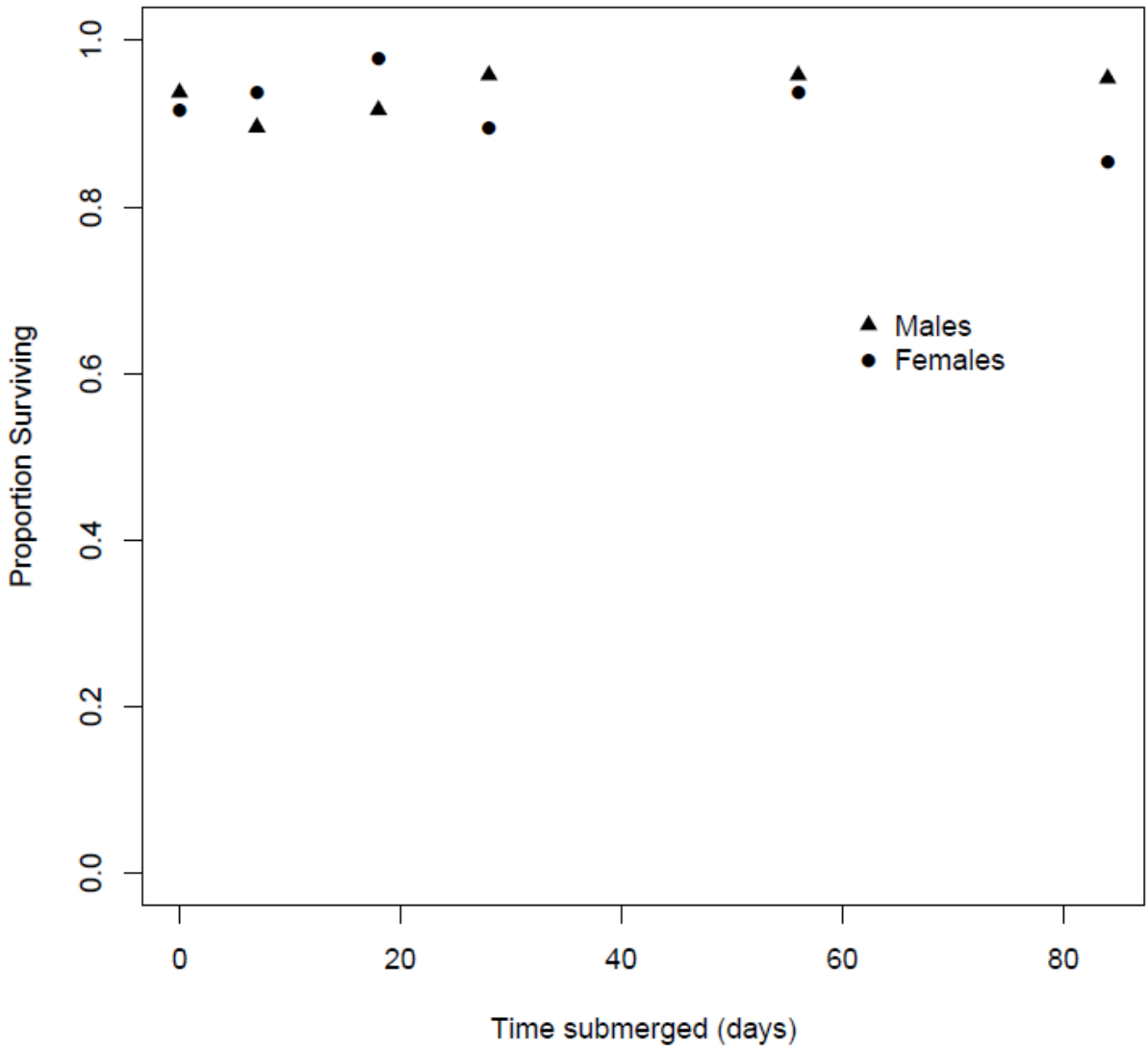


Figure 3.6. Proportion of individuals surviving submersion. As long as gemmae are kept moist, they survive and are capable of germination.

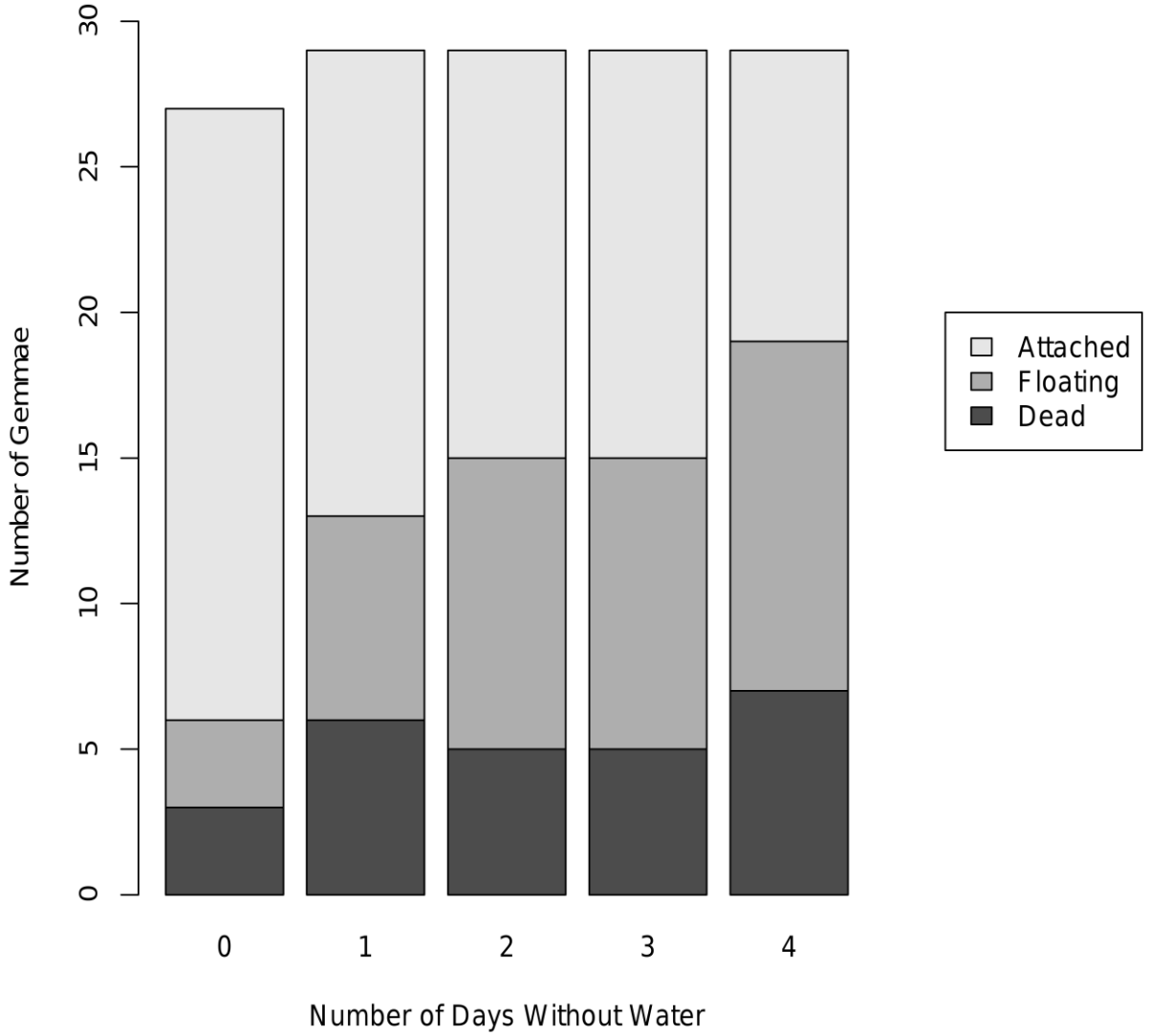


Figure 3.7a. Proportion of individuals germinating after desiccation, males. Individuals not germinating were either dead or were still small and showed no signs of increase in size or production of vegetative material. Germinating individuals ranged from slight germination (gemmae plus the beginning of a vegetative tip) to a greater than tripling in size of the average gemmae. a) Males

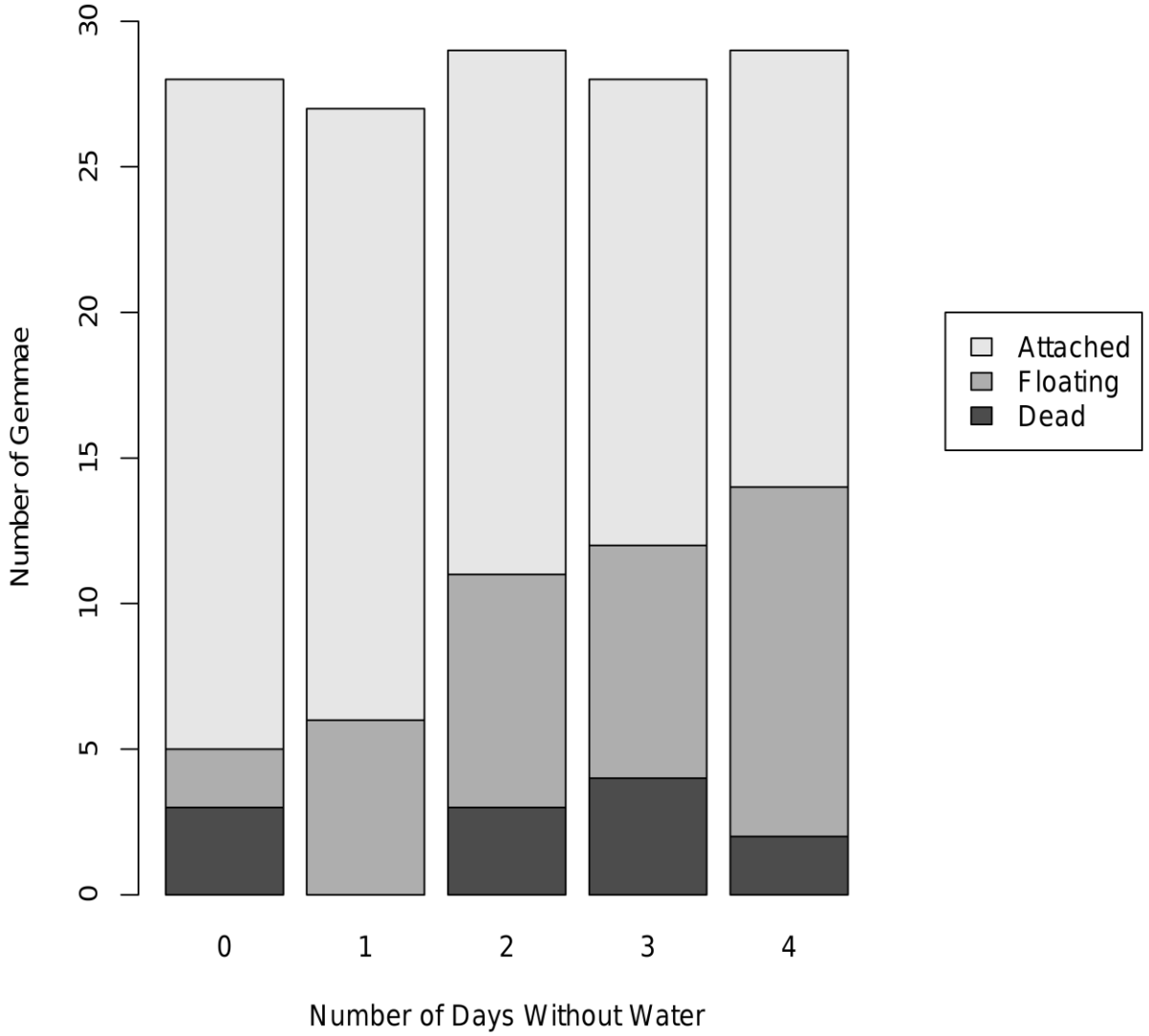


Figure 3.7b. Proportion of individuals germinating after desiccation, females. Individuals not germinating were either dead or were still small and showed no signs of increase in size or production of vegetative material. Germinating individuals ranged from slight germination (gemmae plus the beginning of a vegetative tip) to a greater than tripling in size of the average gemmae. b) Females

Copyright ©Christopher Stieha 2012

CHAPTER FOUR

Maintenance of the sexes: a field test of the effects of population size and distance to nearest neighbor in a clonal plant

Introduction

Understanding the evolution of sexual reproduction and the maintenance of the two sexes is a long-standing problem in ecology and evolution (Williams 1975; Maynard Smith 1978; Bell 1982; Kondrashov 1993; Hurst and Peck 1996, West et al. 1999). Genetically, asexually reproducing parents are contributing all of their genes to each offspring, while only half of the genetic material is passed down from a sexually reproducing parent to offspring (the "two-fold cost of sex" Williams 1975; Maynard Smith 1978; Bell 1982). Individuals that sexually reproduce exclusively have to fend off asexually reproducing invaders. In the presence of the two-fold cost of sexual reproduction and the absence of external influences, asexual individuals will outcompete sexually reproducing individuals. Sexual reproduction increases genetic variation, allowing sexual reproducers to outcompete asexual reproducers in the presence of substantial spatiotemporal variability (Maynard Smith 1978) or an adverse array of parasites (Lively 1987). Inhibiting or preventing sexual reproduction decreases the potential for matching or adapting to unpredictable environments.

Most research on the maintenance of sexual reproduction focuses on asexual reproducers invading a sexually reproducing population (Maynard Smith 1978; Doncaster *et al.* 2000; Pound *et al.* 2002). The ability to reproduce both asexually and sexually allows a clone to persist without the presence of the opposite sex while benefiting from sexual reproduction if the opposite sex is present. Some empirical and theoretical studies indicate that only one sex can reproduce both sexually and asexually (usually a hermaphrodite versus males: Peck 1994; Wolf and Takebayashi 2004; Halkett *et al.* 2006; Chasnov 2009), but there are many examples of systems in which both sexes (or mating types) can asexually and sexually reproduce (bacteria: Lederberg *et al.* 1952, Hayes 1953; protists: Sonneborn 1937, Elliott and Hayes 1953; algae: Smith and Regnery 1950, reviewed in Wreede and Klinger 1988;

fungi: Nelson 1996, Coppin *et al.* 1997; plants reviewed in Eckert 2002; animals: Bell 1982 for a review; Hand and Uhlinger 1992; McGovern 2002).

Clonal organisms with two sexes often have biased sex ratios (Bowker et al. 2000; McGovern 2002) and populations dominated by a single sex (Longton and Schuster 1983; Fuselier and McLetchie 2004). Within populations, competition between the sexes can lead to biased sex ratios and competitive exclusion (Mcletchie et al. 2002; Crowley et al. 2005a,b). However, populations do not exist in isolation but in aggregations (subpopulations that make up a metapopulation; Hanski 1999). Dispersers can migrate among subpopulations within the metapopulation, potentially establishing new subpopulations or rescuing the losing sex.

The spatial configuration of subpopulations within a metapopulation can greatly affect competitive dynamics and may facilitate the persistence of competitors (Kerr et al. 2002). Spatial configuration has only recently been considered in the study of the maintenance of the sexes (Peck et al. 1999; Lomnicki 2001; García-Ramos et al. 2007). In mathematical models, spatial configuration can allow both sexes to coexist for a longer time within subpopulations or to be maintained in the metapopulation (subpopulation size: McLetchie et al. 2002; spatial location of all subpopulations: García-Ramos et al. 2007).

Subpopulations with small carrying capacities should quickly reach carrying capacity, causing the competitive exclusion of one sex. Subpopulations with larger carrying capacities should take longer to reach the upper limit, thereby delaying competitive exclusion (*r*- versus *K*- selection of MacArthur and Wilson 1967, but see also McLetchie et al. 2002; Crowley et al. 2005a,b). Immediately after colonization, resources are abundant and competition is not required to secure resources. As the subpopulation grows and approaches carrying capacity, resources become limiting (including space or substrate itself), and competition between the sexes increases. During this time of population growth, the sex that dominates is best able to colonize unoccupied space (Crowley and McLetchie 2002). When the

subpopulation has reached carrying capacity, the sexes directly compete with one another, leading to competitive exclusion (McLetchie et al. 2002; Crowley et al. 2005a). The winning sex is able to obtain space by interference (e.g. overgrowth; Crowley and McLetchie 2002). Even though one sex may eventually be lost through competition, the time until the loss of one sex is much greater for a larger subpopulation, increasing the amount of time that both sexes are present and the probability of observing the subpopulation with both sexes.

Immigrants can disperse into an existing subpopulation or can colonize a new area and start a new subpopulation. Dispersal into an existing subpopulation can prevent competitive exclusion by rescuing the losing sex. The shorter the distance between subpopulations, the greater the probability that individuals will disperse between the two subpopulations. As the distance to the nearest neighbor increases, subpopulations will be less likely to receive dispersers, and the losing sex will not be rescued. The colonization of empty areas by both sexes allows coexistence until patch filling and the onset of competitive exclusion (see description above; García-Ramos et al. 2007). The process of subpopulation extinction and recolonization can maintain competitors (Hanski 1999) and, in particular, the two sexes within the metapopulation (García-Ramos et al. 2007). In many systems, the sexual products are thought to be the main forms of dispersal (Hansson et al. 1992; Starfinger and Stöcklin 1995). Recently, asexual products (whether vegetative material or distinct asexual propagules) have been shown to disperse beyond the source subpopulation (Johanssen and Nilsson 1993; Pohjamo et al. 2006) and contribute to population dynamics (Johanssen and Nilsson 1993). Although the predictions of mathematical models are well developed, they have not been tested in the field. My goal is to test these predictions in a system that has variation in the spatial configuration of subpopulations and variation in the subpopulations' sex ratios.

I use spatial configuration (subpopulation size and distance to nearest neighbor) in four naturally occurring metapopulations to test the predictions that: 1) As subpopulation size increases,

both sexes are more likely to be maintained and 2) As distance to nearest neighbor increases, both sexes are less likely to be maintained. I assume that the main form of dispersal between subpopulations is the product of sexual reproduction, and not asexual propagules, although my analysis tests this assumption.

Model Organism

Marchantia inflexa Nees et Mont is a New World bryophyte found on rocks and banks along creeks and streams from the southern United States to Venezuela (Bishler 1984). Males and females are separate individuals, chromosomally determined (Bishler 1986). In the field, sexes can be differentiated during periods of sex expression. Both sexes are capable of sexual and asexual reproduction; sexual spores are wind dispersed, while asexual gemmae are water dispersed. In *M. inflexa*, inter-subpopulation dispersal can occur either by asexual or sexual propagules.

Many subpopulations can be found along the streams, forming a metapopulation. Variation exists in the ratio of males to females both across streams and within streams. Within a metapopulation, subpopulations can be either all male, all female, or contain both sexes with male biased to female biased sex ratios (McLetchie and Puterbough 2002; Fuselier and McLetchie 2004; Stieha, unpublished data). Stream systems (metapopulations) can consist of only females, only males, or contain both males and females at various proportions (Bishler 1984; McLetchie and Puterbough 2002; Fuselier and McLetchie 2004; Stieha, unpublished data).

Males invest more energy in asexual reproduction, while females invest more energy in vegetative growth (McLetchie and Puterbough 2002). This life history difference gives competitive advantage to one sex over the other (McLetchie *et al.* 2002; Crowley *et al.* 2005). In weakly disturbed environments, the subpopulation is predicted to be dominated by the female's ability to vegetatively grow over competing individuals (McLetchie *et al.* 2002; Crowley *et al.* 2005). In highly disturbed environments, males are able to successfully colonize newly cleared areas via asexual propagules, while the female's vegetative growth is too slow for this purpose (McLetchie *et al.* 2002; Crowley *et al.* 2005).

In mathematical models, single isolated subpopulations are predicted to lose one sex due to competitive exclusion (McLetchie *et al.* 2002; Crowley *et al.* 2005). Both sexes can be maintained within a metapopulation, although individual subpopulations within the metapopulation may lose one sex (García-Ramos *et al.* 2007).

Within a subpopulation, dense overlapping growth forming mats of plants inhibits myability to discern individuals; therefore I define subpopulation size as the total area occupied by the plants, as opposed to the number of individuals.

Methods

Survey

Subpopulations from four stream metapopulations of *Marchantia inflexa* were surveyed from January 2007 to June 2007 (Quare River, 5 times; eastern Turure, 8 times; western Turure upstream, 7 times; western Turure downstream, 6 times; Stieha, unpublished data). The four metapopulations vary widely with respect to spatial configuration, from small subpopulations at high density to large subpopulations separated by large distances (Figure 4.1a and b). For each subpopulation, I measured the size of subpopulation (denoted as area because of *M. inflexa*'s clonal growth), determined the spatial location of each subpopulation with respect to other subpopulations, and counted male and female sex structures throughout the field season. Individuals and subpopulations containing these structures are considered to be sex expressing. From the location data, I determined distances between subpopulations of *M. inflexa* along the stream. Subpopulations may be near one another in two dimensional space, but be farther from one another following the bends of the stream. Dispersing asexual and sexual propagules are thought to travel exclusively along the stream course.

Distance

I computed distance to nearest neighbor in two ways by using two different classifications of nearest neighbor. The first determined the distance from the focal subpopulation (a subpopulation that was expressing sex and has a known sex ratio) to its nearest neighbor, independent of whether or not the nearest neighbor was expressing sex. Even if a subpopulation is not expressing sex and therefore does not have the potential to produce spores, the subpopulation could be dispersing asexual propagules. The second classified distance to nearest neighbor as the distance between the focal subpopulation and the nearest subpopulation that was expressing sex and contained both males and females. These subpopulations could produce spores, which disperse to other subpopulations. For the analysis, focal subpopulations always had sex expression, as I could not determine the number of females and males without expression.

Sexual propagules in bryophytes are thought to be the main form of inter-population dispersal (although see Laaka-Lindberg et al. 2003). Using the two methods to define nearest neighbor attempts to distinguish the effects of sexual propagules (Distance to Nearest Neighbor containing both males and females) and asexual propagules (Distance to Nearest Neighbor, independent of sex expression), although the Distance to Nearest Neighbor containing both males and females inherently includes asexual propagules.

Predicting One-Sex versus Two-Sex Subpopulations

To test whetherI could use spatial configuration to predict whether a subpopulation would lose a sex, I used the sex ratio to determine whether a subpopulation contained both sexes, classified by a 0 (both sexes present) or contained only a single sex, classified by a 1 (one sex is lost). I then analyzed the results using an unweighted generalized linear model with a logit link function and a quasibinomial error distribution (Wilson and Hardy 2002). I looked at the effects of subpopulation size (area), distance to nearest neighbor, metapopulation identity, all two-way interactions, and the three-way interaction on the probability a sex is lost (classified as 1). Stepwise model reduction was performed by removing non-

significant terms and comparing the change in the deviance between a model with the term and a model without the term with a *F* test (Crawley 2007). Given variation inherent in any field study, I included terms in my model when $P < 0.1$. I wanted to produce models that were first explanatory, then predictive. Explanatory models require significant terms and significant corresponding coefficients; predictive models require only significant terms (Berry and Feldman 1985).

Predicting Subpopulation Sex Ratios (Sex Bias Index)

The sex ratio (proportion of males) for each subpopulation was computed by two different methods. The first method used the *total number of sex structures* counted within a subpopulation throughout the whole field season. The second method determined the *maximum number of sex structures* within a subpopulation counted during a single survey for both males and females. For many subpopulations, the maximum number of sex structures for females occured at a different point in time (generally later) than the maximum number of male sex structures (Stieha, unpublished data). Using the maximum number of sex structures counted during a single sample removed the possibility of counting the same sex structures across survey times. Subpopulations contained both sexes, only males, only females, or were of unknown sex ratio due to lack of sex expression.

Because I am interested in the maintenance of the sexes, and single-sex subpopulations could be either all male or all female, I compute the Sex Bias Index (SBI) for the subpopulation. SBI represents the deviation from a 1:1 male to female ratio; a SBI of 1 represents a single sex subpopulation (either male or female) while a SBI of 0 represents a 0.5 sex ratio (Fig. A). Sex ratio is converted to SBI using the conditional formula

$$SexBiasIndex = \begin{cases} 1-2s & s \leq 0.5 \\ -1+2s & s > 0.5 \end{cases} \qquad \text{(eq. 1)}$$

where *s* is the sex ratio (proportion male) of the focal subpopulation.

I analyzed the effects of subpopulation size (area), metapopulation identity, distance to nearest neighbor, all two-way interactions, and the three-way interaction on the SBI using an unweighted generalized linear model with a logit link function and a quasibinomial error distribution due to underdispersion with a binomial error distribution. I used logistic regression because SBI is between 0 and 1 inclusive and is computed from the sex ratio, a proportion. Model reduction was performed as explained in *Predicting One-Sex versus Two-Sex Subpopulations*.

Analyses were performed and graphs were produced in R 2.10.1 (R Development Core Team 2009) using the beeswarm package (Eklund 2010) and the akima package (Akima et al. 2012 from Akima 1976, 1996). Subpopulation area and distance to nearest neighbor were $\log_{10}$ transformed to account for the differences in magnitudes of the values. Metapopulation identity (a categorical variable) and the interactions of metapopulation identity with area and distance were included to document and validate variation among the streams. The proportion of males computed from the total number of observed sex structures did not show spatial correlation within metapopulations when analyzed with Moran's I and Geary's C as computed by the spdep package in R with an inverse distance weight matrix ($p > 0.65$ for Quare, Eastern Turure, and Western Turure upstream, $p > 0.073$ for Western Turure downstream; Moran 1950; Geary 1954; Dale 1999; Fortin and Dale 2005). Subpopulations expressing sex along the Quare River are randomly distributed (see Appendix 1). Both these results suggest that spatial autocorrelation of the proportion of males is minimal.

Results

Predicting One versus Two Sex Subpopulations

Regardless of whether the nearest neighbor had individuals expressing sex or not, only area was significant after model reduction ($F_{1,69} = 25.52$, $P < 0.001$, for both analyses; Table 4.1). The coefficient

for the intercept was non-significant (t = 1.49, P = 0.140). Given the negative coefficient for the area (-2.12±0.56, Mean±SE, t = -3.80, P < 0.001), the likelihood that a subpopulation contains only one sex decreases as subpopulation size increases (Figure 4.3).

Predicting Subpopulation Sex Ratio (Sex Bias Index)

The Sex Bias Index (SBI) calculated from both the maximum sex structures counted at a single sampling event and the total sex structures counted throughout the sampling period gave very similar results when I classified the nearest neighbor as any other subpopulation of *Marchantia inflexa* and did not require both sex expressing males and females (Table 4.2, for both analyses of the response variable, total and maximum SBI). The best model contained the all three explanatory variables (area, distance, and stream) and the interaction between stream and distance (Table 4.2). All terms, two-way interactions, and three-way interactions were significant (see Table 4.2). However, none of the coefficients in the model were significant, suggesting multicollinearity between the terms. After model reduction, the final model contains the interaction between distance and stream, stream, area, and distance (see Table 4.2). Coefficients for SBI models using both computations of SBI are presented in Table 3.

Given the similarity between the two computations of SBI, I show graphs of the effects of explanatory variables on SBI determined using the total number of sex structures. As population size increases, the SBI decreases (sex ratio approaches 1:1, coefficient -0.67±0.30 Mean±SE; Figure 4.4a) for both maximum number of sex structures (t = -2.264, P = 0.027) and total sex structures (t =-2.208, P = 0.031). As distance between the focal subpopulation and the nearest neighbor increases, the effect on SBI varies with respect to the stream (interaction between stream and distance to nearest neighbor, $F_{3,62}$=2.79, P=0.0477 for the total sex structures and $F_{3,62}$=4.105, P = 0.010 for the maximum number of sex structures; Table 2). As distance increases, subpopulations along the downstream branch of the western Turure River are more likely to have a sex ratio bias and contain only one sex (higher SBI). The

upstream subpopulations of the western branch Turure River also shows this effect, but the coefficient is not significant for either computation of SBI (W. Turure Upstream * Distance to NN in Table 2). For the other two metapopulations, subpopulations are more likely to contain both sexes as distance increases (lower SBI; Figure 4.4b, Table 4.2). To determine how well the model works, Iplotted the difference between the observed and predicted SBI versus the observed SBI, where zero is a perfect fit (Figure 4.5). The predicted value and observed value differed from anywhere between -0.4 to 0.4. When the observed SBI is small (representing a less biased sex ratio), the model predicts a larger SBI (a more biased sex ratio). When the observed SBI is large (representing a more biased sex ratio), the model predicts a smaller SBI (a less biased sex ratio). No statistics were performed because the observed SBI was used to compute the difference value.

Nearest Neighbor with Both Sexes

When I focus only on the distance between nearest neighbors that are sex expressing and contain both males and females, the different calculations of SBI (total vs. maximum) gave us different results. When the total number of sex structures is used to compute SBI of a focal subpopulation, the only significant term in the final model is area ($F_{1,69}$ = 8.24, P = 0.005; Table 4.2). As the area increases, SBI decreases; subpopulations approach an equal sex ratio (Intercept 1.65±0.29 (Mean±SE), t = 5.69, P < 0.001; area -0.73±0.27 (Mean±SE), t = -2.68, P = 0.009). When the maximum number of sex structures of each sex counted during a single survey is used to compute SBI, I get a model that includes stream, area, distance, the interaction between stream and area, and the interaction between area and distance (Table 4.2). Coefficients for the final model are presented in Table 4.4. Figure 4.6 shows a contour plot fit using observed data displaying both the stream-area interaction and the distance-area interaction.

Discussion

As predicted by mathematical models, spatial configuration is predictive of 1) whether the focal subpopulation losses a sex and 2) the Sex Bias Index (SBI) in naturally occurring metapopulations. Area is a significant predictor of the maintenance of both sexes of the focal subpopulation (Figure 4.3; Table 4.1) and the SBI of the focal subpopulation (Figure 4.4a; Table 4.2). When nearest neighbor is defined as the next closest subpopulation of *Marchantia inflexa* (independent of whether the neighbor has sex expression), area, distance, stream, and a stream by distance to nearest neighbor interaction affect the SBI of the focal subpopulation (Figure 4.4b; Table 4.2). When the nearest neighbor is defined as the next closest subpopulation of *M. inflexa* containing both males and females, the results vary depending on the method of computing SBI.

Effects of Size

With respect to subpopulation size, a threshold for one or two sexes appears around 1 m^2. Below the threshold, a subpopulation will contain only one sex; above the threshold, a subpopulation will contain both sexes (Figure 4.3, 4.4a, and 4.6). Competitive exclusion appears to be occurring faster in smaller subpopulations compared to larger subpopulations. Another possible explanation is that smaller subpopulations are more likely to be colonized by a single dispersing propagule, leading to a subpopulation containing individuals of the same sex and genotype. Colonization events have been observed on sandbars along the streams, but sandbars are not stable enough to maintain a subpopulation (McLetchie, pers. obs.). On many of these sandbars, there are many individuals of sufficient size to suggest they are recent colonizations and not colonizations from previous years (McLetchie, pers. obs.). Many new permanent subpopulations (observed one year and persist and are larger the next year) have been observed to be colonized by multiple individuals (Stieha, unpublished data). My analysis assumes that all subpopulations have been occupied about the same amount of time. It is possible that smaller subpopulations are found in habitats with higher turnover rates compared to

larger subpopulations. Due to the higher turnover rate, the subpopulations would be newer and have had less time to lose one sex. In this case, I would expect smaller subpopulations to be more likely to contain both sexes, which I do not observe.

Effects of Distance to Nearest Neighbor

Independent of the classification of nearest neighbor (all subpopulations or only subpopulations containing both males and females), the distance to nearest neighbor and the metapopulation identity affect the SBI for the focal subpopulation. The various streams show different patterns with respect to the effects of the nearest neighbor, suggesting that dispersal capability varies among streams. I predicted that SBI would increase (more biased sex ratios/single sex subpopulations) as distance to nearest neighbor increased. Only one stream, downstream of the western Turure River, matched my prediction that SBI would increase as distance to nearest neighbor increased (Figure 4.4b). The upstream segment of the western Turure River appeared to follow this trend, but the coefficient was not significant (Table 4.3), possibly due to the low number of subpopulations actually observed along that segment (11 subpopulations with only 5 expressing; Figure 1b). Conversely, subpopulations along the Quare River and the eastern fork of the Turure River decreased in SBI (more even sex ratio) as distance between subpopulations increased. With subpopulations that were farther apart, subpopulations were more likely to maintain both sexes than to lose a sex. Subpopulations that were close to another subpopulation were likely to contain only one sex, suggesting that the subpopulations, despite being physically separate, acted as a single population. In these streams, I expect that asexual propagules are able to disperse easily between subpopulations, suggesting that asexual propagule dispersal occurs and strongly affects population dynamics (Laaka-Lindberg et al. 2003). It is unclear why subpopulations at similar distances from nearest neighbors across multiple streams would act differently unless dispersal capabilities were fundamentally different. In the Quare and eastern fork of the Turure River, subpopulations could be more likely to contain one sex if I could get very large

distances between nearest neighbors, as suggested by the 2 single sex subpopulations of the eastern Turure that are separated from their nearest neighbor by almost 300m (Figure 4.6).

When SBI is computed using the maximum number of sex structures, the distance to nearest neighbor with expressing males and females may be influencing the maintenance of both sexes in the focal subpopulation. Both the interaction between area and stream and the interaction between area and distance to nearest neighbor significantly affect SBI. Small subpopulations very close to other subpopulations are more likely to contain only one sex (Figure 4.6). Subpopulations greater than 50m from the nearest neighbor are lacking in my metapopulations (Figure 4.6) which limits testing at this scale.

Because colonization occurred in the past, it is not necessarily the subpopulations currently expressing sex (or even the subpopulations currently existing!) that influence a neighboring subpopulation's SBI. Other currently extinct subpopulations might have had past influence. Distance between subpopulations independent of whether the neighbor contained both sexes significantly explained the SBI in the focal subpopulation (Figure 4.4b; Table 4.2) - but in the opposite direction of my prediction in three out of 4 metapopulations. As distance to nearest neighbor increased, the maintenance of both sexes increased. The effects of distance to nearest neighbor became more complicated when neighbors contain both males and females. Using my data, I may be only picking up the effects of dispersal from asexual propagules, which can come from any subpopulation, whether the individuals within the subpopulation are sexually expressing or not. Greater distances between subpopulations could make colonization by spores (and therefore both sexes) more likely than colonization by gemmae (potentially only one sex). In many bryophytes, sexual propagules are thought to be the main dispersal mechanism, although effects of asexual propagules on population dynamics are being documented (Johanssen and Nilsson 1993; Laaka-Lindberg et al. 2003; Pohjamo et al. 2006). Although not conclusive, my research helps support the idea of interpopulation dispersal of asexual

propagules in bryophytes specifically, but also suggests the importance of asexual dispersal in any clonal system.

Maintenance of Competitors

In the context of reproductive assurance, Eppley and Pannell (2007) found conceptually similar results in the plant *Mercurialis annua,* where focal population size and density of subpopulations affects mating type and proportions of mating types. Reproductive assurance hypothesizes that small, widely spaced subpopulations are more likely to be monomorphic (containing only one sex, a selfing hermaphrodite in *M. annua*), while large, more common subpopulations will be sexually dimorphic (containing multiple sexes). Dimorphic subpopulations of *M. annua* contained more plants and occupied a greater proportion of the sampling area, while monomorphic subpopulations contained fewer plants and sparsely occupied the area. Similar to my results, population size in androdioecious populations was positively correlated with the proportion of males. My system differs from the *M. annua* system as both males and females produce asexual dispersing propagules, creating monomorphic populations containing either males or females. *Mercurialis annua* has selfing hermaphrodites as asexual reproducers. My monomorphic and dimorphic subpopulations are also found within the same stream, as opposed to having different geographic regions. Reproductive assurance is not driving my system as both sexes can produce asexual propagules and are therefore assured; lack of both sexes means sexual propagules cannot be produced.

Spatial configuration influences whether a subpopulation is monomorphic or dimorphic (Eppley and Pannell 2007) and also affects the sex ratio. Therefore, spatial configuration can influence spatial segregation of the sexes, producing biased or single sex subpopulations through competition and dispersal (García-Ramos et al. 2007), as opposed to requiring environmental partitioning by the sexes (Bierzychudek and Eckhart 1988). Sexual reproduction cannot occur in subpopulations containing only one sex, which affects genetic variation and population growth. Even when both sexes are present,

biased sex ratios in plants and animals can have extreme impacts on population growth (Rydgren et al. 2010; Miller and Inouye 2011) and genetics (Vandepitte et al. 2010, Hilfiker et al. 2004). If sperm availability is not limiting (all females are fully fertilized) and Allee effects are not present (where one sex can find the other sex despite its low population size), an equal sex ratio or female-biased sex ratio produces a higher population growth rate than a male biased subpopulation or a female dominated subpopulation. Understanding the effects of spatial configuration on the sex ratios is vital for maintaining sexual reproduction (genetic diversity) and population viability (by maintaining a positive population growth rate).

Explaining coexistence of the sexes due to spatial configuration is similar to explaining the maintenance of competitors. Spatial configuration can also influence biodiversity and variation among individuals. For example, in the cyclic rock-paper-scissors game (rock beats scissors, scissors beats paper, and paper beats rock) of antibiotic production, resistance, and sensitivity in bacteria (antibiotic producing beat sensitive, sensitive beat resistant, resistant beat antibiotic producing), spatial structure stabilizes the population dynamics and maintains all three strategies (Kerr et al. 2002). In a completely mixed environment, one strategy dominates while the other two persist at low levels. Producing a two dimensional arena forces competition between neighbors and produces stable waves of strategies that move across the arena. Similarly, in a series of rock pools, competition and dispersal may maintain three species of *Daphnia*, where pools that have lost a species can regain it through dispersal from a surrounding rock pool (Pajunen and Pajunen 2003). The distance between pools as well as the size of the pool can determine when and how often dispersal occurs. Finally, predator-prey systems have been shown to perish in spatially simple environments and persist in spatially complex environments (Huffaker 1958). Many of the results from this paper correspond to these patterns of coexistences due to spatial arrangement.

Subpopulation area and distance to nearest neighbor are simplified measurements of the natural complexity of the system (Moilanen and Nieman 2002). The size of the nearest neighbor and the number of neighbors in close proximity to the focal population may better represent the potential for immigration into a focal subpopulation and may be a better predictor of the maintenance of the sexes and sex ratios. More advanced spatial measurements are available (Moilanen and Nieman 2002), but these require extra levels of parameterization, that, if done incorrectly, could give the wrong results. Given the similarities between stream systems, I may be able to use one metapopulation to parameterize the more advanced spatial measurements (such as the α and β in the Incidence Function Model; Hanski 1994) and use the parameters to compute the advanced spatial measurements for the other metapopulations. Environmental information, such as light availability, moisture, humidity, may be required to predict which sex will win, but the importance of spatial configuration to the maintenance of the competitors (males and females in my case) is clear. I must take this into consideration as human-induced habitat fragmentation increases, disrupting the maximum population an area can support as well as the dispersal between populations.

Table 4.1. Deviance table for the effects on whether the subpopulation has lost a sex. Grey boxes represent terms included in the final explanatory model.

	stream*area*distance	stream*area	stream*distance	area*distance	stream	distance	area
One or Two Sexes							
nearest neighbor	Dev = 6.01	Dev = 6.02	Dev = 10.33	Dev = 3.21	Dev = 0.45	Dev = 0.36	Dev = 29.26
all subpopulations	$F_{3,55} = 1.63$	$F_{3,58} = 1.14$	$F_{3,61} = 3.17$	$F_{1,64} = 2.97$	$F_{3,65} = 1.63$	$F_{1,68} = 0.32$	$F_{1,69} = 25.52$
	p = 0.193	P = 0.340	p = 0.030[a]	p = 0.090[a]	p = 0.95	p = 0.575	p<0.0001
nearest neighbor	Dev = 10.06	Dev = 9.83	Dev = 12.95	Dev = 1.99	Dev = 0.09	Dev = 1.44	Dev = 29.262
with males and females	$F_{3,55} = 3.64$	$F_{3,58} = 2.48$	$F_{3,61} = 4.41$	$F_{1,64} = 1.59$	$F_{3,65} = 0.02$	$F_{1,68} = 1.20$	$F_{1,69} = 25.52$
	P = 0.018[a]	P = 0.070[a]	P = 0.007[a]	P = 0.212	P = 0.995	P = 0.277	p<0.0001

a – F statistic significant, but no significant or trending coeffcient values for the term

Table 4.2. Deviance table for the effects on the Sex Bias Index (SBI). Grey boxes represent terms in the final model. The final model is similar for total number of sex structures and maximum number of sex structures when the neighboring subpopulation can be any subpopulation of *Marchantia inflexa*. When neighboring subpopulations must contain both sexes, the final models for the total number of sex structures and the maximum number of sex structures differ.

	stream*area*distance	stream*area	stream*distance	area*distance	stream	distance	area
Nearest Neighbor, any subpopulation							
Total number of sex structures	Dev = 4.95 F3,55 = 3.92 P = 0.013[a]	Dev = 0.91 F3,58 = 0.65 P = 0.584	Dev = 3.83 F3,62 = 2.79 P = 0.048	Dev = 0.13 F1,61 = 0.28 P = 0.602	Dev = 4.46 F3,62 = 3.24 P = 0.028[b]	Dev = 0.0036 F1,65 = 0.0071 P = 0.933[b]	Dev = 2.47 F1,62 = 5.39 P = 0.024[b]
Maximum number of sex structures	Dev = 3.71 F3,55 = 2.98 P = 0.0394[a]	Dev = 1.21 F3,59 = 0.91 P = 0.44	Dev 5.41 F3,62 = 4.10 P = 0.010	Dev = 0.13 F1,58 = 0.30 P = 0.586	Dev = 6.47 F3,62 = 4.90 P = 0.004[b]	Dev = 0.015 F1,65 = 0.029 P = 0.865[b]	Dev = 2.49 F1,62 = 5.66 P = 0.0204[b]
Nearest Neighbor with males and females present							
Total number of sex structures	Dev = 2.58 F3,55 = 2.12 P = 0.108	Dev = 2.24 F3,58 = 1.77 P = 0.163	Dev = 4.09 F3,62 = 3.03 P = 0.0360[a]	Dev = 1.07 F1,61 = 2.44 P = 0.123	Dev = 1.95 F3,66 = 1.31 P = 0.278	Dev = 0 F1,65 = 0 P = 1.0	Dev = 4.02 F1,69 = 8.24 P = 0.005
Maximum number of sex structures	Dev = 2.27 F3,55 = 1.83 P = 0.153	Dev = 3.87 F3,62 = 2.72 P = 0.0523	Dev = 1.97 F3,58 = 1.55 P = 0.212	Dev = 1.65 F1,61 = 3.74 P = 0.0578	Dev = 2.65 F3,66 = 1.79 P = 0.157	Dev = 0.153 F1,65 = 0.30 P = 0.585	Dev = 3.92 F1,69 = 7.76 P = 0.00688

a – F statistic significant, but no significant or trending coeffcient values for the term

b – Computed F statistic by comparing a model containing stream, distance, and area to a model missing the term in question

Table 4.3. Coefficients for the final models when nearest neighbor is closest neighboring subpopulation. These coefficients correspond to the models presented in the top half of Table 4.2.

	Total Number of Sex Structures			Maximum Number of Sex Structures		
	Estimate(Standard Error)	t value	P	Estimate(Standard Error)	t value	P
Intercept – East Turure	3.24(1.58)	2.05	0.0445*	3.48(1.66)	2.09	0.041*
Quare	-0.51(1.69)	-0.3	0.764	-0.80(1.76)	-0.45	0.652
West Turure – Downstream	-3.32(1.77)	-1.88	0.0652†	-4.31(1.86)	-2.32	0.023*
West Turure – Upstream	-3.34(2.53)	-1.32	0.192	-2.72(2.53)	-1.07	0.288
Area	-0.67(0.30)	-2.21	0.031*	-0.67(0.29)	-2.26	0.0271*
Distance to nearest neighbor (NN)	-1.61(1.29)	-1.25	0.214	-2.03(1.48)	-1.37	0.175
Quare*Distance to NN	0.24(1.58)	0.16	0.878	0.72(1.72)	0.42	0.677
W. Turure Downstream*Distance to NN	3.38(1.67)	2.02	0.048*	4.51(1.88)	2.39	0.0197*
W. Turure Upstream*Distance to NN	2.67(2.94)	0.91	0.368	1.89(2.97)	0.64	0.527

† means P < 0.1

* means P < 0.05

Table 4.4. Coefficients for the final model when nearest neighbor must contain both males and females. These coefficients correspond to the SBI computed from the maximum number of sex structures. The final model when SBI is computed from the total number of sex structures contains only area.

	Estimate(Standard Error)	t value	P
Intercept – East Turure	7.31(2.94)	2.49	0.0155*
Quare	-3.73(2.33)	-1.6	0.114
West Turure – Downstream	-6.67(2.67)	-2.5	0.015*
West Turure – Upstream	-3.24(2.91)	-1.12	0.269
Area	-5.40(2.23)	-2.42	0.0185*
Distance to nearest neighbor (NN)	-2.02(1.06)	-1.92	0.0601†
Quare*Area	3.29(1.77)	1.86	0.0684†
W. Turure Downstream*Area	6.51(2.43)	2.68	0.00935*
W. Turure Upstream*Area	1.84(2.36)	0.78	0.437
Area*Distance to NN	1.61(0.91)	1.77	0.0811†

† means P < 0.1

* means P < 0.05

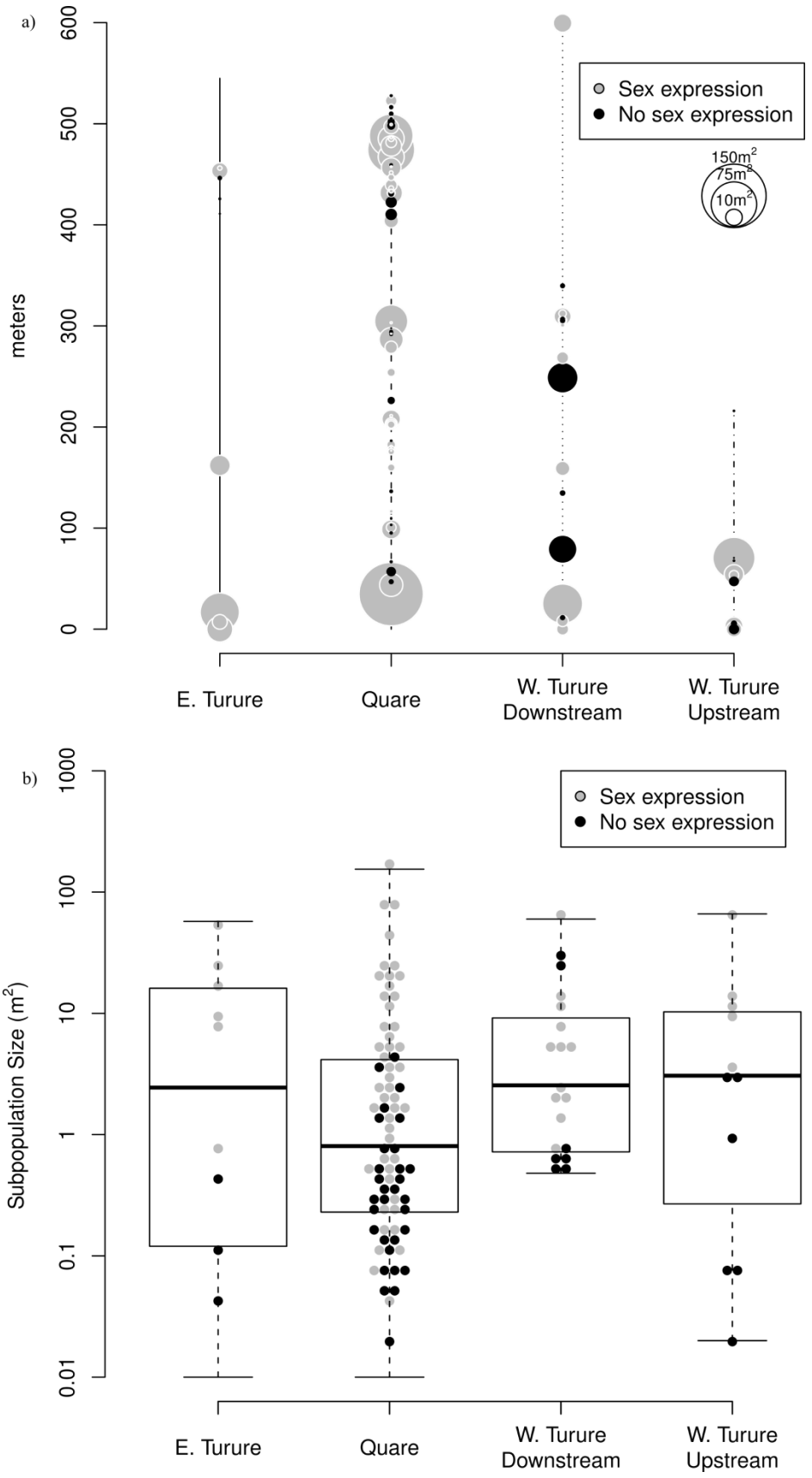

a)
meters
0
100
200
300
400
500
600
Sex expression
No sex expression
150m2
75m2
10m2
E. Turure
Quare
W. Turure Downstream
W. Turure Upstream
b)
Subpopulation Size (m2)
0.01
0.1
1
10
100
1000
Sex expression
No sex expression
E. Turure
Quare
W. Turure Downstream
W. Turure Upstream

Figure 4.1. Spatial Configuration of the Four Metapopulations. 1a) Location and size of subpopulations along 4 streams. Grey circles show subpopulations that were expressing sex and have an observed sex ratio. Black circles represent subpopulations that were not expressing sex; therefore the sex ratio is unknown. All grey subpopulations are focal subpopulations. Streams flow from the top of the graph to the bottom (from 600 meters to 0 meters on the y axis). 1b) Subpopulation size distribution for the 4 metapopulations. Grey circles represent subpopulations of the metapopulations that were expressing sex and are focal subpopulations. Filled in circles are subpopulations that were not expressing sex and had unknown sex ratio.

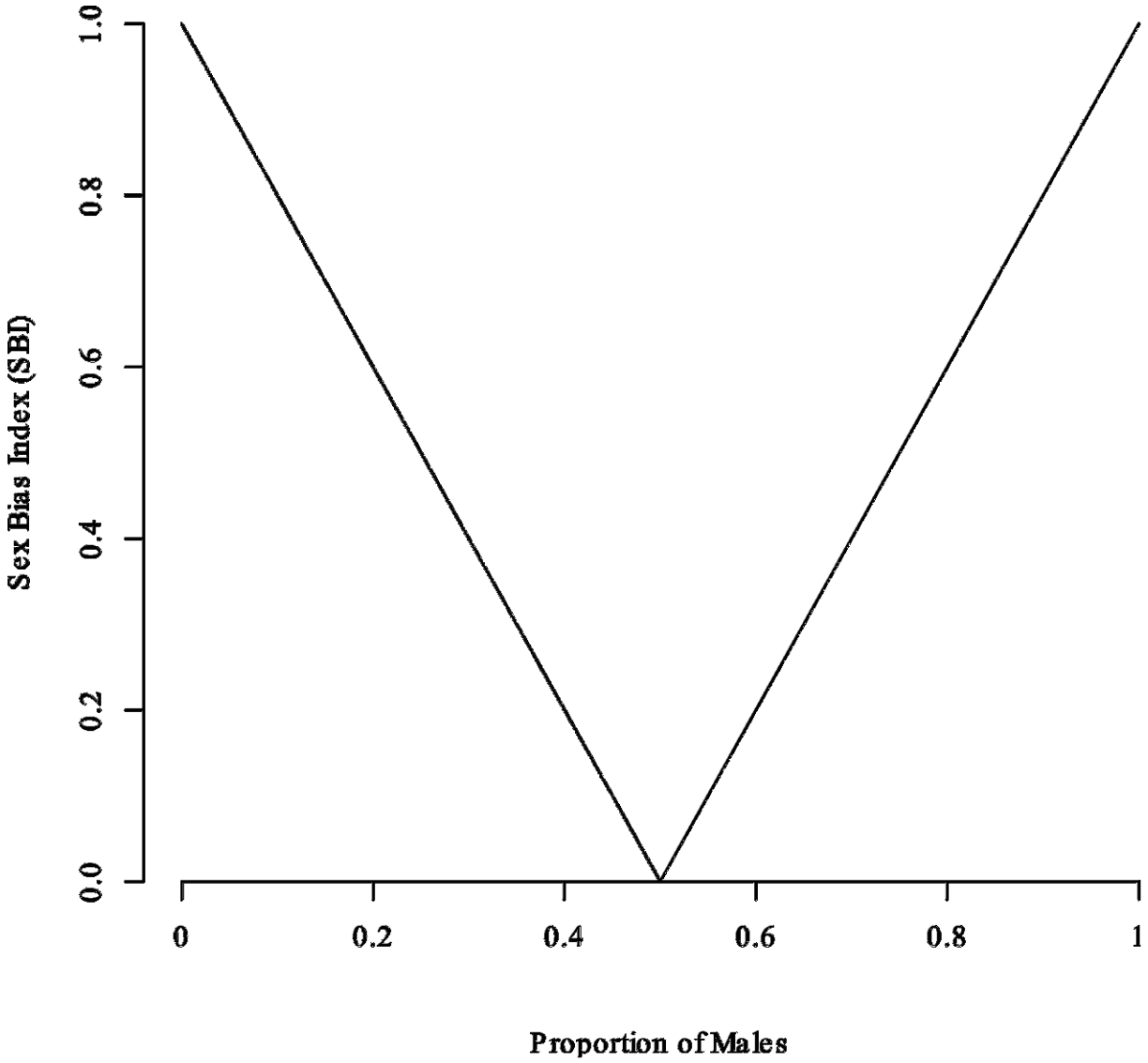


Figure4.2. Relationship between sex ratio as proportion of males and Sex Bias Index (SBI). SBI is equivalent to the number of sex structures of the dominant sex within a subpopulation divided by the total number of sex structures within a subpopulation, scaled from [0.5, 1] to [0,1].

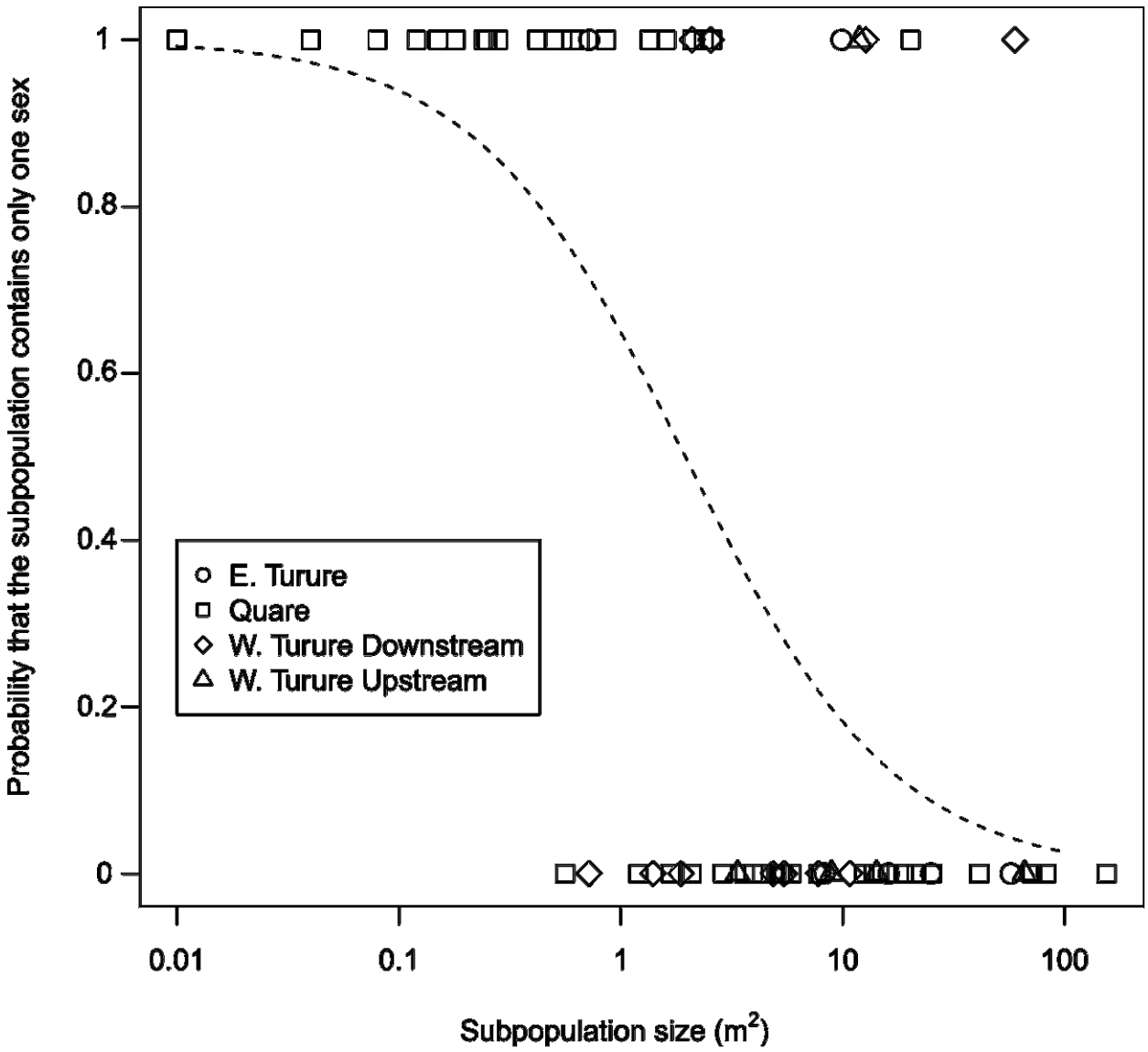
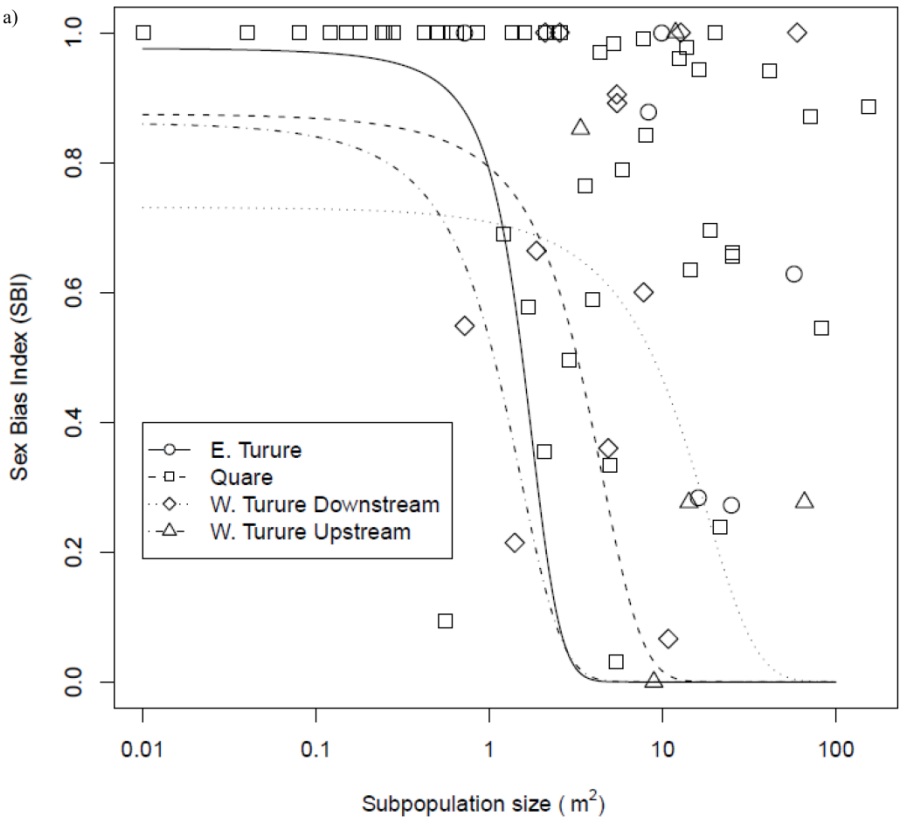


Figure 4.3. Loss of One Sex versus Subpopulation Size. A subpopulation has a value of 1 when it contains only one sex. A value of 0 signifies that a subpopulation has not lost a sex. The dashed line represents the likelihood the subpopulation contains only one sex.

a)


Sex Bias Index (SBI)
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
Subpopulation size (m2)
E. Turure
Quare
W. Turure Downstream
W. Turure Upstream

b)

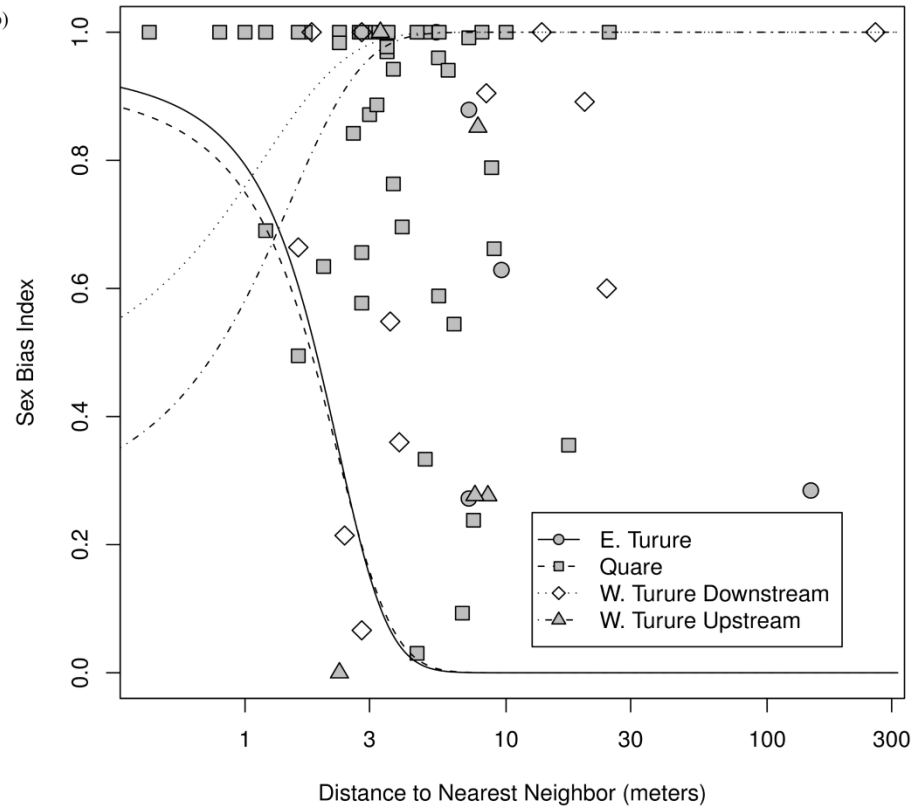

Sex Bias Index
1.0
0.8
0.6
0.4
0.2
0.0
1
3
10
30
100
300
Distance to Nearest Neighbor (meters)
E. Turure
Quare
W. Turure Downstream
W. Turure Upstream

Figure 4.4. The Effects of Spatial Configuration on Sex Bias Index. 4a) The effect of subpopulation size on the Sex Bias Index (SBI). A value of 1 signifies a subpopulation that has lost one sex. A value of 0 signifies a subpopulation with an equal number of males and females. A value between zero and 1 represents a bias in the ratio of males to females. SBI was computed using the total number of sex structures counted. To draw the lines for the graph, distance to nearest neighbor was included as the average distance within a stream. 4b) the effects of distance to nearest neighbor on Sex Bias Index (SBI). Grey shapes represent the three stream systems that were not significantly different from one another. The coefficient for the upstream section of the Western Turure River is similar to the coefficient for the downstream section, but is not significantly different from zero (Table 3). SBI is computed using the total number of sex structures counted. To draw the lines for the graph, mean subpopulation size for each stream was included.

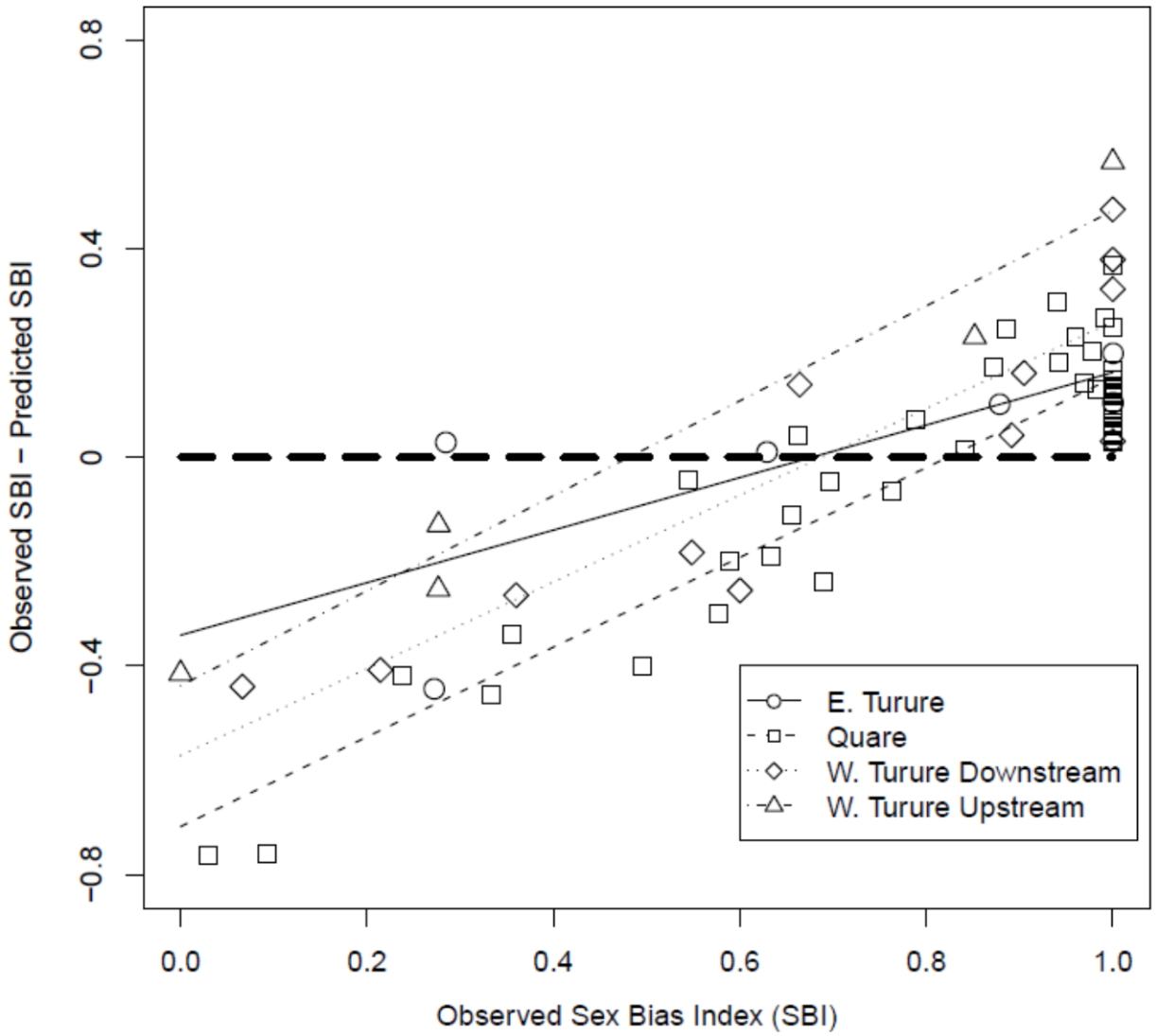


Figure 4.5. Comparison of model predictions and observed data. Values close to zero represent subpopulations where the observed SBI and predicted SBI were similar. Lines are for visual representation only – no statistics were performed given the use of the observed SBI in both the x value and y value.

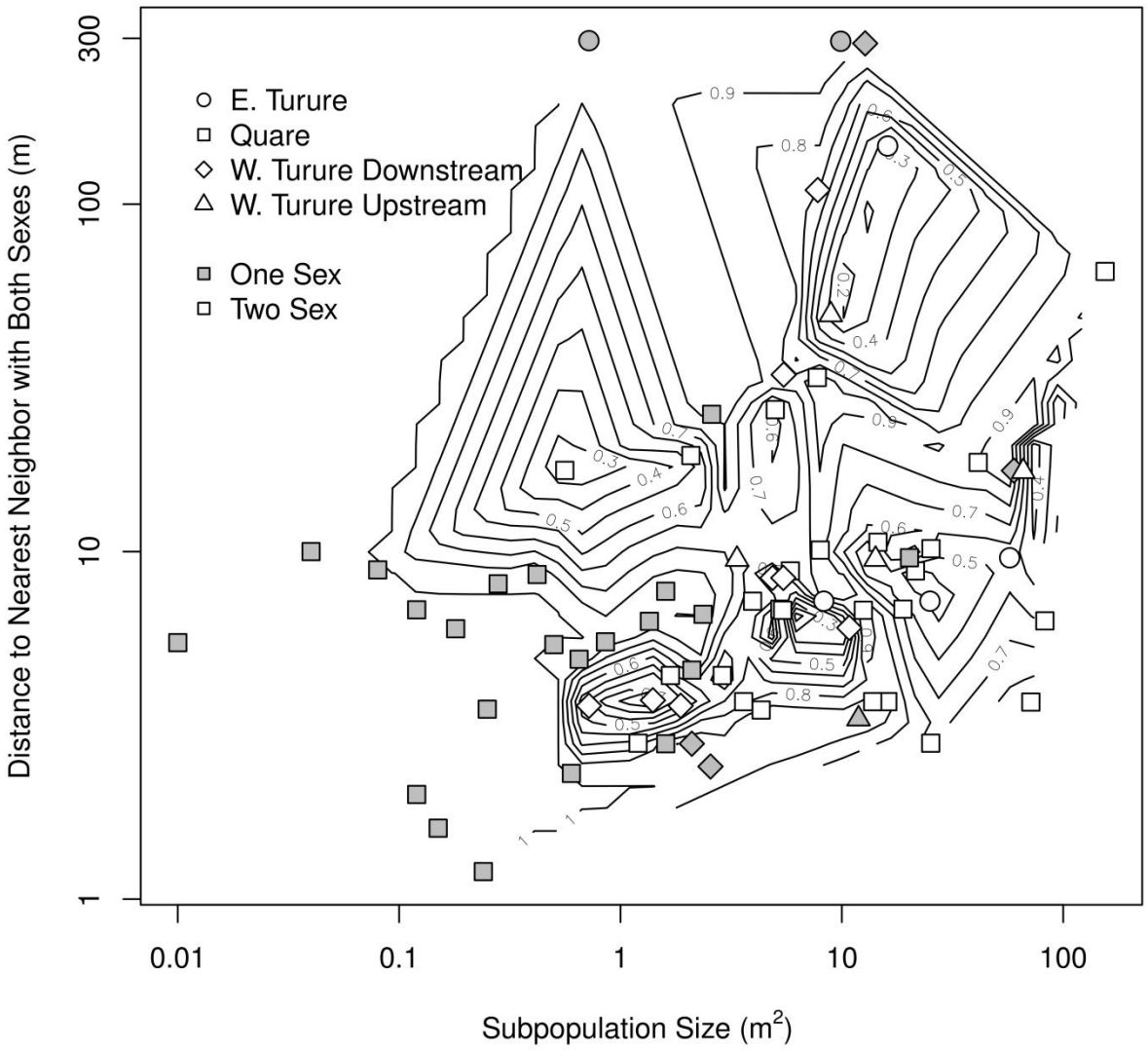


Figure 4.6. The Interaction of Subpopulation Size and Distance to Nearest Neighbor on Sex Bias Index (SBI). Distance to nearest neighbor only includes neighbors that were expressing sex and contained both males and females. Contours represent the SBI computed from maximum number of sex structures of each sex counted during a single survey. Grey symbols show subpopulations that contained only one sex; Open symbols show subpopulations that contained both sexes. Contour lines were fit with the observed SBI, not the predicted SBI. Subpopulations are more likely to contain only one sex if distance to nearest neighbor is small (<10m) and subpopulation size is small (<1m^2). Subpopulations contain both sexes when subpopulation sizes are greater than 1m^2. Independent of subpopulation size, the subpopulations contain only one sex when the distance to the nearest neighbor is almost 300 meters.

Copyright ©Christopher Stieha 2012

CHAPTER FIVE

Maintenance of the Sexes and Persistence of a Clonal Organism in Spatially Complex Metapopulations

Introduction

Migration between populations (or the lack thereof) can greatly affect local population dynamics as well as global (multi-) population dynamics and persistence (Koelle and Vandermeer 2005; García-Ramos et al. 2007; Abbott 2011). Migration can be directly affected by the densities (Hanski 1994, 1999), spread (Hanski 1994, 1999), and the environment between populations (Wiens 1997). Populations exist as aggregations, forming metapopulations of subpopulations (Hanski 1999). Spatial configuration of the subpopulations, such as spatial displacement (spread) between subpopulations and sizes of neighboring subpopulations, can greatly affect the population dynamics of a species (Hanski 1994). Spatial configuration influences dispersal between populations and persistence of populations (Flather and Bevers 2002), as well as competitive (Kerr et al. 2002), host-parasite (Nicholson and Bailey 1935; Hassell et al. 1991), and predator-prey (Huffaker 1958; Pascual et al. 2002) interactions. As human-driven habitat fragmentation increases and spatial arrangements are modified, we need to understand the effects of spatial arrangements on population dynamics and persistence to be able to predict the fate of species subjected to these habitat alterations and (where necessary) manage them appropriately.

In competition between two competitors in a single habitat, there are three basic outcomes: 1) stable coexistence, 2) exclusion of one competitor, and 3) initial condition dependence (Volterra 1926; Lotka 1932; Gotelli 2001). Here I assume that other effects like intraguild predation, which might introduce the possibility of limit cycles, is negligible. Under scenarios of competitive exclusion, maintenance of competitors can be facilitated by metapopulation dynamics and spatial configuration (Kerr et al. 2002; García-Ramos et al. 2007). When the competitors are the two sexes or various mating types of the same species, maintenance of competitors is vital for ensuring sexual reproduction and genetic diversity (Maynard Smith 1978). Competition between males and females can drive population

structure and dynamics (Nanami et al. 2005), potentially producing single-sex populations (McLetchie et al. 2002; Crowley et al. 2005a,b). Single-sex populations are often described as “evolutionary dead ends” and are expected to go extinct (Maynard Smith 1986 but see Judson and Normark 1996; Martens et al. 2003).

In clonal systems, the dispersal capabilities of asexual propagules can differ greatly from sexual propagules (Kimmerer 1991; Laaka-Lindberg et al. 2003; Cousens et al. 2008), which can substantially affect metapopulation dynamics and persistence. In most cases, sexual propagules are thought to be the main form of interpopulation dispersal (Hansson et al. 1992; Starfinger and Stöcklin 1995); therefore, single-sex metapopulations are assumed to be ephemeral. But asexual propagules are also known to disperse between subpopulations (Johansson and Nilsson 1993; Laaka-Lindberg et al. 2003; Pojamo et al. 2006; Cousens et al. 2008) and thus have the potential to influence population and metapopulation dynamics, presumably allowing single-sex metapopulations to persist.

Dispersal is a 'double-edged sword' (Hudson and Cattadori 1999); dispersal can support the persistence of species, but too much dispersal can synchronize subpopulations and potentially lead to a global extinction (Crowley 1981). Understanding the relationship between spatial synchrony (positive correlation between subpopulation dynamics) and dispersal-induced stability (persistence of a metapopulation due to dispersal among subpopulations), whether positive (Abbott 2011) or negative (Murdoch et al. 1992) is vital to ensure persistence and prevent extinction (Abbott 2011).

The dispersal differences and genetic differences between asexual propagules (being the same sex as the parent) and sexual propagules (being a mixture of the two sexes) can differentially affect metapopulation dynamics, greatly affecting the persistence of both sexes within the metapopulation and the persistence of the metapopulation itself. With regard to spatial synchrony and sex ratios in clonal organisms, asexual propagules will increase the correlation between subpopulations, while sexual propagules should decrease the correlation between subpopulations; asexual propagules will lead to a

loss of a sex while sexual propagules will help maintain both sexes. With regard to dispersal-induced stability, sexual and asexual propagules can both contribute to stability, assuming that subpopulations are close enough that dispersal among subpopulations is likely.

Using mathematical models, I will determine the effects of 1) the distance between neighboring subpopulations, 2) the arrangement of the subpopulations, and 3) directional bias in the dispersal of asexual propagules on the maintenance of the sexes and the persistence of the metapopulation.

Subpopulations can be separated by various distances that make 1) dispersal between subpopulations likely for both asexual and sexual propagules, 2) dispersal between subpopulations unlikely for asexual propagules but likely for sexual propagules, and 3) dispersal between subpopulations unlikely for either asexual or sexual propagules. I predict that metapopulations with subpopulations at distances allowing both asexual and sexual migrants will become synchronized, acting as a single uniform population, with the loss one sex (McLetchie et al. 2002; Crowley et al. 2005a,b) but persisence as a single-sex population. Metapopulations with subpopulations separated by distances where migration by either asexual or sexual propagules is unlikely will lose one sex. Unlike metapopulations where dispersal between subpopulation via asexual propagules is possible, these metapopulations will not persist. Arrangement and clumping of the subpopulations within the metapopulation could allow single-sex metapopulations that would otherwise go extinct to persist. I also determine the effects of asymmetrical asexual propagule dispersal on the maintenance of the sexes and the persistence of the metapopulation.

Model Organism

Marchantia inflexa Nees & Mont. is a New World non-vascular plant found along streams from northern Venezuela to the southern United States (Bishler 1984). Populations occupy rocks or the banks of streams in discrete patches. Stream systems containing multiple populations are considered metapopulations (sensu Freckleton and Watkinson 2002). Sex is chromosomally determined, with

seperate male and female individuals (Bishler 1986). Metapopulations may contain only one sex (either males or females) or both sexes (Fuselier and McLetchie 2004; Stieha, unpub data). In metapopulations containing both sexes, subpopulations run the gamut from containing all males, to some mixture of males and females, to all females (McLetchie and Puterbaugh 2000 Stieha, unpublished data).

Migrants to other subpopulations or unoccupied habitable patches can either be the products of asexual mechanisms or of sexual reproduction. Asexual dispersal can occur via two modes: 1) living vegetative material or 2) distinct asexual propagules. Vegetative material can be broken off and float downstream, possibly reaching suitable substrate. Although frequent in other organisms (Johansson and Nilsson 1993), this is thought to be rare. Both males and females produce asexual propagules (gemmae) in specialized structures known as gemmae cups. Gemmae are splashed out of the cups by falling rain (Brodie 1951). Gemmae are about 200 µm in diameter (Stieha, personal observation) and are dispersed by water (Schuster 1992). Sexual propagules (spores) are about 28 µm in diameter and are wind dispersed (Schuster 1992). The difference between the modes of dispersal (water versus wind) gives asexual propagules and sexual propagules different dispersal capabilities. Asexual propagules are thought to stay mainly within the source subpopulation, while sexual propagules are the primary form dispersal between subpopulations (Hansson et al. 1992; Starfinger and Stöcklin 1995; Laaka-Lindberg *et al.* 2003).

Mathematical models predict competitive exclusion of one sex by the other in *M. inflexa* (McLetchie *et al.* 2002, Crowley *et al.* 2005b), driven by life history differences between the sexes (McLetchie and Puterbaugh 2000 McLetchie *et al.* 2002). Males invest more in asexual reproduction than females, while females invest more in vegetative growth than males (McLetchie and Puterbaugh 2000). In undisturbed isolated populations, females vegetatively overgrow male plants, slowly driving them extinct (McLetchie et al. 2002; Crowley et al. 2005a,b). In frequently disturbed isolated populations, males are capable of persisting and can drive females extinct due to their ability to colonize

locally cleared habitat with asexual propagules (McLetchie *et al.* 2002, Crowley *et al.* 2005a,b). Metapopulation dynamics have been shown to allow both sexes to persist in a metapopulation, even though one sex may be gradually eliminated within individual subpopulations (García-Ramos et al. 2007). The previous models did not incorporate spatially complex configurations of subpopulations, which can influence the coexistence of both sexes within the metapopulation and the persistence of the metapopulation itself.

Model

I have extended the mathematical model focusing on within-subpopulation male and female competition (McLetchie et al. 2002) and maintenance of males and females in simplified metapopulations (García-Ramos et al. 2007) to include spatially complex metapopulations. I define patches to be habitable substrate. When a patch is occupied, I define it as a subpopulation.

Subpopulation Model

The life cycle of *Marchantia inflexa* is formulated as seven coupled ordinary differential equations describing seven life history stages (Figure 5.3, Table 5.1: 4 female stages and 3 male stages; McLetchie et al. 2002; García-Ramos et al. 2007). Both males and females have non-reproductive stages and asexually reproductive stages. Males have one sexually reproductive stage, while females have two sexually reproductive stages, unfertilized and fertilized. Differential equations ignore the within-patch spatial distribution of individuals and assume segments from each stage are randomly distributed within a patch (Table 5.1). A single population model that explicitly incorporates the within-patch spatial distribution of individuals gives similar results to the differential equation subpopulation model (Crowley et al. 2005b).

In nature, spores are wind dispersed and can stay within the subpopulation or disperse to other subpopulations or patches. I assume spore dispersal decays exponentially (Figure 5.2: Nathan and

Muller-Landau 2002; Cousens et al. 2008). Fertilized females (stage 1) produce male and female spores at a 1:1 ratio. Spores that land in a suitable area and germinate contribute to the non-reproductive stages of males (stage 2) and females (stage 5) in an equal ratio. Spore germination requires a threshold proportion of empty space within the patch (<0.4) to prevent allelopathic inhibition of spore germination by larger plants (McLetchie, unpublished data), with an upper limit of 1000 units of spores per patch germinating within a time step. The upper limit of the number of spores germinating per patch only affected population and metapopulation dynamics for extreme values (10 units or unlimited units; García-Ramos et al. 2007). Spores are assumed to germinate immediately and not persist in an ungerminated state (McLetchie, unpublished data).

The transitions between non-reproductive stages and reproductive stages are seasonally dependent. Non-reproductive tissue (stages 2 and 5, males and females, respectively) transitions to asexually reproductive tissue (stages 3 and 6) during the rainy season, September through December. Non-reproductive tissue (stages 2 and 5) transitions to sexually reproductive material (stage 4, males) and sexually reproductive non-fertilized tissue (stage 7, females) during the dry season, January through June (McLetchie, unpublished data; see García-Ramos et al. 2007). If both sexually reproductive male and female tissue (stages 4 and 7) is present within a subpopulation, unfertilized female tissue (stage 7) may become fertilized (stage 1) based on the amount of sexually reproductive male tissue (stage 4) in the subpopulation.

Similar to spore production by sexually reproductive stages, the asexually reproductive stages produce gemmae. The gemmae can contribute to the dynamics of the source subpopulation or disperse to neighboring subpopulations and unoccupied patches. The dispersal capabilities of the water-dispersed gemmae are greatly reduced compared to the wind-dispersed spores (Figure 5.2). Like spores, gemmae colonize unoccupied areas of a patch and are assumed to germinate immediately. Unlike spores, gemmae are not inhibited by adult plant tissue, although the amount of gemmae germinating is

directly proportional to the amount of unoccupied area within the patch. Competition can occur through colonization of unoccupied area and overgrowth competition of occupied area. Non-reproductive stages (stages 2 and 5, males and females, respectively) invest the most in vegetative growth and therefore are the most effective at overgrowth competition. Asexually reproducing stages for both males and females (stage 3 and 6, respectively) are capable of some vegetative growth. Sexually reproducing stages (stages 4,7, and 1) invest all energy into sexual reproduction and none into vegetative expansion.

Disturbance is a patch-independent phenomenon that removes a proportion of the individuals within the patch. In my model, I assume that within-patch disturbances occur on average of once every five months, but independently between months. When within-patch disturbance occurs, 20% of the individuals in each stage within the patch are removed. To convert the probability of within-patch disturbance from per month to per time step, I computed the probability of a disturbance during a timestep as $p'_{\Delta t} = 1 - (1 - p_d)^y$, where p_d is the probability of a disturbance per time interval T (which is a month) and y is the fraction of one month represented by the timestep duration determined by $\Delta t/T$. Time steps are one tenth of a month. For each subpopulation, I track the number of units in each stage. These units are either male or female and used to compute a sex ratio of the subpopulation, represented as proportion of males within the subpopulation.

Metapopulation Model

The metapopulation model (García-Ramos et al. 2007) is an extension of the subpopulation model (McLetchie et al. 2002); multiple subpopulations are simulated simultaneously, with interaction between subpopulations via dispersing asexual or sexual propagules. Subpopulations can be independently affected by extinction events, where all plant material is removed from a patch, leaving a completely unoccupied area. All patches are assumed to be homogeneous with respect to

environmental variables affecting growth rates, transition rates, and other parameters (Table 5.2). Patches differ in distances to particular neighboring patches (see *Spatial Model*).

I track the number of individuals of stage *i* in subpopulation *j* and determine the proportion of males in subpopulation *j*. Asexual and sexual propagules influence the source subpopulation and neighboring subpopulations, as well as recolonize unoccupied patches. The number of immigrants into a focal subpopulation is the sum of all emmigrants from neighboring subpopulations reaching the focal subpopulation. For each subpopulation, the number of dispersing units is computed by multiplying the amount of tissue in specific life history stages capable of producing propagules (stages 3 and 6 for asexual propagules, stage 1 for sexual propagules) by the production rate of the propagules per unit tissue (F_1 for sexual propagules, A_3 or A_6 for asexual propagules). Only a proportion of these propagules land in suitable environments (other subpopulations or patches) determined by the dispersal probability function of the propagule type (gemma or spore) and the distance from the subpopulation of origin to the target patch (Figure 5.2). Propagules are also limited by the amount of unoccupied space in the target patch. The proportion of occupied space directly decreases the number of asexual propagules germinating within a target patch. Sexual propagules are also affected by the proportion of unoccupied space and require a threshold level before germination can occur (>0.4 unoccupied space within a patch). The maximum number of sexual propagules to germinate within a patch is 1000. Upon germination, asexual propagules contribute to the non-reproductive life stage of the sex that produced them (males stage 2, females stage 5). Sexual propagules are assumed to be a 1:1 (male:female) ratio and contribute to both males (stage 2) and females (stage 5).

The metapopulation contains 30 patches that are 1 m^2, representing a carrying capacity of $2x10^4$ units of plant tissue. Assuming that the frequency distribution of spore dispersal distances is exponential, the fraction of spores dispersing from the focal subpopulation and landing in a patch *x* meters away is $f_{jk}(x) \approx [2h^2/(\pi\lambda_1|x|)]\exp(-|x|/\lambda_1)$, where *h* is half the distance of patch side (0.5 meters)

and λ_1 is the spore dispersal parameter. Due to wind dispersal, spores are assumed to be capable of dispersing in two dimensions in all directions from the source subpopulation (Higgins and Cain 2002; Levin et al. 2003) For gemmae, I assume two different types of dispersal: one-dimensional and two-dimensional. One-dimensional dispersal ignores the rapid increase in area that could potentially be reached in moving away from the source population. The fraction of gemmae dispersing from the focal subpopulation to a patch *x* meters away is $a_{jk}(x) \approx (h/z)\exp(-|x|/z)$, where *h* is half the distance of the side of the patch and *z* is the gemmae dispersal parameter value. Two-dimensional dispersal incorporates the increase in area which decreases the proportion of gemmae reaching a patch *x* meters away compared to one-dimensional dispersal. The equation for two-dimensional gemmae dispersal is the same as for spore dispersal, but replacing the spore dispersal parameter λ_1 with the gemmae dispersal parameter, z. In the model, distance between patches affects the number of dispersing propagules landing in a patch; as distance between patches increases, number of propagules decreases. Including both dispersal parameters makes the analysis comparable to previous work (see García-Ramos et al. 2007) and increases realism.

Subpopulations can experience within-subpopulation disturbance that removes some individuals from the subpopulation. To be a metapopulation, the possibility of a disturbance that removes all individuals is required (Hanski 1999; Freckleton and Watkinson 2002). I define this patch level disturbance as an extinction event, with a default average of occurring once in 40 years (p_e; Table 5.2; García-Ramos et al. 2007). Extinctions are spatially independent, affecting only the subpopulation and not its neighbors. The probability of an extinction was computed in the same manner as the probability of a disturbance, but using the patch extinction rate, p_e, as opposed to the patch disturbance rate, p_d. An extinction removes all individuals within the subpopulation as well as all immigrating propagules.

Spatial Model

Subpopulations are not isolated in space, but found in discrete locations with respect to other subpopulations within the metapopulation. The spatial configuration of the subpopulations with respect to one another could greatly influence the maintenance of the sexes within subpopulations and within the metapopulation and could influence whether a metapopulation persists or goes extinct (Hanski and Simberloff 1993). In my attempts to understand the effects of spatial configuration on the maintenance of the sexes, I explore 6 different spatial configurations (Figure 5.1): 1) Island Model (5.1A), 2) Linear Model of single patches (5.1B), 3) Linear model of pairs of patches (Doubles; 5.1C), 4) Linear Model of triplets of patches (Triplets; 5.1D), 5) a 5 by 6 arrangement of patches (Grid Model; 5.1E) and 6) a Randomized Network Model based off the Grid Model (5.1F, 5.1G). All metapopulations contain 30 patches that are capable of supporting a subpopulation of *M. inflexa*. I assumed an equal dispersal distribution of asexual propagules for all the spatial configurations. I looked at the effect of directional bias on the dynamics of Linear Model of single, pairs and triplets of patches and the grid model.

The island model is the simplest scenario where all dispersing propagules are equally likely to reach all other patches within the metapopulation (Levins 1969; Hanski 1991). The island model idealizes the concept of a minimum amount of dispersal required for maintenance of the sexes and persistence of the metapopulation.

The 3 linear models differ with respect to existence of patches to the left and right of one another and the number of these neighboring patches. The linear model of single patches contains all patches placed along a line, where all patches are upstream or downstream of one another. The linear model of pairs of patches (Doubles) conceptualizes a metapopulation along a stream where there are patches directly across from one another on both the left side and right side of the stream. The linear model of triplets of patches (Triplets) is an idealized stream with patches on both banks and one on an island in the middle of the stream. Streams had a fixed width of 5m, which is close to an observed

average of stream width (Stieha, personal observation) and within the assumed dispersal capabilities of asexual propagules (Figure 5.2).

I also look at the effects of a grid spatial arrangement on the maintenance of the sexes. Using a 5 by 6 arrangement of patches, I first run simulations where patches are found at intersections of the grids (Grid Model). To produce the Randomized Network Model, I begin with a 5 by 6 arrangement of patches, then I take *n* patches and randomly reassign them a location within an area defined by the initial 5 by 6 grid but without overlap between patches. When $n = 0$, I have the simple Grid Model. The Randomized Network Model allows me to look at the effect of randomness within spatial configuration on the maintenance of the sexes.

I am interested in the effects of distance between subpopulations, a proximate measure of the dispersal capabilities between subpopulations, on the maintenance of the sexes in the metapopulation and the persistence of the metapopulation. In Figure 1, I modified the distance labeled *x* to range from 1m (where all patches are right next to one another) to 200m (where dispersal of sexual propagules between subpopulations is unlikely). I modified the distance between subpopulations as opposed to the dispersal parameters (z and λ; Table 5.2) to allow my model to be compared to previous models (McLetchie et al. 2002; García-Ramos et al. 2007).

Because gemmae are water dispersed, I also incorporated a directional dispersal component. I looked at three scenarios: 1) no directional bias in dispersal, 2) strong directional bias, and 3) weak directional bias. Under no directional bias, gemmae could disperse uninhibited upstream or downstream. Strong directional bias allowed gemmae to disperse only downstream. According to Vuillemier et al. 2010, strong dispersal asymmetries can reduce a metapopulation's viability. Differences in dispersal of asexual and sexual propagules could influence the persistence of the metapopulation to an even greater degree. I tested directional bias in only a subset of spatial configurations of subpopulations within a metapopulation as presented under the heading *Spatial Model*. I determined if

a patch was classified as upstream or downstream of the source subpopulation. If the patch is upstream of the source subpopulation, gemmae are unable to disperse from the source subpopulation into that patch. If the patch is downstream of the source subpopulation, the number of gemmae reaching the patch was multiplied by two to compensate for the non-directional dispersal kernel described above. If the patch was perpendicular relative to the flow direction from the focal patch (across the stream), I allowed the gemmae from the source subpopulation to disperse into the patch. Weak directional bias allowed a fraction of the gemmae to disperse upstream (only 5% of the predicted), while the rest of the gemmae dispersed downstream. Dispersal capabilities to patches perpendicular to the flow direction from the focal patch were not affected.

Analysis

For each spatial configuration and distance between subpopulations, I ran 50 simulations using MATLAB 2011a (MathWorks, Inc. 2011). All simulations were run for 500 years with a time step of a tenth of a month. The first 100 years of a simulation were excluded from analysis to remove transient dynamics. To look at the effects of spatial configuration on the maintenance of the sexes, I started all subpopulations within the metapopulation with 10 units of males and 10 units of females. To understand the persistence of metapopulations once one sex had been lost, I ran simulations starting with 20 units of one sex in all patches. For each simulation, I tracked the metapopulation sex ratio (proportion of males) at each time step as the total number of male tissue (Equations 2, 3, and 4) divided by the total amount of plant tissue in the metapopulation. I then averaged the sex ratio of all the time steps after 100 years to get the simulation mean sex ratio. I also tracked whether the metapopulation lost one sex or went extinct. Mean sex ratio for a metapopulation losing one sex was the final proportion of males in the system (0 for an all-female metapopulation, 1 for an all-male metapopulation). When presenting sex ratios, I focus only on simulations that maintained both sexes. If the metapopulation went extinct, the mean sex ratio for that simulation was removed from any sex

ratio analysis. I also tracked the proportion of subpopulations within the metapopulation that contained any individuals at each time step and averaged this over the whole run to compute the average patch occupancy. All graphs were made in R (R Core Development Team 2012).

Results

Spatial Configuration – Island Model

For the Island spatial configuration, all-female metapopulations went extinct when the distance among the subpopulations was 10 meters or more (both 1 dimension (1D) and 2 dimension (2D); data not shown). Island metapopulations containing only males went extinct when the distance among subpopulations was 8 meters or more (both 1D and 2D; data not shown). When the metapopulations were started with both males and females, more than half the simulations lost one sex when the distance among subpopulations was greater than 60m. Many of those single-sex metapopulations went extinct (Figure 5.4a,b). For both 1-dimensional and 2-dimensional dispersal, the proportion of males increased as the distance between subpopulations increased, ranging from about 0.05 to 0.15 depending on the distance among subpopulations (Figure 5.5a,b).

Spatial Configuration – Single Linear System

When linear metapopulations were initialized with only one sex, all-male metapopulations went extinct when distances among subpopulations were 9m and above (both 1D and 2D), while all-female metapopulations went extinct at 20m and above (both 1D and 2D; data not shown). When metapopulations were initialized with both sexes and the distance between neighboring patches was >= 20m, the metapopulations lost one sex (Figure 5.4c,d) and went extinct (Figure 5.5c,d). For distances of less than 10m between neighboring patches, the simulations were likely to lose a sex, but this decreased as distance increased (Figure 5.4c,d). These single-sex metapopulations did not go extinct (Figure 5.4c,d). If both sexes were present at the end of the simulations, the proportion of males increased as

distance between neighboring patches increased until 20m (Figure 5.5c,d). After 20 meters, all metapopulations lost one sex before the end of the simulations (Figure 5.5c,d). When metapopulations started with both sexes, directional bias of dispersal had no observable effect on the maintenance of the sexes, the sex ratio, or persistence of the metapopulation in a linear arrangement (data not shown).

Spatial Configuration – Linear system, Double

When a metapopulation with patches arranged in pairs (Doubles) started with a single sex (either male or female), the metapopulation did not go extinct, even at distances of 100 and 200 meters between pairs of patches (results not shown). When the metapopulations with doubles were initialized with both sexes, metapopulations with doubles (pairs) near each other (Figure 5.4e) or with doubles (pairs) far from each other (Figure 5.4e, f) were more likely to produce single-sex metapopulations. These single-sex metapopulations did not go extinct (Figure 5.4e,f). For intermediate distances between doubles, 1-dimensional dispersal was more likely to produce single-sex metapopulations than 2-dimensional dispersal (Figure 5.4e,f). For both 1-dimensional and 2-dimensional dispersal of asexual propagules, sex ratio increased as distance between subpopulations increases (Figure 5.5e,f). Metapopulations with distances of 20m and 30m between doubles did not maintain both sexes in the 1-dimensional dispersal scenario, but did maintain both sexes under the 2-dimensional dispersal scenario (Figure 5.5e,f).

When metapopulations were initialized with both sexes, directional bias affected the persistence of single-sex metapopulations at distances greater than 20m. When there was no directional bias, the single-sex metapopulation persisted. In both scenarios with directional bias, the single-sex metapopulation went extinct. For scenarios with and without directional bias, the dynamics did not appear to differ for distances less than 20m. The sex ratios of the metapopulations with both sexes were similar under all dispersal scenarios (data not shown).

Spatial Configuration - Linear system, Triplets

Similar to the metapopulations with doubles, single-sex metapopulations with triplets (three patches near one another) did not go extinct, even at distances between triplets of 200m (results not shown). When triplets were initialized with males and females, the dynamics of metapopulations changed, depending on the distance between triplets and the dispersal dimension of gemmae (1D or 2D). The one-dimensional dispersal scenario had many simulations at all distances losing one sex (Figure 5.4g), while the 2-dimensional dispersal scenario had metapopulations becoming single-sex when distances between triplets were greater than 10 meters (Figure 5.4h). For 1-dimensional dispersal, sex ratios were between 0.03 and 0.04 males (Figure 5.5g), while for 2-dimensional dispersal, the sex ratios were 0.04 to 0.06 males (Figure 5.5h). Much like the doubles spatial configuration, the triplets spatial configuration was affected by directional bias of dispersal. In the presence of directional bias, when the triplets were greater than 30 meters apart, the metapopulations lost one sex and then went extinct. The transition between persistence of both sexes and loss of one sex occurred at a greater distance for dispersal with a directional bias than with no bias (30m versus 10m). When a metapopulation contained both sexes, the sex ratio was greater for the dispersal with directional bias (ranging from 0.05 to 0.12 male depending on the distance between triplets; not shown) than the dispersal with no directional bias (<0.05 males for all distances; Figure 5.5g,h).

Spatial Configuration – Grid Model

When grid model metapopulations were initialized with only a single sex, female-only metapopulations went extinct when distances were above 20 meters and 8 meters (1-dimensional and 2-dimensional dispersal, respectively; data not shown). Male-only metapopulations went extinct after 9 meters and 7 meters (1D, 2D respectively; data not shown). When grid metapopulations were initialized with two sexes, metapopulations lost one sex and went extinct starting at 40 meters apart (Figure 5.4i,j). In simulations containing both males and females in the metapopulation after 500 years, the proportion

of males increased as distance between subpopulations increased and was as high as 14% males in the metapopulation (at 40 meters between patches; Figure 5.5i,j). Dispersal with a directional bias did not affect the metapopulation dynamics of the grid model (data not shown).

Spatial Configuration – Randomized Grid Model

The randomized grid model started with 30 patches arranged in a 5 by 6 grid separated by distance *d*. I then randomized a set number of patches within the confines of the 5 by 6 grid. For the 1-dimensional dispersal scenario (Figure 5.6a), when patches were close to one another (*d* = 2m), almost half the simulations became single sex for all randomization scenarios. When the randomized grid started with distances from 4 meters to 30 meters between neighboring patches, about ten percent of simulations lost one sex. The number of simulations that lost one sex appeared to increase as the number of patches randomized increased (Figure 5.6a). At a distance of 40 meters, about half of the simulations lost one sex. At distances greater than 40 meters, most simulations lost one sex. The 2-dimensional dispersal scenario was similar to the 1 dimensional scenario for distances greater than 4 meters between neighboring patches (Figure 5.6b). For shorter distances, very few simulations lost a sex. In both the 1-dimensional and 2-dimensional scenarios, the number of simulations that went extinct decreased as the number of randomized patches increased, although the decline appeared to decrease as the number of randomized subpopulations was greater than half the total number of patches (Figure 5.6c,d). Randomization saved metapopulations from extinction, (compare the value of no randomization at 0 to anything greater than 0), but all the patches within the metapopulation did not contain populations - only a fraction of the patches were occupied, sometimes as low as only 10% of patches (results not shown).

Discussion

The spatial configuration of the subpopulations within the metapopulation determines whether both sexes are maintained at the metapopulation level and whether the metapopulation persists once one sex is lost. In many scenarios, when subpopulations were close enough to one another where they could easily exchange asexual propagules, the subpopulations were synchronized and acted as a single population. Single populations (McLetchie et al. 2002; Crowley et al. 2005a,b) and strongly connected metapopulations (García-Ramos et al. 2007) will lose one sex when sexes compete intensively. Whether gemmae dispersal was 1-dimensional or 2-dimensional greatly influenced the number of propagules reaching other subpopulations and the synchrony of the system. Given the rectangular nature of streams (not simply linear due to banks and rocks within the stream but not truly 2 dimensional as the plants cannot live far from the stream), the actual dispersal function falls in between the two dispersal scenarios.

If patches were far apart and migration was unlikely to occur via asexual or sexual propagules, the metapopulation lost one sex through competitive exclusion. Because the metapopulation contained only one sex and could not produce sexual propagules capable of long distance dispersal (Figure 5.2), the patches remained empty as subpopulations went extinct. As more subpopulations went extinct and were not recolonized, the metapopulation slowly went extinct. If subpopulations exhibited some form of clumping (doublets and triplets in Figure 5.1 or through the randomization process), the metapopulation could persist. Despite long distances between clumps of subpopulations, the metapopulation persisted because the neighboring subpopulations were capable of recolonizing empty neighboring patches. These clumps may be able to support patches prone to extinction by contributing migrants (Vulleumier et al. 2010) or may be the only patches within the metapopulation that persist (see Results of *Spatial Configuration – Randomized Grid Model*).

Doubles and Triplets spatial configurations are substantially affected by directional dispersal. At greater distances between patches, dispersal without a directional bias would produce single sex metapopulations that persisted. But when the same spatial configurations were combined with a directional biased dispersal, the metapopulation lost one sex and then went extinct. Horizontal dispersal (dispersal perpendicular to the biased dispersal direction) can increase the likelihood that a metapopulation will persist (Vuilleumier et al. 2010). In my case, dispersal with a directional bias appears to decrease the ability of a subpopulation to recolonize its neighboring patch, increasing the probability both will go extinct and increasing the speed of the metapopulation's extinction.

The model assumes that extinctions are not correlated in space and time. If extinctions were correlated in space and time, the metapopulations with clumped patches may be less likely to persist, as an extinction event would affect all subpopulations in the area and not leave any subpopulations to recolonize the empty patches. When distances between patches are such that asexual propagules are not likely to reach other subpopulations but sexual propagules are likely to do so, both sexes are more likely to persist. In this range, dispersal-induced stability maintains both sexes and allows the metapopulation to persist. At short distances, where dispersal of asexual propagules is likely, spatial synchrony leads to the loss of sex, but the metapopulation still persists. Stability of the metapopulation is maintained in both cases, but synchronization leads to the loss of a sex, adding another level to the spatial synchrony – dispersal-induced stability debate (Abbott 2011).

Increasing distance between subpopulations decreases the proportion of habitat occupied relative to the amount of area simulated. The proportion of habitat occupied has been shown to be the driving force of population dynamics, but habitat arrangement, such as length of habitat edge and size of largest patch, also affects population dynamics (Flather and Bevers 2002). Despite controlling for subpopulation number as opposed to proportion of available habitat, I show that spatial arrangement affects population dynamics between two competitors. For example, when subpopulations are 20

meters apart, 30 subpopulations in a linear arrangement are along 600 meters of stream. When I look at paired subpopulations, the same number of subpopulations (15 pairs of subpopulations) simulates 300 meters of stream. The stability of the two systems is completely different. Both linear and double spatial configurations lose one sex, but the linear configuration goes extinct while the double configuration persists. If extinctions are correlated in space and time (as speculated above), the second configuration could go extinct as quickly as, or even faster than, the first (as multiple subpopulations go extinct due to a single extinction event), although work is needed on autocorrelation of extinction events (González-Megías et al. 2005; Kallismanis et al. 2005).

Interactions between individuals can greatly affect the synchronization of populations. Interactions in the *M. inflexa* system are competitive in nature, with one sex being driven extinct through competitive exclusion. Both sexes, however, are required for sexual reproduction, which introduces an interdependence in the system. In simple linear multitrophic systems, decreasing the distance (the likelihood of dispersal) between two communities decreases spatial synchrony of the populations within the communities, dependent on the migrating species (Koelle and Vandermeer 2005). With competition between sexes, decreasing distances between subpopulations increases synchrony, leading to a loss of one sex. Complex multiptrophic systems with competition may exhibit competitive loss as well.

In this paper, I have confirmed results found in García-Ramos et al. (2007) and extended the model to include a greater variety of spatial configurations, distances between patches (which affects dispersal of both asexual and sexual propagules), and dispersal with directional bias. Spatial configuration contributes to maintaining the sexes and sexual reproduction in the face of asexual competitors (Peck et al. 1999). My results suggest that spatial configuration is important in maintaining both sexes but can also facilitate the loss of one sex (depending on the dispersal capabilities of migrants) and increase the extinction probability of the metapopulation as a whole. Although not explicitly tested

here, distance to nearest neighbor has been shown to affect the maintenance of the sexes within subpopulations (Stieha, unpublished manuscript). Compared to metapopulations containing both sexes, single-sex metapopulations appear to contain a greater density of subpopulations within a stream reach and be dominated by a single large subpopulation (or aggregate of subpopulations) that could produce source-sink dynamics (Stieha, personal observation). A more extensive comparison of single-sex versus two-sex metapopulations from nature is required, as well as an analysis of the relationship between variation of sex ratios in subpopulations and the spatial arrangement of those subpopulations.

Table 5.1. Equations describing the population dynamics of male and females stages of M. inflexa.

Stage	Stage Dynamics	Eqn. no.
Fertilized Females	$\frac{dN_{1j}}{dt}=\frac{M_7 N_{4j} N_{7j}}{K}-T_{51}N_{1j}-N_{1j}\sum_{i=2,i\neq 4}^{6} G_i \frac{N_{ij}}{K}$	eqn 1
Non-reproductive Males	$\frac{dN_{2j}}{dt}=(F_1\sum_{k=1}^{n} f_{jk}N_{1k}+A_3\sum_{k=1}^{n} a_{jk}N_{1k})(1-\sum_{i=1}^{7}\frac{N_{ij}}{K})+T_{23}N_{3j}+T_{24}N_{4j}$ $-(T_{32}+T_{42})N_{2j}+G_2 N_{2j}(1-\frac{N_{2j}}{K})-N_{2j}\sum_{i=3,5,6} G_i\frac{N_{ij}}{K}$	eqn 2
Asexually reproductive males	$\frac{dN_{3j}}{dt}=T_{32}N_{2j}-T_{23}N_{3j}+G_3 N_{3j}(1-\frac{N_{3j}}{K})-N_{3j}\sum_{i=2,5,6} G_i\frac{N_{ij}}{K}$	eqn 3
Sexually reproductive males	$\frac{dN_{4j}}{dt}=T_{42}N_{2j}-T_{24}N_{4j}-N_{4j}\sum_{i=2,i\neq 4}^{6} G_i\frac{N_{ij}}{K}$	eqn 4
Non-reproductive females	$\frac{dN_{5j}}{dt}=(F_1\sum_{k=1}^{n} f_{jk}N_{1k}+A_6\sum_{k=1}^{n} a_{jk}N_{6k})(1-\sum_{i=1}^{7}\frac{N_{ij}}{K})+T_{51}N_{1j}+T_{56}N_{1j}$ $+T_{57}N_{7j}-(T_{65}+T_{75})N_{5j}+G_5 N_{5j}(1-\frac{N_{5j}}{K})-N_{5j}\sum_{i=2,3,6} G_i\frac{N_{ij}}{K}$	eqn 5
Asexually reproductive females	$\frac{dN_{6j}}{dt}=T_{65}N_{5j}-T_{56}N_{6j}+G_6 N_{6j}(1-\frac{N_{6j}}{K})-N_{6j}\sum_{i=2,3,5} G_i\frac{N_{ij}}{K}$	eqn 6
Sexually reproductive females	$\frac{dN_{7j}}{dt}=\frac{-M_7 N_{4j} N_{7j}}{K}T_{75}N_{5j}-T_{57}N_{7j}-N_{7j}\sum_{i=2,i\neq 4}^{6} G_i\frac{N_{ij}}{K}$	eqn 7

Table 5.2. Parameter values of the default model used to predict sex ratio and sex bias index.

Symbol	Definition	Magnitude	Units
Life-history			
G_2, G_5	Growth rates for Non-reproductive stages 2 and 5	0.585, 0.605	$month^{-1}$
G_3, G_6	Growth rates for asexually reproductive stages 3 and 6	0.220, 0.233	$month^{-1}$
T_{42}, T_{75}	Stage transition rates 2→4 and 5→7	0.714	$month^{-1}$
T_{23}, T_{24}, T_{56}, T_{57}	Stage transitions from 3→2, 4→2, 6→5, and 7→5	2.14	$month^{-1}$
T_{32},T_{65}	Stage transition rates 2→3 and 5→6	1.11, 0.833	$month^{-1}$
T_{51}	Stage transition rate 1→5	0.638	$month^{-1}$
M_7	Fertilization rate	10	$month^{-1}$
A_3, A_6	Asexual reproduction rates by stages 3 and 6	1.112, 0.519	$month^{-1}$
F_1	Sexual reproduction rate by stage 1	442	$month^{-1}$
Dispersal and establishment			
λ_1, λ_2	Spore dispersal parameters – decay rates	0.7, 10	m
p	Proportion of spores dispersing by λ_1	0.27	
z	Gemma dispersal parameter – decay rate	0.83	m
q	Uncovered threshold for spore germination	0.4	
r	Maximum spore establishment rate	1000	unit $month^{-1}$
Patch System			
$4h^2$	Patch size	1.11, 0.833	m^2
u	Size of a unit of M. inflexa tissue	5×10^{-5}	m^2 $unit^{-1}$
K	Patch carrying capacity, $K=4h^2/u$	20 000	units
n	Number of patches	30	
x	Distance between patches	varies	m
Disturbance and extinction			
p_d	Probability of a patch disturbance in a month	0.2	
p_e	Probability of a patch extinction in a month	0.002	

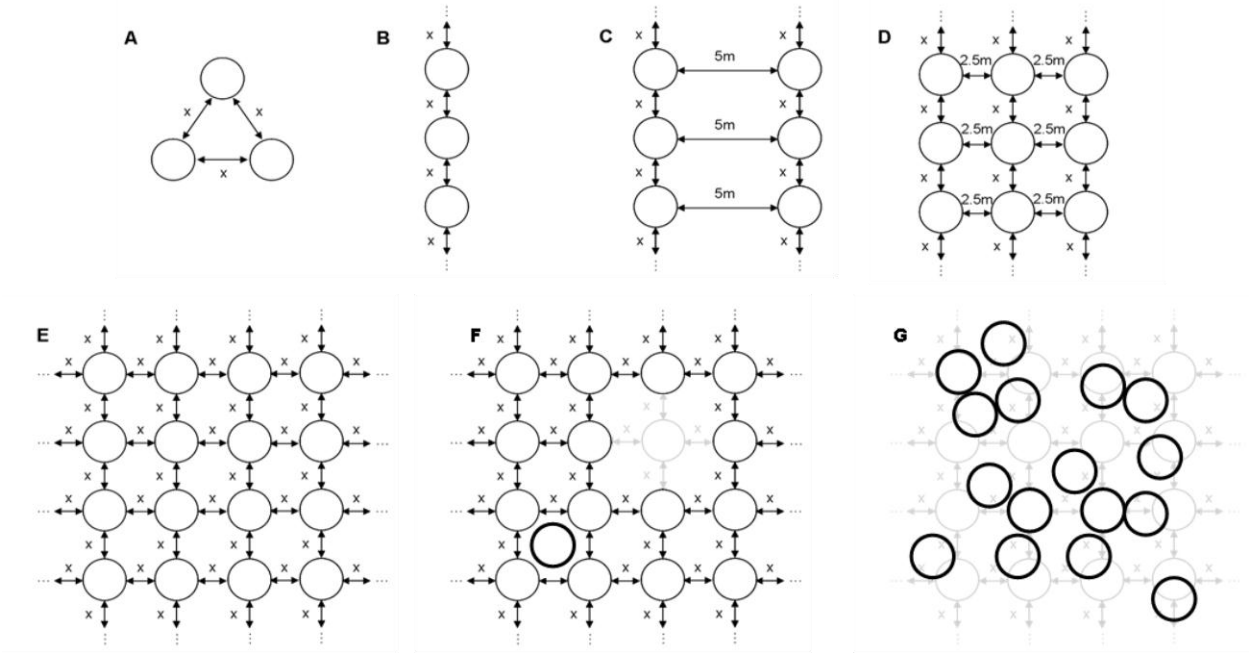


Figure 5.1. Diagram of subpopulation arrangements. The variable x represents the distance that can change across simulations for a specific scenario. Within one scenario for a single simulation, all x values are the same. Dots represent directions where other subpopulations occur. For each spatial arrangement, I modeled 30 patches. The spatial arrangements are: A) Island model, B) Linear model of single patches, C) Linear model of pairs of patches (doubles), D) Linear model of triplets of patches (triples), E) Grid model, and G&F) Randomized Network Model. The Grid model is the Randomized Network Model when no patches are redistributed on the landscape. Grayed out area in Figure G and F show the previous location of subpopulations uniformly distributed (Figure E). Darker circles represent the new locations of the subpopulations after randomizations (Figure G and F only).

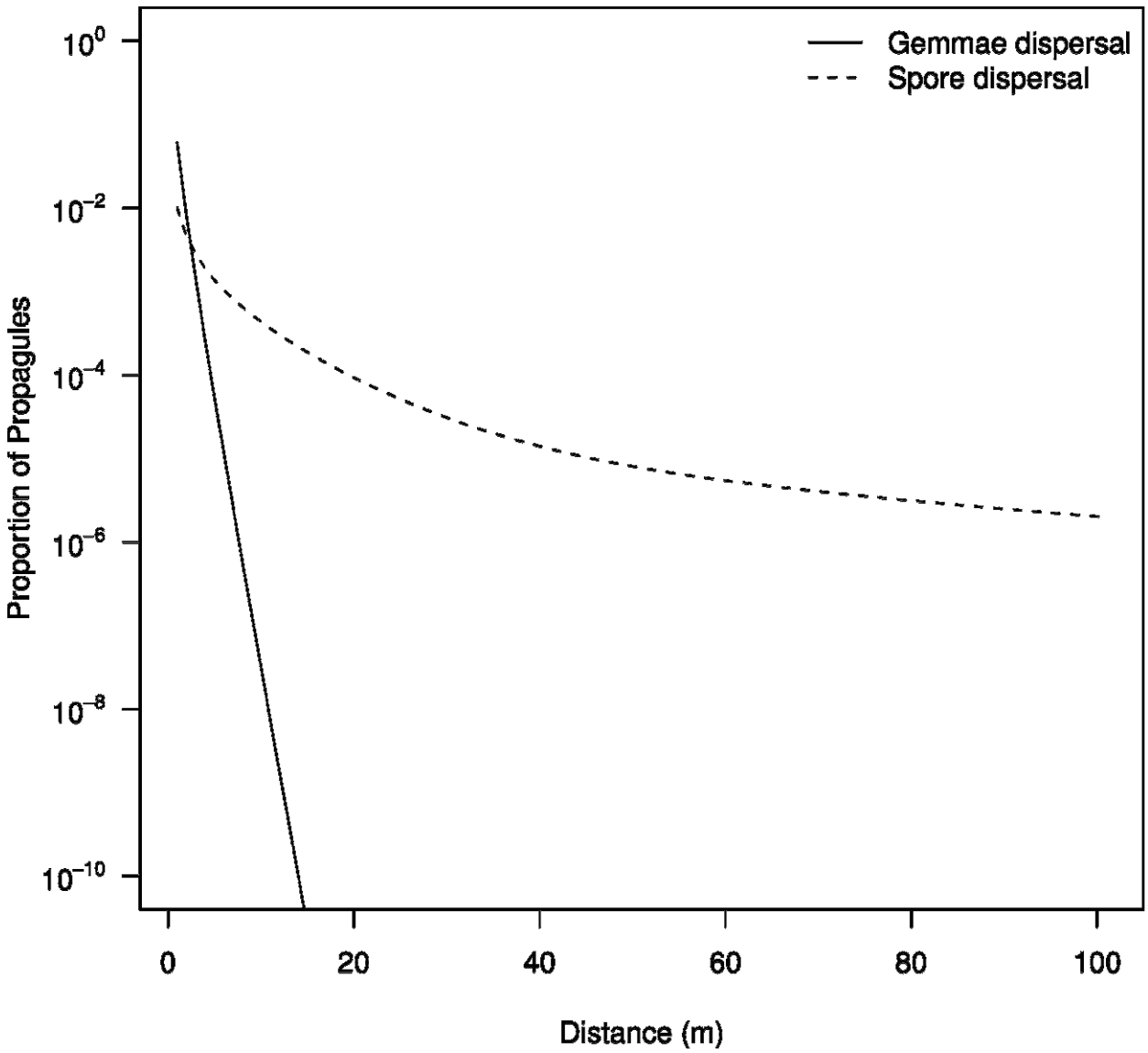


Figure 5.2. Dispersal capabilities of sexual and asexual propagules. The x axis is the distance from the source population. The y axis represents the proportion of gemmae leaving the source population that land in a $1m^2$ patch a specific integer distance *x* from the source population. The y axis is a log plot. The dashed line represents the proportion of sexual propagules reaching a specific patch *x* meters away. The solid line represents the dispersal of asexual propagules, most of which stay within the source subpopulation. For this graph, I assume 1-dimensional dispersal of gemmae and 2-dimensional dispersal of spores.

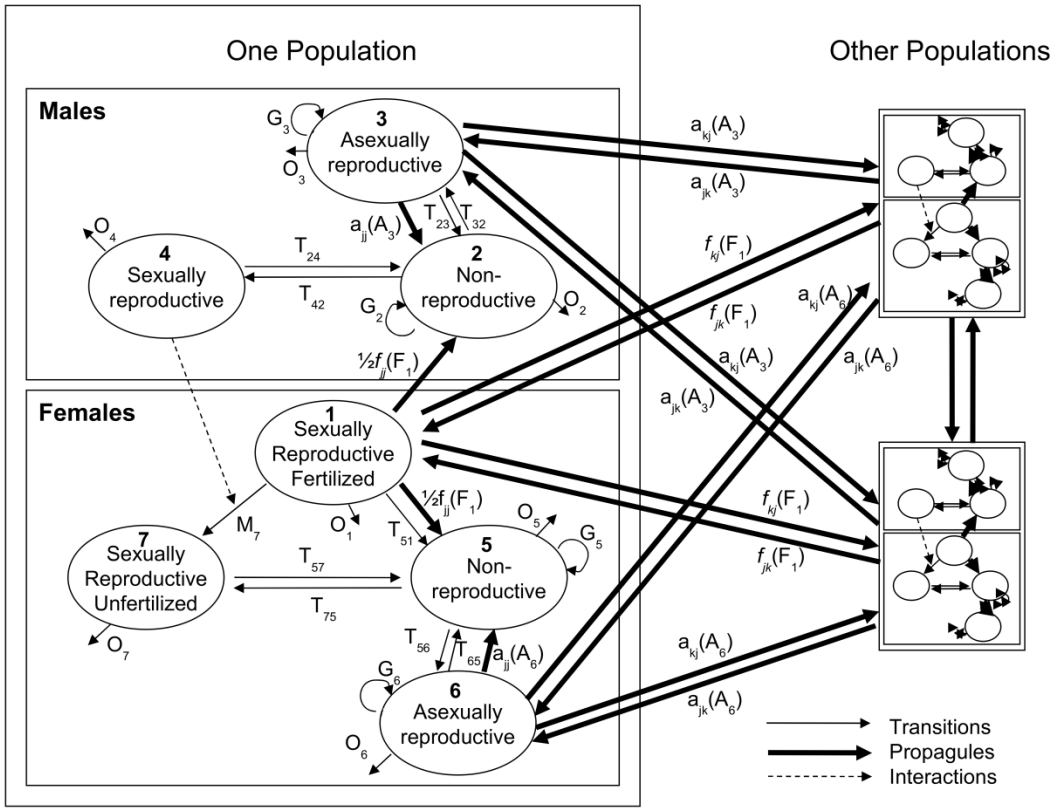


Figure 5.3. Transition diagram of the model. Ovals represent the various stages described in the *Model* section. The diagram shows the interactions and transitions within a single subpopulation and the interactions between subpopulations within a metapopulation. Normal lines represent possible transitions between the various stages. T's represent transitions between the various stages. G's represent addition to the tissue through growth. O's represent loss of tissue through overgrowth competition. All stages can experience death and be removed through disturbance events. The dashed line represents the interaction between the male sexually reproductive stage and the female unfertilized sexually reproductive stage to transition the female from unfertilized to fertilized. Bold lines represent the influence of propagules, either sexual or asexual, on the various stages. Asexual propagules are produced at a rate of A_3 (males) or A_6 (females) per unit tissue. Only a subset of the asexual propagules stays within the source subpopulation (a_{jj}) while the remaining subset migrates out of the focal subpopulation. Migrating asexual propagules can reach other patches and subpopulations, denoted by a_{kj}. Sexual propagules disperse similarly as asexual propagules, but are produce by only one stage, the female fertilized sexually reproductive stage, at a rate F_1. Unlike asexual propagules, sexual propagules are assumed to contribute to the non-reproductive stages of males and females equally. This represents the 1:1 sex ratio of spores. The proportion of sexual propagules that stay in the focal subpopulation is denoted by f_{jj} while the proportion that disperse from the focal subpopulation to a neihgboring subpopulation is f_{kj}. Distance between the two subpopulations affects the dispersal rate.

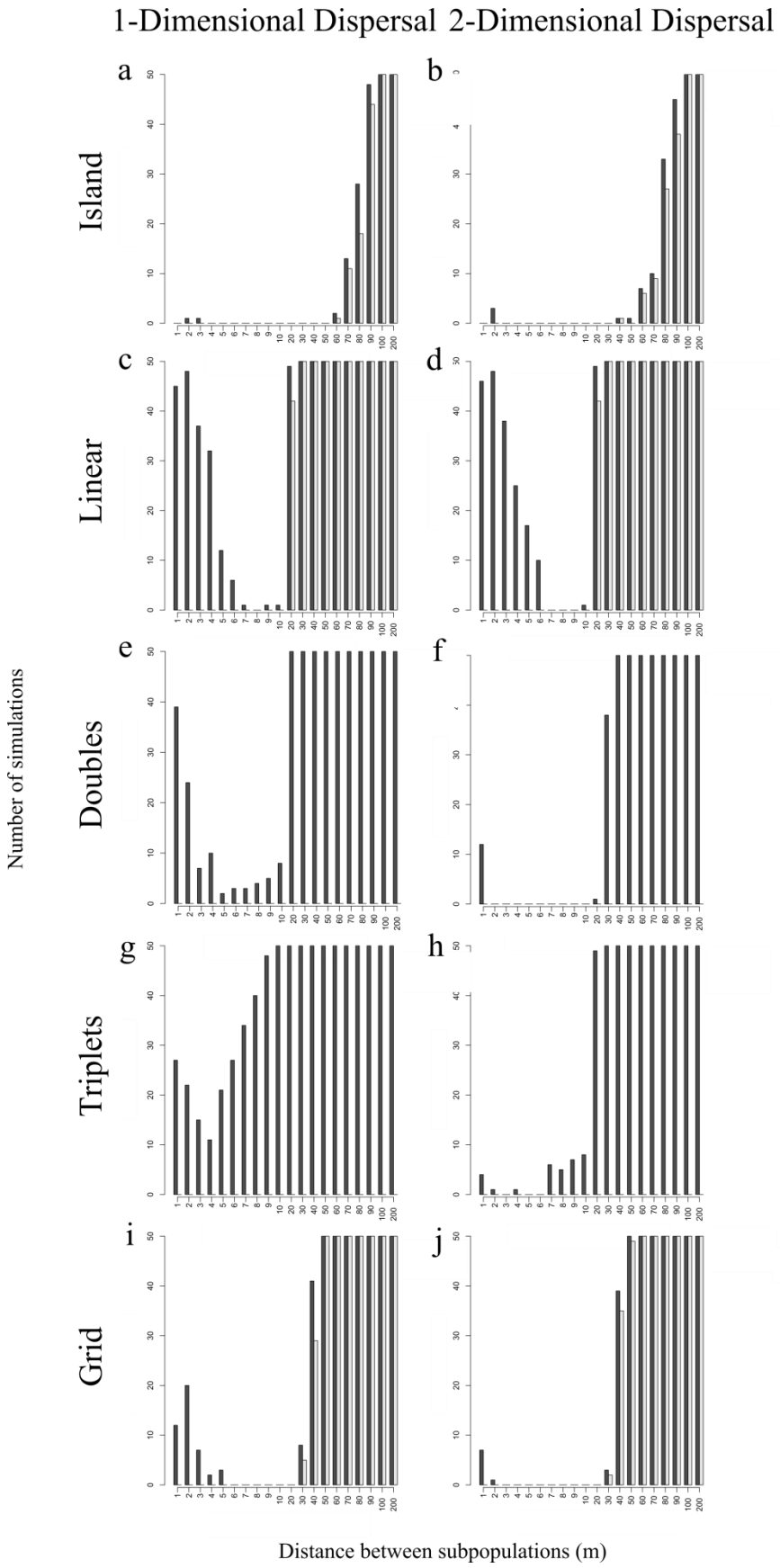

1-Dimensional Dispersal
2-Dimensional Dispersal
Island
Linear
Doubles
Triplets
Grid
a
b
c
d
e
f
g
h
i
j
Number of simulations
Distance between subpopulations (m)

Figure 5.4. Effects of spatial configuration and dispersal on the maintenance of the sexes and persistence of single-sex metapopulations. Dark bars represent the number of simulations out of 50 that had lost one sex at the end of the 500 simulated years. Grey bars represent the number or metapopulations that were extinct at the end of the simulations. Given my parameters (McLetchie et al. 2002; García-Ramos et al. 2007), single-sex metapopulations were all female. Of the simulations that lose one sex (the dark bars), some fraction of these metapopulations then go extinct (gray bars). Persisting single-sex metapopulations could be going extinct, but were not extinct by the end of the simulation. Metapopulations that contain both sexes do not go extinct. The asexual dispersal function could be described in either 1 dimension (1-dimensional; Figures a,c,e,g,i) or 2 dimensions (2-dimensional; Figures b,d,f,h,j), denoted by the heading above the two columns. Rows represent each of the five spatial arrangements that were simulated: Island model (a,b), Linear model of singles (Linear; c,d), Linear model of pairs (Doubles; e,f), Linear model of triplets (Triplets; g, h), and the Grid model (Grid; i, j). Distance between subpopulations in meters (as discussed in Figure 1) were:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200. These values represent a wide range of the distances observed in nature (Stieha, unpublished data) while also testing the extreme limits of the dispersal parameters (Figure 2).

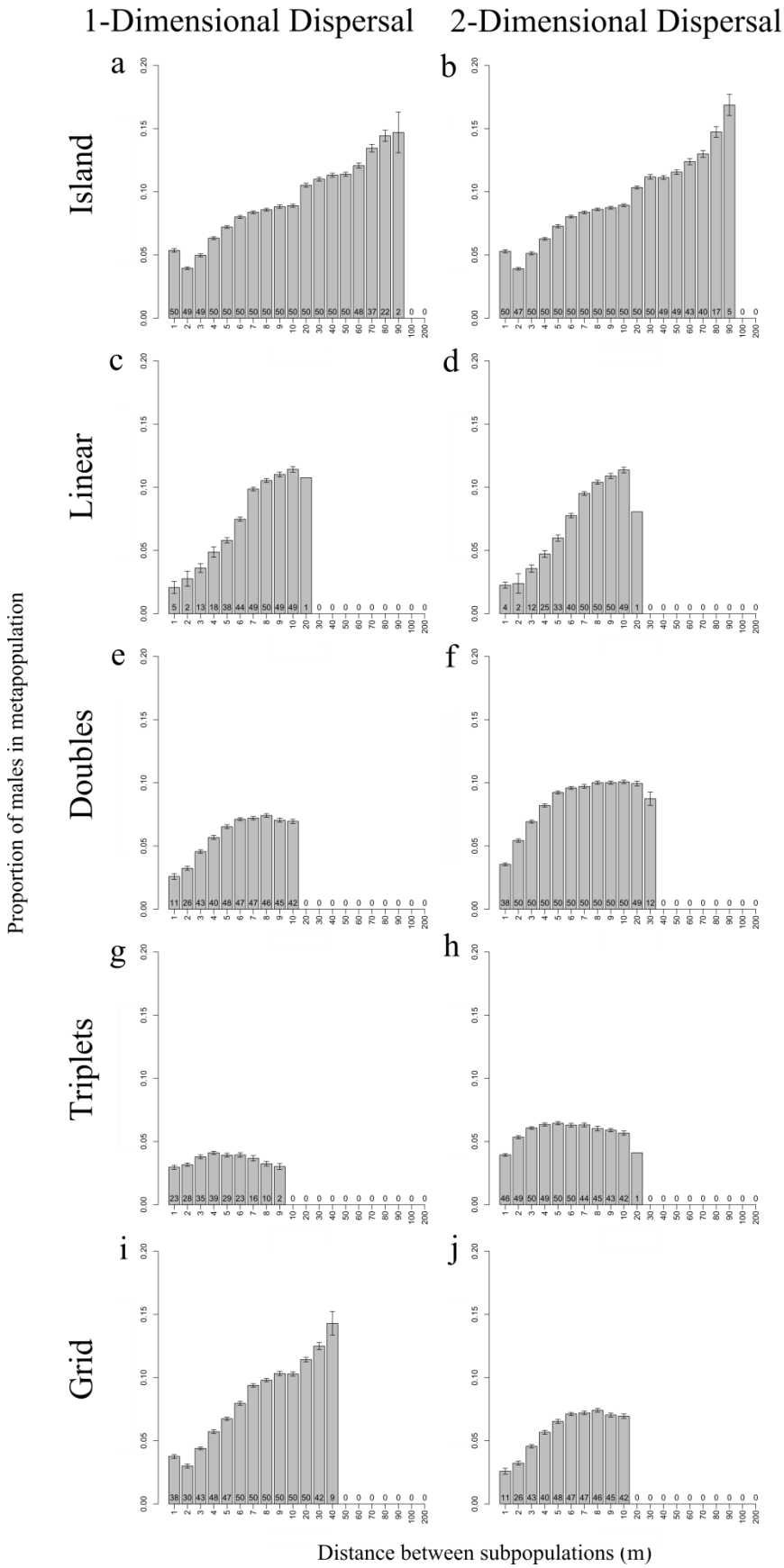

1-Dimensional Dispersal
2-Dimensional Dispersal
Island
Linear
Doubles
Triplets
Grid
a
b
c
d
e
f
g
h
i
j
Proportion of males in metapopulation
Distance between subpopulations (m)

Figure 5.5. Sex ratio of metapopulations containing both sexes. The asexual dispersal function could be described as 1-dimensional (Figures a,c,e,g,i) or 2-dimensional (Figures b,d,f,h,j), denoted by the heading above the two columns. Rows represent each of the five spatial arrangements that were simulated: Island model (a,b), Linear model of singles (Linear; c,d), Linear model of pairs (Doubles; e,f), Linear model of triplets (Triplets; g, h), and the Grid model (Grid; i, j). Distance between subpopulations in meters were:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200. Each bar chart represents the proportion of males in the metapopulation averaged over the last 400 years of the simulation. Numbers at the bottom of each column represent the number of simulations out of 50 where both sexes were present at the end of the simulation. Only simulations that contained both sexes at the end of the simulation were used to compute sex ratios for this graph.

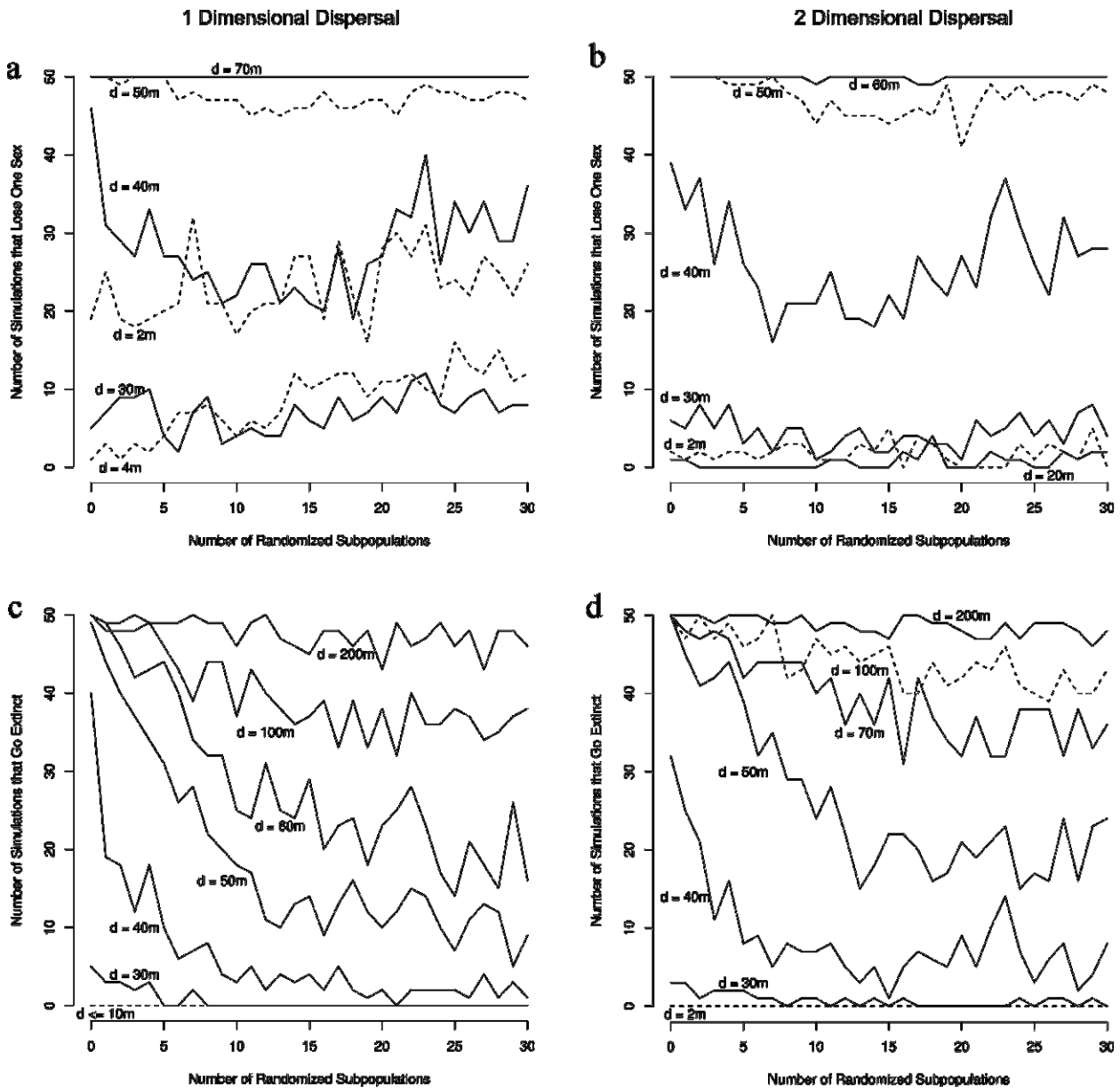


Figure 5.6. Effect of randomization of patch location on the maintenance of the sexes and persistence of single sex metapopulations. Asexual propagules function can either be 1-dimesional (Figures a, c) or 2-dimensional (Figures b, d). Figures a and b show the number of simulations where the metapopulation loses one sex before the end of the simulation (500 years). Figures c and d show the number of simulations where the metapopulation goes extinct before the end of 500 years. Individual lines are labeled with the distance *d* between the patches in the uniform grid before randomization. Dashed lines and solid lines are used to make differences easier to see. Only a subset of all distances are shown for clarity. Even though a metapopulation persists, not all patches necessarily contain individuals, whether it is a temporary extinction or permanent extinction due to no possibility of immigration.

Copyright ©Christopher Stieha 2012

CHAPTER SIX

Predicting the Maintenance of the Sexes in a Clonal Organism using Mathematical Models

Introduction

The spatial arrangement of populations can greatly affect the dynamics of individual as well as neighboring populations (Hanski 1999). Metapopulation theory incorporates the concept that populations do not exist in isolation: they exist as an aggregation of subpopulations that weakly interact with one another through migration (Hanski 1999). The spatial configuration of the subpopulations, defined as the sizes of the subpopulations and the distance between them, can strongly influence the stability and persistence of the metapopulation and the subpopulations within it (Huffaker 1979; Hanski 1994, 1999; Peck et al. 1999; Kerr et al. 2002).

Since metapopulation theory is used heavily in conservation and preserve design, understanding the importance of spatial configuration for subpopulation and metapopulation dynamics is vital for choosing locations for species reintroductions as well as areas to preserve. Reintroducing a species in an aggregation of patches that cannot support a metapopulation is counterproductive and wasteful. As habitat fragmentation affects the spatial configuration of suitable habitat, estimating the stability of metapopulations becomes increasingly important. However, our understanding of the effects of spatial configuration on metapopulation persistence need to be confirmed. We need not only models and predictions, but tests of the model predictions using natural systems. In this paper, I will use mathematical models to make predictions and test those predictions with data from naturally occurring metapopulations.

Testing of mathematical models used to understand the persistence of subpopulations and metapopulations is particularly challenging (Thomas and Hanski 1997), requiring a well-studied system and well developed models. Mathematical models have contributed to our understanding of the maintenance of the sexes in dioecious clonal organisms (McLetchie et al. 2002; Crowley et al. 2005 a,b; García-Ramos et al. 2007) of a well-studied organism (McLetchie and Puterbough 2000; Fuselier and

McLetchie 2004, Fuselier 2008, Groen et al. 2010, Stieha unpublished); predictions of sex ratios generated by these models have yet to be tested against metapopulation data from the field.

The maintenance of the sexes can sometimes be influenced by a unique form of competition, where maintenance occurs when competition does not lead to competitive exclusion. As for other systems with competitive exclusion (Peck et al. 1999; Kerr et al. 200), the maintenance of the sexes depends on metapopulation structure and dynamics, where subpopulations are loosely connected by dispersal (Peck et al. 1999). Subpopulations depend on migrants to re-establish after extinction. On the other hand, populations strongly linked by dispersal to neighboring populations act as a single synchronous population, increasing variability in extinction (Abbott 2011).

Clonal organisms (i.e. those capable of asexual reproduction) are common in many taxa (Eckert 2002; Bell 1982; Avise 2008) and raise some special issues for the maintenance of the sexes. In most cases, if a clonal organism with unisexual individuals loses one sex within a population, the population persists. If one sex is lost from a population of non-clonal organisms, immigration of the other sex is required for population survival.

Focusing on the spatial configuration of subpopulations within metapopulations, I explicitly model four well-studied natural metapopulations of the clonal dioecious organism *Marchantia inflexa*. I use the model to predict the proportion of males found in the metapopulation and in individual subpopulations within the metapopulations. I then compare the predicted results to the observed proportion of males found in the metapopulations and the subpopulations within each of the four metapopulations. I explicitly test the predictive powers of the model as well as the effects of spatial configuration on the maintenance of competitors. I also determine whether incorporating spatial structure alone is enough to produce accurate predictions of natural systems.

Model Organism

Marchantia inflexa Nees & Mont. is a New World non-vascular clonal plant found along streams from northern Venezuela to the southern United States (Bishler 1984). Populations are found on rocks within the stream or along the banks of streams in discrete patches. Stream systems containing multiple populations are considered metapopulations (sensu Freckleton and Watkinson 2002). Males and females are separate individuals; sex is chromosomally determined (Bishler 1986). Metapopulations can contain only one sex or both sexes (Fuselier and McLetchie 2004; Stieha, unpub data). Within metapopulations containing both sexes, the proportion of males and females for each subpopulation varies, where a subpopulation can contain only males, only females, or both sexes at varying proportions (McLetchie and Puterbaugh 2000; Stieha, unpublished data).

Dispersal occurs via asexual mechanisms or sexual propagules. Asexual dispersal can occur by two mechanisms: 1) dislodging of living vegetative material or 2) distinct asexual propagules. Dislodged vegetative material floats downstream and may settle on substrate, attach, and continue growing. Although observed in other clonal organisms (Johansson and Nilsson 1993; Stieha, personal observation), this mechanism is thought to be rare and is not included in the model. Both males and females produce asexual propagules (gemmae) in specialized structures (gemmae cups). Rain ejects the gemmae from the cups as described in Brodie (1951). Gemmae are about 200 µm in diameter (Stieha and McLetchie, personal observation) and are dispersed by water. Propagules that are the product of sexual reproduction (spores) are about 28 µm in diameter and are wind dispersed (Schuster 1992). The difference between the modes of dispersal (water versus wind) gives asexual propagules and sexual propagules different dispersal capabilities. Asexual propagules are thought to mainly stay within the local subpopulation, while sexual propagules are the primary form of long-distance dispersal (Hansson et al. 1992; Starfinger and Stöcklin 1995; Amat et al. 2005; but see Laaka-Lindberg *et al.* 2003, Stieha unpublished manuscript).

Mathematical models predict the loss of one sex in a single isolated population of *M. inflexa* (McLetchie *et al.* 2002, Crowley *et al.* 2005b). The competition between the sexes and subsequent loss of one sex is driven by life history differences between the sexes (McLetchie and Puterbaugh 2002; McLetchie *et al.* 2002). Males and females differ in allocation to the 3-way vegetative growth-asexual reproduction-sexual reproduction trade-off. Males invest more in asexual reproduction than do females, while females invest more in vegetative growth than do males (McLetchie and Puterbaugh 2000). Also, males may invest more in sexual reproduction than unfertilized females but less than fertilized females (McLetchie et al. 2002). Because of the life history differences, the disturbance regime experienced by the subpopulation influences competition between the sexes and determines which sex is excluded (McLetchie et al. 2002). Disturbance is the removal of some fixed fraction of the individuals within the subpopulation. In undisturbed isolated populations, females overgrow male plants via vegetative growth, slowly outcompeting the males and driving them extinct (McLetchie et al. 2002; Crowley et al. 2005a,b). In frequently disturbed isolated populations, males quickly colonize recently cleared habitat with asexual propagules. In this scenario, males are capable of persisting and will outlast females, who cannot recover as quickly from a disturbance event (McLetchie *et al.* 2002, Crowley *et al.* 2005a,b). Populations are rarely found in isolation but instead exist in an aggregation of populations known as a metapopulation (Hanski 1999). Within the metapopulation framework, both sexes can persist at the metapopulation level although either sex may be lost from individual subpopulations within the metapopulation (García-Ramos et al. 2007). The combination of mathematical models and experimental knowledge make the *Marchantia* system an ideal system to test mathematical models.

In this paper, I test the predictive capabilities of the metapopulation model. I determine if the model predicts the observed sex ratio at the metapopulation level and at the subpopulation level. I also use the model to make predictions about the disturbance regimes of individual subpopulations within the various metapopulations.

Methods

Quantifying Natural Metapopulations

Four metapopulations of *Marchantia inflexa* along three streams in Trinidad, West Indies were surveyed multiple times during the 2007 field season (Eastern Turure: 8 times from 25 January to 27 June; Quare River: 5 times from 17 February to 1 June; Western Turure Upstream: 7 times form 22 January to 12 June; and Western Turure Downstream: 6 times from 27 February to 12 June). The two metapopulations along the W. Turure are separated by almost a half kilometer of stream with only two closely neighboring subpopulations of *M. inflexa* and were therefore considered seperate. During the initial survey, all subpopulations of *M. inflexa* were marked and measured for amount of plant material (as size) and location from neighboring subpopulations. The location from other subpopulations was used to compute the distances between all subpopulations. Because *M. inflexa* is found along the stream, and dispersal is assumed to travel only along the stream, distances between subpopulations were computed along the path of the stream as opposed to the distance in 2-dimensional Euclidean space. Despite being airborne, spores are assumed to travel along the stream due to high banks and vegetation obstructing dispersal.

To determine the number of males and females within the subpopulations, sex structures of males and females were counted during visits to the sites. Presence of sex structures is required to determine the proportion of males with a subpopulation. Not all subpopulations within a metapopulation produced sex structures. These subpopulations were used in the model, but could not be used in the test of the model. The number of males and females were turned into sex ratios (denoted as proportions of males within the subpopulation) by 1) using the total number of sex structures counted throughout the field season (total sex ratio) and 2) determining the most sex structures counted in a single survey across all surveys for each sex and using those values to compute sex ratios (maximum sex ratio). I assume that sex ratio computed from the sex structures is similar to sex ratio of

the vegetative tissue. I use two methods of quantifying sex ratio because I do not know if sex structures counted in one survey persisted and were also counted in the next survey. When using the total sex ratio, the lack of independence between surveys could affect the computation of the sex ratios. Total sex ratio and maximum sex ratio are positively correlated (data not shown).

Model

I use the term patch to denote the area where a subpopulation can persist and subpopulation to indicate the individuals living in the patch. Previous research has looked at the maintenance of the sexes in single isolated populations (McLetchie et al. 2002; Crowley et al. 2005a,b) and in simple and complex spatial configurations of habitat (patches) that can support subpopulations within the metapopulation (García-Ramos et al. 2007; Stieha, unpublished manuscript). To vary the spatial configuration of the metapopulation, I modified the spatially explicit metapopulation model presented in Stieha (unpublished, based on McLetchie et al. 2002 and García-Ramos et al. 2007) to include different carrying capacities of individual patches and variation in distances between patches measured in the field.

Subpopulation Model

I describe the life cycle of *Marchantia inflexa* as seven coupled ordinary differential equations describing the seven life history stages (Figure 6.1, Table 6.1; McLetchie et al. 2002; García-Ramos et al. 2007). Males consist of three stages: 1) a non-reproductive stage, 2) an asexually reproductive stage, and 3) a sexually reproductive stage (Figure 6.1). Females consist of 4 stages: 1) a non-reproductive stage, 2) an asexually reproductive stage, 3) an unfertilized sexually reproductive stage, and 4) a fertilized sexually reproductive stage (Figure 6.1). Despite ignoring the explicit spatial distribution of individuals within a subpopulation, the differential-equation single-population models make very similar

predictions to models that incorporate the explicit spatial distribution of individuals within the subpopulation (Crowley et al. 2005b).

Stage transitions are seasonally dependent (McLetchie and Puterbough 2000, García-Ramos et al. 2007). The transition from the non-reproductive stages (stages 2 and 5, males and females, respectively) to asexually reproductive stages (stages 3 and 6, males and females, respectively) occurs from September through December during the wet season. The transition from the non-reproductive stages (stages 2, and 5, males and females, respectively) to the sexually reproductive stage of males (stage 4) and the sexually reproductive unfertilized stage of males (stage 7) occurs from January to June, during the dry season. If sexually reproductive males and females are in the same subpopulation, the female unfertilized sexually reproductive stage (stage 7) can transition into the female fertilized sexually reproductive stage (stage 1).

Sexually reproducing females that are fertilized (stage 1) produce spores at a ratio of 1:1 male:female. Spores are wind dispersed and thought to mainly contribute to interpopulation dispersal. For simplicity in the absence of data, I assume that the frequency distribution of spore dispersal distances is exponential (Nathan and Muller-Landau 2002; Cousens et al. 2008). Due to the presence of allelopathic inhibition of spore germination by vegetative material, spores germinate only if the patch is at least 40% empty (McLetchie, unpublished data). If the spores can germinate, they contribute equally to the non-reproductive stages of the males (stage 2) and females (stage 5). If the spores cannot germinate, I remove them because spores do not persist in an ungerminated state (McLetchie, unpublished data). I assume arbitrarily that only 1000 spores can germinate within a patch per time step. Results of previous models have been shown to be robust to the number of spores allowed to germinate per time step per patch. Only extremely low (10) or high (infinite) values affect the results (García-Ramos et al. 2007).

The asexually reproductive stages can produce asexual propagules (gemmae) capable of intra- and interpopulation dispersal (Stieha, unpublished). Since gemmae are water dispersed, their dispersal capabilities are assumed to be more limited than the dispersal capabilities of the spores. Asexual propagules colonize unoccupied habitat within the patch and are therefore limited by the proportion of unoccupied habitat.

Disturbance removes a proportion of the individuals within a patch and affects all stages equally. For many of the simulations, I assume the probability of a disturbance is 0.2 per month per subpopulation. When a disturbance occurs, 20% of the individuals from each stage are removed from the subpopulation. To convert the probability of within-patch disturbance in a month to the probability of disturbance in a time step Δt, I computed $p'_{\Delta t} = 1 - (1 - p_d)^y$, where p_d is the probability of a disturbance in a time interval *T* (a month) and *y* is the fraction of time represented by the time step duration in the time interval *T*, computed as $\Delta t/T$. For the simulation, I have time steps of 0.1 month.

For each patch, I track the amount of tissue in each stage. From these data, I can compute the sex ratio of the individual subpopulations. Sex ratio is defined as the proportion of males (stages 2, 3, and 4) from the total number of individuals within the subpopulations (stages 1 through 7).

Metapopulation Model

The metapopulation model (García-Ramos et al. 2007) simultaneously simulations multiple subpopulation models of McLetchie et al. (2002). The metapopulation model simulates multiple subpopulations simultaneously while allowing subpopulations to interact through the migration of sexual and asexual propagules. Disturbances occur at the subpopulation level, independent of other subpopulations, thus ignoring any spatial correlations among neighboring patches. At the metapopulation level, extreme disturbances known as extinctions can occur. Extinctions remove all the plant material from an entire patch and affect subpopulations independently of one another. Even though I am simulating multiple patches, I assume that many basic parameters among patches are the

same (Table 6.2). Iam interested in comparing the effects of spatial configuration, such as carrying capacity and distance to nearest neighboring patch) on the predictions of the model (see *Spatial Model*).

As in the subpopulation model, I track the amount of tissue of stage *i* in subpopulation *j* at each time step. With this information, I determine the proportion of males and the variation in the proportion of males observed in subpopulation *j* throughout the simulation.

Asexual and sexual propagules can influence the population dynamics of the source subpopulation and neighboring subpopulations and can recolonize unoccupied patches. The number of propagules immigrating into a focal area (whether an empty patch or subpopulation) is the sum of the total number of dispersing individuals from neighboring subpopulations modified by the distance between the focal area and the neighboring subpopulation. The number of dispersing individuals for each subpopulation is computed by multiplying the amount of tissue in life history stages capable of producing propagules (stages 3 and 6 for asexual propagules, stage 1 for sexual propagules) by the propagule production rate (F_1 for sexual propagules, A_3 or A_6 for asexual propagules). Only the fraction of propagules that land in habitable environments, whether empty patches or patches containing subpopulations, are counted.

I assume dispersal of both asexual and sexual propagules can be described with an exponential frequency distribution of dispersal distance (Cousens et al. 2008). The fraction of spores migrating from the focal subpopulation to a patch *x* meters away is $f_{jk}(x) \approx [2h^2/(\pi\lambda_1|x|)]\exp(-|x|/\lambda_1)$, where *h* is half the length of the patch (when viewed as a square) and λ_1 is the spore dispersal parameter. Spores are assumed to disperse in all directions due to wind dispersal although spores dispersing beyond the stream banks are assumed to be lost. For gemmae, I assume a one dimensional dispersal process similar to García-Ramos et al. (2007) and Stieha (unpublished). The simple one- dimensional dispersal process matches the overall linear nature of stream systems. I assumed an unbiased dispersal of asexual

propagules since directed dispersal (downstream biased due to water dispersal) appears to only affect the persistence of single-sex metapopulations (Stieha, unpublished).

Immigrating asexual and sexual propagules are affected by individuals already within the patch as described in *Subpopulation Model*. Male and female gemmae are tracked independently because asexual propagules contribute to the non-reproductive stages of the sex of their parent (stage 2 males, stage 5 females). Sexual propagules are assumed to have a 1:1 male to female ratio.

The metapopulation concept requires an extinction-recolonization process (Hanski 1999; Freckleton and Watkinson 2002); therefore there must exist some likelihood that a subpopulation goes extinct through the removal of all plant material. Extinctions are assumed to be independent of each other, of spatial location, and of patch size. Following an extinction, I assume the patch persists and can be recolonized. The few extinctions that have been observed in the field have been a mixture of patch-preserving extinctions (scouring of rocks in a flood) and patch-removing extinctions (landslides; McLetchie and Stieha, personal observations). For a time step, I converted the yearly extinction probability as described for disturbances

Spatial Configuration Model

Subpopulations do not exist in isolation but interact through migrating individuals or propagules. The spatial configuration (carrying capacity, distance between neighboring subpopulations) of the habitable patches could greatly affect the occurrence of subpopulations within the metapopulation and determine the fate of the metapopulation (Hanski and Simberloff 1993). Stieha (unpublished) looked at the effects of idealized spatial configurations of patches within a metapopulation to determine the general effects of spatial configuration on the maintenance of the sexes and the persistence of the metapopulation. I use the same mathematical model to simulate natural metapopulations observed along three streams in Trinidad, West Indies. To account for carrying capacities of patches in natural metapopulations of *M. inflexa*, I modified the mathematical model to

include patch size as a patch specific variable. Patches were defined as subpopulations observed during the 2007 field season. Patch size was defined as the subpopulation size measured during the 2007 field season. Patch size has been predicted to delay competitive exclusion (sensitivity analysis in McLetchie et al. 2002), allowing both sexes to persist longer in the same patch. I used the survey data to compute the distances between all patches along the course of the stream.

Analysis

To simulate the four metapopulations of *M. inflexa*, I modified code described in García-Ramos et al. (2007) and Stieha (unpublished). In MATLAB R2011a (Mathworks, Inc), I ran 1000 simulations for each metapopulation. Each simulation ran for 500 years, with 120 time steps per year. All patches within a metapopulation were initially started with 10 units of males (stage 2) and ten units of females (stage 5). I ignored the first 100 years of the simulation to remove transient dynamics and focus on equilibrium dynamics of the metapopulation. Only the last 400 years of the simulations were used to compute sex ratios of the metapopulation as a whole and the subpopulations individually. I assumed p_d, the disturbance probability per month, was 0.2 (Table 6.2). To determine the predicted sex ratios of the metapopulations, I used the total amount of plant material from males and females, independent of the life history stage. For males, this involved adding together Stages 2, 3, and 4. For females, this involved adding together Stages 1, 5, 6, and 7.

Because the model is a simulation of many years of the population dynamics, while the survey is only a snapshot of a single year, comparing the surveyed sex ratio to the mean of the simulations is invalid. I am interested in the mean of the sex ratio, as well as the variation of the sex ratio throughout a simulation. For the last 400 years of each simulation, I computed the mean metapopulation sex ratio and computed the non-parametric 95% confidence interval by taking the mean metapopulation sex ratio at each time step, sorted the means, removed the smallest 2.5% and largest 97.5%, and recorded the minimum and maximum values. I similarly computed the mean and confidence intervals for each

subpopulation within the metapopulation. The non-parametric confidence interval is required because an individual subpopulation will lose one sex, producing a non-normal distribution of sex ratios across the time steps. The confidence intervals allow me to quantify the variation within a simulation and give me an idea of what the sex ratio should be 95% of the time. I can then determine whether my observed sex ratio falls within this interval. I computed the mean and confidence interval of the metapopulation as a whole and the individual subpopulations for each of the 1000 simulations. I then averaged the values from the 1000 simulations to get a single value for the mean, upper confidence interval, and lower confidence interval. If any of the simulations lost one sex before the end of the 500 years, I modified the sex ratios and confidence intervals to account for this. If the metapopulation lost males and became female only, the values were 0 (0 proportion males). If the metapopulation lost females and became male only, the values were 1.

Due to mychoices for the values of the parameters (Table 6.2), subpopulations are predicted to be all female (McLetchie et al. 2002). To account for this bias, I can modify the simulated and observed sex ratios to determine the deviation from a 1:1 male-to-female ratio, defined as the Sex Bias Index (SBI; Stieha, unpublished). SBI allows me to predict whether the subpopulation will lose one sex whether the observed sex ratio is male-only or female-only. SBI is computed by

$$SexBiasIndex = \begin{Bmatrix} 1-2s & s \leq 0.5 \\ -1+2s & s > 0.5 \end{Bmatrix} \qquad \text{(eqn. 1)}$$

where *s* is the observed or simulated proportion of males.

To determine the predictive power of the model for each metapopulation, I compared the simulated sex ratio of the metapopulation to the observed sex ratio of the metapopulation. I computed the predicted proportion of males from the mathematical model in two ways: 1) averaging the predicted sex ratios of all subpopulations within the metapopulation (defined as All) and 2) averaging the

predicted sex ratios of only the subpopulations that also have observed sex ratio data (defined as Exp). For the predicted sex ratios, I only used simulations that contained both sexes at the end of the simulations and ignored simulations where one sex was lost because the observed metapopulations had both sexes. I computed the observed sex ratio in two ways: 1) averaging the observed sex ratios of all subpopulations (defined as Avg) and 2) adding together the total number of sex structures counted as computing a weighted sex ratio (weighted on the number of structures, subpopulations with more sex structures influence the metapopulation sex ratio to a greater degree, defined as Sum).

To determine the predictive power of my model for individual subpopulations, I plotted the observed sex ratios (or SBI values) against the simulated sex ratios (or SBI values). To determine the predictive capabilities of the model, I determined if the predicted range of the sex ratio (or SBI) of a single subpopulation contained the observed sex ratio (or SBI). On my graphs, I looked for overlap between the predicted values and the line $y = x$, where x is the observed sex ratio and y is the predicted sex ratio.

Previous analyses of the *M. inflexa* model determined that the disturbance regime and the growth rate within a patch could influence competition dynamics and determine which sex persists and which is outcompeted (McLetchie et al. 2002). Given the life history differences between males and females and their effect on population dynamics via colonization of space (whether unoccupied or occupied), I ran simulations to determine the disturbance regime for each subpopulation that would produce the observed proportion of males. To predict the disturbance regime, I ran 1000 simulations of the four metapopulations for each disturbance regime from 0 to 0.9 with 0.1 steps. For each subpopulation, I then compared the predicted sex ratio and confidence interval for each disturbance regime to the observed sex ratio. When the confidence interval contained the observed sex ratio, I noted this as a possible prediction. Using this procedure, I determined the predictive power of the

model for the experimentally collected data; but I also tuned the disturbance frequency to otain a best fit to the field data to estimate that frequency.

All graphs and analyses were done using R version 2.10.1 (R Development Core Team 2009).

Results

Overall Metapopulation Dynamics

The Quare metapopulation had the closest match between the predicted sex ratio and the observed sex ratio (Figure 6.2). With respect to the 3 streams from the Turure system (Eastern Turure, downstream Western Turure, and upstream Western Turure), the overall predicted metapopulation sex ratio did not overlap with the observed metapopulation sex ratio (Figure 6.2). In all three cases, the model predicted a female-biased sex ratio where the observed sex ratio was male-biased.

Average sex ratios observed and predicted generally agree with respect to the Quare stream system, but disagree between the observed and predicted sex ratios with respect to the Turure stream systems (Figure 6.3). The model predicted that most subpopulations in the Quare stream would be female biased with less than 20% males. Many of the subpopulations in Quare were observed to have female-only or female-biased sex ratios. For the Eastern Turure, the model predicted that all subpopulations would have an average sex ratio below 0.2 (female-biased), while most of the observed sex ratios had a male bias. For both metapopulations found along the Western Turure, the model predicted female-biased sex ratios while I observed a range of sex ratios, from female-only to male-only.

Subpopulation Dynamics

Eastern Turure River

When the metapopulation along the Eastern Turure River was modeled as a series of 1 m^2 patches, all 1000 simulations lost one sex at the metapopulation level, producing subpopulations containing only females (Figure 6.4*a*). Despite the model predictions, the observed proportion of males

along the Eastern Turure River was not female-only and some subpopulations contained only males (Figure 6.4*a*)! When the proportion of males was converted to the Sex Bias Index (SBI), all subpopulations were predicted to lose one sex (Figure 6.4*b*). In this scenario, neither the predicted proportion of males nor the SBI overlapped with the observed values.

When the observed subpopulation sizes were included in the model as the carrying capacity, the results were different when compared to the 1 m^2 models. About one third of the simulations (337 out of 1000) maintained both sexes after 500 years. When all simulations (both metapopulations that lost one sex and metapopulations that maintained both sexes) were used, the average predicted proportion of males for each subpopulation was about 5%, with a range of 0% to almost 25% male (Figures 6.5*a* and 6.5*c*). The results were similar whether the observed proportion of males was computed using the total number of sex structures or only the maximum number of males and females counted in a single survey (not shown). The predicted and observed sex ratios did not overlap (Figure 6.5*a*). When only simulations that had both sexes persisting in the metapopulation were used to compute sex ratios, the range of the predicted sex ratios for each subpopulation increased, although fewer than half of the subpopulations had a predicted sex ratio that overlapped with the observed sex ratio (Figure 6.5*c*).

When the proportion of males was converted to SBI and no simulations were excluded, about half of the subpopulations had predicted SBIs that overlapped with the observed SBI (Figure 6.5b). Again, predictions did not overlap the observed SBI for subpopulations that had an observed SBI closer to 0 (a more equal ratio of males to females). When the predicted SBI was computed from simulations that maintained both sexes, only one subpopulation's predicted SBI did not overlap with the observed SBI (Figure 6.5*d*).

Quare River

When the carrying capacities of patches along the Quare River were all assumed to be 1 m^2, the model predicted that all subpopulations would have a proportion of males of 0.1 or less (Figure 6.6*a*).

When the predicted proportion of males are plotted against the observed proportion of males (using either the total number of sex structures or the maximum number of sex structures), many of the confidence intervals of the predictions overlapped the line *y=x*, except when the observed proportion of males was 0.6 or greater. When the observed and predicted proportion of males were converted to SBI, the model predictions did not overlap the observed SBI when the SBI was close to zero (and the proportion of males was close to 0.5; Figure 6.6*b*). In the mathematical model, both sexes persisted in 100% of the simulations, and all of the simulations had about a 100% occupancy rate.

When the observed population size is incorporated into the model as the carrying capacity, the results were similar to simulations where carrying capacity was 1 m^2. For the few populations with an observed proportion of males greater than 0.6, the confidence intervals of the predicted value did not contain the observed value (Figure 6.6*c*). When the observed proportion of males was less than 0.6, the confidence interval of the predicted values included the observed values (Figure 6.6*c*). When the proportion of males was converted to SBI, many of the predictions overlap with the observed values (Figure 6.6*d*). Also, both sexes were maintained in 1000 out of 1000 simulations with almost 100% of the patches occupied.

Western Turure downstream

When the Western Turure downstream metapopulation was simulated with the carrying capacity of all subpopulations as 1m^2, the predicted sex ratios of only 3 subpopulations overlap with the observed sex ratios (Figure 6.7*a*). Computing observed sex ratio as either the maximum number of males or females counted at a single time or as the total number of sex structures counted throughout the field season made no difference (data not shown). Using only simulations that contained both sexes at the end of 500 years (237 out of 1000) increased the variationin the predicted sex ratio, but the predicted values of only three subpopulations overlapped the observed values (Figure 6.7*c*). These three subpopulations had either female-only or female-biased observed sex ratios.

When the predicted SBI was compared to the observed SBI, more subpopulations had predicted values that overlapped the observed values. When the predicted SBI was computed using all simulations (both simulations that lose one sex and simulations that maintain both sexes at the metapopulation level), only the subpopulations with an observed SBI close to one (contain mainly one sex) were accurately predicted (Figure 6.7*b*). When only the simulations were used where both sexes are maintained (237 out of 1000 simulations), the variation of the predicted SBIs increased which increased the number of subpopulations where the predicted SBI and observed SBI overlapped (Figure 6.7*d*). The predicted SBI and the observed SBI did not overlap in subpopulations with an observed SBI close to 0 (sex ratio almost 1:1).

When the actual patch size was incorporated into the mathematical model, the predicted sex ratios of only two more subpopulations overlapped the observed sex ratios (Figure 6.8*a*). This result holds whether I compute the predicted sex ratio from all simulations (Figure 6.8*a*) or only simulations that maintained both sexes (Figure 6.8*c*). Only 38 out of 1000 simulations lost one sex. Most of the predicted values of SBI overlapped with the observed SBI independent of how SBI was computed (using the total number of sex structures or maximum number of sex structures, data not shown) or the simulations used to compute the predicted value (simulations that lost one sex or maintained both sexes; Figures 6.8*b* and 6.8*d*).

Western Turure upstream

When the predicted sex ratio of all simulations was compared to the observed sex ratio when carrying capacity is $1m^2$, only one subpopulation had a predicted sex ratio that overlaps the observed sex ratio (Figure 6.9*a*). This subpopulation only had 10% males, while the other subpopulations had greater than 40% males. When the predicted sex ratio was computed using only the simulations that contained both sexes (137 out of 1000), the variation in the predicted sex ratio increased such that two to three subpopulations' predictions overlap with the observed sex ratios (Figure 6.9*c*).

When carrying capacity was $1m^2$ and all simulations were used to compute the predicted SBIs, the number of subpopulations with overlap between the predicted and observed values did not increase (Figure 6.9*b*). Whether the SBI was computed using the maximum number of sex structures or total number of sex structures did not change this result (data not shown). When I only used simulations that contained both sexes at the end of the simulation (137 out of 1000 simulations), the predicted SBI of 4 out of the 5 populations overlapped their observed SBI (Figure 6.9*d*), whether SBI was computed with the total number of sex structures or maximum number of sex structures (data not shown).

When the observed population size was used as the carrying capacity in the mathematical model, 552 out of 1000 simulations maintained both sexes. When all simulations were used to compute the predicted sex ratio (Figure 6.10*a*), only one population had overlap between the predicted sex ratio and the observed sex ratio (two if I compute observed sex ratio as the total number of sex structures counted, not shown). When simulations that contained both sexes were used, the predicted sex ratios of three out of five of the subpopulations overlapped their observed sex ratios (Figure 6.10*c*).

When sex ratio was converted to SBI, the predictive power of the model increased when all simulations are used to compute the SBI: 3 (maximum) to 4 (total) of the subpopulations have predicted SBI that overlap the observed SBI (Figure 6.10*b*, data not shown for maximum sex structures). Only two out of the five subpopulations had overlapping predicted and observed values when the predicted values were computed using the 552 simulations where both sexes were maintained (Figure 6.10*d*).

Predicted Disturbance Regimes

When the model was used to predict the disturbance regime required to produce the observed sex ratio, results matched previous models: male-biased sex ratios required higher disturbance probabilities (McLetchie et al. 2002; Crowley et al. 2005). In all four metapopulations, male-only subpopulations could not be predicted by the model. Subpopulations with an observed sex ratio that could not be predicted by the model are represented by a filled in circle in Figure 6.11.

Eastern Turure River

I only have predictions for three out of the five subpopulations found along the Eastern Turure River (Figure 6.11*a*). The model never predicted a proportion of males greater than 0.9, therefore I have no predictions about the disturbance probability of subpopulations with an observed sex ratio greater than 90% males. In the subpopulations where I could get disturbance regime predictions, the disturbance probability had to be greater than 0.3 (a greater than 30% chance of a disturbance affecting a subpopulation within a month), suggesting that the disturbance rate of the Eastern Turure River is rather high (Figure 6.11*a*). Using all simulations as compared to only using simulations where both males and females were maintained at the end of 500 years produced similar results (results not shown). When I used simulations where both males and females were present, the range of the predicted disturbance probabilities was greater than when I used all simulations to predict disturbance probabilities. For example, a proportion of males of 0.64 was predicted for a disturbance regime between 0.3 and 0.8 when all simulations were used but 0.1 to 0.8 when only simulations that maintained both sexes were used (results not shown).

Quare River

All simulations of the metapopulation found along the Quare River maintained both sexes for all disturbance probabilities. As shown in Figure 6.11*b*, almost all of the predicted disturbance probabilities were below 0.4 (40% chance of a disturbance per month). The model could not assign a predicted disturbance regime for subpopulations that had an observed sex ratio of 1 (all males).

Western Turure River downstream

Using all simulations or only simulations that maintained both sexes produced very similar results for the predicted disturbance regimes for each subpopulation (data not shown). As the observed proportion of males increased, the predicted disturbance regime also increased without an increase in

the variation of the disturbance regime (Figure 6.11*c*). The disturbance regime of four subpopulations could not be predicted. Again, the model could not predict the disturbance regime of most subpopulations with a high proportions of males (>0.77), although one subpopulation with an observed sex ratio of 0.83 could be predicted.

Western Turure River upstream

When all simulations were used to predict disturbance regime, subpopulations in the upstream metapopulation of the Western Turure River had a predicted disturbance range between 0.2 and 0.9 (Figure 6.11*d*). When only simulations that contain both males and females were used to predict disturbance regimes, the subpopulations have a range of 0 to 0.9 (results not shown). As with other metapopulations, as the observed proportion of males increased, the predicted disturbance regime also increased (disturbances have greater chance of occurring in a month). Again, the model could not predict the disturbance regime for male-only subpopulations.

Discussion

My research indicates that spatial configuration is not enough to predict the dynamics of the metapopulation as a whole, let alone the dynamics of individual subpopulations within the metapopulations.

Overall Metapopulation Dynamics

Comparing the predictions from mathematical models and observations from the field produces conflicting results. At the metapopulation level, the predictions for the metapopulation sex ratio were close for Quare River, the metapopulation system used to parameterize the initial models (Figure 6.2; McLetchie et al. 2002; García-Ramos et al. 2007). When I tried to use the same parameters for other metapopulations along two different stream systems, the results were in constant conflict with predictions (Figure 6.2).

When computing the predicted averages, I used the average over the last 400 years of the simulation for many simulations. When computing the observed values, I only used a single snapshot of the metapopulation. The discrepancy between time scales could be influencing the predictive capabilities of the model. For example, competition over unoccupied space is predicted to drive the sex-ratio dynamics of recently colonized patches. Males produce more asexual propagules (Mcletchie and Puterbough 2000), which allows males to quickly occupy the space. Therefore, newly colonized populations are predicted to have a male-biased sex ratio due to intrapopulation colonization by gemmae (McLetchie et al. 2002; Crowley et al. 2005; García-Ramos et al 2007). On the other hand, competition in subpopulations at carrying capacity consists of competition over currently occupied space, requiring an overgrowth strategy. Females invest more energy into vegetative growth (McLetchie and Puterbough 2000). Therefore, they can overgrow and occupy currently occupied areas. In this case, subpopulations that have existed for a longer time and have reached carrying capacity will have a female-biased sex ratio (McLetchie et al. 2002; Crowley et al 2005).

The model represents an average of subpopulations at various ages and occupancy levels. With my studied metapopulations, the densely populated metapopulation along the Quare River may have more subpopulations at various ages and occupancy levels, which produces an overall average situation like the model. The other three metapopulations have a lower population density and may not represent an average scenario. Possibly, the three metapopulations could be recent colonizations, although male-biased sex ratios are possible via other mechanisms, such as abiotic factors. For example, growth rate affects which sex persists in a single population (Mcletchie et al. 2002) which could be influenced by the availability of light. On average, the Turure River systems tend to have less canopy openness than the Quare River (Groen et al. 2010; Stieha and McLetchie, personal observation), potentially affecting growth rates. The overall disturbance regime (and subpopulation extinction regime) could be different for each system, influencing the proportion of males at the metapopulation

dynamics. The Quare metapopulation sits below a reservoir, suggesting regulation and limitation of the stream flow, which could decrease disturbance and extinction rates. On the other hand, the Turure systems do not have this regulatory mechanism. Along the Eastern Branch of the Turure River, evidence suggests that the water level can increase by over two feet during a flood, which obviously produces disturbances and potentially extinctions. These observations give support to the predicted levels of disturbance required to produce the observed proportion of males presented in Figure 6.11.

Subpopulation Dynamics

In many situations, when I tried to predict the sex ratio or sex bias index (SBI) of individual subpopulations, the model gave predicted values that contain the observed value. The predictive values seemed to match the observed values more closely when I removed the simulations that predicted a single sex metapopulation. More subpopulations were characterized by overlap between the predicted SBI and observed SBI than by overlap between the predicted sex ratio and observed sex ratio. Including the actual carrying capacity in the model also increased the predictive power of the model. In all cases, the overlap between the predicted values and the observed values occurred because the variation around the predicted value increased, not because the average predicted value was closer to the observed value. In this case, the model has little or some predictive power at the subpopulation level.

Although subpopulation carrying capacity and distance to nearest neighbor has been shown to influence the observed proportion of males and the sex bias index of individual subpopulations (Stieha, unpublished manuscript), the results apply to a single snapshot in the lifetime of the metapopulation as opposed to an overall average. At the individual subpopulation level, other factors may be driving the observed sex ratios. As presented above, the time since the colonization of the patch can influence the sex ratio. Environmental heterogeneity could also support differential expression of sex by males and females, leading to a biased observation that could not be predicted by the model. I also computed the predicted proportion of male material in the model using all the life history stages, while my observed

proportions were computed from sex structures. A systematic sampling of plant material and the accompanying determination of sex may be required and may support the predictions of the model.

I also warn of using parameters for one metapopulation to predict the dynamics of other metapopulations of the same species. Environmental heterogeneity is a crucial characteristic of patches that affects the life history of the organisms, selection pressures for various life history strategies, and therefore parameterization of the model. Environmental heterogeneity of the patches may be enough to promote the coexistence of competitors within a metapopulation through niche partitioning (Lomnicki 2001) by creating patches within the metapopulation capable of supporting one competitor over the other. In my case, this is spatial segregation of the sexes, a common phenomenon (Bierzychudek and Eckhart 1988). The environment of the patch is not the only environment that can be heterogeneous. Dispersal capabilities of migrating individuals are greatly affected by the habitat through which they have to travel to reach other hospitable patches (Wiens 1997).

The disturbance regime that the subpopulation experiences can also affect population dynamics. Subpopulations that experience infrequent disturbances (with disturbance defined as the probability that a proportion of the individuals will be removed from the subpopulation) are expected to be female biased (McLetchie et al. 2002; Crowley et al. 2005). In this case, the subpopulation reaches carrying capacity due to the low disturbance level. At carrying capacity, competition is by overgrowth, where the females take over areas currently occupied by other individuals. In highly disturbed areas, the resource allocation to vegetative growth is not fast enough to compensate for the removal of material. In highly disturbed areas, individuals are constantly being removed, producing unoccupied area that must be colonized. In this case, the male investment in asexual propagules allows them to quickly colonize the new area. I also assume that disturbance events and extinction events are independent of one another, although extinction and disturbance could be correlated in space and time (González-Megías et al. 2005; Kallismanis et al. 2005). However, despite the single population models predicting all

male sex ratios (McLetchie et al. 2002; Crowley et al. 2005a,b), the metapopulation model could not predict a disturbance regime at the level required for subpopulations to have an all-male sex ratio. In these cases, disturbance may not be driving subpopulation dynamics and environmental factors or stochastic processes (such as a single male colonized the area) may be driving the dynamics.

Growth rates are predicted to affect the sex ratio, where subpopulations with lower growth rates are predicted to be male-biased, while subpopulations with higher growth rates are predicted to be female-biased (García-Ramos et al. 2007). Population growth rate can be affected by many abiotic factors, such as humidity, temperature, and light. Male and female individuals of *Marchantia inflexa* are known to segregate by canopy openness (Fuselier and McLetchie 2004). Even though I have not attempted to correlate any environmental factors with sex ratio in my four metapopulations, the Turure systems tend to have greater canopy cover than Quare River (Groen et al. 2010; Stieha and McLetchie, personal observation). The phenology of *M. inflexa* growing along the various streams is also different, with sex expression early in the year along the Quare River (both males and females observed around mid-February), while occurring late in the year along the upstream portion of the Western Turure River (2 males observed at the beginning of March; Stieha, unpublished data). The difference in phenology could strongly influence the sex ratios (sensitivity analyses in McLetchie et al. 2002; García-Ramos et al. 2007) and should be incorporated into future predictive models.

The use of metapopulation theory in conservation revolves around the persistence of species within an aggregation of subpopulations and the spatial configuration of subpopulations required to postpone or prevent the loss of the species (Thomas and Hanski 1997; Hanksi 1999). Despite focusing on clonal organisms with genetically determined separate sexes, my results can be applied to and offer warnings for any system of competitors. Through tuning, I also show the capabilities of models to make predictions to other metapopulations that can help quantify difficult to measure parameters (such as disturbance) and give testable predictions.

Table 6.1. Equations representing the life cycle of *Marchantia inflexa*.

Stage	Stage Dynamics	Eqn. no.
Fertilized Females	$\frac{dN_{1j}}{dt}=\frac{M_7 N_{4j} N_{7j}}{K}-T_{51}N_{1j}-N_{1j}\sum_{i=2,i\neq 4}^{6} G_i \frac{N_{ij}}{K}$	eqn 1
Non-reproductive Males	$\frac{dN_{2j}}{dt}=(F_1\sum_{k=1}^{n} f_{jk}N_{1k}+A_3\sum_{k=1}^{n} a_{jk}N_{1k})(1-\sum_{i=1}^{7}\frac{N_{ij}}{K})+T_{23}N_{3j}+T_{24}N_{4j}$ $-(T_{32}+T_{42})N_{2j}+G_2 N_{2j}(1-\frac{N_{2j}}{K})-N_{2j}\sum_{i=3,5,6} G_i\frac{N_{ij}}{K}$	eqn 2
Asexually reproductive males	$\frac{dN_{3j}}{dt}=T_{32}N_{2j}-T_{23}N_{3j}+G_3 N_{3j}(1-\frac{N_{3j}}{K})-N_{3j}\sum_{i=2,5,6} G_i\frac{N_{ij}}{K}$	eqn 3
Sexually reproductive males	$\frac{dN_{4j}}{dt}=T_{42}N_{2j}-T_{24}N_{4j}-N_{4j}\sum_{i=2,i\neq 4}^{6} G_i\frac{N_{ij}}{K}$	eqn 4
Non-reproductive females	$\frac{dN_{5j}}{dt}=(F_1\sum_{k=1}^{n} f_{jk}N_{1k}+A_6\sum_{k=1}^{n} a_{jk}N_{6k})(1-\sum_{i=1}^{7}\frac{N_{ij}}{K})+T_{51}N_{1j}+T_{56}N_{1j}$ $+T_{57}N_{7j}-(T_{65}+T_{75})N_{5j}+G_5 N_{5j}(1-\frac{N_{5j}}{K})-N_{5j}\sum_{i=2,3,6} G_i\frac{N_{ij}}{K}$	eqn 5
Asexually reproductive females	$\frac{dN_{6j}}{dt}=T_{65}N_{5j}-T_{56}N_{6j}+G_6 N_{6j}(1-\frac{N_{6j}}{K})-N_{6j}\sum_{i=2,3,5} G_i\frac{N_{ij}}{K}$	eqn 6
Sexually reproductive females	$\frac{dN_{7j}}{dt}=\frac{-M_7 N_{4j} N_{7j}}{K}T_{75}N_{5j}-T_{57}N_{7j}-N_{7j}\sum_{i=2,i\neq 4}^{6} G_i\frac{N_{ij}}{K}$	eqn 7

Table 6.2. Parameter values of the default model used to predict sex ratio and sex bias index.

Symbol	Definition	Magnitude	Units
Life-history			
G_2, G_5	Growth rates for Non-reproductive stages 2 and 5	0.585, 0.605	$month^{-1}$
G_3, G_6	Growth rates for asexually reproductive stages 3 and 6	0.220, 0.233	$month^{-1}$
T_{42}, T_{75}	Stage transition rates 2→4 and 5→7	0.714	$month^{-1}$
T_{23}, T_{24}, T_{56}, T_{57}	Stage transitions from 3→2, 4→2, 6→5, and 7→5	2.14	$month^{-1}$
T_{32},T_{65}	Stage transition rates 2→3 and 5→6	1.11, 0.833	$month^{-1}$
T_{51}	Stage transition rate 1→5	0.638	$month^{-1}$
M_7	Fertilization rate	10	$month^{-1}$
A_3, A_6	Asexual reproduction rates by stages 3 and 6	1.112, 0.519	$month^{-1}$
F_1	Sexual reproduction rate by stage 1	442	$month^{-1}$
Dispersal and establishment			
λ_1, λ_2	Spore dispersal parameters – decay rates	0.7, 10	m
p	Proportion of spores dispersing by λ_1	0.27	
z	Gemma dispersal parameter – decay rate	0.83	m
q	Uncovered threshold for spore germination	0.4	
r	Maximum spore establishment rate	1000	unit $month^{-1}$
Patch System			
$4h^2$	Patch size	1.11, 0.833	m^2
u	Size of a unit of M. inflexa tissue	5×10^{-5}	m^2 $unit^{-1}$
K	Patch carrying capacity, $K=4h^2/u$	20 000	units
n	Number of patches	30	
x	Distance between patches	varies	m
Disturbance and extinction			
p_d	Probability of a patch disturbance in a month	0.2	
p_e	Probability of a patch extinction in a month	0.002	

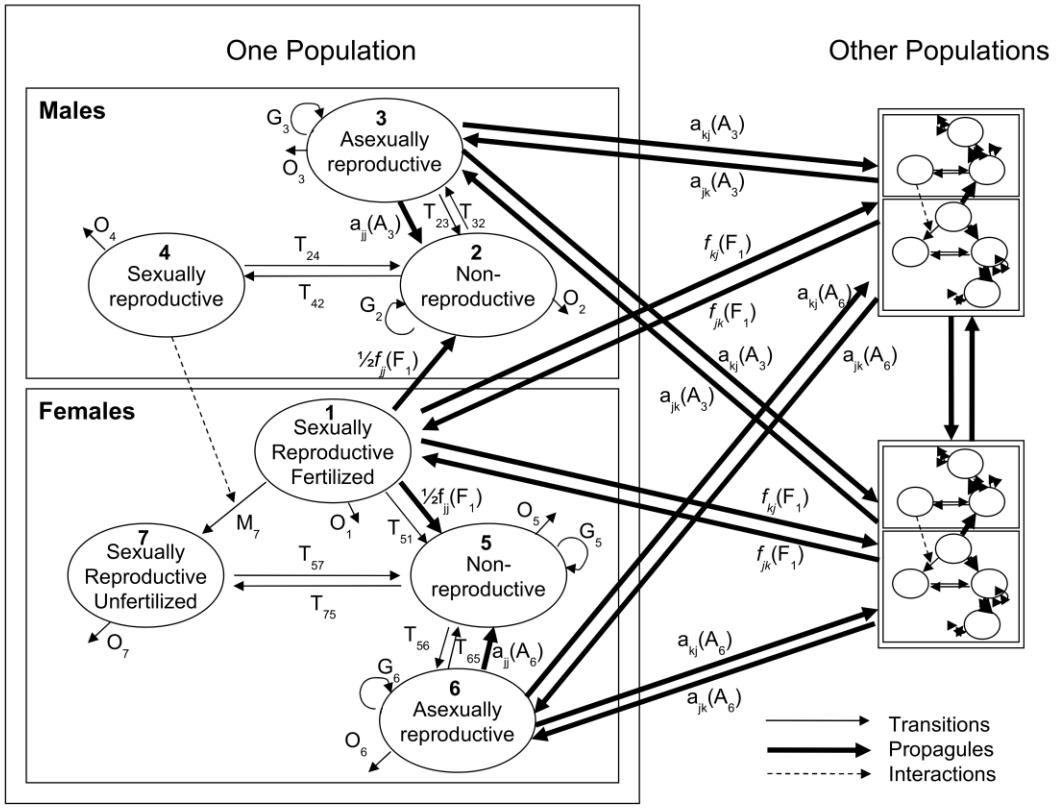


Figure 6.1. Transition diagram of equations. See Methods for a discussion of the various stages and Table 2 for a description of the parameters. Table 1 contains the differential equations derived from this transition diagram.

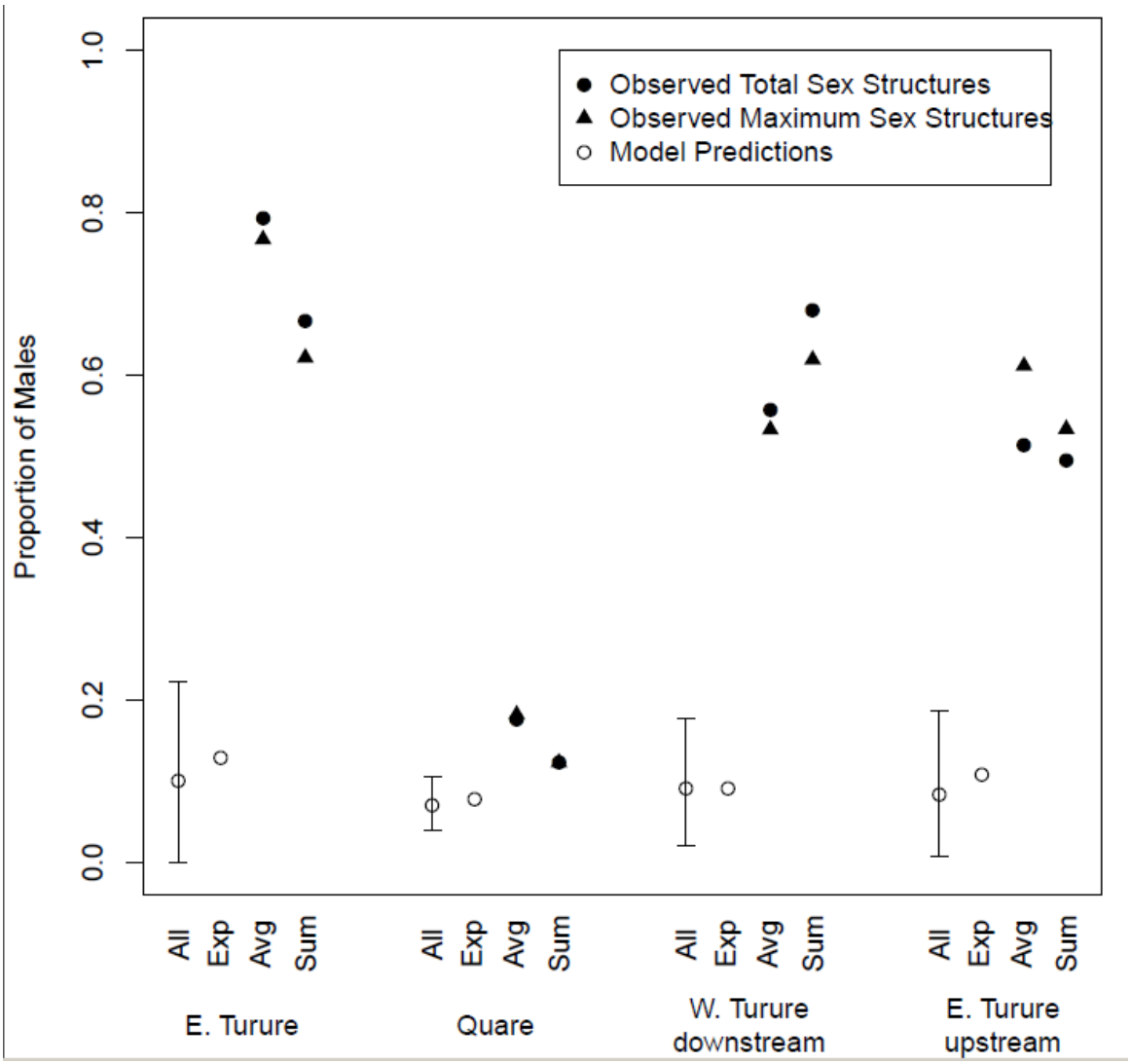


Figure 6.2. Comparison between the predicted and observed metapopulation sex ratios. **All** is computed during the simulation as the average sex ratio of the metapopulation. Non-parametric confidence intervals are determined using simulations that only contain both sexes at the end of the simulations. **Exp** is the predicted sex ratio computed using only subpopulations with an observed sex ratio. The sex ratio was computed after the simulations by determining the predicted mean sex ratio for each subpopulation and then computing the predicted mean of the metapopulation. Observed sex ratio is computed using the total number of sex structures counted during the field season (denoted by the closed circle) and the maximum number of sex structures counted during a single sampling period for each sex (denoted as a closed triangle). **Avg** is the unweighted observed metapopulation sex ratio. The observed sex ratios of all the subpopulations are computed and then averaged together to produce the unweighted metapopulation sex ratio. The weighted average of the sex ratio, **Sum**, computes the sex ratio by adding up all sex structures counted in the metapopulation. Subpopulations with a large amount of sex structures influence the metapopulation sex ratio more than subpopulations with a few sex structures.

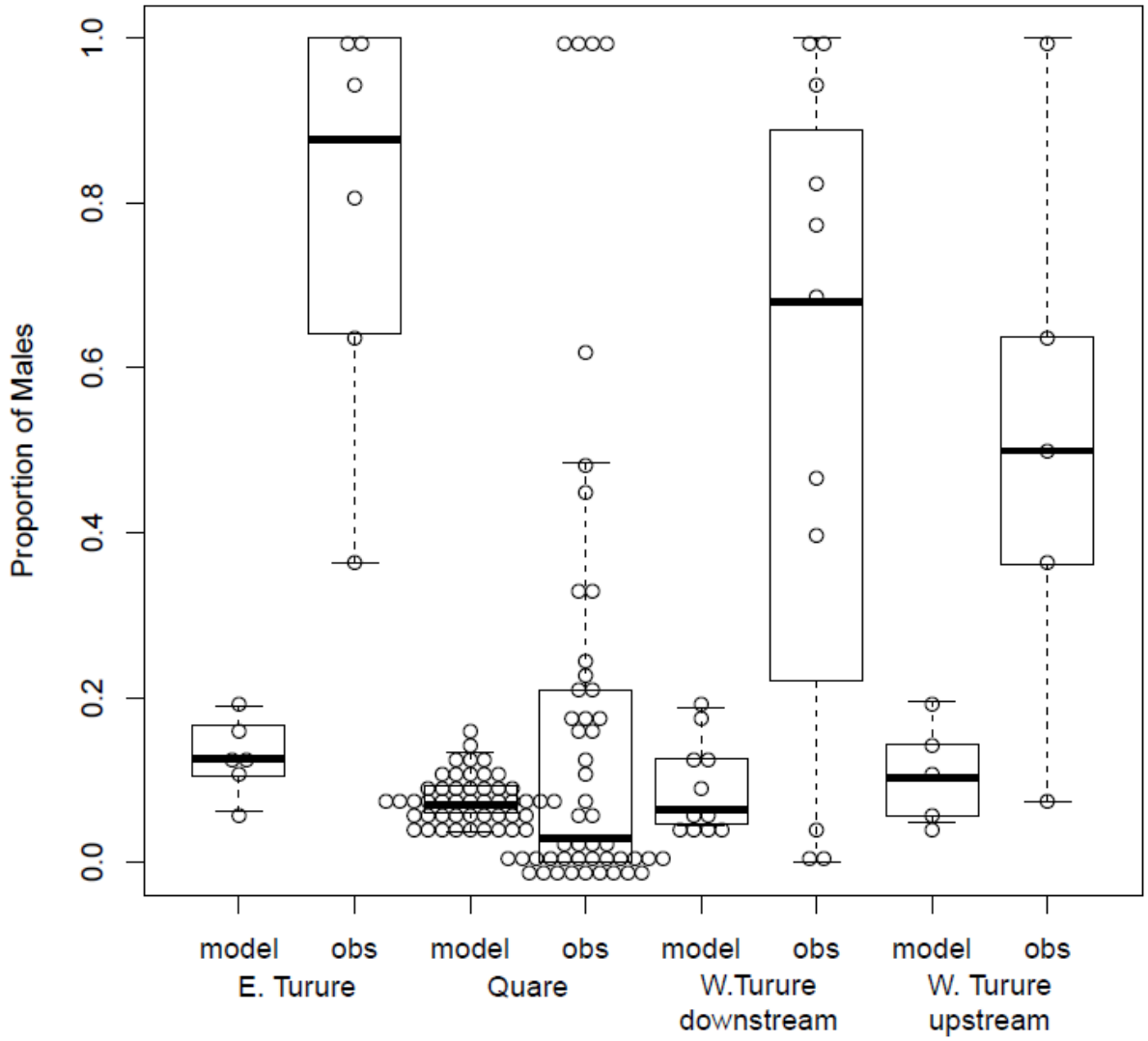
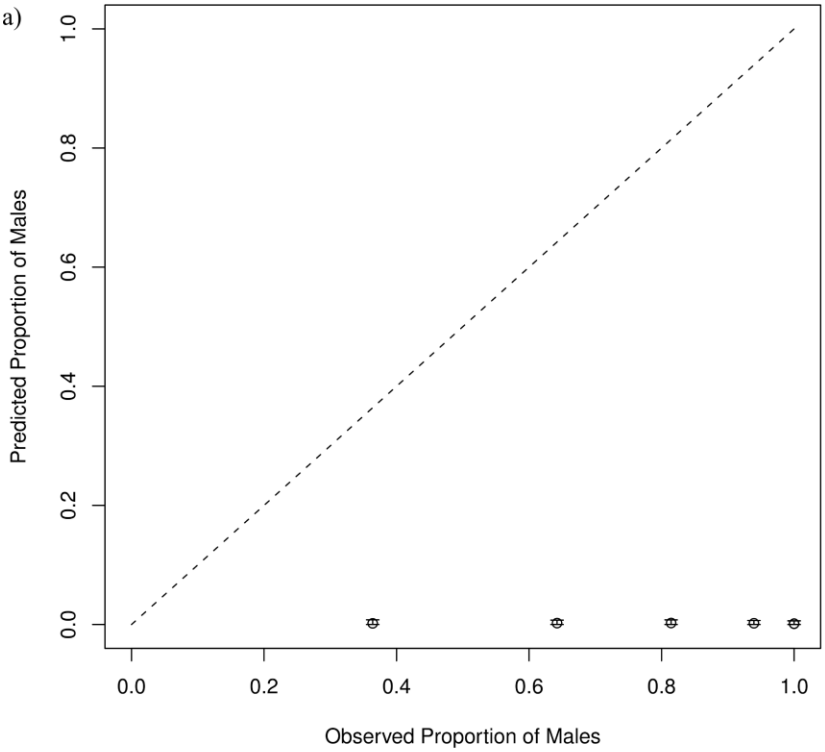


Figure 6.3. Comparison between the observed and predicted distribution of subpopulation sex ratios. Only subpopulations with observed sex ratios are plotted in the graph. **Model** represents the predicted sex ratios of subpopulations. **Obs** display the distribution of observed sex ratios of individual subpopulations. Dark lines represent the median proportion of males of subpopulations within each metapopulation. Boxes contain the subpopulations with a proportion of males in the 25% to 75% quartiles. Whiskers extend 1.5 times the interquartile range.

a)


Predicted Proportion of Males
Observed Proportion of Males
0.0
0.2
0.4
0.6
0.8
1.0

b)

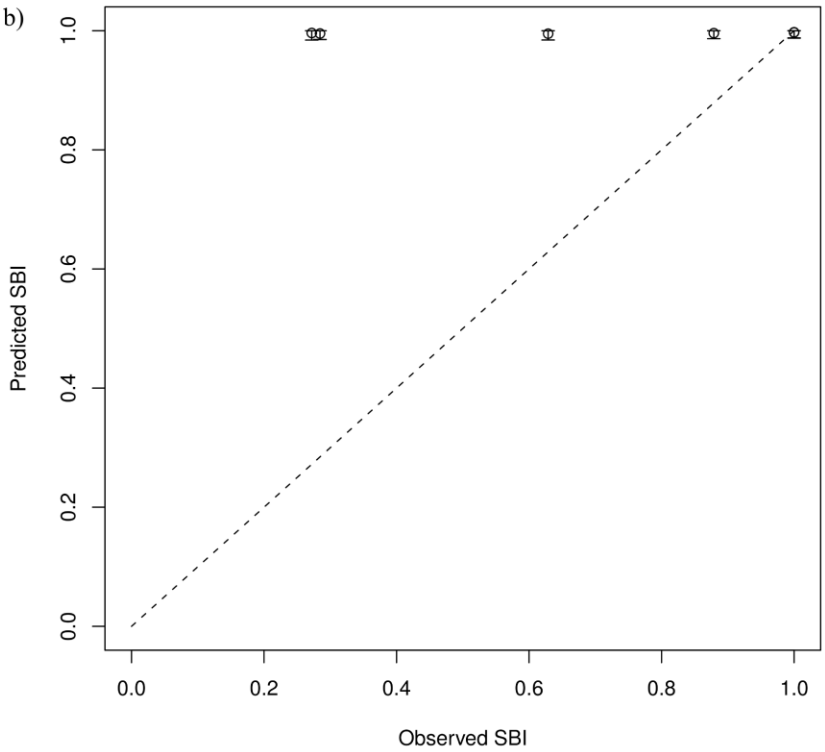

Predicted SBI
Observed SBI
0.0
0.2
0.4
0.6
0.8
1.0

Figure 6.4. Observed versus predicted values for the Eastern Turure Metapopulation, meter. Carrying capacity for all subpopulations was modeled as $1m^2$. a) The observed versus predicted sex ratio denoted as proportion of males. b) The observed versus predicted Sex Bias Index. For all 1000 simulations, the metapopulation was predicted to contain only one sex. All subpopulations were predicted to be female-only and show extreme bias.

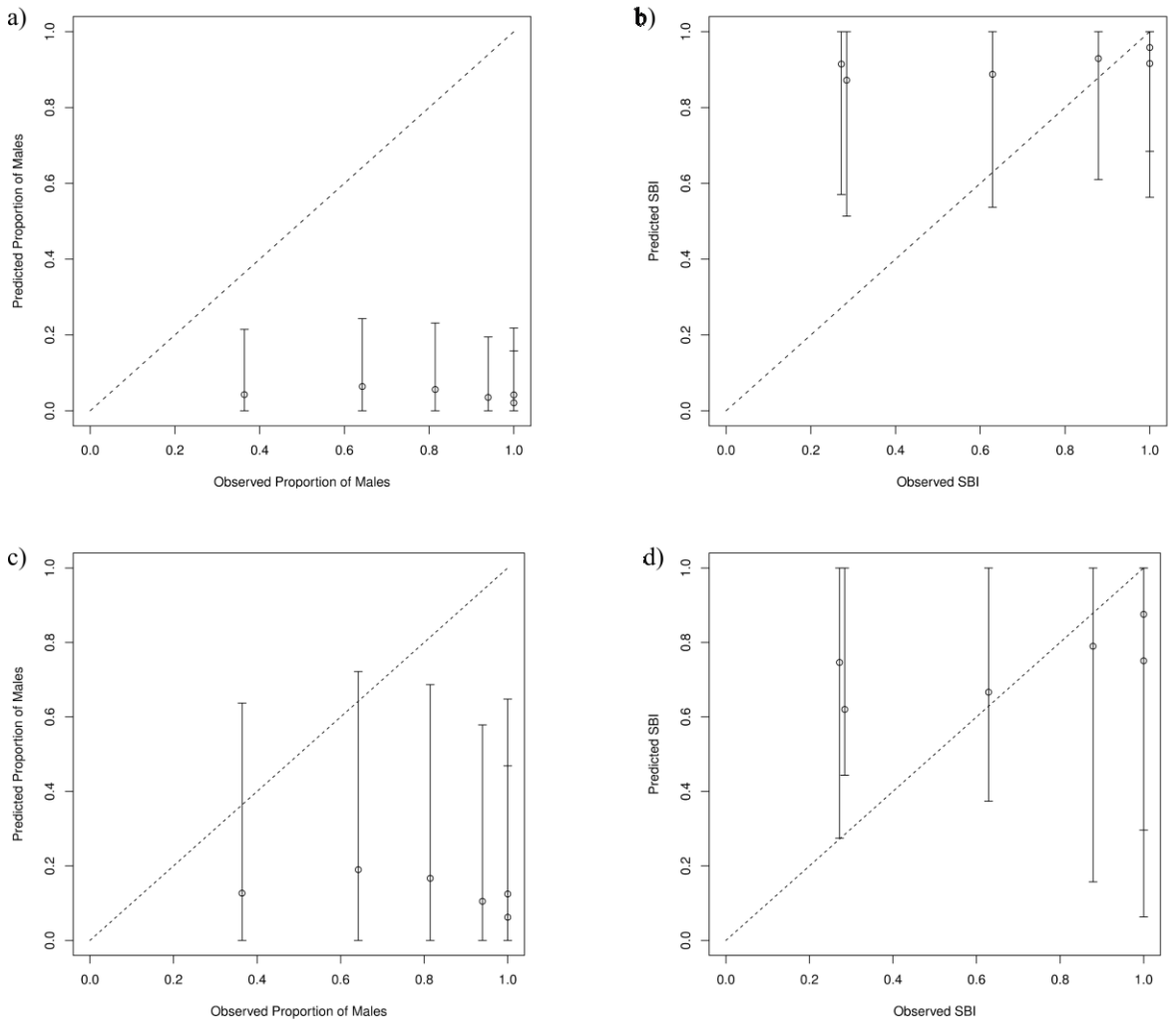


Figure 6.5. Observed versus predicted values for the Eastern Turure Metapopulation, patch. Carrying capacities for all subpopulations were modeled as the observed carrying capacity in the field. Figures a) and c) present the observed versus predicted sex ratio denoted as proportion of males. Figures b) and d) present the observed versus predicted Sex Bias Index. Figures a) and b) compute predicted values using all simulations, while figures c) and d) compute the predicted values using only simulations that contained both sexes at the end of 500 years. 337 out of 1000 simulations contained both sexes at the end of 500 years.

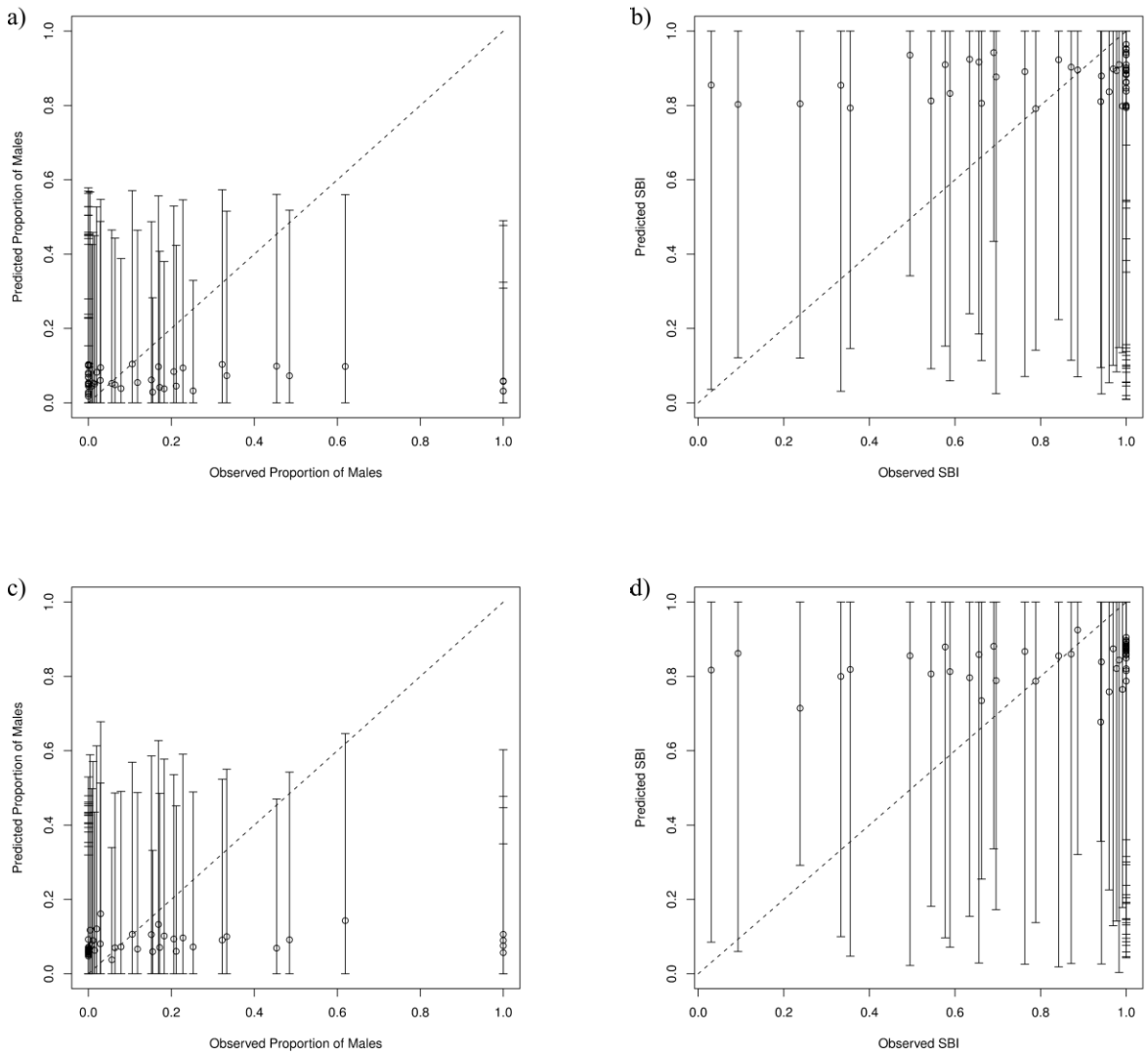


Figure 6.6. Observed versus predicted values for the Quare Metapopulation. All simulations maintained both sexes after 500 years. The predicted values presented in figures a and b are determined from models assuming the carrying capacities of all subpopulations were $1m^2$. Figure a shows the observed versus predicted sex ratio. Figure b shows the observed versus predicted Sex Bias Index. Figures c (sex ratios) and d (Sex Bias Index) present predicted values from simulations that incorporated the observed carrying capacities of the individual subpopulations.

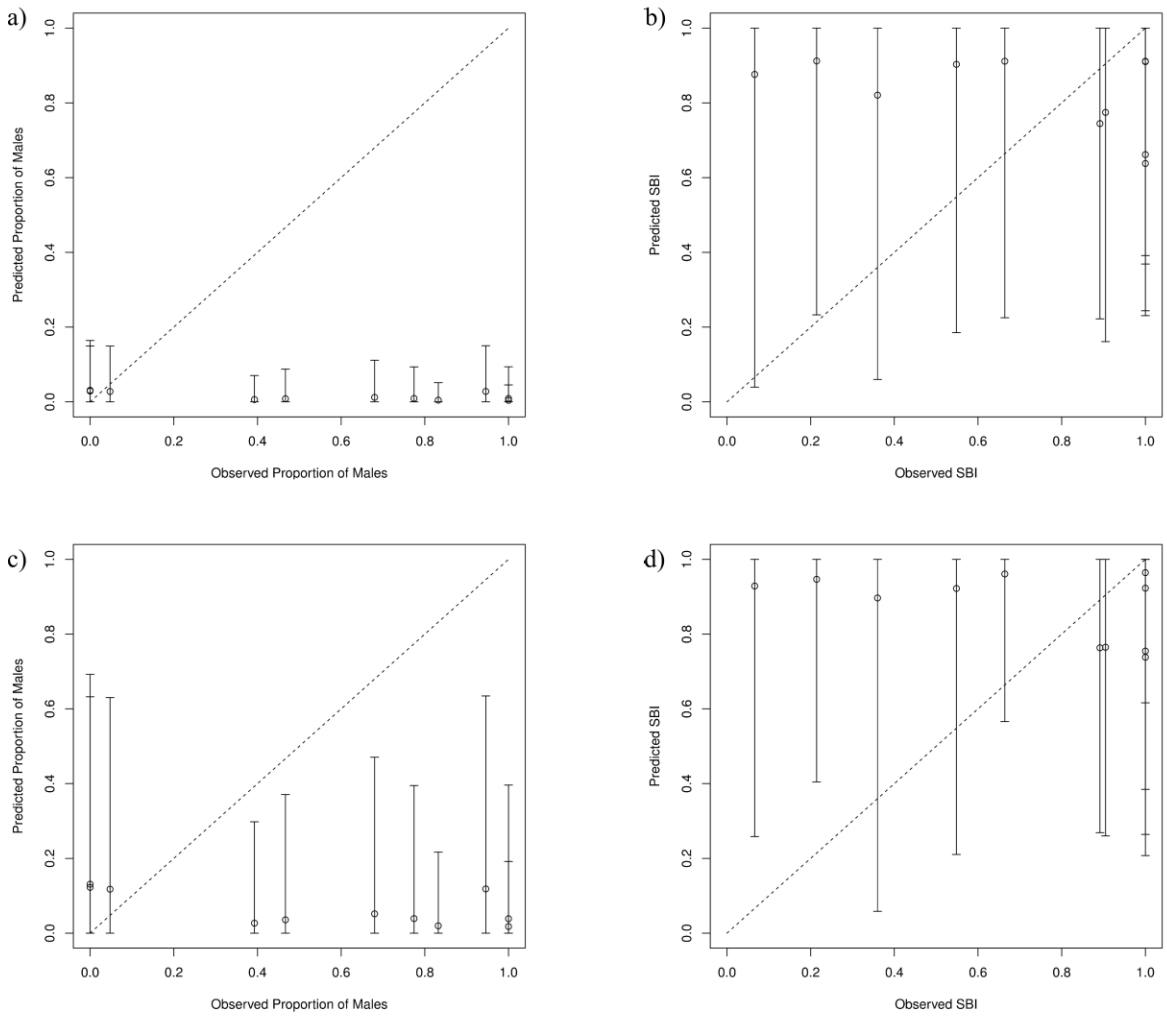


Figure 6.7. Observed versus predicted values for the Western Turure Downstream Metapopulation, meter. Carrying capacities for all subpopulations were modeled as $1m^2$. a) The observed versus predicted sex ratio denoted as proportion of males. b) The observed versus predicted Sex Bias Index. 237 out of 1000 simulations maintained both sexes at the end of 500 years.

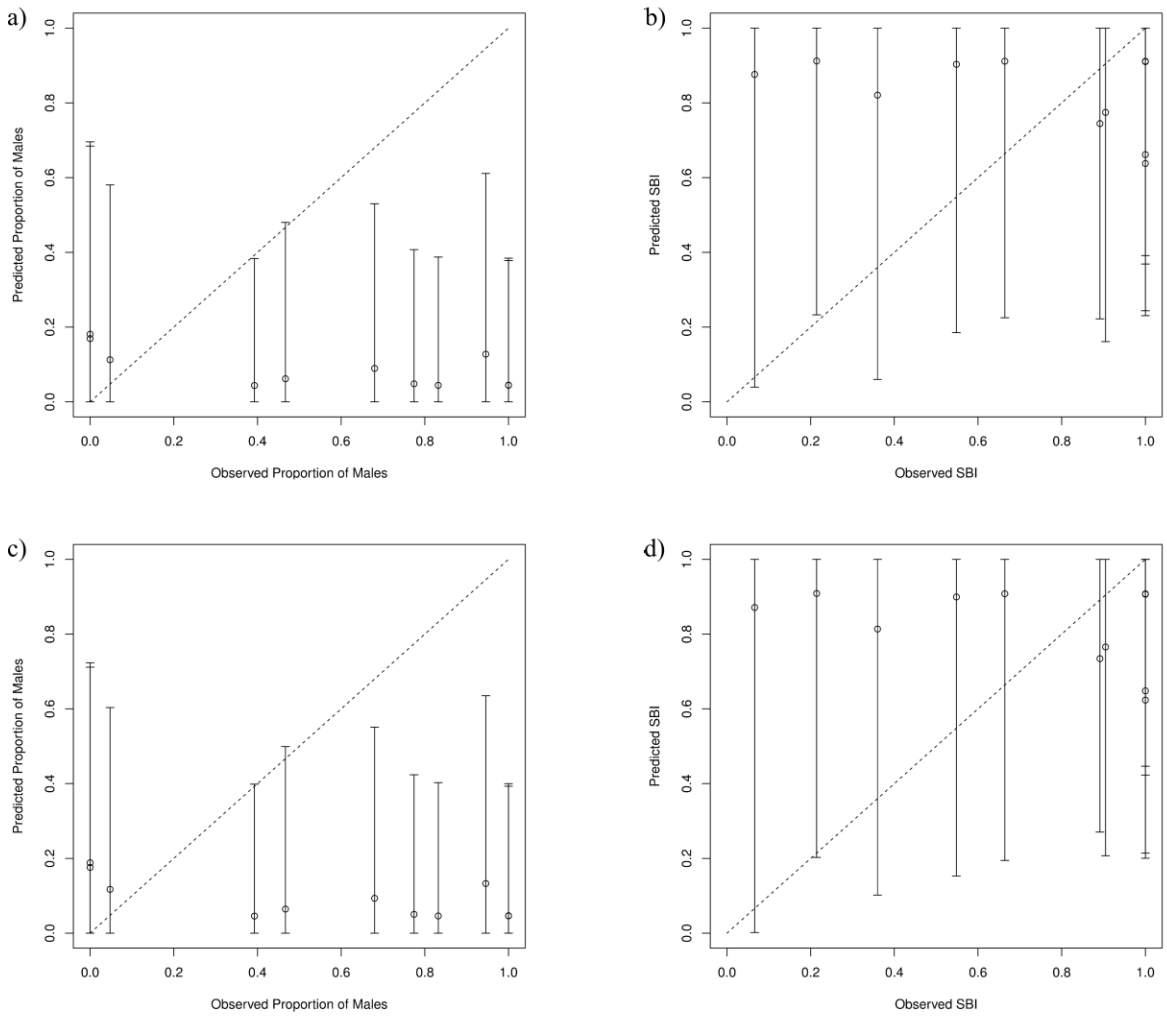


Figure 6.8. Observed versus predicted values for the Western Turure Downstream Metapopulation, patch. Carrying capacities for all subpopulations were modeled as the observed carrying capacity in the field. Figures a) and c) present the observed versus predicted sex ratio denoted as proportion of males. Figures b) and d) present the observed versus predicted Sex Bias Index. Figures a) and b) compute predicted values using all simulations, while figures c) and d) compute the predicted values using only simulations that contained both sexes at the end of 500 years. 962 out of 1000 simulations contained both sexes at the end of 500 years.

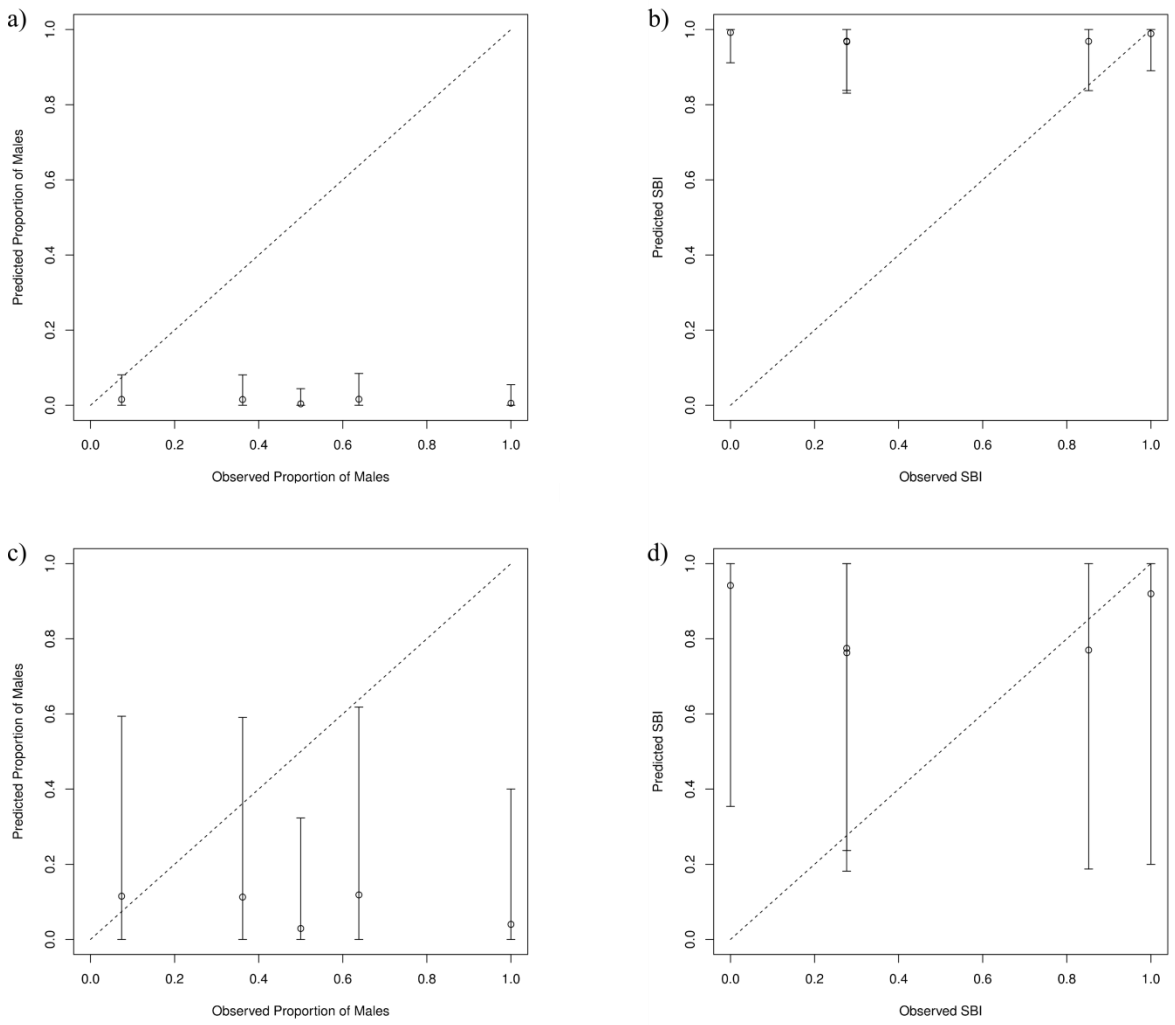


Figure 6.9. Observed versus predicted values for the Western Turure Downstream Metapopulation, meter. Carrying capacities for all subpopulations were modeled as $1m^2$. a) The observed versus predicted sex ratio denoted as proportion of males. b) The observed versus predicted Sex Bias Index. 137 out of 1000 simulations maintained both sexes at the end of 500 years.

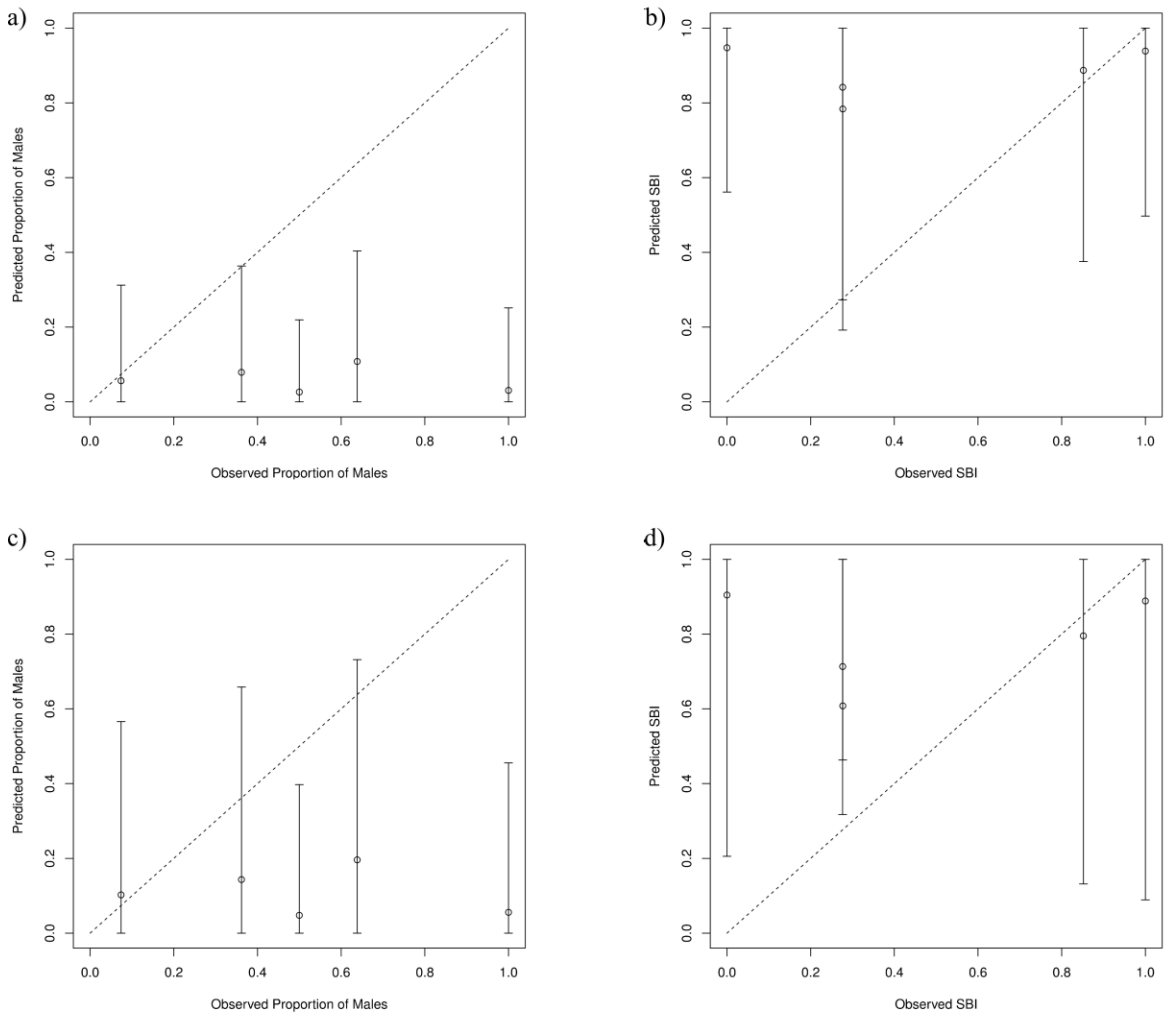


Figure 6.10. Observed versus predicted values for the Western Turure Downstream Metapopulation, patch. Carrying capacities for all subpopulations were modeled as the observed carrying capacity in the field. Figures a) and c) present the observed versus predicted sex ratio denoted as proportion of males. Figures b) and d) present the observed versus predicted Sex Bias Index. Figures a) and b) compute predicted values using all simulations, while figures c) and d) compute the predicted values using only simulations that contained both sexes at the end of 500 years. 552 out of 1000 simulations contained both sexes at the end of 500 years.

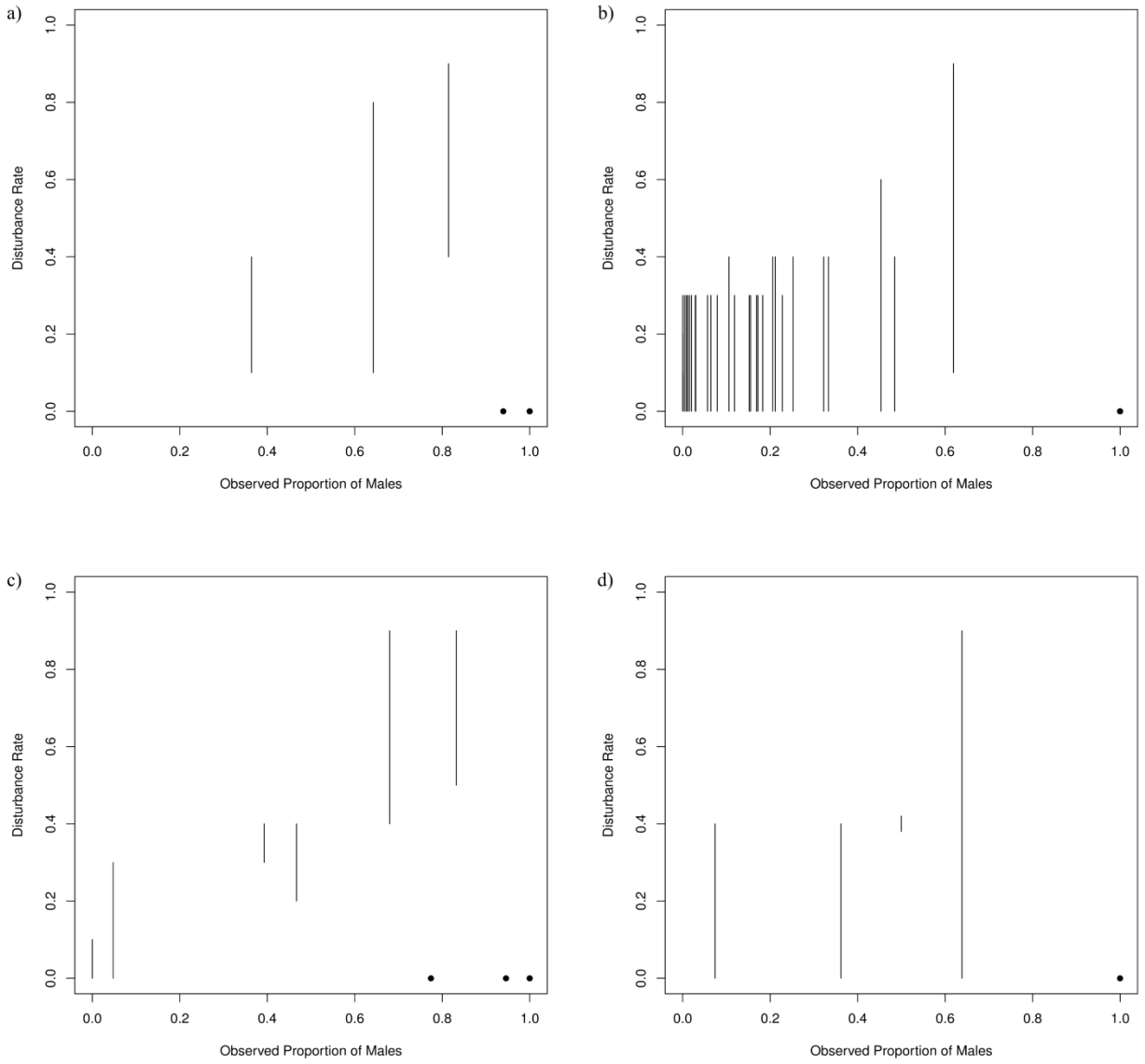


Figure 6.11. Predicted disturbance regime to produce the observed proportion of males. The monthly probability of disturbance was modified for the metapopulation as a whole. I tested disturbance probabilities from 0 to 0.9 in 0.1 increments. Lines on the graphs represent the range of increments where the predicted proportion of males overlapped the observed proportion of males. Filled-in circles represent subpopulations where the observed sex ratio did not overlap any predicted sex ratios for any of the disturbance values. The four different metapopulations show variation in predicted disturbance regimes across and within metapopulations. The metapopulations are: a) Eastern Turure, b) Quare, c) Western Turure, downstream, and d) Western Turure, upstream.

Copyright ©Christopher Stieha 2012

CHAPTER SEVEN

Conclusion and Future Directions

Overall Conclusions

The maintenance of the sexes is required for sexual reproduction. Depending on the research organism, the mechanism for the maintenance of the sexes may give insight into the evolution of sexual reproduction. From my research on the clonal plant *Marchantia inflexa*, dispersal and spatial configuration of subpopulations within a metapopulation contribute to the maintenance of the sexes in subpopulations and the metapopulation as a whole. Depending on 1) the carrying capacities of the subpopulations, 2) the distance to the nearest neighboring subpopulation, and 3) the spatial arrangement of subpopulations with respect to one another, both sexes can be maintained within a subpopulation and the metapopulation. If the subpopulations are close enough that asexual propagules contribute to interpopulation dispersal, single-sex metapopulations can persist. If the subpopulations cannot exchange migrants due to distances between subpopulations, the populations will go extinct once one sex is lost.

Spatial configuration and metapopulation theory is used in conservation to predict the persistence of a species. When comparing predicted values versus observed values, however, mathematical models may not be able to produce subpopulation estimates of population dynamics and persistence and may not be transferable between metapopulations. Thus conservation biologists must consider the influence of spatial configuration in conjunction with other factors, such as life history differences between individuals in different areas and environmental variation among habitats.

Future research directions can confirm and extend results presented here while increasing the understanding of the maintenance of sexes, of population dynamics of clonal organisms, and of the *Marchantia* system specifically. Future directions focus on 1) using other techniques to better observe and understand population and metapopulation dynamics, 2) quantifying dispersal capabilities and 3) extending current mathematical models.

Use of genetic information

Genetic techniques can be used to determine the sex of individuals as well as their genetic makeup. The quantification of sex ratio with sex markers would produce an estimate of the sex ratio that is not dependent on the time of the year or whether individuals within the subpopulation are sex expressing. I collected sex ratio data multiple times over the course of a season. Current field surveys are single instances of the sex ratio, limited by the subpopulations that are expressing and their current point in the expression cycle. When field surveys occur only once a year, the estimation of the sex ratio by sex markers would produce more reliable results that are comparable across years.

By quantifying the genetic makeup of individuals, one would be able to determine how clones contribute to intrapopulation dynamics, the dispersal capabilities of asexual propagules, and the amount of genetic variation for each subpopulation (an estimation of dispersal and sexual reproduction). For example, a newly colonized area could be sampled for all individuals to determine if multiple propagules landed within the patch. If the initial colonization event is observed, obvious by the presence of a small plant or plants, one can determine if the colonizers are the product of sexual or asexual reproduction. Potentially, the neighboring population that produced the propagules could be determined, although the number of individuals and subpopulations that would need to be sampled may be unfeasible. By looking at the genetic variation within and between subpopulations, one could get an estimate on overall dispersal and outcrossing within the metapopulation.

Application of spatial statistics

Spatial statistics are used to determine spatial autocorrelation and patterns of some observed characteristic. While not explicitly testing hypotheses, different spatial patterns are the products of different mechanisms, giving insight into potential influencing mechanisms and driving future research. Given the distribution of subpopulations as points on the landscape as opposed to continuous distribution, two spatial statistical techniques would be applicable to the study of the maintenance of

the sexes: Ripley's K and semivariogram analysis (Dale 1999; Fortin and Dale 2005). Ripley's K determines if some categorical variable is distributed in a random, uniform, or clumped distribution. In the case of *M. inflexa*, the categorical values could either be 1) whether the subpopulation contained both sexes or only one sex or 2) whether the subpopulation contained only males, contained only females, or contained both males and females. In both cases, subpopulations without sex expression would have to incorporated and accounted for. Both designations offer insight into the dynamics. For example, if all female subpopulations are clumped in space, this could be due to a similar colonization time (males have been outcompeted), dispersal of asexual propagules, or environmental homogeneity promoting a female bias.

Semivariograms are used to determine the correlation of a continuous variable between two subpopulations due to distance between the two subpopulations. Correlation between sex ratio would indicate that propagules disperse between the subpopulations (although the effects of asexual propagules and sexual propagule would be confounded) or that environmental homogeneity exists that causes the subpopulations to have similar population trajectories. I have presented initial spatial analyses in Appendix 1.

Dispersal capabilities of sexual propagules

In Chapter Three, I quantified the production, dispersal, and survival of asexual propagules. Although the products of sexual propagules are smaller and more elusive, the same types of experiments could be performed to determine the production, dispersal, and survival of sexual propagules, spores. Production would involve experiments determining the maximum production of spores by females, especially in the case of sperm limitation. Since the transfer of sperm occurs via a splash mechanisms from the male's antheridia to the females archegonia, the probability of a rain drop with sperm landing on a female is low. In the laboratory, dispersal of spores could be quantified in a wind tunnel or with a speed-regulated fan. In the field, Vaseline-covered slides have been used in other

species to capture spores. Interpopulation dispersal of spores could be quantified using similar mathematical models, although dispersal of spores is confounded with dispersal of asexual propagules, which both males and females produce. Spores are dispersed by wind, making a desiccation experiment more important than a submersion experiment. Spore germination within a currently existing population versus new areas needs to be quantified.

Long term subpopulation and metapopulation dynamics

The data in my dissertation were collected during a year-long survey and study of the subpopulation and metapopulation dynamics. A longer-term, fine resolution survey tracking the number of sex structures within subpopulations across years would shed light on the long-term competition between the sexes within subpopulations as well as within the metapopulation. Metapopulation dynamics require colonization and extinction events. Newly colonized subpopulations could be tracked to determine if the competition dynamics match mathematical models. Extinctions could be tracked and a relationship between subpopulation size and extinction rate could be determined.

Enhancement of mathematical model

Given the detailed mathematical model, I have determined that the current model could be enough to predict the proportion of males and females at the metapopulation level, but not necessarily at the subpopulation level. Also, predicting the dynamics of other metapopulations is not as simple as incorporating the spatial configuration of the subpopulations within those metapopulations. Life history differences between the individuals within the metapopulations could have affected my ability to predict the population dynamics. I have observed variation in the time when the two sexes express and the rate at which they express between metapopulations and between subpopulations within a single metapopulation. Environmental heterogeneity between subpopulations and metapopulations could greatly influence the population dynamics. Some habitats may be better suited for males, either through

disturbance regime, light availability, humidity, or some other abiotic factors, which would drive competition dynamics and therefore population dynamics. To make the model quantitatively predictive (predicting whether males or females dominate, not just that one sex is lost) requires the incorporation of environmental heterogeneity.

Copyright ©Christopher Stieha 2012

APPENDIX 1

Spatial Analysis of Proportion of Males

Methods

In 2007, I exhaustively sampled the metapopulation of the plant *Marchantia inflexa* along the Quare River in Trinidad and Tobago (Figure A1.1). I marked all subpopulations of *M. inflexa* within a defined stream reach and calculated each subpopulation's distance from other subpopulations. I also counted the male and female sex structures in each subpopulation over a three month period (see Chapter 4 and 6). I used the total number of male and female sex structures throughout the season to estimate the proportion of both sexes within the subpopulation. Some subpopulations contained individuals that did not express sex. Without sex structures, the sex of the plant could not be determined in the field. Analyses included both expressing and non-expressing populations.

Ripley's K uses spatial point data to determine whether qualitative classes are overdispersed (uniformly distributed), underdispersed (clumped), or randomly dispersed with respect to one another (Ripley 1976, 1977; Dale 1999; Fortin and Dale 2005). Ripley's K is computed as

$$K(d) = (A/n^2)\ \Sigma\ \Sigma\ w_{ij}\ k_{ij} \qquad \text{eq. 1}$$

where d is the distance, A is the area of the study system, n is the number of points within the study system, w_{ij} is a weighted function that corrects for edge effects, and k_{ij} is whether or not the distance between the two populations is less than d. More often, Ripley's K is subtracted from the expected value assuming randomness to give $\hat{L}$ (Dale 1999). $\hat{L}$ is defined as

$$\hat{L}\ (d) = d - (K(d)/\pi)^{(1/2)} \qquad \text{eq. 2}$$

for a 2 dimensional space, where d is the distance between two populations and $K(d)$ is computed from equation 1. With equation 2, $\hat{L} = 0$ corresponds to randomness, $\hat{L} < 0$ corresponds to clumping, and $\hat{L} > 0$ corresponds to overdispersion (Dale 1999).

Using the bivariate Ripley's K and associated $\hat{L}$ (Dale 1999; Dale and Fortin 2005), I determined whether the types of subpopulations exhibited spatial pattern based on 1) whether or not the subpopulation were expressing sex and 2) whether subpopulations contained only males, only females, both sexes, or were not expressing. Confidence intervals were determined through 1000 randomization tests. Because the plant metapopulations were found along streams, the subpopulations naturally exhibited a clumped pattern (found only along the stream). To account for this inherent clumping, location was fixed for each subpopulation but the type of subpopulation was allowed to vary in the randomization test. In this case, observed $\hat{L}$ values below the lower confidence interval suggest clumping, values above the upper confidence interval suggest overdispersion, while values in between the confidence intervals suggest a random distribution.

Semivariogram analysis is used to determine the effect of distance between two subpopulations on the variation in the dependent variable between the two subpopulations (Dale 1999; Fortin and Dale 2005). Semivariance, *S(h)*, (Dale 1999; Fortin and Dale 2005) is defined as

$$S(h) = (1/2)*(1/N(h))\ \Sigma[z(x_i) - z(x_i + h)]^2 \qquad \text{eq. 3}$$

where h is the lag distance, $N(h)$ is the number of pairs distance h from one another, $z(x_i)$ is the value of the dependent variable at position x_i and $z(x_i + h)$ is the value of the dependent variable at the point h distance from x_i. Populations close together should have similar values of the dependent variable ($[z(x_i) - z(x_i + h)]^2$ from equation 3 should be close to 0), while populations far apart should not have similar values of the dependent variable ($[z(x_i) - z(x_i + h)]^2$ from equation 3 should be greater than 0). In the semivariogram analysis, the proportion of males was my dependent variable.

Using semivariogram analysis, I determined the distance that similarity between subpopulations decreased. This distance suggests the potential dispersal capabilities of propagules or the extent of environmental similarity. Semivariance was computed for each pair of subpopulations with known sex ratios. Distances were broken up into 10m categories starting at 0m and ending at 250m, half of the

total longest distance observed between subpopulations. The semivariance of all pairs of subpopulations within a 10m category were averaged together to produce one data point for the category.

For both Ripley's K and semivariogram analysis, distances between subpopulations were computed along the stream as opposed to Euclidean distance in a 2D space. All analyses were performed with the software package PASSaGE Version 2 Beta (Rosenberg and Anderson 2009).

Results and Discussion

Subpopulations that were expressing sex were randomly distributed with respect to other subpopulations that were expressing sex (Figure A1.2a). Non-expressing subpopulations were clumped with respect to other non-expressing subpopulations at distances less than 16m and were randomly distributed at distances greater than 16m (Figure A1.2b). Non-expressing subpopulations were uniformly distributed from subpopulations expressing sex between 8m and 16m (Figure A1.3c). Above 16m and below 8m, the two types of subpopulations were randomly distributed with respect to one another. Subpopulations that were not expressing sex were clumped with respect to other subpopulations not expressing sex. The clumping of non-expressing subpopulations suggests some factor may be limiting sex expression, such as abiotic environment or age of subpopulation. If younger, more recently colonized subpopulations are less likely to express sex, these results suggest a distance for spatial correlation of colonizations and potentially extinctions-recolonization events.

Subpopulations that contained both sexes were randomly distributed across all distances when compared to other subpopulations that contained both sexes (Figure A1.3a). Female-only subpopulations were randomly distributed at all distances (Figure A1.3b). Male-only subpopulations tended to be uniformly distributed at distances less than 18m and were randomly distributed at distances above 18m (Figure A1.2c). The uniform distribution result may be influenced by the small number of male-only subpopulations within the metapopulation (only four out of 80 subpopulations).

The semivariance in the proportion of males increased until subpopulations were greater than 30m apart, at which point the semivariance decreased dramatically (Figure A1.4). After 30m, the semivariance fluctuated, with another drop around 100m. If the proportion of males is environmentally controlled through light, humidity, extinction-recolonization events, these results suggest environmental cycles of 30m and 100m. Spatial correlation of the proportion of males occurred at distances up to 30m between subpopulations. 30m may be the extent of environmental similarity in the system, but may also be the upper limit of dispersal and colonization of propagules.

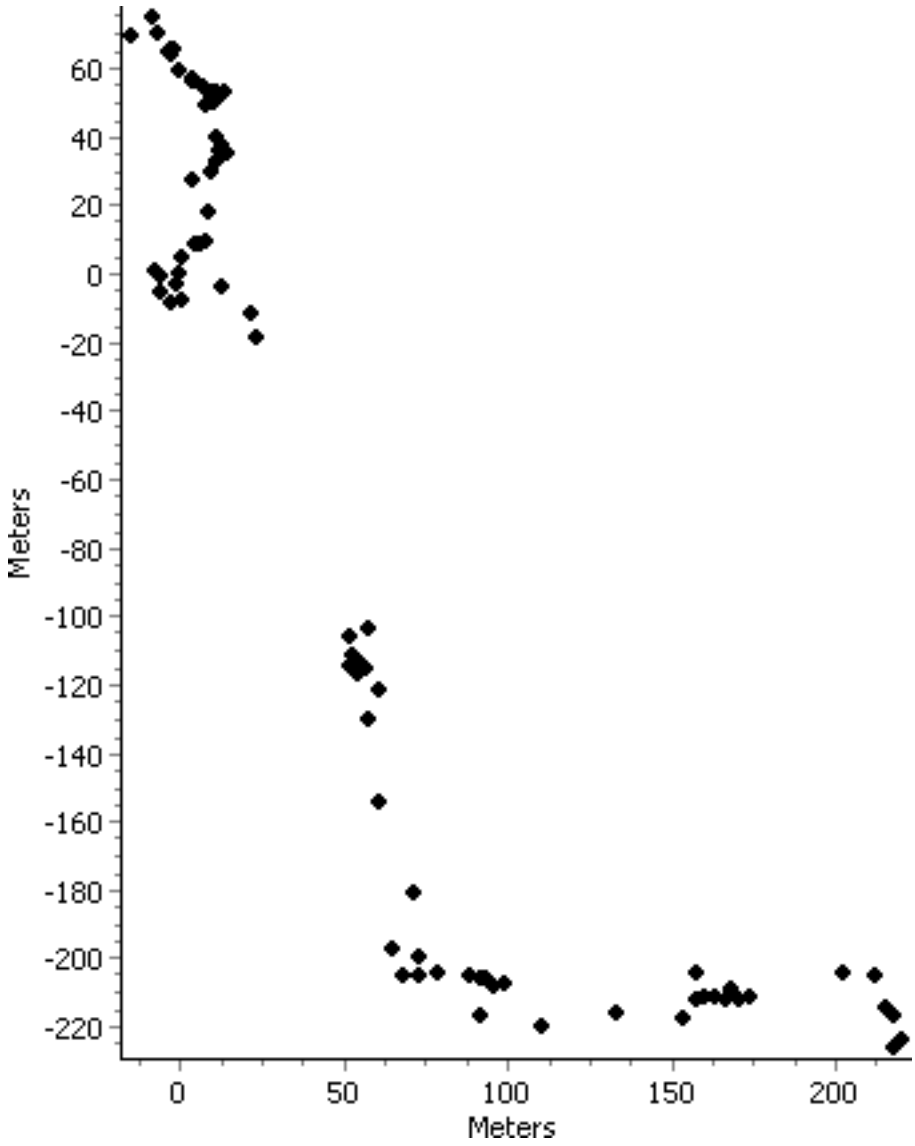


Figure A1.1. Spatial Distribution of Subpopulations along Quare River. Subpopulations exhibited a clumped distribution within the landscape because they all occurred along a stream. All subpopulations were analyzed as a single metapopulation, although subpopulations appeared to be separated into two discrete metapopulations separated by 80 meters.

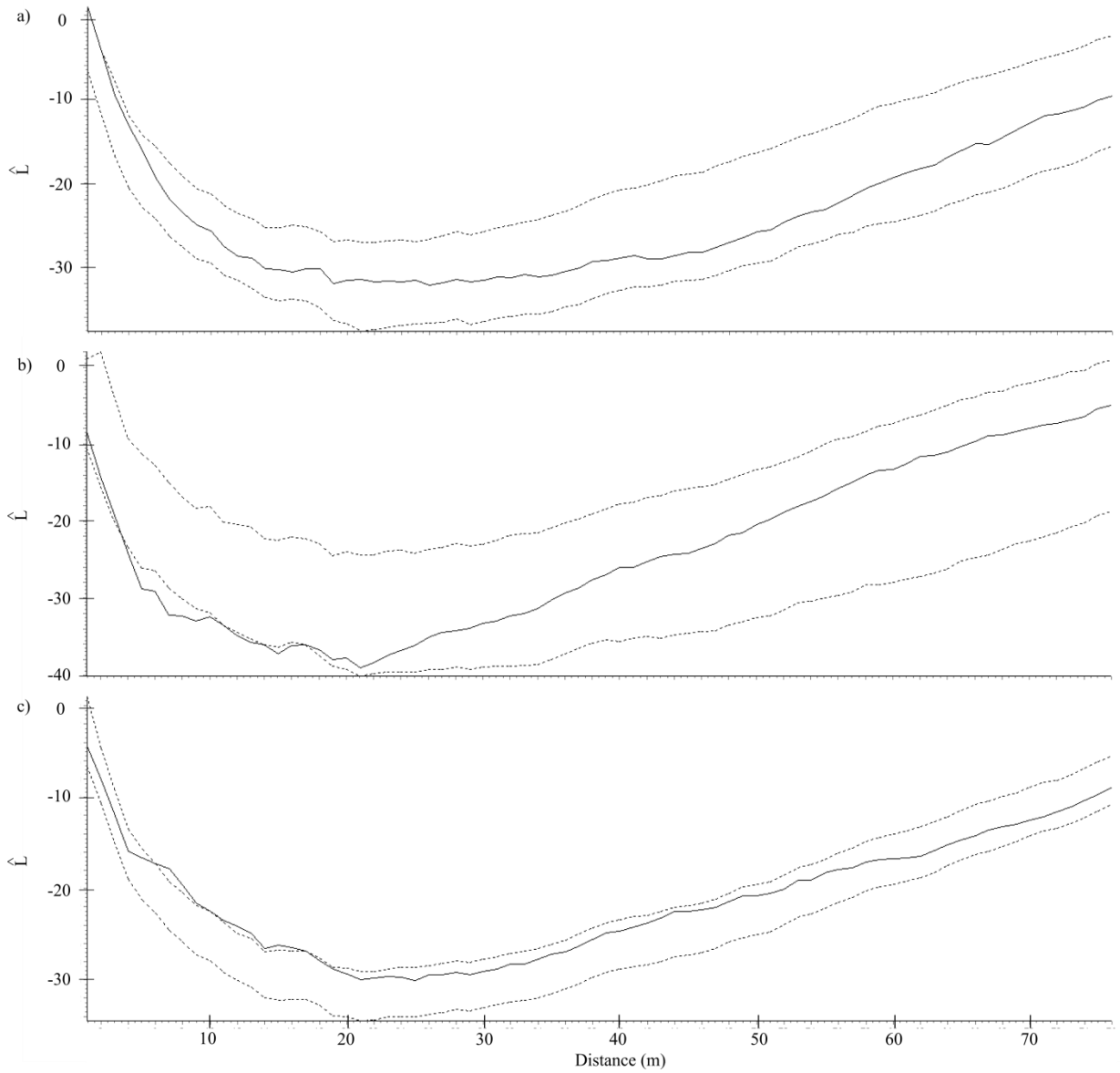


Figure A1.2. Spatial Distribution of Expressing and Non-Expressing Subpopulations. Solid lines represent the $\hat{L}$ values that correspond to the observed values. Dashed lines represent the upper and lower 95% confidence intervals assuming the types of subpopulations are randomly distribution across all locations. If the solid line falls above the upper confidence interval, the subpopulation types exhibit overdispersion and are uniformly distributed within the stream at that distance. If the solid line falls below the lower confidence interval, the subpopulation types are clumped at that distance. If the solid line falls within the confidence interval, the subpopulation types are randomly distributed at that distance. The three graphs show the distributions of a) subpopulations expressing sex with respect to other subpopulations also expressing sex, b) subpopulations not expressing sex with respect to other subpopulation not expressing sex, and c) subpopulations expressing sex with respect to subpopulations not expressing sex.

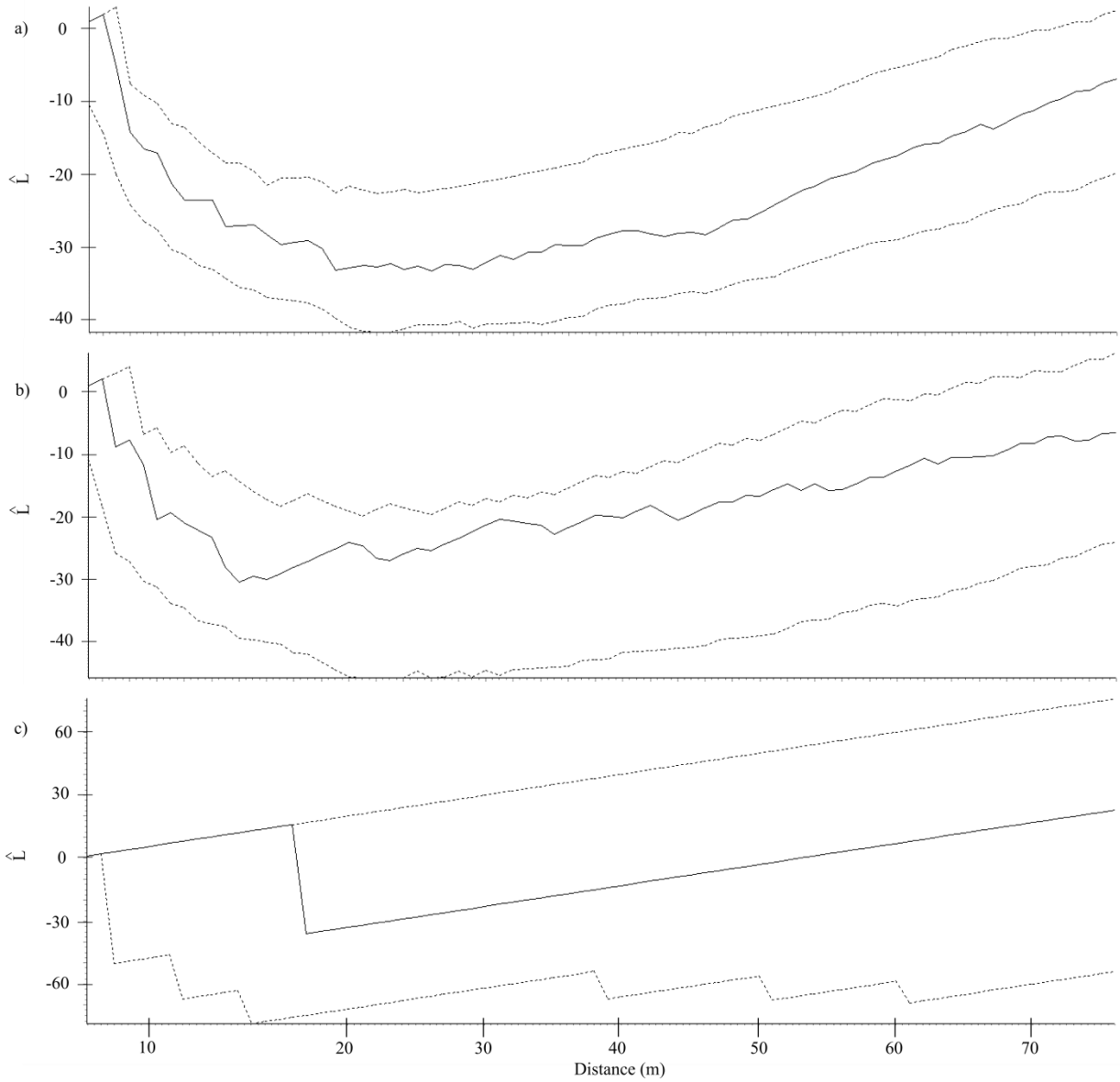


Figure A1.3. Spatial Distribution of Two-Sex, Male-Only, and Female-Only Subpopulations. Solid lines represent the $\hat{L}$ values that correspond to the observed values. Dashed lines represent the upper and lower 95% confidence intervals assuming the types of subpopulations are randomly distribution across all locations. If the solid line falls above the upper confidence interval, the subpopulation types exhibit overdispersion and are uniformly distributed across the stream at that distance. If the solid line falls below the lower confidence interval, the subpopulation types are clumped at that distance. If the solid line falls within the confidence interval, the subpopulation types are randomly distributed at that distance. The three graphs show the distributions of a) subpopulations containing both sexes with respect to other subpopulations containing both sexes, b) subpopulations containing only females with respect to other subpopulations containing only females, and c) subpopulations containing only males with respect to other subpopulations containing only males.

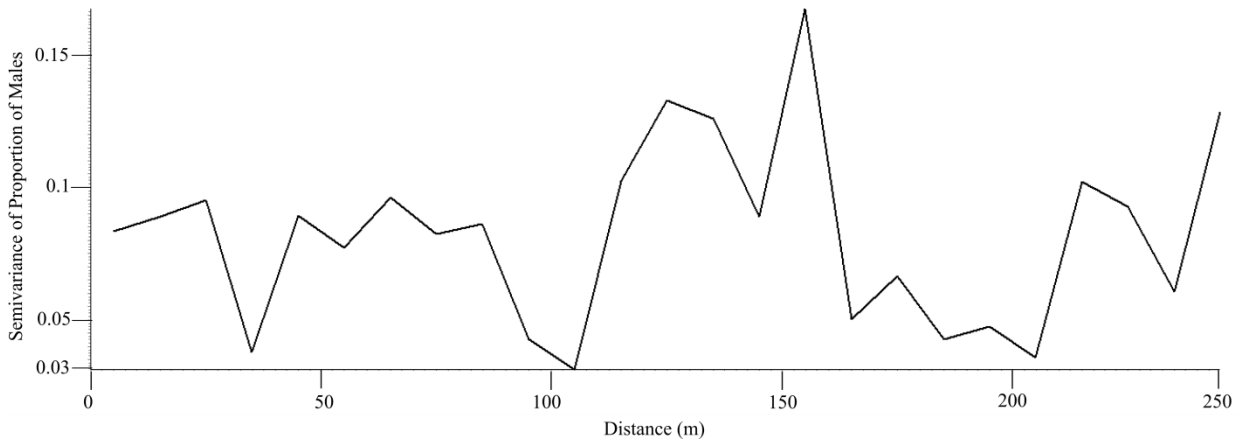


Figure A1.4. Semivariogram of Proportion of Males. Each point represents the mean semivariance of all pairwise comparisons of subpopulations separated by a distance within that 10m interval. Proximity to the neighboring subpopulations should influence the focal subpopulation's proportion of males, where subpopulations closer together should be more similar with respect to proportion of males than subpopulations farther apart. The distance where semivariance levels off suggests the edge of dispersal-influenced dynamics and/or environmental similarity. Leveling off occurs well before 100m with an immediate decline around 35m, suggesting dispersal influences in *M. inflexa* are localized.

Copyright ©Christopher Stieha 2012

REFERENCES

Abbott, K.C. 2011. A dispersal-induced paradox: synchrony and stability in stochastic metapopulations. Ecology Letters. 14(11):1158-1169.

Akima, H. (1978). A Method of Bivariate Interpolation and Smooth Surface Fitting for Irregularly Distributed Data Points. ACM Transactions on Mathematical Software, 4, 148-164.

Akima, H. (1996). Algorithm 761: scattered-data surface fitting that has the accuracy of a cubic polynomial. ACM Transactions on Mathematical Software, 22, 362-371.

Amat, F., Hontoria, F., Ruiz, O., Green, A.J., Sánchez, M.I., Figuerola, J., Hortas, F. 2005. The American brine shrimp as an exotic invasive species in the western Mediterranean. Biological Invasions. 7:37-47.

Anderson, J.L., Morran, L.T., and Phillips, P.C. 2010. Outcrossing and the Maintenance of Males within *C. elegans* Populations. Journal of Heredity. 101:S62-S74.

Ashton P.S., Mitchell D.S. 1989. Aquatic plants: patterns and modes of invasion, attributes of invading species and assessment of control programmes. In: Drake JA, Mooney HA, di Castri F, Groves RH, Kruger FJ, Rejmanek M, Williamson M (eds) Biological invasions: a global perspective. Wiley, Chichester, pp 111–147

Avise, J.C. 2008. Clonality: The Genetics, Ecology, and Evolution of Sexual Abstinence on Vertebrate Animals. Oxford Press, Oxford.

Bataillon, T. 2000. Estimation of spontaneous genome-wide mutation rate parameters: whither beneficial mutations? Heredity. 84:497-501.

Bates, D., Maechler, M., and Bolker, B. 2011. lme4: Package to fit linear and generalized linear mixed-effects models. V0.999375-42 http://lme4.r-forge.r-project.org/

Baguette M. and Van Dyck, H. 2007. Landscape connectivity and animal behavior: Functional grain as a key determinant for dispersal. Landscape Ecology. 22:1117-1129.

Bell, G. 1982. The Masterpiece of Nature. San Fransisco: University of California Press.

Berry, W.D. and Feldman, S. 1985. Multiple regression in practice. Sage University Paper series on Quantitative Applications in the Social Sciences, 07-050. Newbury Park, CA: Sage.

Bierzychudek, P. and Eckhart, V. 1988. Spatial Segregation of the Sexes of Dioecious Plants. The American Naturalist. 132(1):34-43.

Bisang, I., Ehrlen, J. and Hedenaes, L. 2004. Mate limited reproductive success in two dioicous mosses. OIKOS. 104:291-298.

Bischler H 1984. *Marchantia L.* The New World species. Bryophytorum Bibliotheca 26.

Bischler H 1986. *Marchantia polymorpha* L. s. lat. karyotype analysis. Journal of the Hattori Botanical Laboratory 60:105-0117.

Bowker, M.A., Startk, L.R., McLetchie, D.N., and Mischler, B.D. 2000. Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). American Hournal of Botany. 87:517-526.

Brodie, H.J. 1951. The splash-cup mechanism in plants. Canadian Journal of Botany. 29(3):224-234.

Browne, R.A. 1980. Competition Experiments Between Parthenogenetic and Sexual Strains of the Brine Shrimp, *Artemia salina*. Ecology. 61(3):471-474.

Busch, J.W., Neiman, M., Koslow, J.M. 2004. Evidence for maintenance of sex by pathogens in plants. Evolution. 58(11):2584-2590.

Case, T.J. and Taper, M.L. 1986. On the coexistence and coevolution of asexual and sexual competitors. Evolution. 40(2):366-387.

Chaplin, J.A. 1993. The local displacement of a sexually reproducing ostracod by a conspecific parthenogen. Heredity. 71:259-268.

Chapman, H.M., Parh, D., and Oraguzie, N. 2000. Genetic structure and colonizing success of a clonal, weedy species, *Pilosella officinarum* (Asteraceae). Heredity. 84:401-409.

Chasnov, J.R. 2009. The evolution from females to hermaphrodites results in a sexual conflict over mating in androdioecious nematode worms and clam shrimp. Journal of Evolutionary Biology. 23:539-556.

Coppin, E., Debuchy, R., Arnaise, S., and Picard, M. 1997. Mating types and sexual development in filamentous ascomycetes. Microbiology and Molecular Biology Reviews. 61(4):411-428.

Cousens, R., Dytham, C., and Law, R. 2008. Dispersal in Plants: A Population Perspective. Oxford Press, Oxford.

Crawley, M.J. 2007. The R Book. John Wiley & Sons, West Sussex, England.

Crowley, P.H. 1981. Dispersal and the stability of predator-prey interactions. The American Naturalist. 118:673-701.

Crowley, P.H. and McLetchie, D.N. 2002. Trade-offs and Spatial Life-History Strategies in Classical Metapopulations. The American Naturalist. 159(2):190-208.

Crowley, P.H., Davis, H.M., Ensminger, A.L., Fuselier, L.C., Jackson, J.K., and McLetchie, D.M. 2005a. A general model of local competition for space. Ecology Letters. 8:176-188.

Crowley, P.H., Stieha, C.R., and McLetchie, D.N. 2005b. Overgrowth competition, fragmentation and sex-ratio dynamics: a spatially explicit, sub-individual-based model. Journal of Theoretical Biology. 233(1):25-42.

Cutter, A.D. 2005.Mutation and the experimental evolution of outcrossing in *Caenorhabditis elegans*. Journal of Evolutionary Biology. 18:27-34.

Dale, M.R.T. 1999. Spatial Pattern Analysis in Plant Ecology. Cambridge Studies in Ecology. Cambridge University Press, Cambridge.

Daly, M. 1978. The Cost of Mating. The American Naturalist. 112(986):771-774.

Dawson, K.J. 1995. The Advantage of Asexual Reproduction: When is it Two-Fold? Journal of Theoretical Biology. 176:341-347.

Deng, H-W. 1996. Environmental and genetic control of sexual reproduction in *Daphnia*. Heredity. 76:449-458.

Doncaster, C.P., Pound, G.E., and Cox, S.J. 2000. The ecological cost of sex. Nature. 404: 281-285.

During, H.J. 2006. Trends in Bryophyte Population Dynamics. Lindbergia. 31(1/2):6-15.

Ebert, D. and Hamilton, W.D. 1996. Sex against virulence: the coevolution of parasitic diseases. Trends in Ecology and Evolution. 11(2):79-82.

Eckert, C.G. 2002. The loss of sex in clonal plants. Evolutionary Ecology. 15:501-520.

Eklund, A.C. 2010. Package 'beeswarm'. http://www.cbs.dtu.dk/~eklund/beeswarm/

Elliott, A.M. and Hayes, R.E. 1953. Mating Types in *Tetrahymena*. Biological Bulletin. 105(2):269-284.

Engelstädter, J. 2008. Constraints on the evolution of asexual reproduction. Bioessays. 30:1138–1150.

Eppley, S.M. and Pannell, J.R. 2007. Sexual Systems and Measures of Occupancy and Abundance in an Annual Plant: Testing the Metapopulation Model. The American Naturalist. 169(1):20-28.

Equihua, C. 1987. Diseminacion de yemas en *Marchantia polymorpha* L. (Hepaticae). Cryptogamie, Bruol. Lichenol. 8(3):199-217.

Flather, C.H. and Bevers, M. 2002. Patchy Reaction-Diffusion and Population Abundance: The Relative Importance of Habitat Amount and Arrangement. The American Naturalist. 159(1):40-56.

Fortin, M-J and Dale, M. 2005. Spatial Analysis: A Guide For Ecologists. Cambridge University Press, Cambridge.

Freckleton, R.P. and Watkinson, A.R. 2002. The large scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. Journal of Ecology. 90:419–434.

Fuselier, L. 2008. Variation in life history characteristics between asexual and sexual populations of *Marchantia inflexa*. The Bryologist. 111(2):248-259.

Fuselier, L. and McLetchie, D.N. 2004. Microhabitat and Sex Distribution in *Marchantia inflexa*, a Dioicous Liverwort. The Bryologist. 107(3): 345-356.

García-Dorado, A. 1997. The Rate and Effects Distribution of Viability Mutation in *Drosophila*: Minimum Distance Estimation. Evolution. 51(4):1130-1139.

García-Ramos, G., Stieha, C., McLetchie, D.N., and Crowley, P.H. 2007. Persistence of the sexes in metapopulations under intense asymmetric competition. Journal of Ecology. 95(5):937-950.

Gause, GF. 1932. Experimental studies on the struggle for existence. Journal of Experimental Biology 9: 389-402.

Geary, R.C. 1954. The Contiguity Ratio and Statistical Mapping. The Incoporated Statistician. 5(3):115-127+129-146.

Gebhardt, A., Petzoldt, T., and Maechler, M. 2012. akima package: Interpolation of irregularly spaced data. http://cran.r-project.org/web/packages/akima/index.html

González-Megías, A., Gómez, J.M., and Sánchez-Piñero, F. 2005. Consequences of spatial autocorrelation for the analysis of metapopulation dynamics. Ecology. 86:3264–3271.

Gotelli, N.J. 2001. A Primer of Ecology, third edition. Sinauer. Sunderland, MA.

Groen, K.E., Stieha, C.R., Crowley, P.H., and McLetchie, D.N. 2010. Sex-specific plant responses to light intensity and canopy openness: implications for spatial segregation of the sexes. Oecologia. 162(3):561-570.

Halkett, F., Harrington, R., Hulle, M., Kindlmann, P., Menu, F., Rispe, C., and Plantegenest, M. 2004. Dynamics of Production of Sexual Forms in Aphids: Theoretical and Experimental Evidence for Adaptive "Coin-Flipping" Plasticity. The American Naturalist. 163(6):E112-125.

Halkett, F., Plantegenest, M., Prunier-Leterme, N., Mieuzet, L., Delmotte, F., Simon, J.C. 2005. Admixed sexual and facultatively asexual aphid lineages at mating sties. Molecular Ecology 14:325–336.

Halkett, F., Kindlmann, P., Plantegenest, M., Sunnucks, P., and Simon, J.C. 2006. Temporal differentiation and spatial coexistence of sexual and facultative lineages of an aphid species at mating sites. Journal of evolutionary Biology. 19:809-815.

Hand, C. and Uhlinger , K.R. 1992. The Culture, Sexual and Asexual Reproduction, and Growth of the Sea Anemone *Nematostella vectensis*. The Biological Bulletin. 182(2):169-176.

Hanski, I. 1991. Metapopulation dynamics: brief history and conceptual domain. Biological Journal of the Linnean Society. 42:3-16.

Hanski, I. 1994. A Practical Model of Metapopulation Dynamics. Journal of Animal Ecology. 63(1):151-162

Hanski, I. 1999. Metapopulation Ecology. Oxford University Press, New York.

Hanski, I. and D. Simberloff. 1993. The Metapopulation Approach, Its History, Conceptual Domain, and Application to Conservation. In Metapopulation Biology: Ecology, Genetics, and Evolution, eds. I.A. Hanski and M.E. Gilpin. Academic Press, San Diego, CA. P5-26.

Hansson, L., Söderström, L. & Solbreck, C. 1992. The ecology of dispersal in relation to conservation. Pp. 162–200. In: Hansson, L. (ed.), Ecological principles of nature conservation. Elsevier, London.

Hardy, I.C.W. 2002. Sex Ratios: Concepts and Research Methods. University Press, Cambridge.

Hassell, M.P., May, R.M., Pacala, S.W., and Chesson, P.L. 1991. The Persistence of Host-Parasitoid Associations in Patchy Environments. I. A General Criterion. The American Naturalist. 138(3):568-583.

Hayes, W. 1953. Observations on a Transmissible Agent Determining Sexual Differentiation in *Bacterium coli*. Journal of General Microbiology. 8:72-88.

Higgins, S.I. And Cain, M.L. 2002. Spatially realistic plant population models and the colonization-competition trade-off. Journal of Ecology. 90:616-626.

Hilfiker K, Gugerli F, Schutz JP, Rotach P, Holderegger R. 2004. Low RAPD variation and female-biased sex ratio indicate genetic drift in small populations of the dioecious conifer *Taxus baccata* in Switzerland. Conservation Genetics 5:357–365.

Hoagland, D.R. and Arnon, D.I. 1950. The water-culture method for growing plants without soil. Circ. 347. Univ. of Calif. Agric. Exp. Station, Berkley

Holmström, K. and Jensen, H.J. 2004. Who runs fastest in an adaptive landscape: sexual versus asexual reproduction. Physica A. 337: 185-195.

Howard, R.S. 1994. Selection against deleterious alleles and the maintenance of biparental sex. Theoretical Population Biology. 45:313-323.

Howard, R.S. and Lively, C.M. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature. 367:554-557.

Howard, R.S. and Lively, C.M. 1998. The maintenance of sex by parasitism and mutation accumulation under epistatic fitness functions. Evolution. 52(2):604-610.

Hudson, P.J. and Cattadori, I.M. 1999. The Moran effect: a cause of population synchrony. Trends in Ecology and Evolution. 14(1):1-2.

Huffaker, C.B. 1958. Experimental Studies on Predation: Dispersion factors and Predator-Prey Oscillations. Hilgardia: a Journal of Agricultural Science. 27:795-834.

Hurst L.D. and Peck, J.R. 1996. Recent advances in understanding of the evolution and maintenance of sex. Trends in Ecology and Evolution. 11(2):46-52.

Ims, R.A. and Yoccoz, N.G. 1997. Studying Transfer Processes in Metapopulations: Emigration, Migration, and Colonization. In Metapopulation Biology: Ecology, Genetics, and Evolution. eds I.A. Hanski and M.E. Gilpin. Academic Press, New York.

Johansson, M.E. and Nilsson, C. 1993. Hydrochory, Population Dynamics and Distribution of the Clonal Aquatic Plant *Ranunculus lingua*. Journal of Ecology. 81(1):81-91.

Jokela, J., Dybdahl, M.F., and Lively, C.M. 2009. The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. The American Naturalist. 174:S43-S53.

Judson, O.P and Normark, B.B 1996. Ancient Asexual Scandals. Trends in Ecology and Evolution. 11(2):41-45.

Kerr, B., Riley, M.A. Feldman, M.W., and Bohannan, B.J.M. 2002. Local dispersal promotes biodiversity in real-life game of rock-paper-scissors. Nature. 418: 171-174.

Kimmerer, R.W. 1991. Reproductive Ecology of *Tetraphis pellucida* II. Differential success of sexual and asexual propagules. The Bryologist 94(3):284-288.

Kimmerer, R.W. and Young, C.C. 1995. The Role of Slugs in Dispersal of the Asexual Propagules of *Dicranum flagellare*. The Bryologist. 98(1):149-153.

Kimura, M. and Maruyama, T. 1966. The Mutational load with epistatic gene interactions in fitness. Genetics, 54(6):1337-1351.

Koelle, K. and Vandermeer, J. 2005. Dispersal-induced desynchronization: from metapopulations to metacommunities. Ecology Letters. 8(2):167-175.

Kondrashov, A. S. 1993. Classification of hypotheses on the advantage of amphimixis. Journal of Heredity, 84:372-387.

Kronstad, J.W. and Staben C. 1997. Mating in filamentous fungi. Annual Review of Genetics. 31:245–76.

Laaka-Lindberg, S. 1999. Asexual reproduction in a population of a leafy hepatic species *Lophozia silvicola* Buch in central Norway. Plant ecology. 141:137-144.

Laaka-Lindberg, S., Korpelainen, H., and Pohjamo, M. 2003. Dispersal of asexual propagules in bryophytes. Journal of the Hattori Botanical Laboratory. 93:319-330.

Lederberg, J., Cavalli, L.L. and Lederberg, E.M. 1952. Sex compatibility in *Escherichia coli*. Genetics. 37(6):720-730.

Lehtonen, J. Jennions, M.D., and Kokko, H. 2012. The many costs of sex. Trends in Ecology and Evolution. 27(3): 172-178.

Levin, S.A., Muller-Landau, H.C., Nathan, R., and Chave, J. 2003. The ecology and evolution of seed dispersal: a theoretical approach. Annual review of Ecology and Systematics. 34:575-604.
Levins, R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bulletin of the ESA. 15(3):237-240.
Lively, C.M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature. 328:519-521.
Lomnicki, A. 2001. Carrying capacity, competition and maintenance of sexuality. Evolutionary Ecology Research. 3:603-610.
Longton, R.E. 1992. Reproduction and rarity in British mosses. Biological Conservation. 59:89-98.
Longton, R.E. and Schuster, R.M. 1983. Reproductive biology. New Manual of Bryology. ed R.M. Schuster. Hattori Botanical Laboratory, Nichinan, Japan.
Lotka, A.J. 1932. The growth of mixed populations: two species competing for a common food supply. Journal of the Washington Academy of Sciences. 21:461-469.
Lyman, J.C. and Ellstrand, N.C. 1984. Clonal diversity in *Taraxacum officinale* (Compositae), an apomict. Heredity. 53(1):1-10.
Lynch, M. and Gabriel, W. 1990. Mutation load and the survival of small populations. Evolution. 44(7):1725-1737.
MacArthur, R.H., and Wilson, E.O. 1967. The theory of island biogeography. Princeton University Press, Princeton, N.J.
Manoel, D., Carvalho, S, Philips, P.C, and Teotónio, H. 2007. Selection against males in *Caenorhabditis elegans* under two mutational treatments. Proceedings of the Royal Society B. 274:417-424.
Martens, K., Rossetti, G., Horne, D.J. 2003. How ancient are ancient asexuals? Proc. R. Soc. Lond. B. 270:723-729.
MATLAB 2011a, version 7.12.0.635 2011. The Mathworks Inc. Natick, MA.
Matsuda, H. and Abrams, P.A. 1999. Why are equally sized gametes so rare? The instability of isogamy and the cost of anisogamy. Evolutionary Ecology research. 1:769-784.
Maynard Smith, J. 1974. Recombination and the Rate of Evolution. Genetics. 78:299-304.
Maynard Smith, J. 1978. The Evolution of Sex. Cambridge: Cambridge University Press.
Maynard Smith, J. 1986. Evolution: Contemplating life withou sex. Nature. 324(6095):300-301.
McFadden, C.S. 1997. Contributions of Sexual and Asexual Reproduction to Population Structure in the Clonal Soft Coral, *Alycyonium rudyi*. Evolution. 51(1):112-116.
McGovern, T.M. 2002. Sex-ratio bias and clonal reproduction in the brittle star *Ophiactis savignyi*. Evolution. 56(3):511-517.
McLetchie, D.N., García-Ramos, G. and Crowley, P.H. 2002. Local sex-ratio dynamics: a model for the dioecious liverwort *Marchantia inflexa*. Journal of Evolutionary Ecology. 15:231-254.
McLetchie, D.N. and Puterbaugh, M.N. 2000. Population Sex Ratios, Sex-Specific Clonal Traits and Tradeoffs among These Traits in the Liverwort *Marchantia inflexa*. Oikos. 90(2):227-237.
Miller, T.E.X. and Inouye, B.D. 2011. Confronting two-sex demographic models with data. Ecology 92: 2141-2151
Mizuki, I. and Takahashi, A. 2009. Secondary dispersal of *Dioscorea japonica* (Dioscoreaceae) bulbils by rodents. Journal of Forest Research. 14(2):95-100.
Moilanen, A. 1999. Patch occupancy models of metapopulation dynamics: Efficient parameter estimation using implicit statistical inference. Ecology 80:1031–1043.
Moilanen, A. 2004. SPOMSIM: software for stochastic patch occupancy models of metapopulation dynamics. Ecollogical Modelling. 179:533-550.
Moilanen, A. and Nieminen, M. 2002. Simple Connectivity Measures in Spatial Ecology. Ecology. 83(4):1131-1145.
Moran, P.A.P. 1950. Notes on Continuous Stochastic Phenomena. Biometrika. 37(1/2):17-23.

Morran, L.T., Cappy, B.J., Anderson, J.L., and Philips, P.C. 2009. Sexual partners for the stressed: facultative outcrossing in the self-fertilizing nematode *Caenorhabditis elegans*. Evolution. 63(6):1473-1482.

Morran, L.T., Parmenter, M.D., and Phillips, P.C. 2009 Mutation load and rapid adaptation favour outcrossing over self-fertlization. Nature. 462:350-352.

Muller, H.J. 1932. Some Genetic Aspects of Sex. The American Naturalist. 66:118-138.

Murdoch, W.W., Briggs, C.J., Nisbet, R.M., Gurney, W.S.C. & Stewart-Oaten, A.1992. Aggregation and stability in metapopulation models. Am. Nat., 140, 41–58.

Nanami, S., Kawaguchi, H., and Yamakura, T. 2005. Sex ratio and gender-dependent neighboring effects in Podocarpus nagi, a dioecious tree. Plant ecology. 177:209-222.

Nathan, R. and Muller-Landau, H.C. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. Trends in Ecology and Evolution. 15:278-285.

Nelson, M.A. 1996. Mating systems in ascomycetes: a romp in the sac. Trends in Genetics. 12(2):69-74.

Nicholson, A. J. and Bailey, V. A. 1935. The Balance of Animal Populations.—Part I.Proceedings of the Zoological Society of London, 105:551–598.

Otto, S.P. 2009. The Evolutionary Enigma of Sex. The American Naturalist. 174:S1-S14.

Pajunen, V.I. and Pajunen, I. 2003. Long-term dynamics in rock pool *Daphnia* metapopulations. Ecography. 26:731-738.

Papura, D., Simon, J.-C., Halkett, F., Delmotte, F., Le Gallic, J.-F., and Dedryver, C.A. 2003. Predominance of sexual reproduction in Romanian populations of the aphid *Sitobion avenae* inferred from phenotypic and genetic structure. Heredity. 90:397-404.

Parsons, J.G., Cairns, A., Jouhnson, C.N., Robson, S.K.A., Shilton, L.A., and Westcott, D.A. 2007. Bryophyte dispersal by flying foxes: A novel discovery. Oecolgia. 152(1):112-114.

Pascual, M., Roy, M., and Franc, A. 2002. Simple temporal models for ecological systems with complex spatial patterns. Ecology Letters. 5:412-419.

Peck, J.R. 1993. Frequency-dependent selection, beneficial mutations and the evolution of sex. Proceedings of the Roysal Society, London B. 125:87-92.

Peck, J.R. 1994. A Ruby in the Rubbish: Beneficial mutations, deleterious mutations and the evolution of sex. Genetics. 137:597-606.

Peck, J.R., Yearsley, J., and Barreau, G. 1999. The maintenance of sexual reproduction in a structured population. Proceedings of the Royal Society London, B. 266:1857-1863.

Peck, J.R., Yearsley, J.M., and Waxman, D. 1998. Explaining the geographic distribution of sexual and asexual populations. Nature. 391:889-892.

Pohjamo, M., Laaka-Lindberg, S., Ovaskainen, O., and Korpelainen, H. 2006. Dispersal potential of spores and asexual propagules in the epixylic hepatic *Anastrophyllum hellerianum*. Evolutionary Ecology. 20(5): 415-430.

Pound, G.E., Doncaster, C.P., and Cox, S.J. 2002. A Lotka-Volterra model of coexistence between a sexual population and multiple asexual clones. Journal of Theoretical Biology. 217:535-545.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Richmond, R.H. and Hunter, C.L. 1990. Reproduction and recruitment of corals: comparisons among the Caribbean, the tropical Pacific, and the Red Sea. Marine Ecology Progress Series. 60:185-203.

Ripley, B.D. 1976. The Second-Order Analysis of Stationary Point Pattern Processes. Journal of Applied Probability. 13(2): 255- 266.

Ripley, B.D. 1977. Modelling Spatial Patterns. Journal of the Royal Statistical Society, Series B. 39(2): 172-212.

Ronsheim, M.L. 1994. Dispersal Distances and Predation Rates of Sexual and Asexual Propagules of *Allium vineale* L. American Midland Naturalist. 131(1):55-64.
Rosenberg, M.S. and Anderson, C.D. 2009. PASSaGE: Pattern Analysis, Spatial Statistics, and Geographic Exegesis, Version 2 Beta.
Rudolphi, J. 2009. Ant-mediated dispersal of asexual moss propagules. The Bryologist. 112(1):73-79.
Rydgren, K., Halvorsen, R. and Cronberg, N. 2010, Infrequent sporophyte production maintains a female-biased sex ratio in the unisexual clonal moss *Hylocomium splendens*. Journal of Ecology, 98:1224–1231.
Schuster, R. M. 1992. VI. The Hepaticae and Anthocerotae of North America. Field Museum of Natural History, Chicago.
Seger, J. and Stubblefield, J.W. 2002. Models of sex ratio evolution. In Sex Ratios: Concepts and Research Methods. Ed: Hardy, I.C.W. University Press, Cambridge.
Sigmund, K. 1993. Games of Life: Explorations in Ecology, Evolution, and Behaviour. Penguin Books. New York, NY.
Sloan, D.B. and Panjeti, V.J. 2009. Evolutionary feedbacks between reproductive mode and the mutation rate exacerbate the paradox of sex. Evolution. 64(4):1129-1135.
Smith, G.M. and Regnery, D.C. 1950. Inheritance of Sexuality in *Chlamydomonas reinhardi*. Proceedings of the National Academy of Sciences of the United States of America. 36(4):246-248.
Starfinger, U., and J. Stöcklin. 1995. Seed, pollen, and clonal dispersal and their role in structuring plant populations. Progress in Botany 57: 337–355.
Sonneborn, T.M. 1937. Sex, sex inheritence and sex determination in *Paramecium aurelia*. Proceedings of the National Academy of Sciences. 23(7): 378–385
Thomas, C.D. and Hanski, I. 1997. Butterfly Metapopulations. In Metapopulation Biology: Ecology, Genetics, and Evolution, eds. I.A. Hanski and M.E. Gilpin. Academic Press, San Diego, CA. p359-386.
Tobler, M. and Schlupp, I. 2005. Parasites in sexual and asexual mollies (Poecilia, Poeciliidae, Teleostei): a case for the Red Queen? Biology Letters. 1:166-168.
Tomiuk, J. and Köhler, W. 2007. Population Genetics: Evolutionary Features of Asexual Species. Progress in Botany. 68(2):130-150.
Van Valen, L. 1973. A new evolutionary law. Evolutionary Theory. 1(1):1-30.
Vandepitte K, Honnay O, de Mayer T, Jacquemyn H, Rolda´n-Ruiz I, 2011. Patterns of sex ratio variation and genetic diversity in the dioecious forest perennial *Mercurialis perennis*. Plant Ecology 206:105–114.
Volterra, V. 1926. Variations and fluctuations of the numbers of individuals in animal species living together. Reprinted in 1931. In: R.N. Chapman, Animal Ecology. McGraw Hill, New York.
Vuilleumier, S., Bolker, B.M., and Lévêque, O. 2010. Effects of colonization asymmetries on metapopulation persistence. Theoretical Population Biology. 78:225-238.
Walser, J. Molecular evidence for limited dispersal of vegetative propagules in the epiphytic lichen *Lobaria pulmonaria*. American Journal of Botany. 91(8):1273-1276.
West, S.A., Lively, C.M., and Read, A.F. 1999. A pluralistic approach to sex and recombination. Journal of Evolutionary Ecology. 12:1003-1012.
Wiens, J.A. 1997. Metapopulation Dynamics and Landscape Ecology. In Metapopulation Biology: Ecology, Genetics, and Evolution, eds. I.A. Hanski and M.E. Gilpin. Academic Press, San Diego, CA. p43-62.
Williams, G.C. 1975. Sex and Evolution. Princeton, New Jersey: Princeton University Press.
Wilson, K. and Hardy, I.C.W. 2002. Statistical analysis of sex ratios: an introduction. In Sex Ratios: Concepts and Research Methods, ed Hardy, I.C.W. Cambridge University Press, Cambridge, UK.

Wintzer, A.P., Meek, M.H., Moyle, P.B., May, B. 2011. Ecological insights into the polyp stage of non-native hydrozoans in the San Francisco Estuary. Aquatic Ecology. 45:151-161.

Wolf, D.E. and Takebayashi, N. 2004 Pollen limitation and the evolution of androdioecy from dioecy. The American Naturalist. 163(1):122-137.

Wreede, R.E. and Klinger, T. 1988. Reproductive Strategies in Algae. Plant Reproductive Ecology: Patterns and Strategies. Eds. Doust, J.L. and Doust, L.L. Oxford University Press, Oxford.

Xu, J. 2004. The prevalence and evolution of sex in microorganisms. Genome. 47: 775–780.

Yoshida, T., Jones,L.E., Ellner, S.P., Fussmann, G.F., and Hairston Jr., N.G. 2003. Rapid evolution drives ecological dynamics in a predator–prey system. Nature. 424:303-306.

VITA

Christopher R. Stieha

Education

M.S. Biology, University of Kentucky, Lexington, KY 2008
B.A. Chemistry, B.S. Biology, University of Kentucky, Lexington, KY, 2003

Professional Positions

Teaching Assistant, University of Kentucky, Evolution	Fall 2011 – Spring 2012
Teaching Assistant, University of Kentucky, Ecology Laboratory	Spring 2011
Teaching Assistant, University of Kentucky, Biology Laboratory for Non-Majors	2004-2006, 2008-2010

Scholastic and Professional Honors

Finalist for University of Kentucky Provost Teaching Award	Spring 2010
College of Arts and Sciences Certificate of Outstanding Teaching	Spring 2009
Northern Kentucky Alumni Fellowship	Spring 2008
Accomplished Teaching Assistant Award for Biology Lab for Non-Majors	Fall 2007
Fulbright Fellowship for Research in Trinidad and Tobago "Aggressive Interactions Between Two Plant Species"	Fall 2006 – Fall 2007
Wethington Fellowship for Graduate Study	Fall 2004 – Spring 2005

Professional Publications

Rohr, J.R., Sesterhenn, T., Stieha, C. 2011. Will climate change reduce the effects of a pesticide on amphibians?: Partitioning effects on exposure and susceptibility. Global Change Biology. 17:657-666, doi: 10.1111/j.1365-2486.2010.02301.x

Groen, K.E., Stieha, C., Crowley, P.H., and McLetchie, D.N. 2010. Sex-specific plant responses to light and canopy openness: Implications for spatial segregation of the sexes. Oecologia. 162(3): 561-570.

Groen, K.E., Stieha, C., Crowley, P.H., and McLetchie, D.N. 2010. Sex-specific plant responses to two light levels in the liverwort Marchantia inflexa. Bryologist. 113(1): 81-89.

García-Ramos, G., Stieha, C., McLetchie, D.N., and Crowley, P. 2007. Metapopulation Sex Ratio Dynamics and Coexistence of the Sexes under Asymmetrical Competition. J. Ecology. 95:937-950

Crowley, P.H., Stieha, C.R., and McLetchie, D.N. 2005. Overgrowth Competition, Fragmentation and Sex-Ratio Dynamics: A Spatially Explicit, Sub-Individual Based Model. J. Theor. Biol. 233:25-42.