ANTAGONISTS AND MIXED MATING: CONSEQUENCES FOR THE DEMOGRAPHY OF *IMPATIENS CAPENSIS* (BALSAMINACEAE)

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Given the prevalence of intermediate levels of outcrossing among angiosperms, a general mechanism explaining the evolution and maintenance of this condition is needed. Although numerous theoretical models predict mixed mating to be evolutionarily stable, the conditions favoring intermediate selfing are often very stringent and have limited applicability. Here I investigate the role of two plant antagonists, vegetative herbivores and intraspecific competitors, in influencing the mixed mating system of *Impatiens capensis* (Balsaminaceae). This species exhibits an obligate mixed mating system by producing heteromorphic flowers (i.e., individuals produce both selfing, cleistogamous and facultatively-outcrossing, chasmogamous flowers). Thus, these antagonisms may affect mating system at the level of relative heteromorphic flower production, outcrossing within chasmogamous flowers and whole-plant outcrossing. In a comparative study exploring how herbivory and intraspecific competition jointly affect mating system expression, I found that these antagonisms affect plant growth and mating system traits differently, and thus the mating system response could not be accurately predicted from plant growth response. Using surveys of wild populations and experiments manipulating herbivory under field conditions, I found that herbivory reduced outcrossing by increasing proportional cleistogamous reproduction. In the field, I found that herbivory increased outcrossing among

chasmogamous flowers due to effects on flower display, pollinator visitation rate and pollinator fauna composition. Overall, herbivory slightly lowered whole-plant outcrossing.

To understand further the consequences of mixed mating, I manipulated herbivory in two wild *I. capensis* populations to explore the demographic consequences of mixed mating, herbivory, and the interactive effects of mating system and herbivory. I found that selfed individuals had higher rates of germination and survival and lower fecundity than did their outcrossed counterparts. Herbivory also had demographic consequences as it reduced population growth rate due to its effect on vital rates of selfed individuals.

Overall, the results presented in this dissertation offer important insight to the ecological factors that cause variation in mating system as well as the long-term consequences of variation in mating patterns. Furthermore, these findings have implications for population genetic diversity and structure and point to the role of natural enemies in contributing to the maintenance of a mixed mating system.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would foremost like to thank my advisor, Tia-Lynn Ashman, for her unending encouragement and support of my research and career. Tia-Lynn's creativity, positive nature, wealth of knowledge, and ability to challenge me intellectually has enhanced the quality of my research and helped me to develop into an independent scientist. I am also thankful to the other members of my dissertation committee, David Carr, Susan Kalisz, Jeffrey Lawrence, and Stephen Tonsor, who have provided guidance throughout my dissertation. I would also like to thank my collaborators James L. Hamrick and Tiffany Knight for their assistance with outcrossing rate estimation and demographic analyses, respectively. This work also significantly benefited from discussions and comments provided by Josh Auld, Sarah Berman, Emily Bond, Julia Butzler, Andrea Case, Jon Chase, Denise Cole, Chris Ivey, Tiffany Knight, Patrick Lorch, Cassie Majetic, John Paul, Andrea Quesada, April Randle, and other members of the Evolution and Ecology group at the University of Pittsburgh.

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1. INTRODUCTION

Because plants are immobile, both biotic and abiotic factors play vital roles in determining their reproductive success. One component relevant to plant fitness is the mating system, the relative production of selfed versus outcrossed seeds. Understanding the causes and consequences of mating system variations has been a major focus of research in plant population biology because changes in mating patterns have profound effects on the fitness of individuals (Charlesworth and Charlesworth 1987), the genetic structure of populations (Hamrick and Godt 1990), as well as the likelihood of speciation (Barrett 1990). While a variety of factors likely influence mating system expression and evolution, most theoretical and empirical examinations have focused primarily on genetic aspects (e.g., Lande and Schemske 1985, Campbell 1986, Uyenoyama 1986, Charlesworth and Charlesworth 1990, Latta and Ritland 1993, Ronfort and Couvet 1995, Chang and Rausher 1999) or the role of pollination biology (e.g., Holsinger 1991, Schoen et al. 1996, Kalisz et al. 2004). To have a comprehensive understanding of the factors shaping the evolution of this complex phenotypic trait, it is crucial that we begin to investigate other ecological agents that cause variation in mating patterns. Two of the most important factors influencing plant reproduction are vegetative herbivory and plant competition (Harper 1977, Marquis 1992), and yet their effects on the mating system have gone almost unstudied (but see Levri and Real 1998, Elle and Hare 2002, Steets and Ashman 2004, Ivey and Carr 2005).

Although researchers have long-studied the consequences of mating system variation, the demographic significance of mixed mating has been widely overlooked (but see Oostermeijer

2000). However, there clearly exists a link between mating system and demography as inbreeding can lead to population extinction (Saccheri et al. 1998). Further, given that selfed and outcrossed individuals often differ in fitness measures that could affect population dynamics (reviewed in Charlesworth and Charlesworth 1987) it is likely that mating types will differentially contribute to population growth. However, to understand the population-level consequences of mating system, demographic models must begin to incorporate mating system structure. In doing so, we can then begin to evaluate how alterations in mating system due to changes in ecological condition (e.g., herbivory, competition) will influence population growth and persistence.

In this dissertation, I present an examination of the role of two plant antagonists, vegetative herbivores and intraspecific competitors, in influencing mating system expression of *Impatiens capensis*. I further extend this work to investigate the demographic consequences of mixed mating as well as the interactive effects of vegetative herbivory and mating system for the population dynamics of *I. capensis*. This species is well suited to address these objectives as it exhibits an obligate mixed mating system by producing heteromorphic flowers, i.e., individuals produce both obligately-selfing cleistogamous (CL) flowers and facultatively-outcrossing chasmogamous (CH) flowers.

In Chapter 2, I present the results of a study that examines of the role of simulated and natural herbivory in influencing heteromorphic flowering of *I. capensis*. In addition, this study explores the effect of herbivory on correlates of the mating system of CH flowers. I conducted this study in collaboration with Dr. Tia-Lynn Ashman at the University of Pittsburgh and published the findings in the American Journal of Botany (Steets and Ashman 2004).

In Chapter 3, I report the results of an experiment investigating the independent and combined effects of two plant antagonists, vegetative herbivores and intraspecific competitors, for plant growth, architecture and mating system expression of *I. capensis*. This experiment was conducted with the help of Rhiannon Salla and Dr. Tia-Lynn Ashman and is in revision at *The American Naturalist* (Steets et al. 2005).

In Chapter 4, I present the results of a study investigating the effects of vegetative herbivores on the outcrossing rate of *I. capensis*. This study is an expansion of the work presented in Chapters 2 and 3, as it quantifies the total effect of vegetative herbivory for CH flower and whole-plant outcrossing. This work was conducted in collaboration with Dr. James L. Hamrick at the University of Georgia and Dr. Tia-Lynn Ashman and is being prepared for submission to *Ecology*.

Chapter 5 extends the findings of the previous chapters to understand the demographic consequences of mixed mating. In particular, I present the results of a study investigating the interactive effects of vegetative herbivory and mixed mating for the population dynamics of *I. capensis*. This work was conducted in collaboration with Dr. Tiffany M. Knight at Washington University and Dr. Tia-Lynn Ashman and is being prepared for submission to *The American Naturalist*.

Finally in Chapter 6, I review my findings and discuss the overall significance of my dissertation work. In addition, I highlight potential directions for future research.

2. HERBIVORY ALTERS THE EXPRESSION OF A MIXED MATING SYSTEM

2.1. Abstract

The direct and indirect effects of vegetative herbivory on the mating system of *Impatiens capensis* were analyzed through a survey of herbivory in natural *I. capensis* populations and manipulation of leaf damage in the field. Across 10 wild populations of *I. capensis* proportion of cleistogamous flowers had a significant positive exponential relationship with natural levels of herbivory. Similarly, experimental leaf damage increased the proportion of flowers and seeds that were cleistogamous. Leaf damage also reduced the biomass of cleistogamous progeny more severely relative to that of chasmogamous progeny. The cumulative effect of leaf damage was to increase plant reliance on fitness derived from cleistogamous progeny. Leaf damage indirectly affected mating system traits by reducing chasmogamous flower size, leading to a reduction in pollinator visitation. Under these experimental conditions, herbivory did not significantly reduce the number of simultaneously open flowers and potential for geitonogamy, nor did it result in significant changes in the composition of the pollinator fauna. These findings are among the first to demonstrate that herbivory has consequences for mating system and should be considered a factor shaping mating system evolution.

2.2. Introduction

Mating system (the proportion of selfed vs. outcrossed seeds) is a complex trait that reflects interactions among floral traits, demography, genetics, population structure, and

numerous environmental factors that affect pollination (Barrett and Eckert 1990). Understanding the causes and consequences of shifts in mating system is of primary importance to evolutionary biologists because changes in mating patterns can have profound effects on the reproductive fitness of individuals (Charlesworth and Charlesworth 1987), the genetic variation within populations (Hamrick and Godt 1990) and speciation (Barrett 1990). Recently much attention has been paid to how stochasticity in pollinator availability affects plant mating system (e.g., Eckert and Schaefer 1998, Vogler and Kalisz 2001, Culley 2002, Kalisz et al. 2004) and how herbivory influences plant pollinator interactions and plant fitness (e.g., Schemske and Horvitz 1988, Strauss et al. 1996, Krupnick et al. 1999, Mothershead and Marquis 2000, Adler et al. 2001). However, there is a void in our current understanding of how vegetative herbivory (hereafter, herbivory) influences mating system (see Ashman 2002).

Herbivory can affect plant mating system in at least two ways. First, by reducing plant resources, herbivory may have direct consequences on mating system. Resource limitation caused by herbivory can affect flower production (e.g., Quesada et al. 1995, Lehtila and Strauss 1997, Mothershead and Marquis 2000), flowering phenology (Juenger and Bergelson 1997, Agrawal et al. 1999), and seed mass and number (e.g., Stephenson 1981, Koptur et al. 1996, Agrawal 2001). Herbivory can influence traits that reflect the quality of offspring, including progeny size, reproduction and herbivore resistance; thus, it can have transgenerational consequences (Agrawal 2001). Despite the evidence that maternal herbivory generally can affect offspring traits, there is little information on whether such stress can differentially affect the production and/or vigor of selfed vs. outcrossed progeny. In one of the only studies to investigate the direct effect of leaf damage on mating system, plants with greater fungal damage had reduced production of selfed relative to outcrossed progeny, and the former had lower

survival (Levri and Real 1998). In addition, in plants that produce heteromorphic flowers — large, biotically pollinated (chasmogamous, CH) and small, self-pollinated (cleistogamous, CL) flowers — herbivory may directly alter the mating system by changing the relative production of these flower types. Specifically, if herbivory reduces resources, I expect a decline in the more costly CH flowers, resulting in a shift in the mating system toward selfing.

Second, herbivory can affect mating system via its effects on floral display and subsequent pollinator visitation. For instance, leaf damage can reduce the number of simultaneously open flowers on a plant (Strauss et al. 1996, Elle and Hare 2002), and thus decrease the potential for pollinators to affect geitonogamy (selfing among-flowers on a plant) (Harder and Barrett 1995). Herbivory can also reduce flower morphology and reward, which in turn may reduce pollinator visitation (Strauss et al. 1996, Mothershead and Marquis 2000) and may increase autogamy in plants capable of this mode of selfing. Moreover, because floral morphology largely determines the composition of the pollinating fauna (Baker and Hurd 1968), herbivory-induced changes in floral phenotype could influence the composition of the pollinator pool. However, because we have only begun to understand how herbivory alters floral traits and the abundance and composition of the pollinator fauna, we are ill-equipped to address its impact on mating system.

Because the direct and indirect effects of herbivory may have opposing influences on mating system (i.e., they may increase and decrease the selfing rate) they need to be addressed in concert. In this study, I addressed these issues in *Impatiens capensis*, which produces heteromorphic flowers. I conducted a survey of vegetative herbivory and mating system in natural *I. capensis* populations and performed a controlled field experiment in which I artificially damaged leaf tissue to address the following questions: (1) Does leaf damage lead to greater

production of CL relative to CH flowers and seeds in *I. capensis*? (2) Does leaf damage decrease floral display size and, thus, the potential for geitonogamous selfing in CH flowers? (3) Does leaf damage alter the size or shape of CH flowers? (4) Does leaf damage alter the abundance or composition of insects visiting CH flowers? (5) Does leaf damage affect the quality of CL or CH progeny or their contribution to total plant fitness?

2.3. Materials and Methods

2.3.1. Study system

Impatiens capensis Meerb. (Balsaminaceae) is a common native annual throughout moist forests in eastern North America (Schemske 1978). The CH flowers are orange and produce copious nectar that attracts various species of bees and occasionally the ruby-throated hummingbird (Rust 1977). These flowers are self-compatible, but strong protandry prevents autogamy. Outcrossing rates for CH flowers have been estimated to range between 0.29 - 0.71 (Waller and Knight 1989). In contrast, the obligately self-pollinating CL flowers have reduced petals, anthers and sepals and lack nectaries. CL and CH flowers are easily distinguished by their positions on the plant and pedicel structure (Schemske 1978).

A variety of herbivores feed on *I. capensis* (see Schemske 1978). Vegetative damage at the populations studied here was caused by chrysomelid beetles, grasshoppers, leaf miners, aphids, and occasionally by white-tailed deer (J. A. Steets, personal observation).

2.3.2. Survey of populations

To determine whether there is a relationship between mating system and herbivory, I surveyed 10 natural populations in Crawford and Lawrence Counties in northwestern Pennsylvania, USA. In each population, I collected one *I. capensis* plant at 5-m intervals along a transect, for a total of 20 plants. I measured total number of leaves and number of leaves

damaged by herbivores. As in other studies (Waller 1980, Le Corff 1993, Gross et al. 1998), I estimated total reproduction by counting the number of CL and CH pedicels. In *I. capensis*, flower production is a good predictor of fruit production for both flower types (CL fruits = 0.55*CL flowers + 0.14, $P < 0.0001$, $r^2 = 0.76$, N = 23; CH fruits = 0.57*CH flowers - 0.70, $P < 0.0001$, $r^2 = 0.81$, N = 23), therefore, I estimated plant mating system as the proportion of CL flowers. Because the plants experienced a killing frost a few days after collection, the estimates of CL and CH flower production represent total lifetime reproduction.

For each population, I calculated the mean proportion of CL flowers and mean proportion of leaves damaged. To explore the relationship between proportion CL flowers and leaf damage, I fit the data to two functions: 1) a linear function (PROC REG, SAS Institute, 2001) and 2) an exponential function reaching an asymptotic maximum at approximately one (PROC NLIN, SAS Institute, 2001). This latter function was chosen because the mating system metric, proportion of CL flowers, is bounded between zero and one.

2.3.3. Experimental manipulation of leaf damage

To explore the effects of leaf damage on plant mating system, pollinator visitation and composition of pollinator fauna, I transplanted 50 seedlings from each of three wild populations in Crawford County, Pennsylvania, USA into 11.4-cm^2 pots of Fafard #4 soil (Conrad Fafard, Inc., Agawam, Massachusetts, USA). Seedlings were situated along an edge of a wooded area at the Pymatuning Laboratory of Ecology, Crawford County, Pennsylvania, USA. I matched plants from each population according to their height, pre-existing CL flowers, and leaf damage. One member of each pair was randomly assigned to the damaged treatment. The other served as an undamaged control. After the initial assignment of treatments, plants were not treated as paired individuals. All plants were intermixed in a single random block and watered twice daily.

Although natural herbivory was not controlled, every week plants in the damage treatment had an additional 50% of leaf area removed from all new leaves by manually clipping each leaf in half (perpendicular to the midrib) with scissors. Although this level of damage is high, it is still within the range of damage observed in wild populations (0 – 75% of leaf tissue removed per plant, J. A. Steets, personal observation).

2.3.3.1. Effect of leaf damage on plant size and mating system

I measured plant size (height, stem diameter at first internode, number of leaves, nodes and branches) and CL and CH flower production at the beginning (prior to treatment application) and end (3 d prior to a killing frost) of the experiment. In addition, one to five CL and CH seed capsules were collected from a subset of the plants (see later) and the mean number of seeds within each type of capsule was determined. For each flower type, I estimated seed production as the product of the mean seeds per capsule and flower number. The mating system of each plant was calculated as the proportion of CL flowers and seeds.

I analyzed data on initial (plant size, number of CL and CH flowers) and final (plant size, number of CL and CH flowers and proportion CL flowers) traits using multivariate analyses of variance (PROC GLM, MANOVA option, SAS Institute, 2001) with treatment, population and their interaction designated as fixed effects, followed by individual ANOVAs on each variable. Population was treated as a fixed effect in all analyses because the three populations were located very close to one another and, therefore, do not represent a random sample of all *I. capensis* populations. To determine if damage affected seeds per capsule or proportion of CL seeds per plant, I performed ANOVAs with treatment, population and their interaction as fixed effects. Proportion of CL seeds per plant and seed production per capsule were not analyzed in the MANOVA on final vegetative and reproductive traits because I lacked seed production data

for some individuals. Proportion CL flowers and seeds were arcsine transformed to improve normality (Zar 1999).

2.3.3.2. Effect of leaf damage on floral display and geitonogamy

I counted the number of open CH flowers per plant (i.e., CH floral display size) and noted their sexual phase on three dates during peak CH flowering. I calculated the mean daily CH floral display for each plant and analyzed this data using ANOVA with treatment, population and their interaction as fixed effects. To determine if damage affected the potential for geitonogamous self-pollination, plants were categorized as follows: (1) no potential for geitonogamy: one open CH flower or more than one open CH flower but all flowers in the same sexual phase and (2) potential for geitonogamy: more than one CH flower open and flowers in both sexual phases. Data were analyzed using log-likelihood goodness-of-fit *G* tests (Zar 1999), where the null hypothesis was equal potential for geitonogamy between damaged and undamaged plants. No heterogeneity was detected among survey dates, therefore, only the analysis of pooled data is reported.

2.3.3.3. Effect of leaf damage on floral traits

For plants that had CH flowers open on collection days, one to four CH flowers on the central axis were measured for nectar spur length, lateral petal length and width, flower opening length and width, porch (side) petal length and androecium/gynoecium length. When more than one flower was measured per plant, I used the mean of these in the analysis. Because floral traits can be highly correlated, I performed a principal components analysis (PROC FACTOR, SAS Institute, 2001) on the correlation matrix of the seven floral traits. This allowed me to create several orthogonal composite variables, the first two of which describe flower size and shape

(see later). I explored the effect of leaf damage on these using ANOVAs with treatment, population and their interaction as fixed effects.

2.3.3.4. Effect of leaf damage on pollinator abundance and composition

I observed pollinator visitation on 3 days during peak CH flowering and pollinator activity (1000 – 1600 h). As commonly occurs in *I. capensis* wild populations, flowers of neighboring plants intertwine, thus rather than separating plants, I observed flowers in both treatments simultaneously by observing small patches composed of 10 – 30 flowers. During 8 h of observation (twenty-four 20-min sessions) I recorded a total of 441 visits. Visitation by bumblebees, honey bees, and miscellaneous small bees were recorded separately. I analyzed all data using log-likelihood goodness-of-fit *G* tests (Zar 1999), in which the null hypothesis was equivalent visitation to flowers of damaged and undamaged plants. No heterogeneity was detected among observation days, therefore only the analysis of pooled data is reported. Tests of heterogeneity were performed to determine if visitation pattern differed among pollinator types (bumblebees, honey bees, and small bees).

2.3.3.5. Effect of leaf damage on progeny

To assess the potential for transgenerational effects of herbivory, I collected CL and CH seeds from damaged and undamaged plants late in the season. As a result of early season mortality among the experimental plants (22 out of 150 plants died), I was unable to collect seeds from all individuals. In total, 377 CL seeds from 100 plants and 541 CH seeds from 113 plants were collected. Seeds were stored in distilled water in cell culture trays at 4°C for approximately 4 months to break dormancy (Leck 1979). Once germinated, seeds were planted in 96-well plug trays with Fafard #4 soil (Conrad Fafard, Inc.) and moved to a growth chamber at 5°C with an 8-h daylength to simulate early spring germination conditions. When seedlings

emerged and cotyledons reflexed, I estimated cotyledon size as the product of length and width of the largest cotyledon. Two months after the first seed germinated, I harvested, dried and weighed aboveground biomass for one CL and one CH seedling per maternal plant. I calculated percentage germination, mean number of days to germination and mean cotyledon size for CL and CH seedlings from each maternal plant. To determine if maternal damage affected percentage germination or dry biomass, I performed individual ANOVAs with maternal treatment, population, flower type and their interactions as fixed effects. In the biomass analysis germination date was included as a covariate. To determine if maternal damage affected days to germination or cotyledon size, I performed a MANOVA with maternal treatment, population, flower type and their interactions as fixed effects, followed by individual ANOVAs on each trait.

2.3.3.6. Effect of leaf damage on cumulative female fitness

I calculated the cumulative fitness from cleistogamy and chasmogamy as the product of flower production, seeds per fruit, percentage germination and seedling dry biomass for each flower type. The sum of these two products reflects total cumulative plant fitness. Although these cumulative fitness functions falsely assume full fruit set, they are still useful parameters for comparing treatment effects because flower production is a good estimator of fruit production in *I. capensis* (see above). To determine if leaf damage affected total cumulative fitness, cumulative fitness from cleistogamy or chasmogamy or the proportion of fitness derived from CL progeny, I performed ANOVAs with treatment, population and their interaction designated as fixed effects. The proportion of fitness from CL progeny was arcsine transformed to improve normality (Zar 1999).

2.4. Results

2.4.1. Survey of populations

I found a significant increase in proportion CL flowers with increasing natural leaf damage (Figure 2.1; mean proportion CL = 1.23*mean proportion leaves damaged + 0.44, $r^2 = 0.48$, $F_{1, 8} = 7.36$, $P = 0.027$). However, the exponential function of damage explained twice the variance in proportion CL flowers as the linear function (Figure 2.1; mean proportion CL = 1.08*[1 - exp (-5.03*mean proportion leaves damaged)], $r^2 = 0.98$, $F_{2, 8} = 189.89$, $P < 0.0001$).

2.4.2. Experimental manipulation

2.4.2.1. Effect of leaf damage on plant size and mating system

Prior to the damage, vegetative traits were similar in control and treatment groups (MANOVA overall treatment effect: $F_{5, 136} = 0.09$; $P = 0.99$). Overall, damage reduced vegetative traits and flower production (MANOVA overall treatment effect: $F_{8, 115} = 7.81$; $P < 0.0001$). Damage reduced plant height by 10% and stem diameter by 9% ($F_{1, 122} = 4.79, 16.2$; $P = 0.03, 0.0001$, respectively). However, the number of nodes, leaves and branches did not differ significantly between treatments ($F_{1, 122} = 0.80, 0.19, 2.16$; $P = 0.37, 0.67, 0.14$, respectively). Damage reduced total and CH flower number, but not CL flower number (Table 2.1). Therefore, damaged plants produced fewer flowers in their lifetime and a greater proportion of their flowers were CL, resulting in a shift in the mating system toward greater selfing (Table 2.1). Although populations varied in terms of vegetative traits and flower production (MANOVA: $F_{16, 230} = 15.64$; $P < 0.0001$), all populations responded to the damage treatment similarly (MANOVA treatment*population effect: $F_{16, 230} = 1.29$; $P = 0.20$). Damaged and undamaged plants produced similar numbers of CL and CH seeds per capsule (Table 2.1). However, because damaged plants made fewer total CH flowers, a greater proportion of seeds produced by a damaged plant were CL (Table 2.1).

2.4.2.2. Effect of leaf damage on floral display and geitonogamy

The mean daily number of open CH flowers did not differ significantly between damaged and undamaged plants (1.52 ± 0.10 vs. 1.76 ± 0.17; $F_{1, 73} = 1.41$; $P = 0.24$). The potential for geitonogamous selfing also did not differ significantly between damaged and undamaged plants (13% vs. 22% of plants had simultaneously open female- and male-phase flowers; $G_{1, 114} = 1.51$; $P > 0.2$).

2.4.2.3. Effect of leaf damage on floral traits

Combined, the first two principal components explained 68% of the variance in floral traits among plants (Table 2.2). The first principal component (PC1) explained 54% of floral trait variance and had large positive associations with all flower dimensions, and thus represents overall flower size. The second principal component (PC2) explained an additional 14% of floral trait variance and had large positive correlations with spur length and androecium/gynoecium size, but negative ones with lateral petal dimensions, thus it reflects floral shape. The remaining principal components explained less than 12% each and were not investigated further. Damage significantly reduced overall flower size by 197% ($F_{1, 65} = 26.60$; $P < 0.0001$). This reduction reflects a 10 to 15% reduction in most flower dimensions (data not shown). There was no significant effect of population or an interaction between population and damage with respect to PC1 ($F_{1, 65} = 2.07, 1.63$; $P = 0.13, 0.20$, respectively). Damage, population and their interaction did not explain a significant amount of the variation in flower shape as reflected by PC2 ($F_{1, 65} = 1.87, 1.66, 0.20$; $P = 0.18, 0.20, 0.82$, respectively).

2.4.2.4. Effect of leaf damage on pollinator abundance and composition

Pollinators visited flowers of damaged plants half as frequently as those of undamaged plants (Figure 2.2; $G_{1, 441} = 44.84$; $P < 0.0001$), and this was true for all three pollinator types (Figure 2.2). Furthermore, there was some indication that visitation by bumblebees was

depressed more by damage than visitation by small bees or honey bees (Figure 2.2); however, this pattern of heterogeneity was not statistically significant ($G_{H\,(2,\,441)} = 2.57$; $P = 0.25$).

2.4.2.5. Effect of leaf damage on progeny

While there was little effect of damage on germination rate or timing ($F_{1,\,165} = 1.66$, 0.43; $P = 0.20$, 0.51, respectively), damage marginally reduced cotyledon size ($F_{1,\,165} = 2.89$; $P = 0.09$) and seedling biomass ($F_{1,\,164} = 2.86$; $P = 0.09$). However, the latter trait depended significantly on seed type (treatment*seed type effect: $F_{1,\,164} = 4.61$; $P = 0.03$). Specifically, damage reduced cotyledon size by 4%, and reduced CL seedling biomass by 8%, but CH seedling biomass by only 7%. There were no other main effects of seed type or interactions with damage (all $P > 0.12$).

2.4.2.6. Effect of leaf damage on cumulative female fitness

Damage reduced cumulative female fitness by 37% ($F_{1,\,65} = 5.36$; $P = 0.024$). Further, the cumulative fitness from chasmogamy was significantly reduced by damage, whereas the fitness derived from cleistogamy was not significantly affected by leaf damage (47% reduction vs. 23% reduction; $F_{1,\,85} = 7.65$, $F_{1,\,89} = 2.53$; $P = 0.007$, 0.11, respectively). As a result, the overall effect of damage was to increase the proportion of fitness achieved through CL progeny (0.66 ± 0.04 vs. 0.55 ± 0.04; $F_{1,\,65} = 4.23$; $P = 0.04$).

2.5. Discussion

2.5.1. Direct effects of leaf damage

This study demonstrates that both natural and experimental leaf damage affect the mating system of *I. capensis*. In response to natural leaf damage, plants increased the proportion of flowers that were CL (Figure 2.1). Furthermore, there appears to be a threshold at approximately 35% leaf damage above which plants are nearly entirely selfing. Thus, modest changes in the

herbivore environment can have dramatic effects on mating system. Experimental leaf damage also resulted in a striking increase in the proportional production of CL flowers (Table 2.1). However, the plant response to the experimental manipulation with respect to the mating system was weaker than the response to natural leaf damage. All leaves on plants in the simulated leaf herbivory treatment were damaged (i.e., 100% damage), yet on average, this resulted in 78% CL flower production. This lower response in the experimental plants could be due to effects of simulated leaf damage (Baldwin 1990) or the fact that these plants were grown in pots. Nevertheless, both experiments show that plants respond to herbivory by decreasing production of outcrossing flowers.

The results of this study support other investigations that have found that mixed mating systems can be modified by environmental factors. For instance, low soil moisture (Schemske 1978, Waller 1980, Bell and Quinn 1987), low light intensity (Schemske 1978, Waller 1980, Bell and Quinn 1987), low nutrient availability (Le Corff 1993) and high plant density (Lu 2000) all decrease CH flower production but not CL flower production. Taken together, these data indicate that not only abiotic, but also biotic stress can result in a shift in mating system toward greater selfing. Furthermore, my ongoing research suggests that this herbivory response may be quite general and that other forms of antagonism (i.e., deer grazing, intraspecific competition) can have more severe effects on mating system than insect feeding (J. A. Steets, unpublished data).

This study is among the first to demonstrate that leaf damage has significant effects on plant mating system (see also Levri and Real 1998, Elle and Hare 2002). Levri and Real (1998) found that fungal leaf damage altered the mating system of *Kalmia latifolia*; diseased plants produced proportionally more outcrossed progeny because they set a lower proportion of selfed

fruits relative to outcrossed fruits and produced selfed seedlings with lower survival than outcrossed seedlings. However, the present study adds to the findings of Levri and Real (1998) in that leaf damage can also alter mating system at the level of flower production in plants with heteromorphic flowering systems. I found that damaged plants would produce the small, selfing flowers over the more expensive CH flowers. Based on the findings of Levri and Real (1998), selective abortion of selfed fruits may offset the increase in proportion CL flower production found here. I am currently investigating this hypothesis.

Both the within-generation (i.e., relative seed production) and between-generation (i.e., relative seedling vigor) effects of leaf damage on mating system may have consequences for demography and population genetic diversity. For example, I found that damage reduced the biomass of CL seedlings more than that of the CH seedlings. Because initial plant size is an important determinant of survival in dense stands of *I. capensis* (Schmitt et al. 1987b), my results suggest that the smaller, CL seedlings of damaged plants may have higher mortality than the larger CH seedlings or either seedling type from undamaged plants. Thus, a population experiencing moderate levels of herbivory may have higher genetic diversity as a result of increased selfed seedling mortality. Further, if herbivore damage changes other demographic parameters (e.g., fecundity) of selfing and outcrossing progeny, then their contribution to population growth rate may also change. To address this hypothesis, life table response experiments are needed (see Chapter 5).

2.5.2. Indirect effects of leaf damage

Leaf damage indirectly affected some traits associated with mating system (CH floral traits and pollinator visitation), but not others (CH floral display, potential for geitonogamy and composition of the pollinator fauna). These findings add to the growing body of work

demonstrating that herbivory decreases flower production, floral attraction, pollinator visitation and female reproductive success (e.g., Strauss et al. 1996, Mothershead and Marquis 2000). These effects might be expected to result in changes in the outcrossing rate of CH flowers because pollinator type, fit and efficiency are believed to affect pollen removal and deposition. However, a test of this idea requires direct estimation of CH flower outcrossing rate in damaged and undamaged plants. Such work is currently underway.

In the only other study to investigate the effect of herbivory on mating system, Elle and Hare (2002) reported that leaf damage reduced floral display and selfing rate of *Datura wrightii*, the latter of which was likely caused by a decrease in geitonogamy. Conversely, I found that damage had no significant effect on CH floral display size and potential for geitonogamy in *I. capensis.* The contrasting findings between this study and those of Elle and Hare (2002) are likely due to the extreme differences in floral display size of the study organisms. *Datura wrightii* can produce over 100 flowers in a single night (Elle et al. 1999), whereas in the present study, *I. capensis* produced fewer than five CH flowers per day because resources were limiting in pots. However, in natural populations of *I. capensis*, removal of natural insect herbivores results in a large increase in CH floral display (see Chapter 4) suggesting that geitonogamy may contribute to mating system in wild populations of this species.

If we wish to understand how leaf damage affects a whole plant mating system, we need to consider its effects on the proportional production of CL and CH flowers, fruits and seeds as well as on CH outcrossing rate. Based on the mean CL and CH flower and seed production (Table 2.1) and an average estimate of 0.5 for CH outcrossing rate taken from the literature (Waller and Knight 1989), I estimate that undamaged plants have an overall plant outcrossing rate of 0.26, whereas damaged plants have an outcrossing rate of 0.20 in the absence of any

indirect effects. However, if the smaller CH floral display size, lower potential for geitonogamy and greater proportion of small bee pollinators translate into reduced actual geitonogamy in damaged plants, then the outcrossing rate of their CH flowers could increase. Given the above estimates, the outcrossing rate of CH flowers on damaged plants would have to increase from 0.50 to 0.78 to oppose the direct effects of herbivory. Therefore, the indirect effects of herbivory on outcrossing rate of CH flowers would have to be substantial to counter the direct effects of herbivory that favor selfing.

2.5.3. Consequences for mating system evolution

Whereas the majority of mating system evolution models have incorporated factors such as inbreeding depression and population structure to predict equilibrium levels of selfing (e.g., Lande and Schemske 1985, Uyenoyama 1986, Charlesworth and Charlesworth 1990), variation in the pollination environment may also influence mating system evolution. For example, in an unpredictable pollination environment selection favors the ability for plants to both self and outcross (Schoen and Brown 1991, Schoen et al. 1996). Although often overlooked, herbivores and other antagonists (e.g., competitors) are likely to serve as selective agents in the evolution of mating systems. In a model by Schoen and Lloyd (1984), a heteromorphic flowering system will be maintained when individuals are able to produce the appropriate flower type in response to heterogeneity in the parental environment that is an indicator of variation in pollinator activity (i.e., individuals produce CL flowers under conditions of low pollinator activity and CH flowers when pollinator activity is high). This study provides support by showing that leaf damage both reduces pollinator activity and increases proportional CL flower production. In contrast, in a low herbivory environment, pollinator activity is more dependable and plants reproduce more via CH flowers. My results combined with those from other studies (Levri and Real 1998, Elle and Hare

2002) indicate that herbivores are likely to have a role in shaping the evolution of the mating system. However, further work is needed to determine whether plasticity to herbivory with respect to mating system is adaptive and whether heterogeneity in the herbivore environment maintains it.

Table 2.1 The effect of treatment (undamaged, damaged) on the mean number of cleistogamous (CL), chasmogamous (CH), and total flowers (N = 66, 62), CL seed production per capsule (N = 47, 53), CH seed production per capsule (N = 49, 31) and proportion CL flowers (N = 66, 62), and seeds (N = 38, 36) produced by *Impatiens capensis* (mean ± SE).

	Treatment				
Trait	Undamaged	Damaged	d.f.	*F*	*P*
CL Flowers	44.6 ± 1.48	42.4 ± 1.55	1, 122	0.79	0.38
CH Flowers	21.0 ± 1.34	13.9 ± 1.21	1, 122	14.64	0.0002
Total Flowers	65.7 ± 1.88	56.3 ± 2.26	1, 122	9.14	0.0030
CL seeds/capsule	1.33 ± 0.06	1.21 ± 0.05	1, 94	2.29	0.13
CH seeds/capsule	2.59 ± 0.18	2.47 ± 0.18	1, 79	0.01	0.91
Proportion CL Flowers	0.69 ± 0.02	0.78 ± 0.02	1, 122	13.88	0.0003
Proportion CL Seeds	0.57 ± 0.03	0.70 ± 0.04	1, 68	6.41	0.014

Table 2.2 Principal components analysis of floral traits of *Impatiens capensis*. Correlation between floral dimensions and principal components (PC) and percentage variance explained by each.

	Floral Dimension	
Floral Trait	PC1	PC2
Androecium/Gynoecium Length	0.472	0.706
Lateral Petal Length	0.834	-0.293
Lateral Petal Width	0.732	-0.472
Opening Width	0.868	-0.033
Opening Length	0.836	0.149
Porch Petal Length	0.792	-0.039
Spur Length	0.460	0.416
Eigenvalue	3.746	1.005
Percent Variance Explained	54%	14%

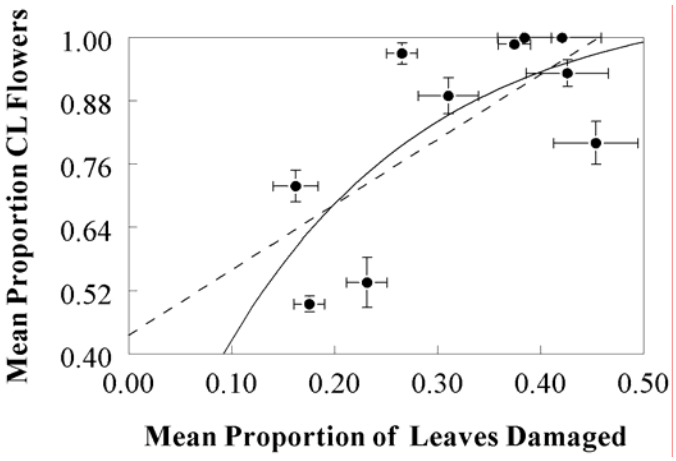


Figure 2.1 Mean proportion of cleistogamous (CL) flowers of *Impatiens capensis* as a function of mean proportion of leaves damaged. Points represent mean ± SE for individual populations. The dashed line is the linear regression (mean proportion CL = 1.23*mean proportion leaves damaged + 0.44, $r^2 = 0.48$; $P = 0.027$). The solid line represents the nonlinear regression (mean proportion CL = 1.08 * [1 - exp(-5.03*mean proportion leaves damaged)]; $r^2 = 0.98$; $F_{2, 10} = 189.89$; $P < 0.0001$).

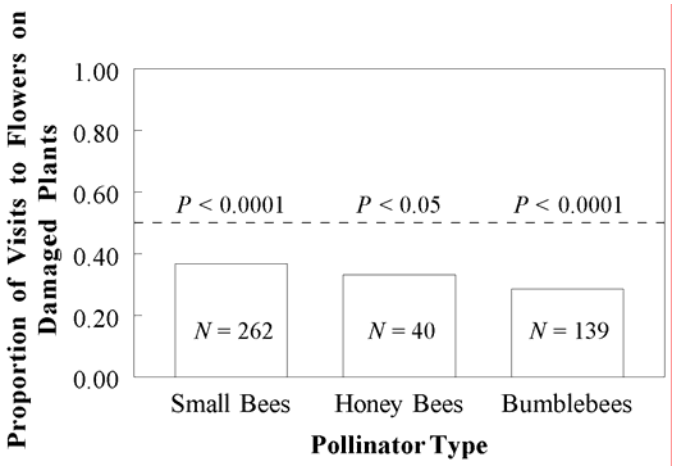


Figure 2.2 Proportion of visits to flowers on damaged plants of *Impatiens capensis* by each pollinator type. The line ($y = 0.50$) reflects the null hypothesis of equal visitation to flowers on damaged and undamaged plants.

3. COMPETITION-DEPENDENT EFFECTS OF HERBIVORY FOR MATING SYSTEM EXPRESSION IN *IMPATIENS CAPENSIS*

3.1. Abstract

As a first step towards understanding how community context shapes mating system evolution, I investigated the combined role of two plant antagonists, vegetative herbivory and intraspecific competition, for mating system expression (relative production of selfing, cleistogamous and facultatively-outcrossing, chasmogamous flowers and fruits) of *Impatiens capensis*. In a greenhouse experiment where leaf damage and plant density were manipulated, I found that multispecies interactions had dramatic effects on mating system. Specifically, the combined antagonisms had weaker than additive effects for mating system expression and chasmogamous reproduction whereas they had synergistic effects for chasmogamous flower size and cleistogamous flower production. These results indicate a trade-off between resource allocation to chasmogamous flower size and number. Further, these results show that competitive interactions between plants influence the effect of herbivory on components of fitness and mating system, and thus, antagonisms may have unforeseen consequences for mating system evolution, population genetic diversity and persistence.

3.2. Introduction

In recent years, researchers have become aware that the outcome of pairwise-interactions can be altered by the presence of other interacting species, and thus the net ecological and evolutionary effect of multispecies interactions cannot be predicted from their independent

effects (e.g., Strauss 1991, Agrawal 2004). Two primary antagonistic interactions faced by plants are consumption by herbivores (Marquis 1992) and competition with other plants for above- and below-ground resources (Harper 1977). Because human-induced fragmentation and disturbance has lead to native plants experiencing increased incidence of herbivory (Lienert and Fischer 2003, Rand and Louda 2004) and competition with invasive species (Vila and Weiner 2004) it is imperative that we evaluate how multiple antagonisms affect interacting species. In addition to affecting plant fitness (e.g., Harper 1977, Marquis 1992) and community composition (e.g., Hairston et al. 1960, Wardle and Barker 1997, Carson and Root 2000), these antagonisms may have consequences for population genetic diversity (reviewed in Linhart and Grant 1996), however, this is still largely unexplored. One mechanism by which these antagonisms might affect the genetic diversity and structure of plant populations is through their joint effect on mating system expression.

There is mounting evidence that vegetative herbivory (hereafter, herbivory) and competition independently influence mating system expression. For example, leaf damage can increase outcrossing rate by inducing selective abortion of selfed seeds (Levri and Real 1998) and leaf damage or intraspecific competition can reduce daily floral display size and consequently geitonogamous selfing (Karron et al. 1995, Elle and Hare 2002). Alternatively, in species that produce heteromorphic flowers on a single individual (i.e., large, facultatively outcrossing and small, obligately selfing flowers), both herbivory and competition can alter the relative production of flower types, shifting the mating system towards selfing (Schmitt et al. 1987a, Steets and Ashman 2004). Given that plants regularly experience both herbivory and competition and individuals are becoming more prone to these antagonisms (Lienert and Fischer 2003, Rand and Louda 2004, Vila and Weiner 2004), understanding how they jointly influence

mating system will provide insight into their effect on population genetic diversity and the evolution of mating systems.

Mating system plasticity may be a generalized plant response to resource stress such that a plant subject to multiple antagonisms will exhibit altered mating system in a manner that is predictable from effects on plant growth. However, the largely additive effects of herbivory and competition seen for plant growth (e.g., Fowler and Rausher 1985, Mutikainen and Walls 1995, Reader and Bonser 1998, Erneberg 1999; but see Fowler 2002, Agrawal 2004, Haag et al. 2004) may not translate into additive effects on mating system for a few reasons. First, in species with heteromorphic flowers, the demands of one antagonism may affect a plant's ability to maintain a certain level allocation to outcrossing flowers when a second antagonism is introduced, and thus the two antagonisms will have a synergistic effect on mating system. Given that multiple antagonists can have synergistic effects on plant reproduction (Friedli and Bacher 2001), a similar response might be expected for mating system. In addition, non-additive mating system effects may also occur if herbivory and competition differentially affect the architecture of heteromorphic flowering species. For instance, competition for light can cause plants to decrease branch production (Schmitt et al. 1987b) whereas herbivory reduces plant height but has little effect on branching architecture (Steets and Ashman 2004). If selfing and outcrossing flowers are produced at different locations on a plant, then differential effects of these antagonists on branching patterns could result in non-additive effects on mating system. Additionally, because the realized mating system of the facultatively outcrossing flowers is a function of both intrinsic (i.e., flower morphology) and extrinsic (i.e., pollinator behavior, population genetic structure) factors, antagonistic interactions may also affect these and ultimately the mating system. Currently no study has investigated any of the above mechanisms,

so as a first step at addressing this, I isolate the effects of two antagonisms on plant resources, architecture and mating system expression in *Impatiens capensis* Meerb. (Balsaminaceae). Specifically, I manipulated levels of leaf herbivory and intraspecific competition in a greenhouse experiment to address the following questions: (1) do plants respond to multiple antagonistic interactions additively or non-additively?, (2) do growth, reproduction and mating system traits respond to multiple antagonists similarly?, and (3) is mating system response to multiple antagonists predictable from growth response?

3.3. Materials and Methods

3.3.1. Study system

Impatiens capensis (jewelweed, touch-me-not) is a common native annual throughout moist forests in eastern North America (Schemske 1978) that is ideally suited to the objectives of this study because individuals exhibit a mixed mating system by producing both large, facultatively outcrossing (chasmogamous flowers, CH) and small, obligately self-fertilizing (cleistogamous, CL) flowers (i.e., heteromorphic flowering system). The CH flowers are self-compatible, but strong protandry prevents autogamy. In populations in northwestern Pennsylvania, outcrossing rates for CH flowers range between 0.29 – 1.00 (mean: 0.57) and varies with display size (see Chapter 4).

3.3.2. Experimental design and data collection

I collected *I. capensis* seeds from a population in Crawford County, PA. Seeds were stored in distilled water in cell culture trays at 4EC for approximately four months to break dormancy (Leck 1979). Once germinated, seeds were planted (treatments below) in 10 cm square pots filled with Fafard™ #4 soil (Conrad Fafard, Agawam, Massachusetts, USA) and transferred to a greenhouse with supplemental heating and lighting.

I employed a randomized complete block design consisting of 50 blocks, with treatments factorially applied to plants as follows:

(1) competition treatment — focal plants were grown in the absence (-C) or presence (+C) of another *I. capensis* plant. The +C treatment simulates a density of 200 plants/m^2, which is within the range of natural densities (juvenile density: 88 – 340 *I. capensis*/m^2; adult density: 4 – 300 *I. capensis* /m^2; J. A. Steets, unpublished data).

(2) herbivory treatment — focal plants were either undamaged (-H) or damaged (+H). I aimed for 30% leaf damage, as this is the average experienced by *I. capensis* in the wild (J. A. Steets, unpublished data), by applying generalist land snails, *Helix aspersa* Müller (Helicidae) and *Mesodon ferrissi* Pilsbry (Polygyridae), to individual leaves on each plant. Damage began pre-flowering and continued through the end of the experiment. Snails were contained on leaves in small plastic cups attached to stakes placed in the soil. A single snail remained on a leaf until some feeding occurred (range 1 – 14 d), after which it was moved to an undamaged leaf on the plant. As snail feeding was a slow process, I augmented this damage with simulated herbivory, removing 20 - 40% of an individual leaf's area using a hole punch. Plants in the undamaged treatment had a snail-free plastic cup placed on a leaf; cups were moved to new leaves in a manner similar to that in the +H treatment.

I watered plants daily and hand-pollinated all open CH flowers using pollen collected from the competitors twice per week. I measured plant growth (height, production of branches, aboveground dry biomass) and CL and CH flower production 77 days after transplanting. I also quantified CL and CH fruit production on a subset of the plants (10 plants/treatment from 10 blocks). I calculated the mating system of each focal plant as the proportional production of CL flowers (or fruits). In addition, I quantified traits known to be related to the mating system of the

CH flowers (J. A. Steets, unpublished data), including: (1) CH floral display size as the number of simultaneously open CH flowers on three days during peak flowering and (2) several floral dimensions (lateral petal length and width, porch petal length, upper petal length, upper and lower flower length, spur length and opening length) of the first female-phase CH flower on each plant using Optimus digitizing software (Media Cybernetics, Silver Spring, Maryland, USA). Very few plants in the +C+H treatment produced CH flowers (12/50 plants); thus, to minimize bias in sampling intensity among treatment classes, I measured CH floral traits on all possible individuals in the +C+H treatment as well as 10 - 15 randomly chosen plants from each of the other treatments.

3.3.3. Statistical analyses

To determine if herbivory and competition affected plant growth (height, number of branches, aboveground dry biomass), reproduction (CL and CH flower and fruit production, mean CH floral display size) or mating system (proportion CL flowers and fruits) traits in additive or non-additive ways, I conducted mixed model analyses of variance for each trait (PROC MIXED, SAS Institute, 2001) in which competition, herbivory and their interaction were considered fixed effects and block was designated a random effect. The mating system metrics were arcsine transformed prior to analyses to improve normality (Zar 1999). The presence of a significant interaction term in the ANOVA indicated that the effects of herbivory and competition were non-additive (Figure 3.1). To determine if this non-additivity was greater than (i.e., synergistic) or less than (i.e., substitutive) predicted by the pairwise interactions, I investigated the interaction plots and performed Tukey's multiple comparison tests (Figure 3.1).

To determine if herbivory or competition interacted to affect CH floral traits, I performed a principal components analysis (PROC FACTOR, SAS Institute, 2001) on the correlation

matrix of the eight floral dimensions. The first principal component explained 84% of the variance among plants in floral traits and all traits loaded positively (all loadings > +0.83), indicating that it reflects overall flower size. I explored the effect of herbivory and competition on flower size using ANOVA (PROC GLM, SAS Institute, 2001) with herbivory, competition and their interaction as fixed effects. Block was not considered in this model due to the random sampling of individuals across blocks.

To determine if herbivory and competition affected plant growth, reproduction and mating system traits in similar ways, I performed two separate mixed model multivariate analyses of variance (PROC MIXED, SAS Institute, 2001) with trait and its interactions with competition and herbivory as fixed effects and block designated as a random effect. First, I conducted a MANOVA using the subset of plants for which CL and CH fruit production was measured (N = 40) and included nine dependent variables in the model (height, number of branches, aboveground dry biomass, CL and CH flower and fruit production and proportional production of CL flowers and fruits). In the second analysis, I included all focal plants. However, as fruit production was only measured on a subsample of individuals in the experiment, I eliminated all fruit production traits from the dependent variables in the model. CH floral display size and CH flower size were not included in either model as these traits were quantified on only a small subset of plants. A significant herbivory*competition*trait term indicates that traits showed differential responses to the antagonisms. The results of the two analyses did not differ, so I only report those from the second analysis in the *Results* section.

To determine if mating system response is predictable from growth response to herbivory and competition, I performed a mixed model MANOVA (as above) with only two dependent variables, aboveground dry biomass (as an estimate of plant growth) and proportional production

of CL flowers (as an estimate of mating system). A significant herbivory*competition*trait term indicates that the growth and mating system traits are differentially affected by the antagonisms, and thus one cannot accurately predict the mating system response from the growth response.

3.4. Results

3.4.1. Do plants respond to multiple antagonistic interactions additively or non-additively?

Herbivory and competition had an additive effect on plant growth (Table 3.1A; Figure 3.2A). In addition, two of the six reproductive traits, CH floral display and CL fruit production, and one mating system trait, proportion CL fruits, responded additively to the effects of the joint antagonisms (Table 3.1B, C; Figure 3.2C, F, I).

Although the combined effects of herbivory and competition were additive with respect to plant growth, for some of the reproductive and mating system traits I found that the effects of herbivory were dependent upon the competitive environment. For example, the combined antagonisms were weaker than additive for mating system measured as proportion CL flowers as well as CH flower and fruit production (Table 3.1B, C; Figure 3.2D, E, H). In contrast, herbivory and competition had synergistic effects on CL flower production and CH flower size (Table 3.1B; Figure 3.2B, G). In all cases, competition had more dramatic effects on these plant traits than herbivory (Figure 3.2).

3.4.2. Do growth, reproduction and mating system traits respond to multiple antagonists similarly?

Growth, reproductive and mating system traits responded differently to the multiple antagonisms (herbivory*competition*trait: $F_{5, 911} = 15.7$, $P < 0.0001$). In addition, plant traits

were differentially affected by herbivory (herbivory*trait: $F_{5,\ 911} = 14.7$, $P < 0.0001$) and competition (competition*trait: $F_{5,\ 911} = 79.2$, $P < 0.0001$).

3.4.3. Is mating system response to multiple antagonists predictable from growth response?

Growth and mating system traits responded differently to herbivory and competition (competition*trait: $F_{1,\ 143} = 293.3$, $P < 0.0001$; herbivory*trait: $F_{1,\ 143} = 6.8$, $P = 0.01$; herbivory*competition*trait: $F_{1,\ 143} = 2.66$, $P = 0.1$), indicating that the mating system response cannot be predicted from the growth response.

3.5. Discussion

There are three primary results from this study. First, plant mating system and reproductive trait responses to herbivory were dependent upon the competitive environment. In addition, reproductive and mating system traits responded differently to antagonists than growth traits, and thus the mating system response cannot be accurately predicted from plant growth response. Finally, plants experiencing multiple antagonisms preferentially maintain CH flower size while reducing flower number indicating an allocation trade-off between these traits. I expand on each of these findings below and discuss the implications of these findings for population genetic diversity and mating system evolution.

3.5.1. Combined effects of herbivory and competition

Although this study found that the combined effects of herbivory and competition were additive with respect to plant biomass and other growth traits, I detected non-additive effects of the joint antagonisms for CL and CH reproduction as well as mating system expression (proportion CL flowers). Given the design of this experiment, non-additive effects of competition and herbivory cannot be due to competition altering the magnitude of herbivory a

plant experiences (but see Agrawal 2004). Rather, it is likely that the non-additive responses reflect changes in plant resource allocation. Because CH flowers are more costly to produce than CL flowers (Schemske 1978), CH reproduction is often reduced when plants are grown in stressful environments (e.g., Schemske 1978, Waller 1980, Bell and Quinn 1987, Le Corff 1993, Lu 2000), thus I expected a similar response in plants experiencing herbivory and competition. Indeed, I found that CL flower production was only reduced under the most stressful conditions whereas small increases in antagonism (i.e., herbivory or competition alone) had very large effects on CH flower and fruit production. When total antagonism became more severe (i.e., herbivory and competition co-occur) plants did not have the ability to reduce CH flower and fruit production farther as these are bounded by zero. Because CH flower and fruit production are components of the mating system metrics, it follows that both mating system estimates should also respond in a less than additive manner to the antagonisms. However, my results indicate that mating system estimated as the proportional production of CL flowers responded non-additively to the antagonisms whereas mating system measured as proportional production of CL fruits responded additively. The different response of the two mating system metrics to the joint antagonism is likely due sample size limiting the detection of a significant interaction in the analysis of proportion CL fruits (competition*herbivory $P = 0.13$), as this metric was measured on a subsample of plants. Further, a power analysis substantiated that the sample size used in this analysis provided insufficient power to detect an interaction (analysis not shown, power (β) < 0.35). The non-additive mating system response I detected is corroborated by field data from Steets and Ashman (2004) who found a similar non-additive response for proportional production of CL flowers along a natural herbivory gradient (i.e., modest increases in herbivory

greatly depressed outcrossing). Overall, these results indicate that antagonistic interactions have more severe effects for mating system and reproductive traits than for plant growth.

Although not explored in this study, the outcrossing rate of CH flowers may also be modified by environmental conditions. For example, I found that competition reduced CH floral display size, which could lead to an increase in outcrossing rate of the CH flowers as a result of reduced geitonogamy. Karron and colleagues (1995) found evidence of this in *Mimulus ringens*; plants grown at high densities had significantly higher outcrossing rates than those grown at low densities because of a reduction in geitonogamous selfing in the former. My finding that herbivory did not affect CH floral display size is in line with that of Steets and Ashman (2004). In the present study, competition may have had a greater effect than herbivory on CH display because the former antagonism reduced plant resource status (i.e., plant growth) to a greater degree. However, other researchers have found that herbivory can cause a decrease in floral display size and selfing rate (Elle and Hare 2002). The conflicting results of this study and those of Elle and Hare (2002) with respect to the effect of herbivory for floral display size is likely due to differences in the average floral display of the study organisms. Specifically, *I. capensis* plants in the present study had display sizes that were over an order of magnitude smaller than *Datura wrightii* studied by Elle and Hare (2002). Taken together, my results in conjunction with those of other researchers (Karron et al. 1995, Elle and Hare 2002) indicate that plant antagonists often cause a reduction in floral display size that can then lead to a reduction in selfing rate; however, more work is needed to determine the generality of this response.

3.5.2. Allocation to flower size vs. flower number

Plant-antagonist interactions, such as competition and herbivory, can reduce plant resources and flower size (Cresswell et al. 2001, Iwaizumi and Sakai 2004). I also found that

antagonists reduced CH flower size, but this reduction occurred only when plants were subject to both antagonisms. This result, in conjunction with my finding that CH flower production is greatly reduced with small increases in the antagonism environment (i.e., herbivory or competition alone), suggests that there is a trade-off between allocation to CH flower size and number — flower size is more highly conserved than flower number with increasing antagonism. The conservation of flower size over number has also been demonstrated by other researchers (Cresswell et al. 2001) and could reflect the importance of maintaining the fit between flower and pollinator for proper transfer of gametes.

3.5.3. Consequences of antagonists for population genetic diversity

This study shows that small increases in the antagonism environment greatly increase selfing at the level of relative heteromorphic flower production, suggesting that plant antagonists are likely to reduce population genetic diversity. Given the increased incidence of herbivory and competition in natural plant populations (Lienert and Fischer 2003, Rand and Louda 2004, Vila and Weiner 2004), small or isolated populations may be losing genetic variation at rates higher then expected from changes in population size alone. Declines in genetic diversity can increase a population's risk of extinction above that due to demographic and environmental effects (Newman and Pilson 1997). What remains to be seen is whether the antagonist-mediated changes in CH floral display that favor increased outcrossing are strong enough to offset the effects of antagonists on relative heteromorphic flower production. If these antagonisms are to increase, then outcrossing of CH flowers may be a mechanism of genetic rescue. Data from another experiment (J. A. Steets and T.-L. Ashman, unpublished data) suggests that this genetic rescue via CH outcrossing can occur for plants subject to one antagonism, however, data from this study suggests that this is unlikely when plants are subject to two antagonisms.

3.5.4. Consequences of plant antagonists for mating system evolution

Understanding how multispecies interactions affect mating system is the first step in discerning how community context influences mating system evolution. The majority of studies exploring the effect of multispecies interactions on plant fitness or selection have investigated herbivore-herbivore and herbivore-pollinator interactions (reviewed in Strauss and Irwin 2004, but see Tiffin 2002) whereas those investigating the effect of ecological context on mating system have mainly studied plant-pollinator interactions (e.g., Kalisz et al. 2004). The current study adds significant breadth to these bodies of research by demonstrating that competitive interactions between plants influence the effect of herbivory on components of fitness and mating system. Further, the antagonism-induced change in mating system that I report may be an adaptive plastic response to living in heterogeneous ecological or environmental conditions. Thus, plant antagonists may serve as selective agents on the mating system. To understand whether herbivory and competition exert selection on the mating system or its plasticity, we need to know how these antagonists affect the relationship between mating system and fitness and the degree to which these interaction vary in space and time. Theory predicts that mixed mating systems will evolve under variable environmental conditions (e.g., Schoen et al. 1996, Masuda et al. 2001). Although this environmental variation is primarily considered in terms of pollinator visitation, the large degree of spatial and temporal variation that exists in the intensity of herbivory and competition (Louda 1989, Kadmon 1995, Rand 2002) is also likely to contribute to the stabilization of mixed mating systems. Future studies should aim to incorporate these prevalent ecological interactions into our view of mating system evolution.

Table 3.1 Effect of herbivory, competition and their interaction on (A.) growth, (B.) reproduction and (C.) mating system traits of *Impatiens capensis* as determined by mixed model analyses of variance. The degrees of freedom (numerator, denominator) and *F*-values are given. Proportion CL flowers and fruits were arcsine transformed prior to analysis. Significance levels for *F*-statistics are denoted as **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	Source					
	Herbivory		Competition		Competition*Herbivory	
Trait	d.f.	*F*	d.f.	*F*	d.f.	*F*
A. Growth						
Biomass	1, 143	6.8**	1, 143	293.3****	1, 143	2.7
Height	1, 143	12.4***	1, 143	174.5****	1, 143	0
Branch production	1, 143	0.03	1, 143	122.0****	1, 143	0.7
B. Reproduction						
CL flowers	1, 143	0.18	1, 143	52.1****	1, 143	7.41**
CL fruits	1, 27	0.84	1, 27	5.45*	1, 27	2.4
CH flowers	1, 143	70.1****	1, 143	217.4****	1, 143	63.8****
CH fruits	1, 27	38.7****	1, 27	94.6****	1, 27	23.9****
CH display size	1, 49	0.26	1, 49	14.28***	1, 49	0.27
CH flower size	1, 49	4.57*	1, 49	6.37**	1, 49	3.38†
C. Mating system						
Proportion CL flower	1, 143	35.0****	1, 143	159.6****	1, 143	36.2****
Proportion CL fruit	1, 27	12.3***	1, 27	43.6****	1, 27	2.42

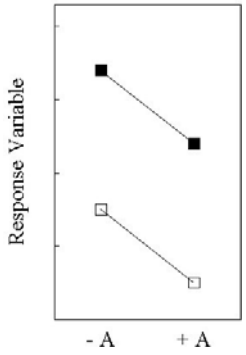
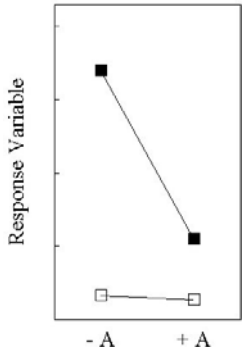
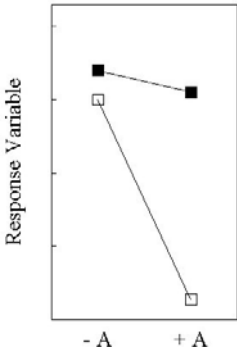
	Method of examination	Type of Interaction		
		Additive	Substitutive	Synergistic
A	Interaction term	Non-significant	Significant	Significant
B	Graphical depiction			
C	Tukey test	Not examined	-A-B treatment differs from -A+B, +A-B, and +A+B treatments. Latter three treatments cluster together.	+A+B treatment differs from -A-B, +A-B, and -A+B treatments. Latter three treatments cluster together.

Figure 3.1 Additive, non-additive substitutive and non-additive synergistic effects of two antagonisms (e.g., herbivory, competition, parasitism) understood through an analysis of variance approach. The expected significance of the interaction term of the ANOVA (A.), graphical depiction of each type of interaction (B.) and Tukey test results (C.) are shown. In the graphs, the *x*-axis represents the absence (-A) or presence (+A) of antagonism A (e.g., herbivory). The presence or absence of antagonism B (e.g., competition) is depicted with open or closed symbols, respectively.

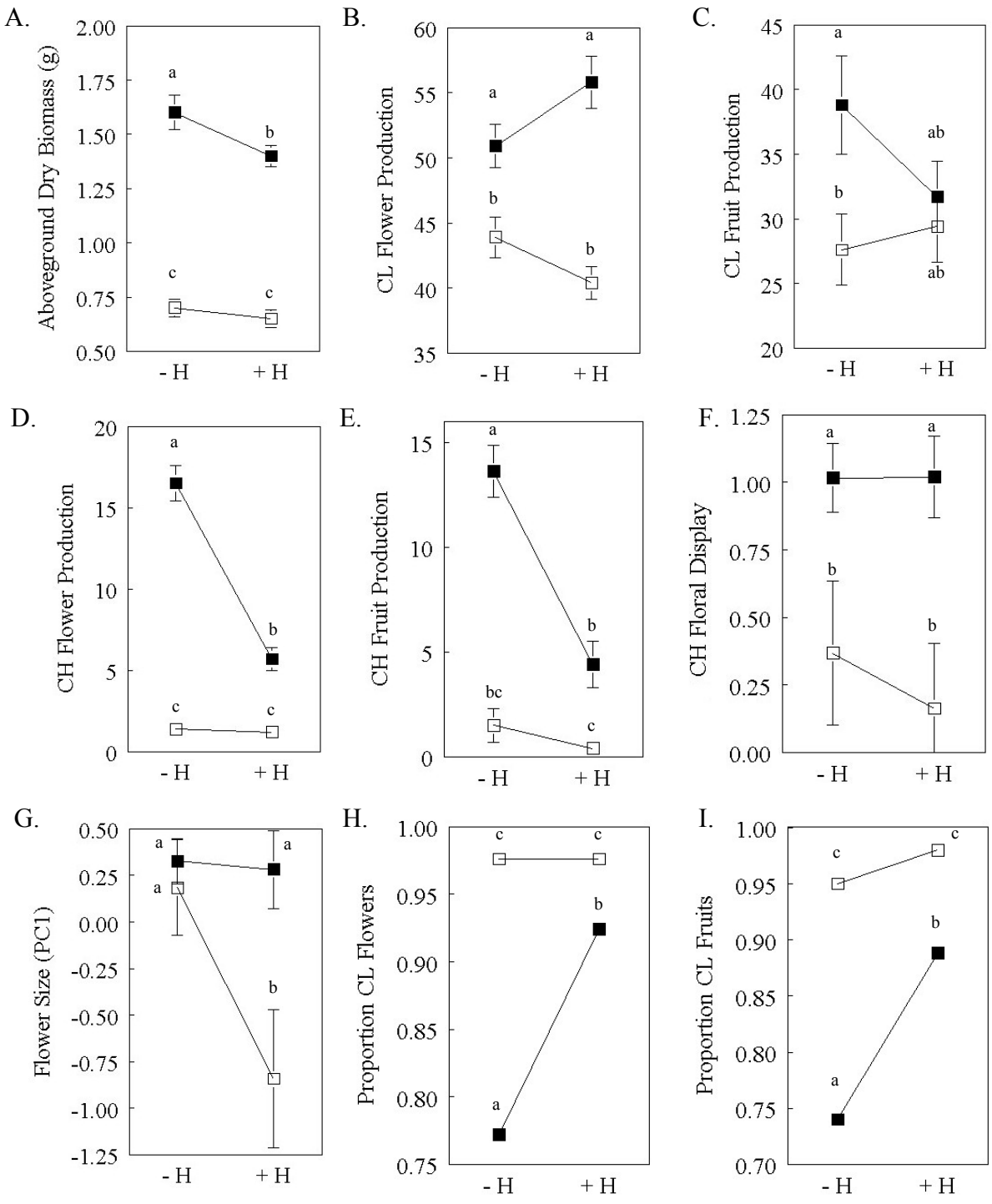


Figure 3.2 Least squares mean (A.) aboveground dry biomass, (B.) cleistogamous (CL) flower production, (C.) CL fruit production, (D.) chasmogamous (CH) flower production, (E.) CH fruit production, (F.) CH daily floral display size, (G.) CH flower size (principal component 1), (H.)

proportional CL flowers and (I.) proportional CL fruits for plants in the herbivore damaged (+H), herbivore undamaged (-H), competitor present (open symbols) and competitor absent (closed symbols) treatments. Back-transformed least squares means are displayed for proportion CL flowers and fruits. Error bars represent ± 1 standard error. Within a trait, means that share letters do not significantly differ from one another as determined by Tukey tests. Refer to the text and Table 3.1 for additional analyses.

4. THE CONSEQUENCES OF VEGETATIVE HERBIVORY FOR THE MAINTENANCE OF INTERMEDIATE OUTCROSSING IN AN ANNUAL PLANT

4.1. Abstract

Given the prevalence of mixed mating systems among plants, a general mechanism explaining the evolution and maintenance of this condition is needed. Although numerous theoretical models predict mixed mating to be evolutionarily stable, the conditions favoring intermediate selfing are often stringent and have limited applicability. Here I investigate the role of vegetative herbivory, a ubiquitous biotic factor limiting plant reproduction, in the mating system expression of *Impatiens capensis* (Balsaminaceae), a species with an obligate mixed-mating system (individuals produce both selfing, cleistogamous and facultatively-outcrossing, chasmogamous flowers). I found that herbivory reduced plant size and altered the fate of floral meristems, which resulted in a reduction in proportional chasmogamous reproduction. Additionally, herbivory reduced chasmogamous flowering display and pollinator visitation rate and altered the composition of the pollinator fauna, which decreased geitonogamous selfing among chasmogamous flowers. Overall, herbivory caused a slight decrease in whole-plant outcrossing, indicating that the opposing effects of herbivory on proportional chasmogamous reproduction and chasmogamous outcrossing for whole-plant outcrossing rate nearly negate one another. These findings are the first to unravel the mechanisms underlying herbivore-mediated changes in mating system. Furthermore, they point to the role of natural enemies in contributing to the maintenance of a mixed mating system.

4.2. Introduction

Nearly 40% of angiosperms surveyed to date exhibit intermediate levels of outcrossing (Goodwillie et al. 2005). Understanding the ecological and genetic causes of this variation is a major focus of research because changes in mating patterns have profound effects on the fitness of individuals (Charlesworth and Charlesworth 1987), the genetic structure of populations (Hamrick and Godt 1990), as well as patterns of speciation (Barrett 1990). While a variety of factors are likely to influence selection for and the maintenance of mixed mating, most theoretical and empirical examinations have focused primarily on genetic aspects (e.g., Lande and Schemske 1985, Campbell 1986, Uyenoyama 1986, Charlesworth and Charlesworth 1990, Latta and Ritland 1993, Ronfort and Couvet 1995, Chang and Rausher 1999) or the role of pollination biology (e.g., Holsinger 1991, Schoen et al. 1996, Kalisz et al. 2004). However, the few models that have explicitly considered interactions between organisms and their natural enemies predict selection for intermediate selfing over much of the parameter space (Lively and Howard 1994, Agrawal and Lively 2001). Despite these predictions, little empirical data exists regarding the effect of enemies on plant mating system (but see Elle and Hare 2002, Steets and Ashman 2004, Ivey and Carr 2005).

Herbivory is the primary antagonism limiting plant reproduction (Marquis 1992) and evidence is accumulating that it can alter mating system related traits (Elle and Hare 2002, Steets and Ashman 2004, Ivey and Carr 2005). However, only one study to date has demonstrated that herbivory has significant consequences for outcrossing rate (Ivey and Carr 2005). Furthermore, we have little understanding of the mechanisms underlying herbivory-induced changes in mating system. A number of possibilities exist. First, in plants that produce heteromorphic flowers — small, selfing (cleistogamous, CL) and large, open (chasmogamous, CH) flowers — leaf damage can alter allocation to flower types, reducing the proportional production of CH flowers (Steets

and Ashman 2004, Steets et al. 2005). This change in relative heteromorphic flower production could have two causes: (1) it may be a passive response to reductions in plant size due to resource limitation with herbivory (van Kleunen and Fischer 2005), such that plants experiencing herbivory are unable to surpass a size threshold necessary for CH flower production (Schmitt et al. 1987a) or (2) it may result from altered floral meristem fate, such that plants experiencing higher levels of herbivory actively differentiate fewer of their primordia to CH flowers. Second, herbivory can cause reductions in floral attractive traits and floral display size (e.g., Strauss et al. 1996, Mothershead and Marquis 2000, Elle and Hare 2002, Steets and Ashman 2004), which in turn may influence outcrossing rate by causing an increase in autogamy (within-flower selfing) and/or a decrease in geitonogamy (among-flower selfing). For example, Elle and Hare (2002) found that herbivory reduced floral display size of *Datura wrightii* and plants with smaller displays had higher outcrossing rates. Third, pollinator abundance and faunal composition may also change with herbivory-induced changes in flowering phenotype (Steets and Ashman 2004), which may have consequences for outcrossing rate if pollinating taxa differ in their propensity to visit multiple flowers on a plant. In addition to these mechanisms, herbivory may also influence post-pollination processes, and thus mating system (Levri and Real 1998). Given that many of the proposed mechanisms of herbivore-mediated changes in mating system oppose one another, it is crucial that we simultaneously study their combined effect on outcrossing, as this will provide insight to the potential role of herbivory in promoting mixed mating. Furthermore, because herbivore pressure varies both spatially and temporally (Louda 1989, Rand 2002) it may be that heterogeneity in this antagonism selects for intermediate and flexible levels of outcrossing.

In this study, I manipulated levels of insect herbivory in natural *Impatiens capensis* populations to determine whether herbivory has consequences for outcrossing rate. Specifically, I addressed three main questions: (1) does herbivory increase selfing by reducing proportional CH reproduction (flowers, fruits or seeds)?, (2) does herbivory alter the outcrossing rate of CH flowers?, and (3) what are the combined effects of herbivory for whole-plant outcrossing rate? In addition, I examined the mechanisms by which herbivory changes the mating system of *I. capensis*. First, I investigated two mechanisms of herbivory-induced changes in heteromorphic flower production: plant size and meristem fate. Second, I investigated the mechanisms by which herbivory alters the outcrossing rate of CH flowers: reductions in CH floral display or flower size and changes in pollinator fauna abundance or composition.

4.3. Materials and Methods

4.3.1. Study system

Impatiens capensis Meerb. (Balsaminaceae) is a native annual that occurs throughout moist forests in eastern North America (Schemske, 1978). It is suited to the objectives of this study as it exhibits an obligate mixed-mating system by producing heteromorphic flowers. Specifically, an individual can produce both closed, obligately-selfing (CL) and facultatively-outcrossing (CH) flowers. The CH flowers are not capable of autogamy because of strong temporal separation of anther and stigma maturation; however, selfing may occur due to geitonogamy. Further, numerous studies have found broad variation in the outcrossing rate of CH flowers among populations (e.g., t_{CH} = 0.29 - 0.71; Waller and Knight 1989).

Herbivory is prevalent among *I. capensis* populations, with numerous insect species feeding on this plant (see Schemske 1978). Vegetative damage at the populations studied here

was primarily caused by chrysomelid beetles, leaf miners, grasshoppers and katydids (J. A. Steets personal observation).

4.3.2. Consequences of herbivory for outcrossing rate

In wild *I. capensis* populations in Crawford County, Pennsylvania near the Pymatuning Laboratory of Ecology (PLE), I randomly selected six 1-m^2 plots in a single population (W: 41E40.6' N, 80E25.6' W) in 2002 and 14 1-m^2 plots in each of three populations (W; L: 41E38.6' N, 80E25.7'W; T: 41E35.7' N, 80E21.2'W) in 2003. Plots were separated from one another by at least 5 m and within each plot, 10 (2002) or 7 (2003) *I. capensis* plants were tagged and assigned to one of two treatments: (1) low or (2) high herbivory. Herbivory was reduced by applying biweekly Conserve™ (Dow AgroSciences LLC., Indianapolis, IN, USA) and Endeavor™ (Syngenta Crop Protection Inc., Greensboro, NC, USA), two insecticides that reduce herbivory without affecting growth or reproduction of *I. capensis* (Appendix A), to plants in low herbivory plots. High herbivory plots were sprayed with water at the same frequency to serve as a control. The insecticide applications began pre-flowering and continued until three weeks prior to a frost that killed the majority of plants.

To determine if herbivory causes a reduction in the proportional production of CH flowers, fruits and seeds, I quantified CL and CH flower production on all tagged plants in each plot and estimated CL and CH fruit and seed production on these plants in 2002 and on three marked individuals per plot in 2003. I also collected up to 15 CL and 15 CH capsules from each tagged plant and calculated total CL and CH seed production as the product of CL and CH fruit production and plot mean CL and CH seed production per capsule, respectively. From these measures, I calculated proportion CH flowers, fruits and seeds. To determine whether herbivory affected CL or CH reproduction or proportion CH flowers, fruits and seeds, I performed separate

ANCOVAs (PROC GLM, SAS Institute, 2001) on the plot means of these traits. I considered each population-year combination a separate replicate in the ANCOVAs. Herbivory treatment, replicate and their interaction were designated as fixed effects. Initial plant height (measured prior to first insecticide application) was included as a covariate to correct for any differences between treatments in initial plant size. Prior to analysis, proportion CL flowers, fruits and seeds were arcsine transformed to improve normality (Zar 1999).

To determine whether outcrossing rate of CH flowers is altered by herbivory, I estimated CH outcrossing rate using allozyme markers. I randomly selected 2 – 15 CH seeds from each tagged plant for allozyme analysis. In addition, the electrophoretic phenotypes of maternal leaf tissue (2002) or 5 selfed CL seeds (2003) were used to infer the maternal phenotype. Enzymes were extracted from fresh or frozen (-70°C) tissues with the extraction buffers of Lu (1995) or Mitton and colleagues (1979), respectively. Extracts were absorbed through Miracloth (Calbiochem, La Jolla, CA, USA) onto filter paper wicks and stored at -70°C until electrophoretic analysis. For plants sampled in 2002, I resolved four polymorphic loci on four, one-locus enzyme systems [aspartate aminotransferase (*Aat*, EC 2.6.1.1), menadione reductase (*Mnr*, EC 1.6.99.2), phosphoglucoisomerase (*Pgm*, EC 5.4.2.2) and uridine diphosphoglucose pyrophosphorylase (*Ugpp*, EC 2.7.7.9)]. For plants sampled in 2003, I resolved two additional loci on one enzyme system, isocitrate dehydrogenase (*Idh*, EC 1.1.1.42). *Idh*, *Pgm* and *Ugpp* were resolved on buffer system 4, *Aat* was resolved on buffer system 7, and *Mnr* was resolved on a modified version of buffer system 8. All gel-electrode buffer systems and enzyme stains are from Soltis and colleagues (1983). Gels were composed of 11.4% hydrolyzed potato starch from Starch Art Corportation (Smithville, TX, USA). A standard of known electrophoretic phenotype was run on each gel.

I used the Newton-Raphson procedure in Ritland's MLTR program (version 2.4) (Ritland 2002) to estimate the multilocus t_{CH} and bootstrapped standard errors in the low and high herbivory treatments of each replicate. The effect of herbivory on t_{CH} in each replicate was examined with a *t*-test. I performed a weighted *Z* test (Whitlock 2005) to determine whether herbivory increased t_{CH} across all replicates.

I calculated the whole-plant outcrossing rate for each plot by taking the product of mean t_{CH} and mean proportional production of CH seeds per plot. The effect of herbivory on whole-plant outcrossing rate in each replicate was examined using *t*-tests. I performed a weighted *Z* test to determine if herbivory affected whole-plant outcrossing rate across replicates.

4.3.3. Mechanisms of herbivory-induced change in heteromorphic flower production

4.3.3.1. Plant size mechanism

If a height threshold must be reached to initiate CH flower production (Schmitt et al. 1987a), then the changes in proportional CH reproduction with herbivory could be a passive consequence of a reductions in plant size with herbivory. To test this hypothesis, I used a series of ANCOVAs to determine whether herbivory-mediated reductions in plant height lead to the reductions in proportional production CH flowers, fruits and seeds. First, I determined whether herbivory had a significant effect on final plant height. Then, in separate ANCOVAs for each of the three mating system metrics (proportion CH flowers, fruits and seeds), I included final plant height, in addition to initial plant height, as a covariate. The mating system metrics were arcsine transformed prior to analysis to improve normality (Zar 1999). For each mating system metric, I compared the proportion of variance explained (Gotelli and Ellison 2004) by herbivory with and without final height in the ANCOVA.

4.3.3.2. Meristem fate mechanism

To determine whether the change in proportion CH flowers, fruits and seeds with herbivory was due to altered floral meristem fate (i.e., a floral meristem converting from CH to CL), I conducted a separate experiment in which one individual from each of 19 pairs of full-sib plants (from six populations) were assigned to either a low or high herbivory treatment (as above). I then recorded the total number of nodes on the central axis and nodal location of the first CH flower. To determine if the node of first CH flower differed with herbivory, I performed an ANCOVA with herbivory treatment, population and their interaction as fixed effects and the total node number as a covariate.

4.3.4. Mechanisms of herbivory-induced change in CH outcrossing rate

4.3.4.1. Floral display and flower size mechanism

I quantified CH floral display size on three days during peak CH flowering on plants in the outcrossing rate experiment. To determine if herbivory reduces CH display size, I conducted a fixed effect ANOVA (PROC GLM, SAS Institute, 2001) on plot mean CH display size with herbivory treatment and replicate. To determine if CH display size is related to t_{CH}, I performed both a linear and non-linear regression (PROC REG and PROC NLIN, SAS Institute, 2001) on plot means of these traits for 2003 plots. The non-linear regression explained more of the variance, thus I only report the results of this analysis.

To determine whether herbivory alters CH flower size, I also measured eight floral traits on one randomly selected female-phase flower per plot and performed a principal components analysis (PROC FACTOR, SAS Institute, 2001) following the procedure of Steets et al. (2005). The first principal component explained 60% of the variance among plants in floral traits and all traits loaded positively (all loadings > +0.46), indicating that it reflects overall flower size. I explored the effect of herbivory on this principal component using ANOVA, with herbivory

treatment, replicate and their interaction as fixed effects. To determine if CH flower size is related to t_{CH}, I performed both a linear and non-linear regression (as above) between plot mean t_{CH} and CH flower size for plots in 2003. The non-linear regression better explained the relationship between t_{CH} and CH flower size, thus I only present the results of this analysis.

4.3.4.2. Pollinator fauna abundance and composition mechanism

To determine whether herbivory leads to altered pollinator abundance or composition, I observed visitation to flowers in the low and high herbivory treatment of the W (2003) replicate of the outcrossing rate experiment. Specifically, across five days, I observed pollinator visitation in five low and three high herbivory plots. On a given day, I recorded pollinator visitation to all open flowers in a plot for 15-minutes and rotated observation periods between herbivory treatments. In a total of16 h of observation, I recorded 194 visits by bumblebees, honey bees and small solitary bees. I used log-likelihood *G* test (Zar 1999) to determine if pollinators undervisit flowers on high herbivory relative to low herbivory plants. In addition, I determined whether the composition of the pollinating fauna differed between herbivory treatments using a *G* test.

To determine whether CH floral display and flower size affect pollinator abundance or composition in ways that could affect t_{CH}, I conducted two separate experiments. To determine if pollinator groups (bumblebees, honey bees, small bees) differ in their foraging behavior and especially in their propensity to visit multiple open flowers on a plant (i.e., affect geitonogamous selfing), I set out arrays of four potted *I. capensis* plants with varying numbers of open CH flowers (1, 2, 3 and 4 or 2, 4, 6 and 8). During 30-minute observation periods on seven days, I recorded the total number of pollinator visits and geitonogamous visits by each pollinator group. I performed linear regressions (PROC REG, SAS Institute, 2001) between mean number of geitonogamous visits and CH display size for each pollinator group.

To determine if the abundance of the different pollinating fauna is related to CH flower size, I reanalyzed data from Steets and Ashman (2004) on pollinator visitation and CH flower size of *I. capensis* plants. I performed linear regressions (PROC REG, SAS Institute, 2001) between visitation rate and CH flower size for each pollinator group (bumblebees, honey bees and small bees).

4.4. Results

4.4.1. Does herbivory increase selfing by reducing proportion CH reproduction?

Overall, the insecticide applications reduced herbivory by 50% (15% vs. 30% leaf damage; $P < 0.0001$). Herbivory significantly reduced all components of CH reproduction (Tables 4.1 and 4.2), whereas it only significantly reduced CL flower production (Tables 4.1 and 4.2). Combined, the differential effects of herbivory on CL and CH reproduction resulted in approximately a 60 - 66% reduction in the proportional production of CH flowers, fruits and seeds with increasing herbivory (Figure 4.1A, Tables 4.1 and 4.2).

4.4.2. Does herbivory alter the outcrossing rate of CH flowers?

Although only one replicate showed a statistically significant increase in t_{CH} with herbivory (W 2002: $t = 1.9$, d.f. = 13, $P_{1\text{-tail}} = 0.04$; other replicates $P_{1\text{-tail}} > 0.15$; Figure 4.1B), all replicates showed a trend to increase and the general pattern across replicates was statistically significant ($Z = 1.9$, $P_{1\text{-tail}} = 0.03$; Figure 4.1B).

4.4.3. What are the combined effects of herbivory for whole-plant outcrossing rate?

When I considered the consequence of herbivory for whole-plant outcrossing, high herbivory plots had lower whole-plant outcrossing rates than low herbivory plots in three out of four replicates; however, this trend was not significant within any replicate (Figure 4.1C; all $P > 0.15$). Across replicates, herbivory caused a marginally significant decrease in whole-plant

outcrossing rate (Figure 4.1C; $Z = 1.6$, $P = 0.1$). This result indicates that the opposing effects of herbivory on t_{CH} and heteromorphic flower production for whole-plant outcrossing rate nearly negate one another.

4.4.4. Mechanisms of herbivory-induced change in heteromorphic flower production

4.4.4.1. Plant size mechanism

Herbivory significantly reduced final plant height (Tables 4.1 and 4.2), and when final plant height was included in the analyses of proportional CH reproduction, the proportion of variance explained by the herbivory treatment was reduced by 77 - 82% (proportion CH flowers: 14.0% vs. 3.2%; proportion CH fruits: 13.3% vs. 2.4%; proportional CH seeds: 14.0% vs. 3.1%). These results indicate that herbivory-induced change in mating system is largely, but not entirely, due to effects on plant height.

4.4.4.2. Meristem fate mechanism

High herbivory plants produced their first CH flower at an earlier node than low herbivory plants, indicating that the fate of floral meristems also changes with herbivory, but this was only marginally significant (least square means ± s.e.: 11.8 ± 1.07 vs. 14.5 ± 0.99; $F_{1,28} = 3.39$, $P = 0.07$).

4.4.5. Mechanisms of herbivory-induced change in CH outcrossing rate

4.4.5.1. Floral display and flower size mechanism

Herbivory significantly reduced mean CH display size (0.32 ± 0.11 vs. 0.63 ± 0.20 open flowers/plant; $F_{1,38} = 6.35$, P = 0.02) and CH flower size (PC1 ± s.e.: -0.45 ± 0.35 vs. 0.37 ± 0.23; $F_{1, 14} = 5.53$, $P = 0.03$). In addition, there was a significant negative relationship between t_{CH} and CH display size (Figure 4.2A; $t_{CH} = 0.41e^{-0.15*\text{CH display size}}$, $F_{2, 17} = 33.8$, P <0.0001) and

between t_{CH} and CH flower size (Figure 4.2B; $t_{CH} = 0.51e^{-0.13*\text{CH flower size}}$, $F_{2, 15} = 33.5$, $P < 0.0001$), indicating that geitonogamy is reduced in plants with smaller display and flower sizes.

4.4.5.2. Pollinator fauna composition and abundance mechanism

Pollinators visited flowers on low herbivory plants nearly five times as frequently as those of high herbivory plants (1.64 ± 0.24 vs. 0.36 ± 0.16 visits/flower/hour; $G = 66.7$, d.f. = 1, $P < 0.0001$). Furthermore, visitation by bumblebees was depressed more by herbivory than visitation by small bees and honey bees (Figure 4.3A; $G_H = 6.21$, d.f. = 2, $P < 0.05$). This result is corroborated by the significant linear relationship between abundance of bumblebee visitors and CH flower size (Figure 4.3C; bumblebee visitation = 0.48 + 0.24*CH flower size; $F_{1, 76} = 14.5$; $P = 0.0003$). Visitation by small bees and honey bees did not depend upon CH flower size (Figure 4.3D, E; both $P > 0.4$). The change I observed in pollinator faunal composition with herbivory (Figure 4.3A) has consequences for CH outcrossing rate because the pollinating taxa differ in their geitonogamous foraging behavior. Bumblebees increase number of geitonogamous visits with CH display size (Figure 4.3B; number of geitonogamous bumblebee visits = -0.35 + 0.24*CH display size; $F_{1, 4} = 144.9$; $P = 0.0003$), but small bees and honey bees do not (Figure 4.3B; both $P > 0.75$).

4.5. Discussion

Numerous theoretical models predict mixed mating to be an evolutionary stable strategy, however, the conditions favoring intermediate selfing are often very stringent and likely applicable to a limited number of cases (reviewed in Goodwillie et al. 2005). Given the prevalence of mixed mating systems among plants (Goodwillie et al. 2005), a general mechanism explaining the evolution and maintenance of this condition is needed. The results

presented here point to the role of natural enemies, in particular herbivores, in contributing to the maintenance of a mixed mating system.

4.5.1. Effects of herbivory on CH outcrossing

This study is the first to demonstrate that herbivory causes a significant increase in the outcrossing rate of CH flowers (Figure 4.1B). As selfing in the CH flowers of *I. capensis* can only occur via geitonogamy, the increase in CH outcrossing with herbivory was due to a decrease in this mode of selfing. In addition, these results reveal a few mechanisms for this change. First, herbivory reduced CH flowering display (both CH flower size and number in the display), and thus plants experiencing high levels of herbivory received fewer geitonogamous pollinator visits relative to those experiencing less herbivory. In addition, the herbivory-induced change in CH display caused an overall reduction in pollinator visitation as well as a change in pollinator fauna composition. Heavily herbivorized plants were visited more by small solitary bees and honey bees and less by bumblebees relative to low herbivory plants (Figure 4.3A). Given that honey and small bees tend to visit only a single open flower on a plant, whereas bumblebees forage in a pattern that promotes geitonogamy (Figure 4.3C-D), the change in pollinator fauna composition with herbivory also contributes to increasing CH outcrossing. These findings highlight the need for more detailed studies of the effect of different pollinator species on mating system expression as well as how enemies or other ecological conditions may alter pollinator fauna and thus mating system.

Only a few other studies have investigated the effects of herbivory on outcrossing of open-pollinated flowers (Elle and Hare 2002, Ivey and Carr 2005). Elle and Hare (2002) report findings similar the present study; herbivory reduced the floral display of *Datura wrightii* and plants with smaller displays had higher outcrossing rates. On the other hand, Ivey and Carr

(2005) found that herbivory reduced outcrossing in *Mimulus guttatus*. However, the mechanism of change was unclear. If we are to gain a more general understanding for the role of herbivores in plant mating system expression, future work should aim to determine the relative importance of the various mechanisms by which herbivory affects outcrossing for other plant species.

The finding of increased CH outcrossing with herbivory is in accord with one of the widely held theories regarding the evolution of outcrossing from asexuality, namely that natural enemies select for genetically variable progeny in their hosts. A recent analysis by Busch and colleagues (2004) found a positive relationship between the outcrossing rate of a species and the number of pathogens attacking that species, providing empirical support for pathogen-mediated selection maintaining outcrossing in angiosperms. What remains to be seen is whether increased CH outcrossing with herbivory is an adaptive response or a passive consequence of resource-mediated change in flowering phenotype.

4.5.2. Effects of herbivory on heteromorphic flowering

Although herbivory increased CH outcrossing, I found that in terms of relative heteromorphic seed production, herbivory shifted the mating system toward selfing. The reduction in proportion CH seeds was primarily due to herbivory reducing CH reproduction more than CL reproduction (Tables 4.1 and 4.2). I have previously demonstrated that interactions with community members, such as herbivores and competitors, reduce CH flower production and proportional CH reproduction (Steets and Ashman 2004, Steets et al. 2005). In addition, other studies have demonstrated that stressful abiotic conditions can also cause plants to reduce energetically-expensive CH reproduction (Schemske 1978, Waller 1980, Bell and Quinn 1987, Le Corff 1993, Lu 2000). However, prior to the current study, the mechanism for change in relative heteromorphic flower production with stress was unclear. I found evidence that both

passive and active processes caused this change in mating system. In accord with a previous study on *I. capensis* demonstrating that CH flower production is size dependent (Schmitt et al. 1987a), I found that herbivory reduced plant height, which in turn reduced CH flower production. However, when I controlled for final plant height in the analyses, there was still a marginally significant difference between herbivory treatments in proportional CH reproduction, indicating that other mechanisms may also play a role in this change. For example, I found that high herbivory plants produced their first CH flower at an earlier node than low herbivory plants. Given that the herbivory treatments did not differ in total node production (data not shown, $P > 0.05$) and high herbivory plants produced fewer total CH flowers than low herbivory plants, the former must have stopped CH flower production at an earlier node than the latter. Together, these results indicate that floral meristem fate differed between herbivory treatments. Future work is planned to further explore this hypothesis by comparing the fate of floral meristems at each node for individuals experiencing different herbivore pressure.

4.5.3. Consequences for the evolution and maintenance of intermediate outcrossing

The overall lowering of whole-plant outcrossing with herbivory depended upon the relative effect of herbivory on proportional CH reproduction versus CH outcrossing rate. Given that a large degree of spatial and temporal variation can exist in the intensity of herbivory (Louda 1989, Rand 2002), heterogeneity in this antagonism may select for and maintain mixed mating. Models that have incorporated ecological condition into understanding mating system evolution find that resource limitation and the trade-offs imposed by it (e.g., Schoen and Lloyd 1984, Iwasa 1990) as well as variable pollinator visitation (e.g., Schoen et al. 1996) select for mixed mating. Given the ubiquity of herbivory and its effect on both plant resources (reviewed in Crawley 1989) and pollination environment (e.g., Strauss et al. 1996, Steets and Ashman 2004,

present study) this antagonism may drive heterogeneity or stochasticity in resource and pollination environments that can select for mixed mating. Thus, this antagonism may contribute more to the evolution and maintenance of intermediate outcrossing than is currently appreciated.

This study also provides evidence that herbivory influences traits related to the stability of mixed mating systems. For example, our findings support the predictions of a model by Masuda and colleagues (2001) exploring the evolution of heteromorphic flower production, a mixed mating strategy. According to this model, mixed mating systems are an evolutionary stable strategy when geitonogamy among CH flowers increases with increasing floral display size. The finding of reduced geitonogamy with smaller CH display sizes provides support for this model; however, other parameters, such as inbreeding depression, must be quantified to fully test the model and whether herbivory can maintain heteromorphic flower production in *I. capensis*.

4.5.4. Consequences for population structure and biparental inbreeding

One consequence of herbivory altering mating system is a change in population genetic structure. If a population is sufficiently structured, then breeding with close relatives (i.e., biparental inbreeding) may result. Although data from this experiment (not shown) and those of Ivey and Carr (2005) indicated that biparental inbreeding did not differ with herbivory, over different temporal and spatial scales this may not be the case. Specifically, if herbivore pressure is patchy in a population and this heterogeneity is maintained over time, then genetic structure is likely to result and could lead to biparental inbreeding in some patches. Given that biparental inbreeding may stabilize intermediate outcrossing (Ronfort and Couvet 1995), it is crucial that researchers begin to study whether herbivory has consequences for this mode of inbreeding, and thus the stability of mixed mating.

4.5.5. Conclusions

Overall, the results of this study shed light on an intensively studied topic in evolutionary biology, the evolution of mixed mating. I have shown that herbivory alters several components of plant mating system. This result coupled with the knowledge that herbivore pressure is very common (Marquis 1992) and often heterogeneous (Louda 1989, Rand 2002), suggest that enemies contribute to the maintenance of intermediate outcrossing. Empirical studies measuring selection on the mating system in different herbivory environments would greatly add to our understanding of the role of natural enemies in the evolution of mixed mating.

Table 4.1 Least-squares means (± 1 standard error) of *Impatiens capensis* vegetative and reproductive traits by herbivory treatment (low, high). Traits include plant height (cm), cleistogamous (CL) flower, fruit and seed production, chasmogamous (CH) flower fruit and seed production and proportion CH flowers, fruits and seeds. Back-transformed mean proportion CH flowers, fruits and seeds are presented.

Trait	Herbivory Treatment	
	Low	High
Height (cm)	62.1 ± 1.38	56.0 ± 1.15
No. CL Flowers	23.1 ± 1.17	19.3 ± 0.98
No. CL Fruits	17.5 ± 1.58	13.4 ± 1.36
No. CL Seeds	20.5 ± 2.05	16.0 ± 1.77
No. CH Flowers	13.5 ± 1.33	5.5 ± 1.12
No. CH Fruits	10.6 ± 1.37	3.7 ± 1.18
No. CH Seeds	23.5 ± 3.90	7.2 ± 3.37
Proportion CH Flowers	0.20 ± 0.002	0.08 ± 0.002
Proportion CH Fruits	0.20 ± 0.003	0.07 ± 0.002
Proportion CH Seeds	0.27 ± 0.004	0.09 ± 0.003

Table 4.2 The effect of treatment, replicate and their interaction on plant height, cleistogamous (CL) flower, fruit and seed production, chasmogamous (CH) flower, fruit and seed production and proportion CH flowers, fruits and seeds produced by *Impatiens capensis*. The degrees of freedom (numerator, denominator) and *F*-values are given. Significance levels for *F*-statistics are denoted as **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\dagger} P < 0.10$. Initial height was included as a covariate in all analyses (all $P < 0.01$). Proportion CH flowers, fruits and seeds were arcsine transformed prior to analysis.

	Source					
	Treatment (T)		Replicate (R)		T*R	
Trait	d.f.	*F*	d.f.	*F*	d.f.	*F*
Height	1,37	10.9**	3,37	6.5***	3,37	4.7**
No. CL Flower	1,37	5.8*	3,37	1.0	3,37	5.3**
No. CL Fruit	1,34	3.8†	3,34	0.1	3,34	2.0
No. CL Seed	1,34	2.8	3,34	0.07	3,34	2.0
No. CH Flower	1,37	20.1****	3,37	9.4****	3,37	5.5**
No. CH Fruit	1,34	14.3***	3,34	3.9*	3,34	5.4**
No. CH Seed	1,34	9.6**	3,34	3.1*	3,34	4.6**
Prop. CH Flower	1,37	8.5**	3,37	9.2***	3,37	1.3
Prop. CH Fruit	1,34	7.6**	3,34	4.7**	3,34	0.75
Prop. CH Seed	1,34	8.0**	3,34	5.1**	3,34	1.1

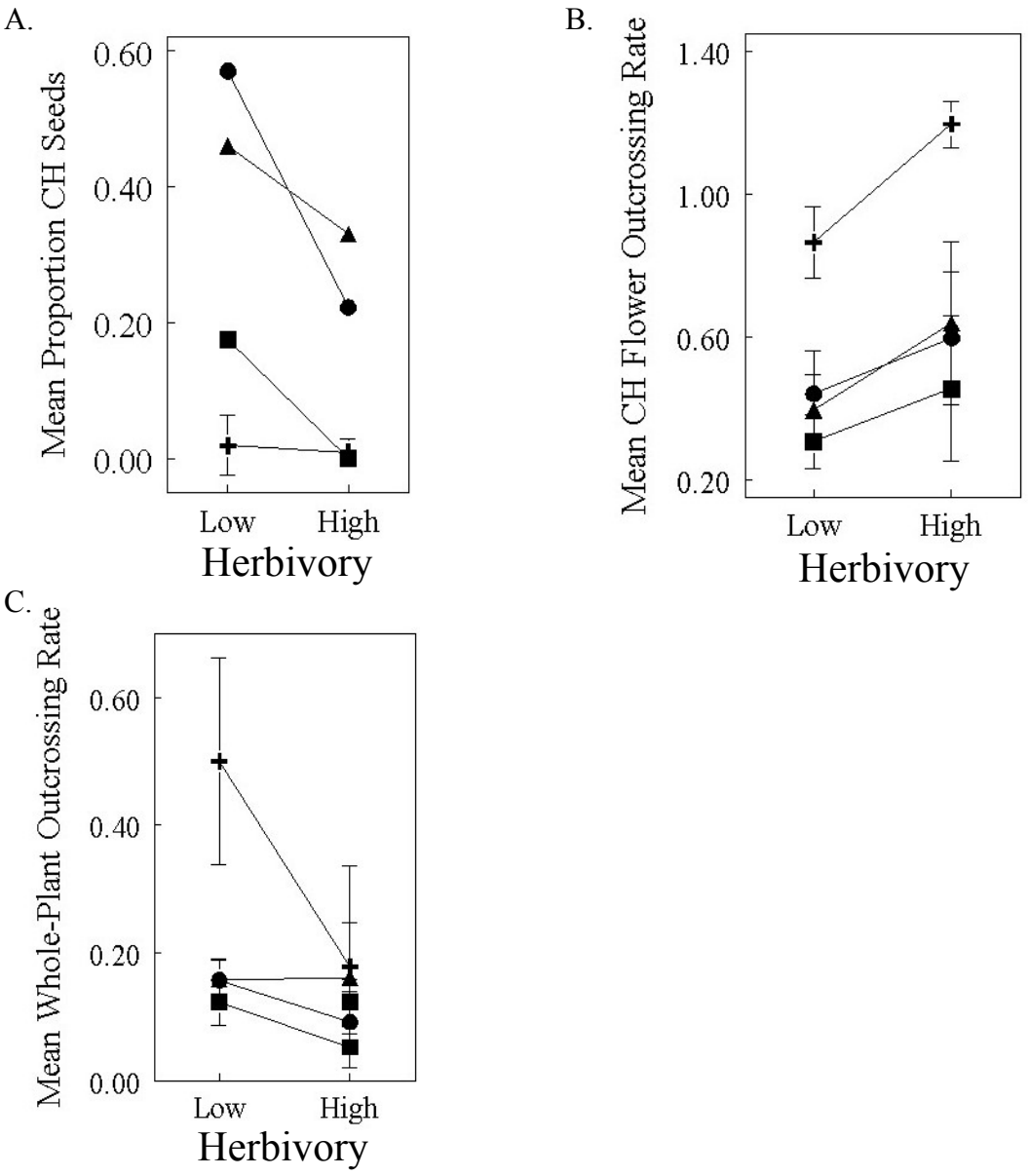


Figure 4.1 (A.) Back-transformed least-squares mean proportional production of CH seeds per plant, (B.) mean CH flower outcrossing rate, and (C.) mean whole-plant outcrossing rate for *Impatiens capensis* individuals experiencing low or high herbivory. Symbols represent different population replicates: plus = W (2002); circle = L; triangle = T; square = W (2003). Error bars represent ± 1 standard error. Refer to text and Table 4.2 for statistics.

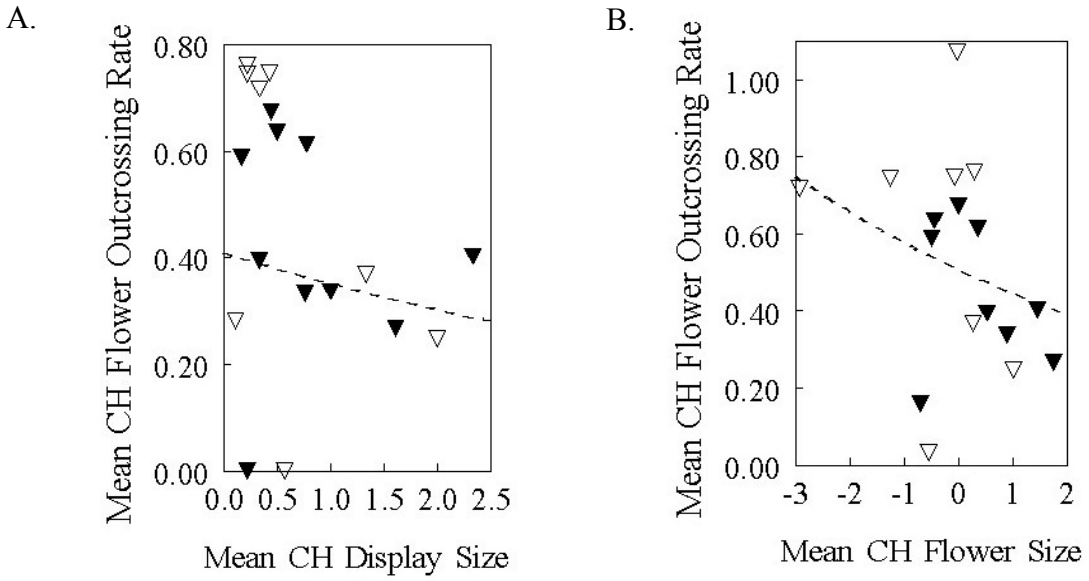


Figure 4.2 Relationship between plot mean CH flower outcrossing rate and (A.) plot mean CH display size (CH outcrossing rate = $0.41e^{-0.15*\text{CH display size}}$, $F_{2, 17} = 33.8$, $P < 0.0001$) or (B.) CH flower size (CH outcrossing rate = $0.51e^{-0.13*\text{CH flower size}}$, $F_{2, 15} = 33.5$, $P < 0.0001$). Dashed lines represent significant regression lines. Symbols represent different herbivory treatments: open triangles = high herbivory; closed triangles: low herbivory.

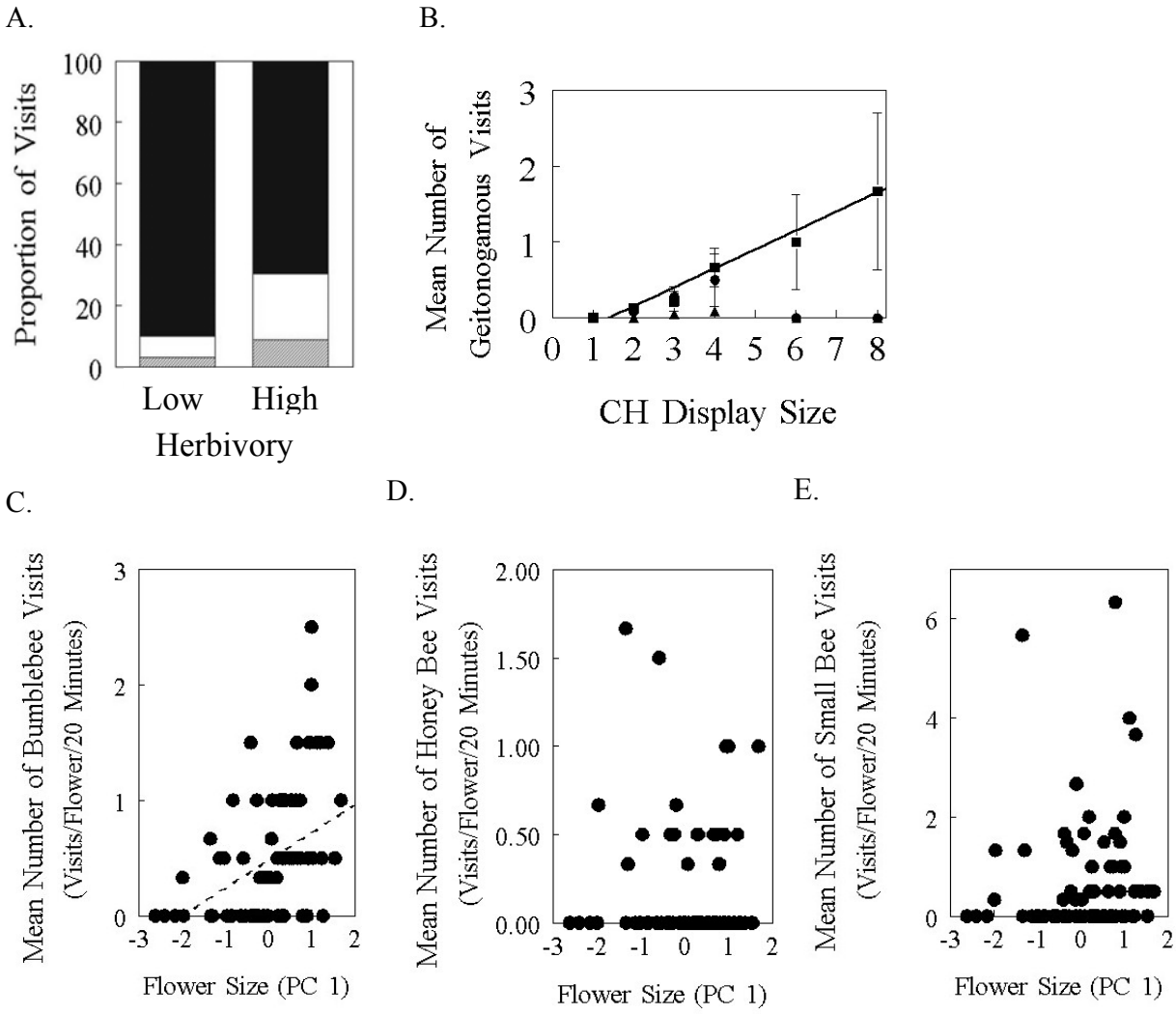


Figure 4.3 (A.) Proportion of pollinator visits to low and high herbivory plants in the W (2003) population. Shading represents different pollinator classes: solid bar = bumblebees; open bar = honey bees; hatched bar = small bees. (B.) Mean number of geitonogamous pollinator visits made by bumblebees (squares), honey bees (triangles), and small bees (circles) to plants of varying display sizes. (C.) Mean number of bumblebees visits, (D.) honey bee visits and (E.) small bee visits as a function of CH flower size (principal component 1).

5. THE INTERACTIVE EFFECTS OF HERBIVORY AND MIXED MATING FOR THE POPULATION DYNAMICS OF *IMPATIENS CAPENSIS*

5.1. Abstract

In this study I examine the demographic consequences of mixed mating and explore the interactive effects of vegetative herbivory and mating system for the population dynamics of *Impatiens capensis*, a species with an obligate mixed mating system (i.e., individuals produce both obligately-selfing, cleistogamous and facultatively-outcrossing, chasmogamous flowers). In two natural *I. capensis* populations, I followed seeds derived from cleistogamous and chasmogamous flowers subject to different herbivory levels throughout their life cycle. Using a mating system-explicit matrix model for *I. capensis* I found that mating system types differed in important vital rates. Cleistogamous individuals had higher rates of germination and survival than did chasmogamous individuals, whereas chasmogamous individuals expressed a fecundity advantage over cleistogamous individuals. In addition, population growth was most sensitive to changes in vital rates of cleistogamous individuals. Herbivory also had demographic consequences; a 33 – 49% reduction in herbivory caused the population growth rates to increase by 104 – 132%. The reduction in population growth rate with herbivory was primarily due to effects on vital rates of selfed individuals. This study is the first to consider the demographic consequences of mixed mating and to address the combined effect of herbivory and mating system for population dynamics. Further, these results have important implications for mating system evolution.

5.2. Introduction

Both genetic factors and environmental condition influence the structure of plant populations (Hamrick and Godt 1990, Linhart and Grant 1996), and thus are likely important regulators of population dynamics. Among genetic factors, the mating system (relative production of selfed and outcrossed individuals) is of utmost importance to population structure as it largely determines the amount of genetic variation observed in a population. One of the primary ecological factors affecting plant populations is herbivory (Crawley 1989). This ubiquitous ecological interaction is known to affect numerous vital rates of plants, including growth, survivorship and fecundity (reviewed in Crawley 1989, Huntly 1991), which can scale up to affect population dynamics and growth rate (e.g., Bastrenta et al. 1995, Ehrlen 1995, Rooney and Gross 2003, Knight 2004). In recent years, evidence has mounted that herbivory also has consequences for mating system expression (Elle and Hare 2002, Steets and Ashman 2004, Ivey and Carr 2005, Steets et al. 2005; see also Chapter 4). Yet, to date, no one has merged these lines of research to understand the effect of mating system structure on population dynamics, nor whether herbivory has consequences for population growth due to its effect on mating system.

Although evolutionary biologists have long-studied mating system expression, paying particular attention to its consequence for individual fitness (Charlesworth and Charlesworth 1987), population genetic structure (Hamrick and Godt 1990) and speciation (Barrett 1990), the demographic significance of mixed mating has been largely ignored (but see Oostermeijer 2000). To have a greater understanding of the population-level consequences of mixed mating we must first account for contributions of selfed and outcrossed individuals throughout the life cycle to population dynamics. For example, selfed individuals may germinate at a lower rate and experience reduced survival and fecundity compared to outcrossed individuals due to inbreeding

depression (reviewed in Charlesworth and Charlesworth 1987). Alternatively, in highly structured populations, the opposite pattern may hold due to outbreeding depression (e.g., Pelabon et al. 2005). Although numerous researchers have measured vital rates of selfed and outcrossed individuals (e.g., Waller 1984, Luijten et al. 2002), no one has accounted for this type of population structure in demographic models, thus we currently are ill-equipped to address whether mating system types differentially contribute to population dynamics. Furthermore, given that environmental conditions are changing at alarming rates (Vitousek 1992) and such conditions greatly influence mating system expression (e.g., Waller 1980, Elle and Hare 2002, Steets and Ashman 2004), it is imperative that we evaluate the demographic consequences of mating system in order to understand how changes in mating system will effect population persistence.

Herbivory is known to affect mating system expression (Elle and Hare 2002, Steets and Ashman 2004, Ivey and Carr 2005, Steets et al. 2005; see also Chapter 4) and can have differential effects on selfed and outcrossed individuals, which may scale up to influence plant population dynamics. There are several reasons herbivory-induced changes in mating system may affect population dynamics. First, herbivory may increase (Steets and Ashman 2004, Ivey and Carr 2005, Steets et al. 2005; see also Chapter 4) or reduce (Elle and Hare 2002) the relative numbers of selfed versus outcrossed individuals in a population by affecting outcrossing rate. These herbivory-induced changes in the mating system may have demographic effects if vital rates (e.g., germination, survival, fecundity) differ between mating system types. Second, herbivory may increase the expression of inbreeding depression (Carr and Eubanks 2002, Hayes et al. 2004), which may further skew the relative performance of selfed and outcrossed progeny, alter the population dynamics, and reduce the proportion of selfed individuals in the population.

Finally, herbivory may have transgenerational effects (*sensu* Agrawal 2001) that alter the realized mating system and the demographic value of selfed versus outcrossed seeds. For instance, given that herbivory more greatly reduces the quality (i.e., seedling size) of selfed relative to outcrossed progeny in *I. capensis* (Steets and Ashman 2004), selfed seeds produced by maternal plants experiencing high herbivore pressure may have reduced performance relative to outcrossed seeds produced in a similar environment. As a first step in understanding the avenues by which herbivory and mating system interact to affect population dynamics, I investigate such effects within a generation here and address the existence of mating system-dependent transgenerational effects of herbivory for population dynamics in future work.

In this study, I address the demographic effects of mating system and herbivory in *Impatiens capensis*, a species with an obligate mixed mating system (i.e., individuals produce both selfing, cleistogamous (CL) and facultatively-outcrossing chasmogamous (CH) flowers). In natural populations I followed seeds derived from CL and CH flowers (hereafter, CL and CH individuals) subject to different herbivory levels throughout their life cycle to address the following questions: (1) how do mating system and herbivory affect vital rates (i.e., germination, survival, fecundity)?, (2) how does herbivory affect population dynamics of *I. capensis*?, (3) how sensitive are the population dynamics of *I. capensis* to cleistogamy and chasmogamy and does herbivory change the relative contributions of CL and CH individuals to population growth?, and (4) what is the relative contribution of each vital rate to changes in population growth rate between herbivory levels?

5.3. Materials and Methods

5.3.1. Study system

Impatiens capensis Meerb. (Balsaminaceae) is an annual herb native to North America (Schemske 1978). This species only reproduces sexually via CL and CH flowers. The flower types are easily distinguished by their positions on the plant and pedicel structure (Schemske 1978). The obligately self-pollinating CL flowers have reduced petals, anthers and sepals and lack nectaries. In contrast, the CH flowers are showy and open to pollination by numerous species of bees (e.g., *Bombus* spp., *Apis mellifera*, *Dialictus rohweri*) as well as the ruby-throated hummingbird (*Archilochus colubris*) (J. A. Steets, personal observation). The CH flowers are self-compatible, but strong protandry prevents autogamy. In populations in northwestern Pennsylvania, outcrossing rates for CH flowers range between 0.29 – 1.00 and differ with herbivory (see Chapter 4). In northwestern Pennsylvania, plants usually emerge in the spring (March - May), begin production of CL flowers in early summer (mid-June) and CH flowers in late summer (August) and senesce in fall (October). Seeds produced in the fall germinate the following spring, i.e., there is no persistent seed bank (Antlfinger 1989).

Numerous insect species feed upon the vegetative tissue of *I. capensis* (see Schemske 1978). In the populations studied vegetative herbivory averaged 50% of leaves damaged per plant, but varied among individuals (0 – 100%) and was primarily caused by chrysomelid beetles, leaf miners, grasshoppers and katydids (J. A. Steets, personal observation).

5.3.2. Experimental design

During the summer of 2003, I haphazardly collected CL and CH seeds from individuals in two *I. capensis* populations in Crawford County, Pennsylvania (L: 41E38.6'N, 80E25.7'W; W: 41E40.6'N, 80E25.6'W). Both populations occurred in deciduous forests in which the

overstory was predominately composed of oak (*Quercus* spp.), beech (*Fagus sylvatica*) and maple (*Acer* spp.). In the L population, *I. capensis* occurred in monospecific stands, whereas in the flood-prone W population, *I. capensis* was found among other understory herbs, including skunk cabbage (*Symplocarpus foetidus*), jack-in-the-pulpit (*Arisaema* spp.) and large-flowered trillium (*Trillium grandiflorum*). Seeds were stored in distilled water at 4EC until planting in November 2003. I randomly set out 12 1-m^2 plots in each population in locations where *I. capensis* occurred. Within each plot, I cleared other vegetation and planted 400 native seeds (200 CL and 200 CH seeds) in a randomized grid 5 cm apart, for a total of 2,400 CL and 2,400 CH seeds per population. This planting design produced a seed density that was similar to that found in natural *I. capensis* populations (Antlfinger 1989). Seeds were planted to a depth of 1 cm into sections of 1 cm diameter plastic straws and covered with sand and leaf litter. Once seedlings emerged in the spring, plots were randomly assigned to either a low (LH) or high (HH) herbivory treatment. Herbivory was reduced by applying biweekly Conserve™ (active ingredient: spinosad), an insecticide that reduces herbivory without affecting *I. capensis* growth or reproduction (Appendix A), to plants in LH plots. Plants in HH plots were sprayed with water at the same frequency to serve as a control. In the W population, one LH plot was destroyed by early spring floods, thus the sample size for this population was reduced to 11 plots (5 LH plots and 6 HH plots). Vegetative herbivory was recorded once (mid-flowering season) as the percentage of leaves damaged per plant. To determine if the insecticide spray reduced herbivory, I performed an analysis of variance (PROC GLM, SAS Institute, 2001) with herbivory treatment, replicate, seed type and their interactions as fixed effects and plot mean percent leaf damage of seed types as the response variable.

Ideally, to determine how mating system and herbivory interact to affect population dynamics, one would measure vital rates of known selfed and outcrossed seeds in different herbivory environments. However, obtaining sufficient sample size for such a demographic study would require hundreds of controlled crosses to generate the seeds types. In lieu of this, I took advantage of the heteromorphic flowering system of *I. capensis* and followed known selfed (i.e., CL seeds) and potentially outcrossed (i.e., CH seeds) seeds throughout their life cycle. However, I am confident that the majority of CH seeds used in this experiment were outcrossed as I estimated CH outcrossing rate to be 60% and 82% in the L and W populations, respectively (see Chapter 4).

I recorded seedling germination and survival to reproduction as well as marked the peduncles of developing fruits with non-toxic paint weekly throughout the growing season (April 1, 2004 – October 6, 2004). At the end of the season, I quantified CL and CH fruit production on all surviving plants in each plot by enumerating painted peduncles. To estimate seed production per plant, I collected up to three CL and CH fruits from each individual and enumerated seeds per fruit. For each individual, total CL (or CH) fecundity was calculated as the product of CL (or CH) fruit production and mean CL (or CH) seed production per fruit. For the few plants for which I was unable to obtain seeds, I used plot mean CL or CH seed production per fruit for a CL or CH individual in the above calculation of fecundity. In each plot an average of 354 seeds germinated (range: 286 - 400), 55 individuals survived to reproduce via cleistogamy (range: 13 - 95) and 10 individuals survived to reproduce via cleistogamy and chasmogamy (range: 0 - 26).

5.3.3. How do mating system and herbivory affect vital rates?

The basic model for projection population growth of a structured population is $\mathbf{n}_{t+1} = \mathbf{A}\mathbf{n}_t$, where **n** is a vector with *i* rows representing the number of individuals in each life stage at time *t*

and t+1, and **A** represents the annual projection matrix with i rows and j columns containing all life stage transition probabilities (a_{ij}). I constructed a two-stage (i.e., CL seed and CH seed) matrix model for *I. capensis* with four matrix elements (i.e., CL seed to CL seed (a_{11}), CL seed to CH seed (a_{21}), CH seed to CL seed (a_{12}), and CH seed to CH seed (a_{22})) (Figure 5.1A). The transition probabilities in **A** are a multiplicative function of germination (g), survivorship (p) and fecundity (f) of CL and CH individuals. For each population, I calculated the matrix elements (a_{ij}) within each plot and then averaged across all plots within a treatment to generate a single **A** matrix for each of the four herbivory level-population combinations.

Because *I. capensis* is an annual plant, the above outlined matrix model with a one-year time step (i.e., Figure 5.1A) simultaneously considers many vital rates (germination, survival, fecundity) in a single transition probability, and thus it does not allow for examination of the effects of herbivory on population growth through these underlying vital rates. In contrast, periodic matrix models provide a framework for modeling the demography of annual plants because they emphasize within-year temporal variation, allowing a larger proportion of the information available from demographic observations to be included (Caswell 2001). I created a periodic matrix model that explicitly considers the mating system of *I. capensis* (Figure 5.1B). The complete population cycle consists of 3 phases (m) (Figure 5.1B). The first phase occurs from November to April and includes two vital rates, seed survival and germination of CL (g_{CL}) and CH (g_{CH}) seeds. The second phase occurs from April to June and includes the vital rates of CL (p_{CL}) and CH (p_{CH}) survival to reproduction. The final phase occurs from June to November and includes four fecundities: CL adult CL fecundity ($f_{CL\text{-}CL}$), CL adult CH fecundity ($f_{CL\text{-}CH}$), CH adult CL fecundity ($f_{CH\text{-}CL}$) and CH adult CH fecundity ($f_{CH\text{-}CH}$). The resultant periodic projection matrices **B**(h) (where h = 1, 2,…m) describe within-year variation in these vital rates.

To describe the population dynamics over the entire cycle, the matrix product **A** is calculated by taking the product of all the **B** matrices (i.e., **A** = **B**(3)***B**(2)***B**(1)). I quantified vital rates separately for each herbivory level, and created a separate periodic matrix model for each herbivory level-population combination by first averaging vital rates within a plot and then averaging across plots within a population. As there was no a priori expectation for germination to differ with herbivory levels, all plots in a population were averaged to estimate germination rates of CL and CH seeds.

Because *I. capensis* is a highly selfing species I expected inbreeding depression to manifest at later stages of the life cycle (i.e., fecundity) (Husband and Schemske 1996). To determine if CL and CH transition probabilities (a_{ij}'s) and vital rates (germination (g_{CL}, g_{CH}), survival (p_{CL}, p_{CH}), and fecundity ($f_{CL\text{-}CL}$, $f_{CL\text{-}CH}$, $f_{CH\text{-}CL}$, $f_{CH\text{-}CH}$)) differ and whether inbreeding depression is stronger for fecundity than germination and survivorship, I compared annual transition probabilities (i.e., a_{11} vs. a_{12}, a_{21} vs. a_{22}.) and vital rates (e.g., g_{CL} vs. g_{CH}). To determine if herbivory affected the annual transition probabilities or vital rates, I compared each element between herbivory levels within a population. Finally, to test the hypothesis that CL individuals have reduced vital rates relative to CH individuals when subject to herbivory because of increased expression of inbreeding depression (Carr and Eubanks 2002), I compared the difference in vital rates of CL and CH individuals in each herbivory level. All comparisons were conducted by comparison of bootstrapped confidence intervals (see *Confidence intervals* section).

5.3.4. How does herbivory affect population dynamics of *I. capensis*?

To determine how herbivory affects population dynamics of *I. capensis*, I projected several population-level parameters from **A**. First, I calculated population growth rate (λ) for

each herbivory treatment within each population, as the dominant eigenvalue of **A** (Caswell 2001). I expected λ to be lower with herbivory because this antagonism dramatically reduces fecundity in *I. capensis* (Steets and Ashman 2004). In addition, for each herbivory level-population combination I calculated the stable stage distribution (**w**) as the right eigenvector of **A** (Caswell 2001). The stable stage distribution is the proportion of individuals in each stage class (i.e., CL and CH individuals) once the population reaches equilibrium (Caswell 2001). Given that herbivory increases proportional production of CL seeds per plant (Steets and Ashman 2004), I expected herbivory to shift the stable stage distribution in a similar way. Finally, I calculated the reproductive value (**v**) as the left eigenvector of **A** for each herbivory level-population combination (Caswell 2001). For *I. capensis*, the reproductive value can be interpreted as the present value of the future offspring produced by CH and CL individuals. Because herbivory increases the proportion of CL seeds produced by an individual (Steets and Ashman 2004), I also expected the reproductive value to be altered by herbivory. To determine if λ, **w**, or **v** differ with herbivory treatment, we compared these population-level parameters using bootstrapping (see *Confidence intervals* section below).

5.3.5. How sensitive are the population dynamics of *I. capensis* to cleistogamy and chasmogamy and does herbivory change the relative contributions of CL and CH individuals to population growth?

To determine how sensitive the population dynamics of *I. capensis* are to cleistogamy and chasmogamy, I calculated the elasticities of **A** for the L and W populations. Elasticities (e_{ij})

$$e_{ij} = \frac{a_{ij}}{\lambda}\frac{\partial \lambda}{\partial a_{ij}} = \frac{\partial(\log \lambda)}{\partial(\log a_{ij})}$$

measure the proportional change in λ resulting from a proportional small change in each annual matrix element, a_{ij} (de Kroon et al. 1986, Caswell 2001). Because elasticities range from 0 to 1

and sum to 1, they provide a measure of the relative importance of each matrix element (i.e., matrix elements with large elasticities contribute more to λ than matrix elements with smaller elasticities) (de Kroon et al. 2000). Within each population, to determine if herbivory alters the relative contribution of cleistogamy and chasmogamy to λ, I compared the elasticities between herbivory levels using bootstrapping (see *Confidence intervals* section).

5.3.6. What is the relative contribution of each vital rate to changes in population growth rate between herbivory levels?

Because herbivory occurs on both seedlings and adult plants, it could potentially affect six vital rates (i.e., CL and CH survival and all avenues for adult fecundity). I performed a periodic life table response experiment (LTRE) analysis to determine the contribution of each vital rate to the difference in λ between the high and low herbivory levels. A matrix element will have a large contribution to variation in λ if its magnitude varies greatly among treatments or if λ is highly sensitive to changes in that entry. For the periodic matrix of *I. capensis*, the life table response experiment equation is:

$$\lambda^{LH} - \lambda^{HH} \cong \sum_{hij} (b_{hij}^{LH} - b_{hij}^{HH}) S_{B(h)}$$

where the difference in λ between herbivory treatments is decomposed into contributions of each vital rate in the periodic matrix (Davis et al. 2003). LH and HH designate the low and high herbivory treatments and b_{hij} refers to individual matrix elements of the periodic projection matrices. $\boldsymbol{S}_{\boldsymbol{B}(h)}$ represents the sensitivity of λ to changes in the elements of periodic projection matrix $\mathbf{B}(h)$ and can be calculated using the formula:

$$S_{B(h)} = D^T S_{A(h)}$$

where $\mathbf{D}^{\mathrm{T}}$ represents the transpose of the product of the periodic projection matrices excluding $\mathbf{B}(h)$, and $\boldsymbol{S}_{\mathbf{A}(h)}$ represents the sensitivities of λ to changes in the elements of $\mathbf{A}(h)$ (the annual

projection matrix for the interval beginning at time period *h*)(Caswell and Trevisan 1994). For example, in *I. capensis*, the sensitivity matrix for **B**(2) is $S_{B(2)} = [B(1)B(3)]^T S_{A(2)}$, where **A**(2) = **B**(1)***B**(3)***B**(2). The sensitivities used in the periodic LTRE analysis were calculated from the mean annual matrices across the two herbivory levels.

5.3.7. Confidence Intervals

I calculated the 95% confidence intervals around all annual transition probabilities (a_{ij}'s), vital rates (g's, p's, f's) and matrix projections (λ, **w**, **v**, e_{ij}) for each demographic model using bootstrapping (McPeek and Kalisz 1993, Caswell 2001). A bootstrap dataset was calculated for a given herbivory level-population combination by resampling individuals with replacement at the level of the plot. The sample size of a bootstrap dataset was identical to the original data set (i.e., 400 individuals per plot). This process of generating a bootstrap dataset was repeated 1000 times, to create 1000 bootstrap datasets for each herbivory level-population combination. The matrix elements for individuals in plots in the same population and herbivory level were then averaged to generate mean matrix elements for a treatment with the exception of germination rates (g_{CL}, g_{CH}). For these two elements, I averaged across all plots in a population as the herbivory treatment did not affect these vital rates. From these bootstrap datasets, I calculated the 95% confidence intervals for each matrix element. I then used the bootstrap datasets to generate 1000 values for each matrix projection (λ, **w**, **v**, e_{ij}), from which I calculated the mean matrix projection and 95% confidence intervals. All transition and vital rates, matrix projections, and confidence intervals were calculated using MATLAB (2000). When making comparisons between mating system types (CL vs. CH) or herbivory levels (HH vs. LH) I considered matrix elements (or matrix projections) significantly different if the 95% confidence intervals around each estimate did not overlap.

5.4. Results

5.4.1. How do herbivory and mating system affect vital rates?

5.4.1.1. Mating system effects

Although none of the differences between the transition probabilities of CL and CH individuals for the annual matrix model for each herbivory level-population combination were statistically significant (i.e., a_{11} vs. a_{12} and a_{21} vs. a_{22}; Table 5.1), there was some indication that CL individuals suffered inbreeding depression. For example in three out of the four herbivory level-population combinations, CH seeds had higher transition rates to CH than did CL seeds (Table 5.1). This CH advantage was quite large in some populations, with the CH seed to CH seed transition being up to 73% greater than CL seed to CH seed transition probability. From the periodic matrix model, I found that the higher annual transition probability of CH individuals was due to their fecundity advantage (Figure 5.2, see below), as would be expected if inbreeding depression is expressed late in the life cycle of this highly selfing plant.

In accordance with my prediction, I found evidence of higher inbreeding depression in fecundity measures than germination and survival. Among both herbivory levels in the L and W populations, CH adults produced 14 – 127% more CL and CH seeds than CL adults (Figure 5.2A, B). At earlier stages of the life cycle, CL individuals tended to express an advantage over CH individuals. CL seeds germinated significantly more than CH seeds in the L population (16% CL advantage; Figure 5.2A) and survived to adulthood significantly more in the W population experiencing low herbivory (29% CL advantage, Figure 5.2B). A similar pattern of survival was found in the L population (Figure 5.2A); however, this trend was not significant.

5.4.1.2. Herbivory effects

The insecticide application reduced vegetative herbivory by 48% and 33% in the L and W populations, respectively (L population: 21% vs. 40% leaf damage; W population: 33% vs. 49% leaf damage; $F_{1, 38} = 40.8$, $P < 0.0001$). For both *I. capensis* populations, reducing herbivory tended to increase all transition probabilities of the annual matrix (Table 5.1). For the L population, reducing herbivory significantly increased the CL seed to CL seed transition by 98% and the CH seed to CH seed transition by 269% (Table 5.1A). These changes in transition probabilities of the **A** matrix with herbivory were caused by significant effects on survivorship and fecundity. Specifically, reducing herbivory increased CL seedling survival by 24%, CL adult CL fecundity by 60% and CH adult CH fecundity by 228% (Figure 5.2A).

For the W population, reducing herbivory increased the CL seed to CL seed transition by 127%, the CL seed to CH seed transition by 293%, and the CH seed to CL seed transition by 59% (Table 5.1B). Once again, these changes in transition probabilities of the **A** matrix with herbivory were caused by effects on survivorship and fecundity, however, the fecundities affected by herbivory in the W population differed from those affected in the L population. Specifically, reducing herbivory increased CL seedling survival by 24%, CL adult CL fecundity by 88%, CL adult CH fecundity by 220% and CH adult CL fecundity by 76% (Figure 5.2B).

There was also some support for the hypothesis that inbreeding depression is exacerbated by stressful environmental conditions (e.g., high herbivory levels). In the W population, higher levels of herbivory significantly reduced CL survival rate whereas it had the opposite (but non-significant) effect for CH survival (Figure 5.2B).

5.4.2. How does herbivory affect population dynamics of *I. capensis*?

All herbivory level-population combinations were projected to increase in size (i.e., λ significantly greater than 1; Figure 5.3A). As predicted, the populations experiencing the low

herbivory level were growing at a significantly faster rate than those subject to high levels of herbivory. In particular, reducing herbivory caused a 104% and 132% increase in λ in the L and W populations, respectively (Figure 5.3A). Both populations displayed similar stable stage distributions with CL individuals comprising the majority of the population (77 – 88%) at equilibrium (Figure 5.3B). In both populations, the reproductive value of mating system types was similar (Figure 5.3C). Although I found that herbivory increased the proportion of CL individuals in the stable stage distribution, this trend was not statistically significant (Figure 5.3B). In addition, the reproductive value of CL and CH individuals was not changed by herbivory (Figure 5.3C).

5.4.3. How sensitive are the population dynamics of *I. capensis* to cleistogamy and chasmogamy and does herbivory change the relative contributions of CL and CH individuals to population growth?

For both populations, λ was more sensitive to the CL seed to CL seed transition, with the elasticities of this transition being over four times greater than those for the other transitions (Figure 5.4A, B), indicating that selfing has a greater influence on λ than outcrossing for both populations in the year studied. Herbivory did not alter this pattern (Figure 5.4A, B).

5.4.4. What is the relative contribution of each vital rate to changes in population growth rate between herbivory levels?

Herbivory affected six of the eight vital rates (Table 5.2). Of these vital rates, not all contributed equally to the difference in λ between herbivory levels. In both populations, the survival of CL seedlings and CL fecundity of CL adults contributed the most to the difference in λ between herbivory levels (Table 5.2). Although CL survival did not differ much with herbivory, it had a large contribution because λ was most sensitive to changes in this vital rate

(Table 5.2). On the other hand, CL fecundity of CL adults had a large contribution because this vital rate differed dramatically between herbivory levels (Table 5.2). Despite the large differences in the other fecundity measures ($f_{CL\text{-}CH}$, $f_{CH\text{-}CL}$, $f_{CH\text{-}CH}$) between herbivory levels, they contributed less to the change in λ with herbivory because λ is relatively insensitive to these vital rates (Table 5.2). Finally, of the six vital rates contributing to the change in λ with herbivory, survival of CH seedlings had the lowest contribution despite its high sensitivity because herbivory had little effect on this vital rate (Table 5.2).

5.5. Discussion

Mating system is known to have consequence for individual fitness (Charlesworth and Charlesworth 1987), population genetic structure (Hamrick and Godt 1990) and speciation (Barrett 1990). The present study adds to this work by demonstrating that mating system can also have significant demographic consequences. In particular, I found that selfed and outcrossed individuals differ in important vital rates and differentially contribute to population growth of *I. capensis*. In addition, I found that a prevalent ecological factor, vegetative herbivory, exerted differential effects on selfed vs. outcrossed progeny that influenced population dynamics of *I. capensis*. I expand on each of these findings below and discuss the implications of this work for the evolution of mixed mating.

5.5.1. Demographic consequences of mixed mating

Mating system is an important factor structuring population genetic diversity (Hamrick and Godt 1990). This study demonstrates that it also plays a central role in population dynamics of a species exhibiting mixed selfing and outcrossing. Within the life cycle of *I. capensis*, I found evidence that mating system types differ in important vital rates and these results are consistent with expectations based on inbreeding depression. However, as inbreeding depression

was not explicitly measured in this experiment, my results are not conclusive and other factors, such as maternal effects, may have caused the differences seen in CL- and CH-derived individuals. In highly selfing species, such as *I. capensis*, inbreeding depression is expected to manifest at later stages of the life cycle, such as in growth and reproductive traits (reviewed in Husband and Schemske 1996). In accordance with this prediction, I found that CH adults tended to have higher fecundity than CL adults; however, there was no evidence of a CH advantage early in the life cycle (Figure 5.2). Specifically, CL adults produced up to 50% fewer CL and CH seeds than did CH adults, whereas CL individuals germinated and survived to reproduction at a higher rate than CH individuals (Figure 5.2). Other researchers have demonstrated significant inbreeding depression in reproductive traits of *I. capensis* (Schmitt and Ehrhardt 1987, Schmitt and Gamble 1990, Lu 2002). For example, in populations in Wisconsin and Rhode Island, outcrossed CH individuals produced 22% more total flowers (Lu 2002) and up to 50% more CL flowers (Schmitt and Gamble 1990) than CL plants. In addition, Schmitt and Gamble (1990) found greater inbreeding depression in CL flowering than in seedling emergence, further supporting theoretical predictions of greater inbreeding depression in later stages of development of this mixed-mating species.

The finding of a CL advantage early in life may seem counterintuitive, however another study on this species reports similar findings; CL seed germination was nearly 50% greater than CH seeds (58% vs. 40%) in a natural population (Antlfinger 1986). The higher germination and survival of CL seeds could be due to a few factors. First, there could be maternal environmental effects on mating system types that provide an advantage to the selfed seeds early in life. However, as CL seeds are smaller in size than CH seeds (e.g., Schmitt and Gamble 1990), it seems unlikely that maternal effects on seed types would explain the CL advantage.

Alternatively, CL seeds may express a germination and survival advantage over CH individuals due to ecological factors, such as differential susceptibility to pathogens or attack by seed predators. While the present study is not the first to demonstrate a CL advantage early in life (see also Antlfinger 1986), others have found inbreeding depression in germination and survival in natural *I. capensis* populations (i.e., Mitchell-Olds and Waller 1985). The conflicting results of these studies are likely due to population differences in mating system and environment and should be investigated further by performing a comparative demographic study.

The present study also demonstrates that mating system types differentially contribute to the population dynamics of *I. capensis*. For both *I. capensis* populations studied here I found CL individuals made up the greatest portion of the stable stage distribution, and thus the population dynamics were most sensitive to changes in vital rates of CL plants (Figure 5.4). Thus, the demographic model predicts that factors affecting the vital rates of CL individuals will have the largest effects on population size of *I. capensis*. For instance, genetic factors, such as inbreeding depression, or changes in ecological conditions that affect the fate of CL individuals, such as increasing plant density (Waller 1985) and competitive interactions (Schmitt and Ehrhardt 1990), will dramatically reduce the growth rate of the population. Given that plant density and competition are known to affect mating system in this species (e.g., Waller 1985, Schmitt and Ehrhardt 1990, Lu 2000), these factors are also likely to have mating system-dependent effects on the population dynamics of *I. capensis*. Future works should aim to incorporate density-dependence into demographic models of this species to understand how competitive interactions influence λ.

Although this study indicates that selfing via CL flowers is key to maintaining large populations of *I. capensis* in the sites and year studied here, CH individuals may be contributing

significantly to population dynamics in ways not considered. First, the demographic projections were based on a single year of empirical data. Given that environmental conditions vary greatly from year to year, CH individuals could have greater demographic importance in other years. In particular, in the year I performed this study, western Pennsylvania received record high summer rainfall from two hurricanes, Ivan and Frances, (http://www.depesf.state.pa.us/news/cwp/view.asp?a =1278&q=451909) resulting in flooding in both populations, which lead to early mortality and curtailed continued CH flower production. Alternatively, the primarily outcrossed CH individuals may be favored over selfed CL individuals when they disperse to novel habitats. Work by Schmitt and Gamble (1990) provides support for this hypothesis; inbreeding depression in CL flower production nearly doubled when individuals were planted 12 m from their parental site relative to those planted in the parental site. If we are to gain a better understanding of the importance of outcrossing for population dynamics of *I. capensis*, the demographic differences between CL and CH individuals grown in novel and parental sites must be elucidated.

5.5.2. Demographic consequences of herbivory

This work joins that of others (e.g., Bastrenta et al. 1995, Ehrlen 1995, Rooney and Gross 2003, Knight 2004) in demonstrating major demographic consequences of herbivory. In both *I. capensis* populations studied, λ more than doubled when herbivory was reduced, but even under high herbivory the populations were projected to grow significantly (Figure 5.3A). This study adds a unique aspect to the large body of work investigating the effects of herbivory on population dynamics by demonstrating that the demographic consequences of herbivory are dependent upon the mating system. I found that the vital rates of CL individuals were affected more by this antagonism than those of CH individuals. In particular, the LTRE analysis indicated that the difference in λ between high and low herbivory levels was primarily due to

affects on CL vital rates. Specifically, CL survival contributed greatly to the herbivory-mediated differences in λ because λ is very sensitive to changes in this vital rate (Table 5.2). In addition, CL adult CL fecundity had a large contribution to the herbivory-mediated change in λ because it was greatly reduced by herbivory (Table 5.2). The periodic matrix models also indicated that CL vital rates were affected more by herbivory than CH vital rates. For example, higher herbivory significantly reduced CL survival but not CH survival (Figure 5.2), a result consistent with the hypothesis that herbivory exacerbates inbreeding depression. Increased expression of inbreeding depression with herbivory has been demonstrated by a few researchers (Carr and Eubanks 2002, Hayes et al. 2004, Ivey et al. 2004; but see Nunez-Farfan et al. 1996, Stephenson et al. 2004). For example under field conditions, herbivory increased inbreeding depression for biomass two-fold in *Mimulus guttatus* (Ivey et al. 2004) and for female fitness components two-fold in *Cucurbita pepo* ssp. *texana* (Hayes et al. 2004). My findings add to this work by demonstrating that increased expression of inbreeding depression with herbivory may have demographic consequences.

The LTRE analysis also demonstrated that λ of *I. capensis* is very sensitive to germination of CL seeds (Table 5.2). This finding is very interesting when considered in light of potential transgenerational effects of herbivory. In a greenhouse experiment, Steets and Ashman (2004) found that herbivory more greatly reduced the quality (i.e., seedling size) of CL relative to CH progeny of *I. capensis*. If this differential effect of maternal herbivory on CL and CH offspring translates into reduced germination of CL relative to CH seeds from a high herbivory environment, then mating system-dependent transgenerational effects of herbivory are likely to have dramatic effects on population growth. Experiments testing this hypothesis are currently underway.

5.5.3. Consequences for the evolution of mixed mating systems

Understanding the evolution and maintenance of mixed mating systems is currently an area of intensive study by evolutionary biologists (reviewed in Goodwillie et al. 2005). My study indicates that herbivory may increase inbreeding depression in survivorship and this has demographic consequences (see *Results* section). Given that herbivore pressure often varies spatially and temporally in *I. capensis* (Steets and Ashman 2004, J. A. Steets unpublished data) and other species (Huntly 1991), this will likely lead to variation in inbreeding depression, which can select for stable intermediate rates of selfing (Cheptou and Mathias 2001). Overall, the work presented here as well as that of others (e.g., Carr and Eubanks 2002, Hayes et al. 2004, Ivey et al. 2004, Stephenson et al. 2004) reinforces the need for ecological context to be incorporated into models of mating system evolution.

5.5.4. Conclusions

These findings bring to light the importance of mating system for plant population dynamics in species with mixed mating systems. I have demonstrated that selfed and outcrossed individuals differ in important vital rates and differentially contribute to population growth. Furthermore, I found that vegetative herbivory significantly affects population dynamics due to effects on selfed individuals. These results, when considered with regard to a contemporary model of mating system evolution (Cheptou and Mathias 2001), point to the role of herbivores in the maintaining stable mixed mating.

Table 5.1 Mean transition probabilities (95% bootstrap confidence intervals) for annual matrix models of two *Impatiens capensis* populations, L (A.) and W (B.), experiencing low (LH) or high (HH) herbivory treatment (trt). Refer to text and Figure 5.1A for description of annual matrix model.

		Transition probability			
Pop	Trt	CL seed to CL seed	CL seed to CH seed	CH seed to CL seed	CH seed to CH seed
A. L	LH	2.87 (2.227 – 3.513)	0.68 (0.419 – 0.885)	2.56 (1.945 – 3.280)	1.18 (0.562 – 1.653)
	HH	1.45 (1.158 – 1.783)	0.27 (0.120 – 0.452)	1.66 (1.297 – 2.053)	0.32 (0.163 – 0.507)
B. W	LH	2.70 (2.260 – 3.157)	0.55 (0.313 – 0.845)	2.44 (1.996 – 2.943)	0.51 (0.276 – 0.767)
	HH	1.19 (0.937 – 1.478)	0.14 (0.059 – 0.251)	1.53 (1.256 – 1.853)	0.23 (0.077 – 0.441)

Table 5.2 Periodic life table response experiment (LTRE) for two *Impatiens capensis* populations, L (A.) and W (B.). The sensitivity of population growth rate to changes in each vital rate, the difference in vital rates between low and high herbivory levels, and the total contribution of a vital rate to the observed difference in population growth between herbivory levels are given. Vital rates include CL germination (g_{CL}), CH germination (g_{CH}), CL survival (p_{CL}), CH survival (p_{CH}), CL adult CL fecundity ($f_{CL\text{-}CL}$), CL adult CH fecundity ($f_{CL\text{-}CH}$), CH adult CL fecundity ($f_{CH\text{-}CL}$), and CH adult CH fecundity ($f_{CH\text{-}CH}$). Refer to Figure 5.1B for periodic matrix model and text for description of periodic LTRE analysis.

Pop	LTRE Element	Vital Rate							
		g_{CL}	g_{CH}	p_{CL}	p_{CH}	$f_{CL\text{-}CL}$	$f_{CL\text{-}CH}$	$f_{CH\text{-}CL}$	$f_{CH\text{-}CH}$
A.	Sensitivity	3.25	0.98	9.34	2.84	0.21	0.22	0.04	0.04
L	Difference	0.00	0.00	0.06	0.03	4.04	1.32	3.43	4.14
	Contribution	0.00	0.00	0.42	0.07	0.77	0.28	0.12	0.17
B.	Sensitivity	2.88	0.47	7.70	1.59	0.29	0.27	0.05	0.04
W	Difference	0.00	0.00	0.06	-0.03	3.64	1.11	3.96	1.13
	Contribution	0.00	0.00	0.37	-0.04	0.96	0.32	0.16	0.05

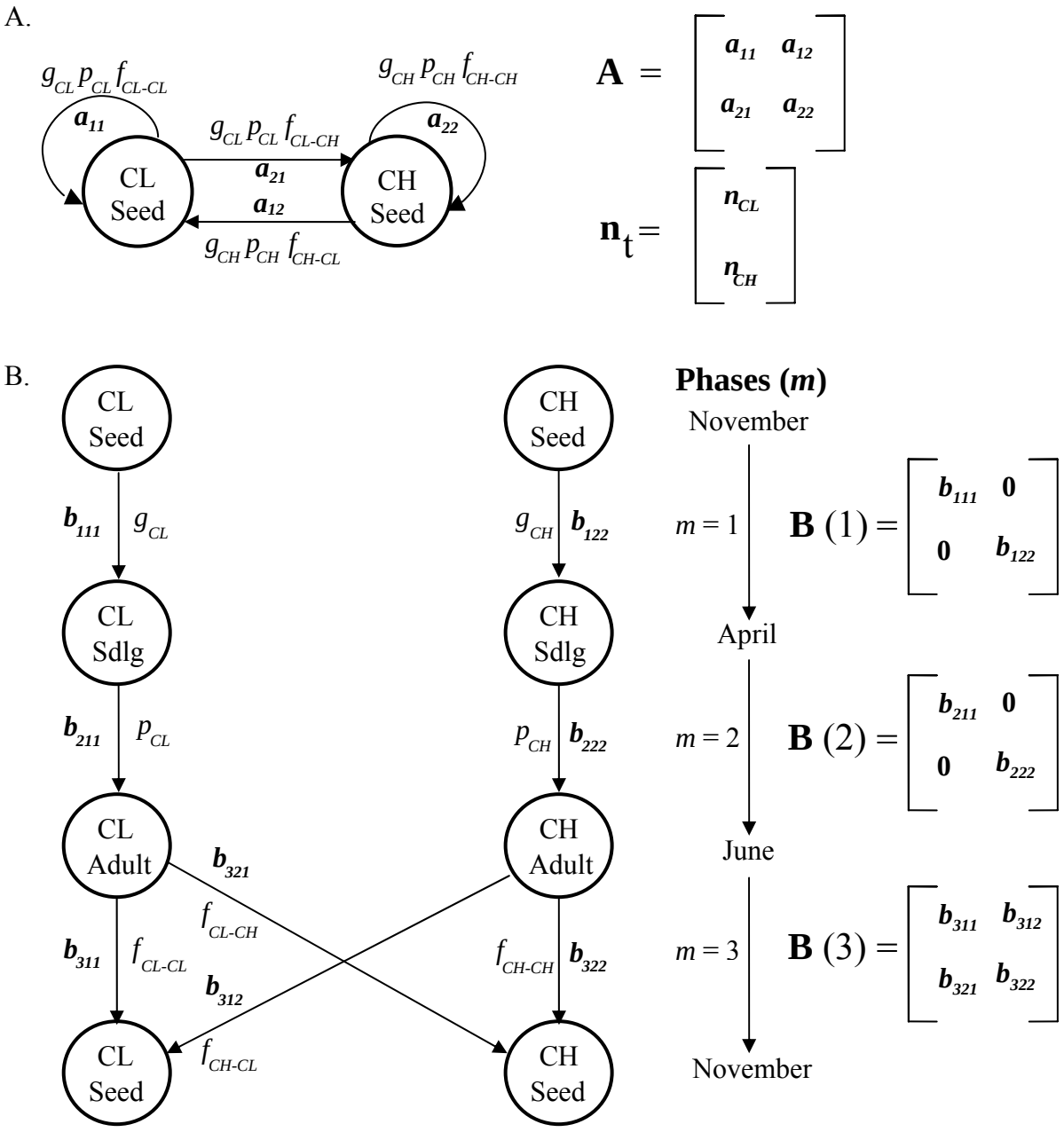


Figure 5.1 (A.) Annual life cycle graph and projection matrix model of *Impatiens capensis* incorporating mating system. The number of cleistogamous (CL) and chasmogamous (CH) individuals in the next generation (n_{t+1}) can be calculated as $n_{t+1} = An_t$, where A represents the annual projection matrix with its four matrix elements, i.e., CL seed to CL seed (a_{11}), CL seed to

CH seed (a_{21}), CH seed to CL seed (a_{12}), and CH seed to CH seed (a_{22}). (B.) Periodic life cycle graph and projection matrix model of *I. capensis* incorporating mating system. The complete population cycle consists of 3 phases (m). The population projection matrices B(h) (where h = 1,2,…m) describe within-year variation in vital rates (b_{hij}). To describe the population dynamics over the entire cycle, the matrix product A is calculated by taking the product of all the B matrices (i.e., A = B(3)*B(2)*B(1)). Vital rates include CL germination (g_{CL}), CH germination (g_{CH}), CL survival (p_{CL}), CH survival (p_{CH}), CL adult CL fecundity ($f_{CL\text{-}CL}$), CL adult CH fecundity ($f_{CL\text{-}CH}$), CH adult CL fecundity ($f_{CH\text{-}CL}$), and CH adult CH fecundity ($f_{CH\text{-}CH}$).

A.

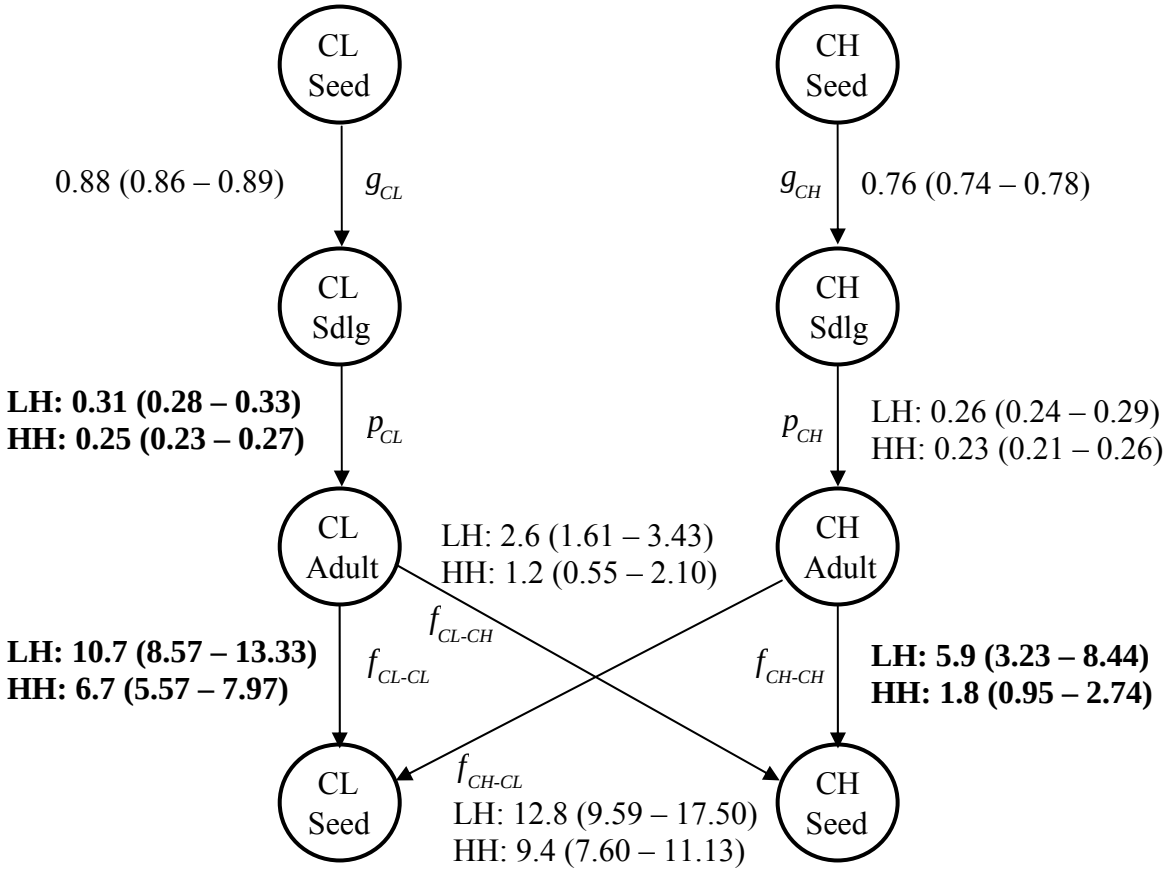


Figure 5.2 Vital rates (95% bootstrap confidence intervals) for periodic matrix model of two *Impatiens capensis* populations, L (A.) and W (B.). Vital rates under high (HH) and low (LH) herbivory are differentiated and those shown in bold were significantly different between herbivory levels. Refer to text and Figure 5.1B for description of periodic matrix model.

B.

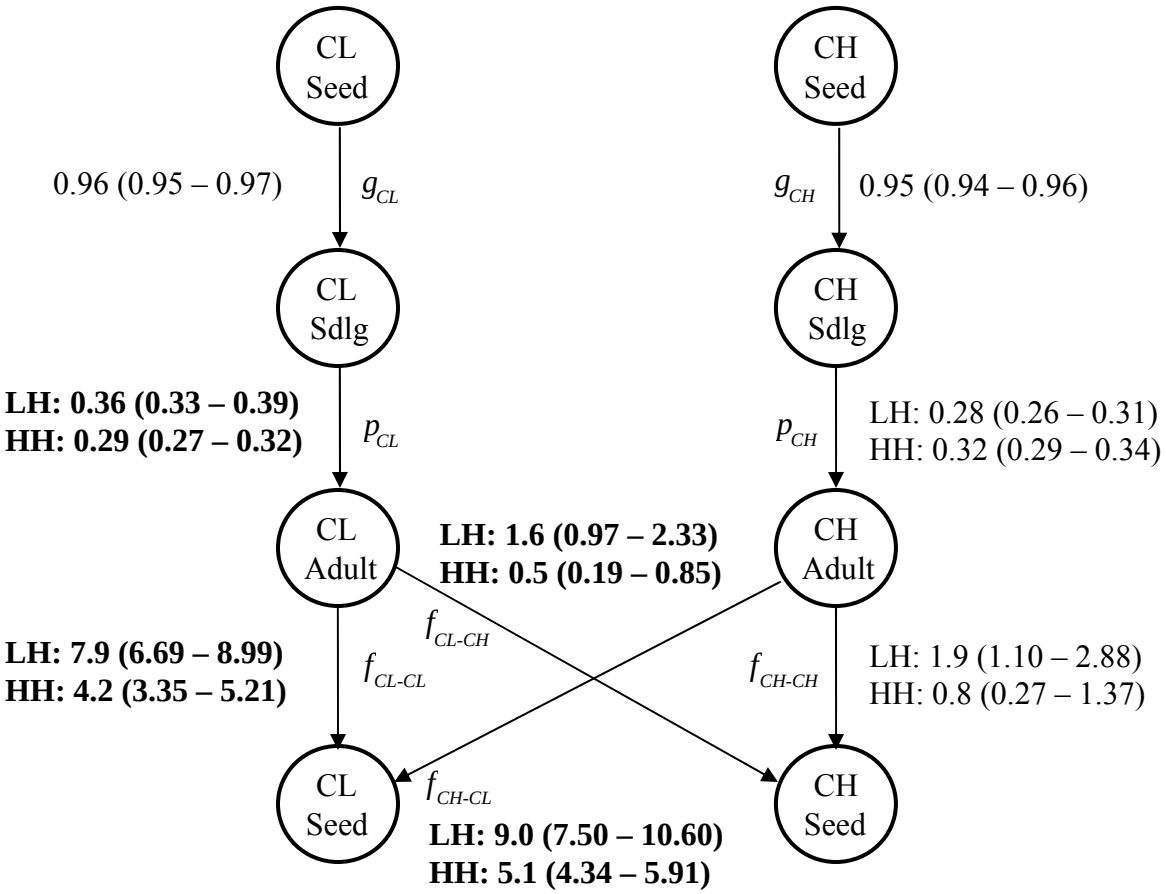


Figure 5.2, continued.

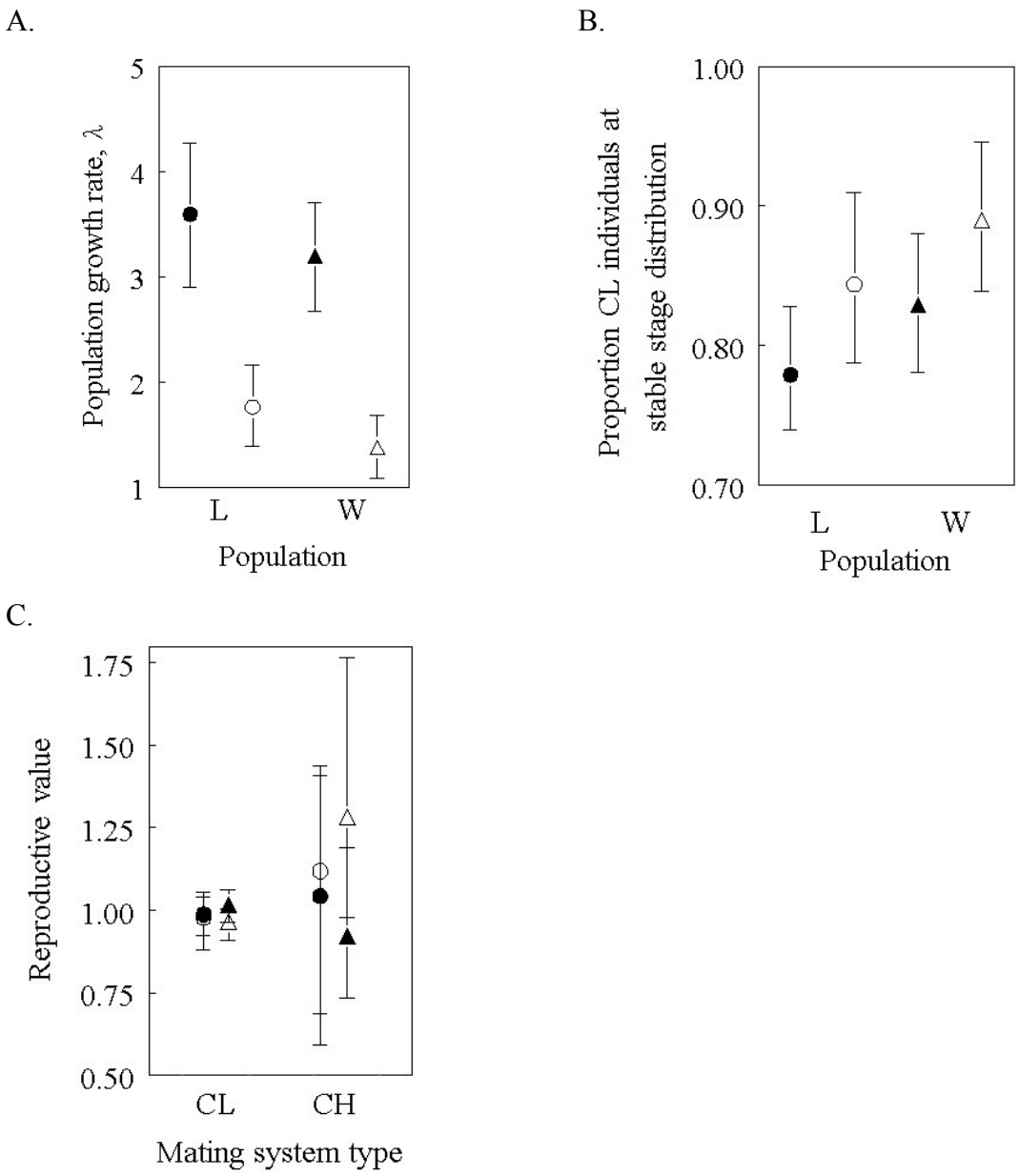


Figure 5.3 Effects of mating system and herbivory on population projections. Population growth rate (A.), proportion of cleistogamous individuals at stable stage distribution (B.), and reproductive value of cleistogamous (CL) and chasmogamous (CH) individuals (C.) for two *Impatiens capensis* populations (L, circles and W, triangles) experiencing high (open symbols) or low (closed symbols) herbivory. 95% bootstrap confidence intervals are displayed.

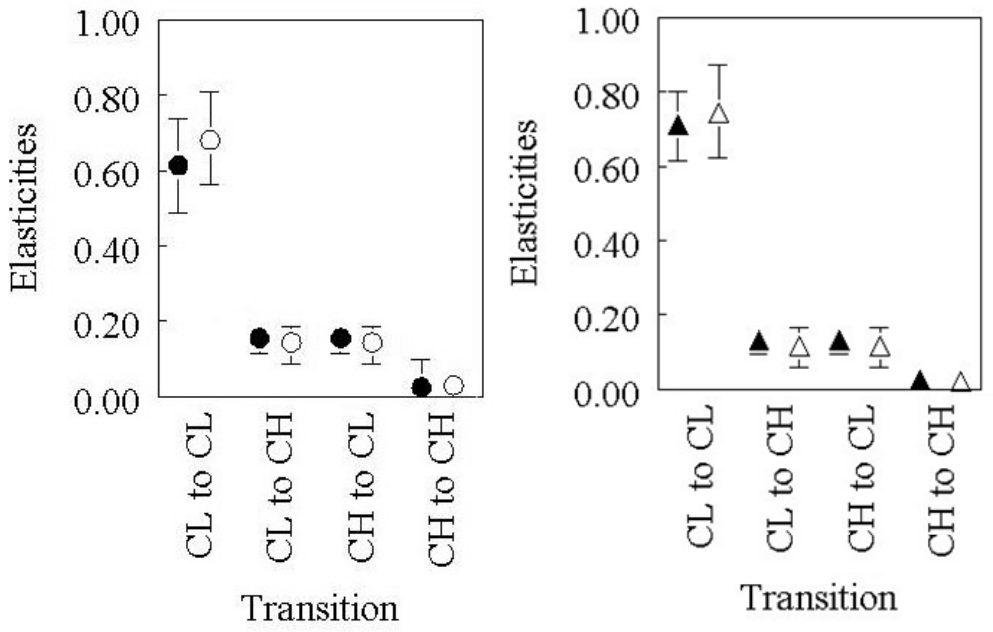


Figure 5.4 Elasticities of cleistogamous (CL) and chasmogamous (CH) transition rates for the annual matrix model of L (A.) and W (B.) *Impatiens capensis* populations experiencing high (open symbols) or low herbivory (closed symbols). Error bars represent 95% bootstrap confidence intervals. Refer to text and Figure 5.1A for annual matrix model description.

6. CONCLUSIONS AND FUTURE DIRECTIONS

This dissertation adds significantly to our current understanding of mating systems by exploring how two ubiquitous ecological factors limiting plant fitness, vegetative herbivory and intraspecific competition, influence the mating system of *Impatiens capensis*. Further, I extend our knowledge of the consequences of mating system variation to show that mixed mating has demographic consequences, and vegetative herbivory can alter population dynamics due to its effects on mating system. In total, these experiments offer important insight to the ecological factors that cause variation in mating system as well as the long-term consequences of variation in mating patterns. In addition, they bring to light the need for ecological context to be incorporated into models of mating system evolution. Below I provide a synopsis of the major findings of each empirical chapter and then discuss future directions of research.

In a preliminary investigation, I identified multiple avenues by which vegetative herbivory (hereafter, herbivory) may affect mating system expression of *I. capensis* (Chapter 2). I found strong evidence that herbivory shifts the mating system towards selfing by increasing the proportional production of selfing (cleistogamous, CL) flowers. Herbivory also reduced the biomass of CL progeny more severely relative to that of progeny derived from facultatively outcrossing (chasmogamous, CH) flowers, indicating that herbivory can have transgenerational consequences that may affect the realized mating system of the population. I also found that herbivory affected traits related to the mating system of the CH flowers. In particular, leaf damage reduced CH flower size and pollinator visitation. Overall, these findings revealed

several pathways by which herbivory may affect mating system of *I. capensis*, and in this way, this experiment served as the stimulus for the subsequent studies I conducted on this system.

As a step towards understanding how community context shapes mating system evolution, I expanded the preliminary investigation to explore how two antagonisms, herbivory and intraspecific competition, affect mating system expression of *I. capensis* (Chapter 3). I found that the combined antagonisms had additive effects for plant growth, weaker than additive effects for mating system expression and CH reproduction, and synergistic effects for CH flower size and CL flower production. These results demonstrate that reproductive and mating system traits respond differently to antagonists than growth traits, and thus the mating system response could not be accurately predicted from plant growth response. Further, these results show that competitive interactions between plants influence the effect of herbivory on components of fitness and mating system, and thus antagonisms may have unforeseen consequences for mating system evolution, population genetic diversity and persistence.

In chapter 4, I extend the work from earlier chapters to understand the effect of herbivory for relative heteromorphic flower production, CH outcrossing and whole-plant outcrossing. Further, I elucidate the mechanisms of herbivory-induced change in mating system. As in earlier studies (Chapters 2 and 3), I found that herbivory increased selfing via an increase in proportional CL reproduction. This change in mating system was due to effects of herbivory on plant size and floral meristem fate. In addition, herbivory reduced CH flowering display and pollinator visitation rate and altered the composition of the pollinator fauna, which decreased selfing among CH flowers. Overall, I found that herbivory caused a slight decrease in whole-plant outcrossing. These findings are the first to unravel the mechanisms underlying herbivore-

mediated changes in mating system. In addition, they point to the role of natural enemies in contributing to the maintenance of a mixed mating system.

Finally, I extended the work from preceding chapters to examine the population-level consequences of mixed mating and explore the interactive effects of vegetative herbivory and mating system for the population dynamics of *I. capensis* (Chapter 5). I found that mating system types differed in ways that affect the population. Selfed (i.e., CL) individuals had higher rates of germination and survival and lower rates of fecundity than did their outcrossed counterparts (i.e., CH individuals). In addition, population growth rate was most sensitive to changes in vital rates of CL individuals. I also found that herbivory had demographic consequences as this antagonism significantly reduced population growth rate due to its effect on vital rates of selfed individuals. This study adds breadth to our understanding plant mating systems by demonstrating that mixed mating also has demographic consequences.

Given the prevalence of mixed mating systems among plants (Goodwillie et al. 2005), a general mechanism explaining the evolution and maintenance of this condition is needed. This dissertation reveals the importance of ecological factors, and in particular herbivory, in the expression and evolution of mixed mating systems. Given that herbivore pressure varies both spatially and temporally (Louda 1989, Rand 2002), heterogeneity in this antagonism may select for and maintain mixed mating. In particular, two of my findings support this claim when considered in light of contemporary theory. First, I found that the decrease in whole-plant outcrossing with herbivory depended upon the relative effect of herbivory on proportional CH reproduction versus CH outcrossing rate (Chapter 4). Given the ubiquity of herbivory and its effect on both plant resources (reviewed in Crawley 1989) and pollination environment (e.g., Strauss et al. 1996, Steets and Ashman 2004) this antagonism may drive the heterogeneity or

stochasticity in resource and pollination environments that is predicted to select for mixed mating (e.g., Schoen and Lloyd 1984, Iwasa 1990, Schoen et al. 1996). Second, my findings support that herbivory increases inbreeding depression, a key element in models of mating system evolution (Chapter 5). If herbivory is variable (Louda 1989, Rand 2002), this will likely lead to variation in inbreeding depression, which can select for stable intermediate rates of selfing (Cheptou and Mathias 2001). Overall, my findings in conjunction with the work of others (e.g., Levri and Real 1998, Carr and Eubanks 2002, Elle and Hare 2002, Hayes et al. 2004, Ivey et al. 2004, Stephenson et al. 2004, Ivey and Carr 2005) clearly indicates the need for ecological context to be incorporated into models of mating system evolution as well as the need for empirical studies to explicitly test the model predictions.

The findings presented in this dissertation also bring to light new areas in need of empirical investigation. First, in all of my studies I found that herbivory increased the proportional production of CL flowers. Further work is needed to determine whether changes in this mating system trait with herbivory is adaptive and whether heterogeneity in the herbivore environment maintains it. However, before this can be evaluated we must first determine if this trait or the plasticity in it is heritable. Second, I found evidence for mating system-dependent transgenerational effects of herbivory (i.e., herbivory more greatly reduces the quality of selfed relative to outcrossed progeny, Chapter 2). Given that mating system types differentially contribute to population growth (Chapter 5), future studies should examine these mating system-dependent transgenerational effects of herbivory in a demographic framework. Finally, the demographic study of *I. capensis* indicates that selfing is important for population growth in the populations and year studied. However, future studies should explore whether outcrossing contributes to population growth under different environmental conditions. For example, a study

examining the demographic differences between mating system types grown in novel and parental sites would be particularly insightful.

APPENDIX

EFFECT OF INSECTICIDE APPLICATIONS ON *IMPATIENS CAPENSIS*

I conducted an experiment to test the effects of two insecticides, Conserve™ (Dow AgroSciences LLC., Indianapolis, IN, USA) and Endeavor™ (Syngenta Crop Protection Inc., Greensboro, NC, USA), on *Impatiens capensis* growth and reproduction and pollinator visitation. I transplanted 35 *I. capensis* seedlings from the L population (41E38.6' N, 80E25.7'W) into 10 cm square pots filled with Fafard™ #4 soil (Conrad Fafard, Agawam, Massachusetts, USA) and randomly assigned them to either a water (control) or insecticide spray treatment. Plants were sprayed with a Conserve™/Endeavor™ insecticide mixture or water biweekly. To remove the potential for differential insect feeding to confound the results, I housed plants in a portable greenhouse that excluded insect herbivores at the Pymatuning Laboratory of Ecology in Linesville, Pennsylvania. I measured plant growth (height, number of branches) and flower production four times during the season. To determine if the insecticide treatment affected plant height, branch or flower production, I performed a repeated-measures analysis of variance (PROC GLM, SAS Institutes, 2001) with insecticide treatment as a fixed effect.

To determine if the insecticide applications affected pollinator visitation, I observed pollinator visitation to plants one day and more than one day (i.e., 2, 5, 6 or 9 days) following the insecticide applications. On observation days, I intermixed equal numbers of control and insecticide sprayed plants (open CH flower number equal between treatments) into arrays (6 – 14

plants/array). I recorded pollinator visitation to all open flowers in an array for 20-minutes. In a total of 12.67 h of observation, I recorded 1315 visits by bumblebees, honey bees, small solitary bees, and syrphid flies. I used log-likelihood *G* tests (Zar 1999) to determine if pollinator abundance differed between control and insecticide treated plants one day or more then one day following insecticide application.

I found that the insecticides had no effect on *I. capensis* growth or reproduction. Specifically, insecticide treatment did not affect plant height (Table A1.1), branching architecture (Table A1.1) or flower production (Table A1.1). In addition, pollinator visitation was not affected by insecticide treatment one day or several days after application (Table A1.2).

Table A1.1 Summary of *F* statistics and significance levels for repeated-measures analyses of variance on plant height (cm) and number of branches and flowers. Numerator and denominator degrees of freedom are as follows: treatment (1, 33), time (3, 99) and treatment*time (3, 99). Significance levels are denoted as follows: **** $P < 0.0001$.

Trait	Source		
	Treatment	Time	Treatment*Time
Height	0.90	0.91	0.95
No. Branches	2.00	47.09****	0.25
No. Flowers	0.01	54.35****	1.64

Table A1.2 Summary of number of pollinator visits to flowers on insecticide treated (N = 65 flowers) and control (N = 65 flowers) plants recorded during 12.67 h of observation, *G*-statistics and *P*-values. Pollinator visitation was recorded one day and more than one day following insecticide application.

Days after treatment	Number of visits		Statistics	
	Control	Insecticide treated	*G*	*P*
1	660	665	0.019	0.89
> 1	393	402	0.102	0.75

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