THE EFFECTS OF COMPETITIVE CONTEXT ON SHADE AVOIDANCE SYNDROME

EVOLUTION IN Impatiens capensis

Brechann McGoey

A thesis submitted in conformity with the requirements for the degree of
Master of Science, Graduate Department of Ecology and Evolutionary
Biology, in the University of Toronto

© Copyright by Brechann V. McGoey (2009)
THE EFFECTS OF COMPETITIVE CONTEXT ON SHADE AVOIDANCE SYNDROME EVOLUTION IN *IMPATIENS CAPENSIS*

Master of Science, 2009.

Brechann McGoe,  
Department of Ecology and Evolutionary Biology, University of Toronto

Abstract

Competition plays a fundamental role in structuring ecological communities, and is a particularly important interaction for sessile organisms such as plants (Goldberg & Fleetwood 1987; Tilman 1994). To mitigate the negative effects of competition on fitness, plants can alter their phenotypes and reproductive traits through plastic responses. For example, decreases in the red to far-red ratio of light signal the presence of competitors, inducing a suite of responses known as shade avoidance syndrome (Franklin 2008).

My thesis examines the impact of the competitive environment on reproductive output, the phenotypes *Impatiens capensis* produce and natural selection acting on shade avoidance responses. I found that heterospecific competitors affect both the phenotypes of *I. capensis*, and selection on shade avoidance traits. I also found evidence of population differentiation in hypocotyl lengths and flowering time. My thesis elucidates the influence of competition on the evolution of phenotypic plasticity in *Impatiens capensis*.
Acknowledgements

This thesis would not exist without tremendous help and encouragement from many people. First, I want to thank my supervisor, John Stinchcombe, and the whole lab. I was very fortunate to have Anna Simonsen and Brandon Campetelli as fellow graduate students. Their advice and support were invaluable. They are both excellent scientists and amazing friends. Katy Heath and Karen Samis were always generous with their time and guidance. It was fantastic to have such wonderful role models. The Stinchcombe lab includes many terrific undergraduate students and I want to thank them all for their help. Particularly, Amanda Stock, who was always patient, resourceful and enthusiastic in the field. Dr. Stinchcombe was an excellent resource and I have learned so much while in his lab. I am sincerely grateful for the many opportunities.

I was extremely fortunate to have Spencer Barrett and Locke Rowe on my committee. I truly appreciate their many insights. Many other faculty offered inspiration and assistance. I want to thank Helen Rodd and Art Weis, in particular, for generously agreeing to sit on my exam committee. Thank you to Bruce Hall and Andrew Petrie, who were very helpful with the greenhouse work. Having access to the Koffler Scientific Reserve was an amazing privilege, and the other students at Willow Ridge made field work an even more rewarding experience.

The Ecology and Evolutionary Biology department is filled with enthusiastic, brilliant, and generous graduate students. Many thanks to Alethea Wang, Rosemary Gibson, Rosalind Murray, Emma Horrigan, Laura Robson and Emily MacLeod, along with Brandon and Anna, for being the best support group a graduate student could ask for. Also, thanks to Jessamyn Manson for sharing an office and her advice.
My family has always been an incredible source of love and support. Thank you to mom and dad for encouraging me to follow my bliss. And thanks for giving me so many wonderful experiences, especially at the cottage where I had my first ecology lessons. Thank you to my siblings, Paul, Lisa and Julia for all the laughs and memories. I am so proud to be your sister. Thank you to Ali for the advice (and yam maki) and thank you to my grandma who is a truly inspiring role model. Thanks most of all to my best friend, Rafal. Your encouragement, cooking and love are all cherished.
Table of Contents

Abstract......................................................................................................................... ii
Acknowledgements....................................................................................................... iii
List of Tables.................................................................................................................. viii
List of Figures................................................................................................................. viii

CHAPTER ONE: INTRODUCTION.................................................................................... 1
Phenotypic plasticity and shade avoidance syndrome................................................. 2
Study species.................................................................................................................. 6
Experiments.................................................................................................................... 8
Field experiment.......................................................................................................... 8
Greenhouse experiment............................................................................................... 9
Synthesis........................................................................................................................ 9

CHAPTER TWO: INTERSPECIFIC COMPETITION ALTERS NATURAL
SELECTION ON SHADE AVOIDANCE PHENOTYPES IN IMPATIENS

CAPENSIS..................................................................................................................... 10
Summary......................................................................................................................... 10
Introduction.................................................................................................................... 12
Materials and methods............................................................................................... 15
Study species................................................................................................................. 15
Experimental design................................................................................................. 15
Data Analysis................................................................................................................. 18
CHAPTER THREE: POPULATION DIFFERENTIATION IN SHADE

AVOIDANCE PHENOTYPES AND PLASTICITY IN RESPONSE TO DENSITY AND R:FR GREENHOUSE TREATMENTS

Summary

Introduction

Methods

Study species and experimental design
Data Analysis........................................................................................................52

Results..................................................................................................................54

Early mortality........................................................................................................54

Light treatment.......................................................................................................54

Population differentiation, treatment effects and full-sib family variation...........55

Selection..................................................................................................................61

Discussion..............................................................................................................61

Population differentiation......................................................................................62

Density and light effects.........................................................................................64

CHAPTER FOUR: CONCLUSIONS AND SYNTHESIS........................................68

Shade avoidance traits...........................................................................................69

Reproductive output in marsh habitat.................................................................70

Selection depends on competitive context............................................................72

Additional future directions for research..............................................................73

Final thoughts.........................................................................................................75

REFERENCES CITED.............................................................................................76

APPENDIX ONE.....................................................................................................83
LIST OF TABLES

Table 1-1: Light parameters...............................................................24
Table 1-2: Mean phenotypic traits......................................................25
Table 1-3: ANCOVA results for relative fitness.................................30
Table 1-4: Directional phenotypic selection gradients........................31
Table 1-5: Stabilizing, disruptive and correlational phenotypic selection gradients...35-36
Table 1-6: Canonical analysis of γ matrices.........................................37
Table 2-1: Least square means of traits..............................................57
Table 2-2: Results of mixed model examining effects of density and light treatments....58
Table A-1: Mean phenotypic traits for experimental and observational plants.........83

LIST OF FIGURES

Figure 1-1: Mean fruit and seed production of Impatiens capensis in marsh experiment, and in natural, monoculture populations.................................................26
Figure 1-2: The effects of intraspecific and total plant density on Impatiens capensis...29
Figure 2-1: Experimental design in the greenhouse..................................51
Figure 2-2: Directional selection on means of shade avoidance traits and their associated plasticities..........................................................59
Figure 2-3: The effect of changing the frequency of the high density environment on plasticity in height..................................................60
Figure 2-4: Average hypocotyl lengths..............................................63
Figure 2-5: R:FR ratio in ambient and reduced R:FR treatments...............67
Table A-1: Heights through time for experimental and observational plants.........84
Chapter One:  

Introduction

The ecological context of a population will have significant effects on its evolutionary trajectory. As Hutchinson elegantly expressed with his 1965 book, *The Ecological Theater and the Evolutionary Play*, the ecology of a species sets the stage for its evolution. To understand the evolutionary dynamics of a population, it is crucial to understand the abiotic and biotic factors in its environment, and their effect on individual phenotypes and fitness. Species interactions are important examples of ecological factors that have significant evolutionary impacts (see Fox *et al.* for reviews *i.e.* character displacement in Schluter; predation in Abrams). For example, competition between plants for resources (*i.e.* light, water) has significant effects on individual fitness (Cipollini 2004, Grace and Tilman 1990), and, therefore, population growth (Haag *et al.* 2004), and natural selection (Weinig 2000).

Just as ecology influences the evolution of a population, the evolutionary history and adaptations of a species have important implications for contemporary ecological interactions (Johnson and Stinchcombe 2007). Ecological interactions can evoke plastic responses, which in turn, can change the magnitude and even the direction of species interactions (Callaway *et al.* 2003, Fordyce 2006). For example, adaptive phenotypic plasticity can mitigate the stress of competition and facilitate coexistence of plant species, maintaining diversity (Callaway *et al.* 2003). In this case, plasticity could be considered a stabilizing force in the ecological community (Miner *et al.* 2005).

My thesis focuses on the effects that ecological factors, specifically competitive context, have on the evolution of phenotypic plasticity. I used the annual plant species
Impatiens capensis (hereafter Impatiens) to examine the impacts that competition has on shade avoidance phenotypes and natural selection on relevant reproductive and morphological traits. To achieve these objectives, I conducted two experiments for my Master’s research. One focused on the effects of interspecific competition on shade avoidance phenotypes in natural Impatiens habitats and natural selection on relevant phenotypes. The second focused on variation among Impatiens in sensitivity to shade avoidance cues, and in phenotypes produced in different density and light treatments. Here I introduce plasticity and the shade avoidance syndrome, provide a brief description of my study organism, and outline my two experiments.

**Phenotypic plasticity and shade avoidance syndrome**

Phenotypic plasticity is the ability of an individual with a given genotype to adjust its phenotype based on environmental variables. Plasticity has been studied for decades and is a ubiquitous phenomenon among organisms (Bradshaw 1965; Sultan 2000; Miner et al. 2005). Pertinent to both the studies of evolution and ecology, plasticity has implications for behavior (Agrawal 2001) and life history (Gotthard and Nylin 1995; Nussey et al. 2007), as well as effects on population dynamics (Fordyce 2006), community interactions (Agrawal 2001) and even speciation (West-Eberhard 1993). Plasticity is typically represented by the reaction norm, composed of phenotypes expressed over a range of environmental conditions (Weis and Gorman 1990; Via et al. 1995), where the elevation is the phenotype in the average environment, and the slope represents plasticity (Nussey et al. 2007).
While it is established that plasticity is a common feature of many species, it is a separate question if plasticity in a specific trait is an adaptation to a given environment (Bradshaw 1965; Cook and Johnson 1968). Plasticity is commonly assumed to be adaptive, but it is necessary to test this hypothesis empirically across a range of ecological settings and contexts (Sultan 2000). A positive impact of plasticity is contingent on the existence of a reliable cue about the relevant environmental parameters. In addition, the consequences of plasticity depend on the environmental context of a population (Givnish 2002; Huber et al. 2004). Plasticity is adaptive if it increases an individual’s fitness relative to non-plastic individuals (Dudley and Schmitt 1996; Schmitt et al. 2003). In an empirical study, there would be evidence of adaptive plasticity if selection on a trait moves in the same direction as plasticity adjusts the trait (Dorn et al. 2000; Bell and Galloway 2008).

Theoretical work has identified a number of factors that can influence the evolution of phenotypic plasticity. Absolute constraints caused by a lack of genetic variation could prevent plasticity from evolving (Via and Lande 1985; Van Tienderen 1991; Dewitt et al. 1998). Likewise, costs of plasticity are expected to slow or prevent populations from attaining their optimal equilibrium (Via and Lande 1985). Dewitt et al. (1998) discuss a number of possible costs and limits of plasticity including maintenance, production, genetic and information acquisition costs. Despite the theoretical support for the importance of costs, empirical evidence for them is scarce (Huber et al. 2004; Van Kleunen and Fischer 2007). Since plasticity is usually studied in one or a few traits at a time, perhaps correlations with other traits that limit plasticity are missed (Valladares et al. 2007). Another explanation for the lack of evidence of costs is that genotypes in
which plasticity is very costly are strongly selected against and so are eliminated from a population (Van Kleunen and Fischer 2007). In addition, most studies focus on costs and limits internal to the individual, but the external factors in the surrounding environment could also explain why organisms are not perfectly plastic (Valladares et al. 2007). For example, individuals are usually involved in many different interactions and may need to respond to multiple cues simultaneously (Valladares et al. 2007).

Theoretical models also demonstrate the importance of the relative frequency of environments for the evolution of phenotypic plasticity (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992). In addition, the strength of selection in different environments is important, and even a rare context can affect the evolution of plasticity depending on the relative average fitness of different habitats (Via and Lande 1985; Donohue et al. 2000a). Differentiation in plasticity among populations is hypothesized to be the result of environment dependent selection leading to adaptive divergence (Donohue et al. 2000a).

As a group, plants are highly plastic and have reaction norms for several ecologically important traits (Sultan 2000; Callaway et al. 2003). Plasticity may be particularly important for plants since they are sessile, and do not have the ability to react with behavioral modification or habitat choice (Schlichting 1986; Donohue 2003). Phenotypic plasticity is generally thought of as leading to phenotypes that better suit their surroundings, and thus allowing plants to maximize their fitness in a range of variable environments (Dudley and Schmitt 1996; Agrawal 2001). For example, when grown in high-density, plants exhibit a suite of plastic responses collectively known as “shade avoidance syndrome” (Dudley and Schmitt 1996; Franklin 2008). Plants use reductions in
the red to far-red ratio (R:FR) of incident light to sense the presence of competitors (Dudley and Schmitt 1996). Their phenotypic response can include elongation of stems and leaves, reorientation of leaves and accelerated reproduction (Donohue et al. 2000a; Franklin 2008).

Despite the volume of empirical work focused on plasticity, the connections between theoretical predictions and observational studies are often weak (Weis and Gorman 1990, Scheiner 2002). However, a number of experimental papers on shade avoidance syndrome have supported theoretical predictions and elucidated the significance of plasticity in the natural environment. There is evidence of adaptive divergence between populations growing in different habitats (Donohue et al. 2000a,b; Donohue et al. 2001; Bell and Galloway 2008). However, von Wettberg et al. (2007) point out that gene flow, low genetic variation, correlations between traits, and genetic drift can all limit the level of local adaptation in plasticity (just as in any other trait). Studies also support the idea that heterogeneous selection in various contexts is important for the evolution of plasticity (Dorn et al. 2000). Work by Sultan (2000, 2003) has suggested that the timing of plastic responses such as elongation is critical.

Several past studies examining *L. capensis* have supported the adaptive plasticity hypothesis for shade avoidance. Dudley and Schmitt (1996) used the R:FR cue to manipulate elongation in jewelweeds. Half of the study plants were elongated and in the other half elongation was suppressed (Dudley and Schmitt 1996), and plants were then transplanted into either high or low density. Using cumulative reproduction as an indicator of fitness, Dudley and Schmitt compared the success of elongated and suppressed plants in the two density environments. Elongation was found to be adaptive
in high-density but not in low-density plots. Their results also suggested an intrinsic cost to elongation (Dudley and Schmitt 1996). In a later study, Donohue et al. (2000a) examined two populations of jewelweeds, one from a wooded area and one from an open-canopy site. They found that plasticity was adaptive in the clearing site, where R:FR was a reliable cue of other jewelweed competitors (Donohue et al. 2000a). Lines from the woodland site were less plastic, and adaptive differentiation between the populations was found (Donohue et al. 2000a).

The optimal levels of plasticity for shade avoidance traits depend on the variability of the R:FR ratio, and the fitness consequences of responses such as elongation for a given population. Since plants are sessile, even small-scale variation in light conditions caused by competitors are relevant for fitness and natural selection (Linhart and Grant 1996; Huber et al. 2004).

Past studies on *Impatiens* have shown that the shade avoidance response is only adaptive when the R:FR cue is a reliable signal of competition (Donohue et al. 2000a). A low R:FR ratio can indicate dense intraspecific competition, in which case, elongation is an adaptive response. However, it could also signal a canopy of trees, or the presence of dominant heterospecific competitors. In these cases, elongation may not be an effective response, or the best time to elongate may differ. In addition, it may be more advantageous to accelerate reproduction in these conditions.

**Study species**

*Impatiens capensis* (Balsaminaceae), commonly called jewelweed, is a native North American annual found in forested areas and wetlands (Kelly 1997; Huber et al.)
2004). Jewelweed has a mixed mating system and produces mostly self-fertilizing cleistogamous flowers (Schmitt 1993; Kelly 1997). Larger individuals also produce chasmogamous flowers, which are showy and highly out-crossing (Schmitt 1993; Kelly 1997). Chasmogamous flowers are protandrous, but can be fertilized by other flowers on the same plant, though chasmogamous selfing rates are estimated to be lower than 10% (Waller 1984; Schmitt et al. 1985). Cleistogamous reproduction is less costly for *I. capensis* plants (Schemske 1978). Fewer chasmogamous flowers are produced in stressful environments (Steets et al. 2006) and, as light intensity decreases, so does the proportion of chasmogamous flowers (Schemske 1978). *Impatiens* plants reproduce in non-overlapping generations and do not have a persistent seed bank (Antlfinger 1989; Kelly 1997; Steets et al. 2007).

*Impatiens capensis* grows in heterogeneous light environments (Schmitt 1993), in monocultures and with other plant species. Seeds typically disperse ballistically 1.5m away from their parental plants (Kelly 1997; Schmitt et al. 1985). Since *I. capensis* seeds float, there is the possibility of secondary dispersal mediated through flooding or animals (Schmitt et al. 1985; von Wettberg et al. 2007). Pollen disperses further than seeds; nonetheless, past studies have found a low level of gene flow between *I. capensis* populations (Knight and Waller 1987; Kelly 1997; but see von Wettberg et al. 2007). *Impatiens capensis* have shallow roots and may be prone to drought stress (Huber et al. 2004; Heschel and Hausmann 2001). Common *I. capensis* herbivores include chrysomelid beetles, leaf miners, katydids and grasshoppers (Steets et al. 2007; Stock and McGoey, pers. Observation).
Experiments

To examine the effect of competitive context on the *Impatiens* shade avoidance syndrome, I conducted two experiments. My field experiment (Chapter 2) focused on phenotypic differences and selection on shade avoidance traits, while my greenhouse experiment (Chapter 3) examined the differences in plasticity to light and density cues of different *Impatiens* populations. Together they provide valuable insights on the impact of the competitive environment on average fitness, the phenotypes *Impatiens* produce and natural selection acting on shade avoidance responses.

Chapter 2: Field Experiment

The goal of this experiment was to determine the impact of interspecific competition on the phenotypes *Impatiens* produce and the patterns of selection on shade avoidance traits. I was also interested in determining if *Impatiens* did relatively poorly in the marsh, making marsh populations sinks for seeds from other habitats. Specifically, I asked the following questions: (1) Do average heights, internode lengths and numbers, reproductive timing, and cleistogamous and chasmogamous flower numbers differ among competitive settings?, (2) Does the per capita reproductive output of plants in a marsh suggest that this habitat is a net population sink? and (3) Do the patterns of selection on shade avoidance traits differ depending on the presence or absence of interspecific competition? I found that light parameters, phenotypes, fitness and selection all differed depending on the presence or absence of heterospecifics, suggesting that competitive context is important for the evolution of phenotypic plasticity. Chapter 2 has been published in *New Phytologist* (McGoey and Stinchcombe 2009).
Chapter 3: Greenhouse Experiment

My greenhouse experiment was focused on plasticity differences in elongation and flowering time in *Impatiens* populations from different competitive habitats. The phenotypic differences seen in the field experiment were presumably caused by plastic responses to light cues. Depending on the variability of R:FR, and the fitness consequences of responses such as elongation, various *Impatiens* habitats would have different optimal levels of both the elevation and slope of their reaction norms for shade avoidance traits. To determine if populations differ in their plasticity and average trait values, I tested the response of plants (descended from different populations) to light quality (R:FR) and intraspecific density. Plastic responses are not necessarily adaptive (Cook and Johnson 1968), and with this experiment, I was also able to examine the effect of both plasticity and shade avoidance trait phenotypes on fitness. I found that the two populations differed in their hypocotyl lengths and flowering times. Both phenotypes and selection on shade avoidance traits were treatment dependent.

Chapter Four: Synthesis and General Discussion

In Chapter 4, I provide an overview of what the two preceding chapters have contributed to our understanding of the evolution of phenotypic plasticity. I review the main conclusions of my thesis and suggest directions for future research.
Chapter Two:

Interspecific competition alters natural selection on shade avoidance phenotypes in *Impatiens capensis*.

*This chapter has been published in New Phytologist (McGoey BV and Stinchcombe JR. 2009. Interspecific competition alters natural selection on shade avoidance phenotypes in *Impatiens capensis*. New Phytologist 183: 880–891). The work described below is the product of collaborative work with John Stinchcombe on writing and analysis, and benefitted from field technical assistance from Amanda Stock.*

Summary

Shade avoidance syndrome is a known adaptive response for *Impatiens capensis* growing in dense intraspecific competition. However, *Impatiens capensis* also grow with dominant interspecific competitors in marshes. Here, I compare the *I. capensis* shade avoidance phenotypes produced in the absence and presence of heterospecific competitors, as well as selection on those traits.

I established two treatments in a marsh; in one treatment all heterospecifics were removed, while in the other, all competitors remained. I compared morphological traits, light parameters, seed output and, using phenotypic selection analysis, I examined directional and non-linear selection operating in the different competitive treatments.

Average phenotypes, light parameters and seed production all varied depending on competitive treatment. Phenotypic selection analyses revealed different directional, disruptive, stabilizing and correlational selection. The disparities seen in both phenotypes and selection between the treatments related to the important differences in elongation
timing depending on the presence of heterospecifics, although environmental covariances between traits and fitness could also contribute.

Phenotypes produced by *Impatiens capensis* depend on their competitive environment, and differing selection on shade avoidance traits between competitive environments could indirectly select for increased plasticity given gene flow between populations in different competitive contexts.
Introduction

Competition plays a fundamental role in structuring ecological communities, and is especially important for plants because of their sessile nature (Goldberg & Fleetwood 1987; Tilman 1994). Competitive interactions between plants often have significant effects on individual fitness (Cipollini 2004; Grace & Tilman 1990), population growth (Haag et al. 2004), community structure and dynamics (Goldberg & Barton 1992; Gurevitch et al. 2006) and natural selection (Weinig 2000). The outcome of competition for light between plants is typically determined by their relative sizes, with larger plants having the advantage (Zobel 1992). Phenotypically plastic changes in size and phenology in response to competitors have been hypothesized as mechanisms that diminish the negative effects of competition, and potentially facilitate the coexistence of competing species (Callaway et al. 2003).

Phenotypic plasticity, the ability of an individual to adjust its phenotype based on environmental variables, has been studied for decades and is a ubiquitous phenomenon among species (Bradshaw 1965; Sultan 2000; Miner et al. 2005). For plants, plastic responses to light cues are particularly important (Neff et al. 2000; Donohue 2003; Gurevitch et al. 2006). Plants sense competitors through decreases in the red to far red ratio (R:FR) of light (see Schmitt et al. 2003 & Franklin 2008 for reviews). This reliable, early warning of competition induces a suite of plastic responses in many plant species known as the “shade avoidance syndrome” (Dudley & Schmitt 1996; Franklin 2008). This includes stem elongation (e.g., elongated hypocotyls and internodes), and if shading by other vegetation continues, accelerated reproduction (Donohue et al. 2001; Franklin 2008).
Several past studies examining *Impatiens capensis* suggest that the shade avoidance syndrome is adaptive (Dudley and Schmitt; Donohue *et al.* 2000a; Huber *et al.* 2004). For instance, Dudley and Schmitt (1996) found that plants that elongated in response to R:FR cues had higher fitness in high density environments but not low density environments. In a later study, Donohue *et al.* (2000a) examined two populations of *I. capensis*, one from a wooded area and one from a clearing site. They found that plasticity was adaptive in the clearing site, where R:FR was a reliable cue of conspecific competitors (Donohue *et al.* 2000a). Lines from the woodland site were less plastic, and there was adaptive differentiation between the populations (Donohue *et al.* 2000a).

Past studies of the shade avoidance syndrome in *I. capensis* have only examined the effects of varying intraspecific competition. However, in addition to growing in dense monocultures in which it dominates the understory and herbaceous layer (Winsor 1983), *I. capensis* also frequently grows with much larger competitor species in marshes and wetlands. These interspecific competitors have diverse resource requirements, growth rates, and flowering times (Firbank and Watkinson 1990), are frequently perennial, and often surpass the maximum height of *I. capensis*, suggesting that the typical *I. capensis* shade avoidance phenotype seen in monocultures is unlikely to be effective. Thus, the presence of heterospecifics may alter natural selection on shade avoidance phenotypes. One approach to testing this hypothesis is to compare the strength and mode of natural selection (Lande and Arnold 1983) between environments with and without heterospecifics.

The habitats where *I. capensis* grow are extremely variable: interspecific competition, intraspecific density and canopy cover all differ among patches. Such spatial
heterogeneity is expected to favour plasticity (van Tienderen 1991). If marsh populations are plastic, the shade avoidance syndrome could allow them to attain a more adaptive phenotype for their competitive surroundings. As suggested by Callaway et al. (2003), plastic responses could mitigate the negative effects of competition and allow *I. capensis* to survive in both dense monoculture and marsh habitats. However, it remains unknown whether marsh and wetland environments are net population sinks, and thus contribute little to the evolution of plasticity in *I. capensis* —theoretical models note that the scale of population regulation and whether populations have above or below average fitness can dramatically affect the evolution of plasticity (Via & Lande 1985; Gomulkiewicz & Kirkpatrick 1992). Marsh habitats may be a sink because *I. capensis* are found in them at much lower densities than in monoculture patches (McGoey and Stinchcombe, pers. obs., see below). If marshes with interspecific competitors are inherently poor or stressful habitats that necessarily lead to low fitness, one would predict plants growing in the marsh habitat would have lower per capita reproductive output than plants in monoculture populations.

To examine the effects of interspecific competition on the evolution of shade avoidance phenotypes, I established experimental blocks in a marsh where plants faced intense interspecific competition and removed heterospecifics from half of each block. From this experiment, I sought to answer the following questions: (1) How does the removal of heterospecific competitors affect shade avoidance phenotypes such as height, total number and lengths of internodes, reproductive timing, and the number of cleistogamous and chasmogamous flowers? (2) Does the per capita reproductive output of plants in a marsh suggest that this habitat is a net population sink? and (3) Does natural
selection on these phenotypes differ depending on the presence or absence of heterospecific competitors?

Materials and Methods

Study Species

*Impatiens capensis* is a native North American annual commonly found in forested areas and wetlands (Schemske 1978; Kelly 1997; Huber et al. 2004; Steets et al. 2006, 2007). *I. capensis* has a mixed mating system (Waller 1980): individuals produce self-fertilizing cleistogamous flowers, and if large enough, orange, showy, outcrossing, chasmogamous flowers. *I. capensis* plants grow in heterogeneous light environments (Schmitt 1993), in both monocultures and in diverse marsh communities. Seeds typically disperse ballistically 1.5m away from parental plants (Schmitt et al. 1985; Kelly 1997), although genetic marker data show evidence for longer distance gene flow, presumably due to secondary water dispersal (von Wettberg et al. 2008). *I. capensis* can reach heights of approximately 2m (Schmitt et al. 1985; Stinchcombe & McGoy pers obs) and competitive hierarchies are established early in the growing season. I studied natural populations of *I. capensis* at the Koffler Scientific Reserve (www.ksr.utoronto.ca; 44° 03' N, 79° 29' W), north of Toronto, Ontario, Canada.

Experimental Design

I used a manipulative field experiment to study how interspecific competition affected shade avoidance traits in the marsh environment where *I. capensis* naturally occurs with a diverse group of heterospecifics; dense monocultures of *I. capensis* are not found in this habitat.
I randomly selected patches for twenty-one blocks throughout a large marsh where *I. capensis* grow with several heterospecifics. The competitors included cattails (*Typha latifolia*), marsh marigold (*Caltha palustris*), stinging nettle (*Urtica dioica*), horsetails (*Equisetum fluviatile*) and a diverse group of unidentified grasses. Each 1 m x 0.5 m block was split into two plots, with each plot randomly assigned to either the removal or interspecific competition treatment. Above-ground interspecific competition was eliminated from removal treatment on a weekly basis with clipping shears. Because the interspecific competition and removal plots are immediately adjacent to each other, and surrounded by the rest of the marsh, my experimental design is likely to be conservative in its ability to detect effects of interspecific competition. Interspecific competitors, plant density, and moisture levels all naturally varied across blocks. Intraspecific density ranged from 20 to 160 plants/m², and heterospecific density ranged from 70 to 200 plants/m².

I tagged *I. capensis* plants in May before they grew true leaves and tracked individuals over the season to assess height, growth rate, flowering time and type, mortality and reproductive success. I measured plant height once a month throughout the five-month growing season, and in late August (approx. 120 days after the start of the experiment), I measured the total number of internodes and internode lengths. To estimate light parameters that affect growth and elongation, at each census I used a Field Scout Red/Far Red Meter and a Field Scout Quantum Meter (both Spectrum Technologies Inc., Plainfield, IL, USA). These measurements gave an indication of light quality (the red:far red ratio of light) and light quantity (total photosynthetic active radiation) for each of the plots. I measured both PAR (photosynthetically active
radiation) and R:FR four times in each plot just above and below the top of the *I. capensis* canopy.

Starting in early July, I censused plants twice a week for flowers to assess the date of first flowering. I tracked mortality over the summer as the percentage of plants that survived between height measurements. I estimated fitness as the total number of seeds produced over a plant’s lifetime. To do this, I marked flowers as either cleistogamous or chasmogamous when they emerged, and counted both types of fruits biweekly throughout August and September to estimate the total number of cleistogamous and chasmogamous fruits per plant. Fruit number was converted to seed number by multiplying these values by the average number of seeds per fruit type, as determined from a sample of 40 cleistogamous and chasmogamous fruits. Plants that died prior to reproduction were assigned a fitness estimate of zero. Importantly, my estimates do not include any possible effects of seed quality such as size, or maternal effects, which could affect the realized fitness of *Impatiens capensis* (McCall *et al.* 1991).

To qualitatively evaluate whether the marsh is poor habitat for *I. capensis* that leads to poor reproductive performance, I compared per-capita reproductive output of plants in the marsh to those of plants in four monoculture, non-marsh populations. For these four populations, I tracked survivorship, flowering, and fruit production as described above for 200 individuals in each population. These four monoculture populations had initial densities ranging from 154 to 350 plants/m² (*i.e.*, at or on the higher end of the marsh densities) and are in sites where *I. capensis* is a habitat dominant (sensu Winsor 1983) in the understory from year to year (J. Stinchcombe, unpub. data). Although drought stress can impose selection on *I. capensis* (Huber *et al.* 2004; Heschel
and Riginos 2005), I did not observe leaf wilting or signs of drought stress in monoculture blocks during the summer.

**Data Analysis**

*Light Measurements*

I used repeated measures ANOVAs to evaluate changes in light quality (R:FR) and quantity (PAR) over the season. Analyses were conducted for both R:FR and PAR measurements taken above and below the canopy of *Impatiens capensis*, for a total of four repeated measures ANOVAs. Each included treatment and time as factors repeated for each block at each census.

*Phenotypic Comparisons between Marsh Treatments*

To assess the effects of treatment and block on height over time, I used a repeated measures ANOVA. In these models, I interpreted an effect of treatment as indicating that vertical position of height profiles differed between the removal and control treatments, while a treatment * time interaction as indicating that treatment effects were variable over time.

For traits for which I had single measurements, I used ANOVA to compare removal and interspecific competition plants. I tested for differences between treatments in the total numbers of chasmogamous and cleistogamous fruits, days to first flower, internode lengths, hypocotyl lengths, and total seed number (my fitness estimate). Because the removal treatment was applied to half a block at a time, rather than individual plants, I tested the treatment effect in my ANOVAs over the Block*Treatment interaction to obtain proper F-statistics for hypothesis testing.
I do not present results for block or block*treatment interactions because: (1) Testing for and generalizing about spatial variation in traits and fitness was not one of my primary goals, and (2) Preliminary inspection of block*treatment interactions revealed that they were universally driven by changes in the magnitude of the treatment effect, rather than reversals in its direction.

Fitness effects and natural selection

Abiotic and biotic factors could have direct effects on the fitness of plants in each plot. To evaluate this, I tested the correlation between light parameters (R:FR and PAR), and total plant density with fitness for each experimental treatment. For these analyses, I used mean fitness of each plot, as the light measures were plot-level rather than individual characteristics.

Phenotypic selection analysis was used to determine which traits were important for fitness in the two treatments. To test the impact of *I. capensis* traits on fitness, as well as any differences in their fitness effects between treatments, I conducted both an ANCOVA and phenotypic selection analyses. In these analyses, the relative fitness was estimated as the total number seeds produced per plant, divided by the mean seed number for all the plants in the marsh. I used mean-standardized values for traits (Hereford *et al.* 2004; Hansen & Houle 2008) to facilitate comparisons between traits with different units.

I performed an ANCOVA for relative fitness with traits that I identified a priori as likely to be under selection. The traits were: maximum height, early growth rate, late growth rate, hypocotyl length, first internode length, and days until flowering. I estimated early growth and late growth rates by calculating the difference in vertical height between the 2nd and 1st census, the 5th and 4th census, respectively, divided by the number of
elapsed days. My approach to estimating growth rate only captures how quickly vertical height changed throughout the season (i.e., cm per day), and not the biomass of these plants— which would have required destructive harvests, and altered the competitive environments— or the developmental rate of plants (which I examine below, using the total number of internodes). Treatment, block, and their interaction, as well as each trait’s interaction with treatment were included in the model. I interpret a significant effect of a trait on relative fitness as evidence for natural selection, and trait*treatment interactions as evidence that selection is affected by competitive context.

To complement the ANCOVA which utilized a priori selected traits, I also used a model-selection approach to characterize the traits that were most important for fitness in the two treatments in an unbiased, systematic manner. Models describing the important traits for relative fitness were developed separately for the interspecific competition and removal treatments. I used mean-standardized traits for all models, and statistical models were selected using Akaike’s Information Criterion (AIC). The coefficients for each trait were taken as directional selection gradients on that trait. To examine quadratic selection, models that included the traits (used in the ANCOVA), squares of the traits, and their cross-products were used. I converted quadratic regression coefficients to selection gradients (γ) by doubling them and their respective standard errors (Stinchcombe et al. 2008). To check if significant nonlinear selection represented true stabilizing selection, or if it was curvilinear selection, I examined partial regression plots and looked for an intermediate minima (cf. Mitchell-Olds and Shaw 1987). Preliminary analysis of variance inflation factors suggested that multi-collinearity did not affect partial regression
coefficients. Because of highly non-normal residuals, I calculated confidence intervals for the coefficients by bootstrapping the residuals.

*Canonical analysis of the $\gamma$ matrix*

To facilitate interpretation of the patterns of stabilizing, disruptive, and correlational selection between treatments, I performed a canonical analysis of the $\gamma$ matrix. Briefly, this approach rotates the $\gamma$ matrix to eliminate correlational selection, leaving only stabilizing/disruptive selection on synthetic traits that are linear combinations of the original traits (Phillips & Arnold 1989; Simms 1990; Blows & Brooks 2003; Stinchcombe et al. 2008). To do this, I applied PCA to the $\gamma$ matrix. The eigenvalues of this PCA are measures of non-linear (stabilizing/disruptive) selection on the synthetic traits; the eigenvectors of each PC describe the contributions of the original traits to the synthetic traits. Similar to traditional PCA, one can estimate the proportion of variation that is associated with a given canonical axis ($m_i$).

To compare the similarity of the two $\gamma$ matrices, I used the sub-space similarity method described by Blows et al. (2004). To do this, I saved the first 3 PC’s of each $\gamma$ matrix as columns in two matrices, $\mathbf{A}$ and $\mathbf{B}$, respectively. I then calculated the matrix $\mathbf{S}$, according to $\mathbf{S} = \mathbf{A}^T\mathbf{B}\mathbf{B}^T\mathbf{A}$, where $^T$ indicates matrix transposition. The eigenvalues of $\mathbf{S}$, in turn, can be used to find the similarity between the two matrices $\mathbf{A}$ and $\mathbf{B}$: the sum of the eigenvalues of $\mathbf{S}$ will range from 0 to $k$ (where $k =$ the number of PC’s entered into the columns of $\mathbf{A}$ and $\mathbf{B}$). Sums close to zero indicate near orthogonality (*i.e.*, complete dissimilarity) of the two matrices, while sums close to $k$ indicate that the two sub-spaces share similar orientations (Blows et al. 2004). I elected to use 3 PC’s of the $\gamma$ matrices because these PC’s explain >99% of the variation in the $\gamma$ matrix, and because subspace
similarity method can only utilize less than half of the PCs of a matrix (Blows et al. 2004)

Data analysis was performed with R (R Development Core Team 2005), SAS (v. 9.1.3, SAS Institute, Cary, NC, USA), and the PopTools add-in for Excel (Hood 2006), depending on ease of use for particular analyses.

**Results**

*Light Characteristics*

There were differences in light parameters in the two treatments, as well as changes through time (see Table 1-1 for May and August measurements, representing the beginning and end of the season). Both above and below the *I. capensis* canopy red:far-red ratios were significantly higher in removal versus interspecific competition plots (for both p<0.0001). In a rm-ANOVA, there were significant effects of time for the R:FR measurements both above and below the *I. capensis* canopy (p< 0.05 for both), with R:FR decreasing over the season in both treatments. For both above and below *I. capensis* canopy R:FR measurements, there were significant interactions between treatment and time, with R:FR declining more rapidly over time in the interspecific competition treatment (Table 1-1).

Total photosynthetically active radiation was also significantly lower in the interspecific competition treatment than in the removal treatment (p<0.0001) (Table 1-1). There was a significant effect of time for both above (p<0.0001) and below (p<0.0001) *I. capensis* canopy PAR measurements, with PAR declining significantly over the season, in both marsh treatments. Only the below canopy measurements showed a significant
interaction between treatment and census (p=0.014); PAR actually increased over the season below the *I. capensis* canopy in the removal treatment.

*Phenotypic Comparisons between Marsh Treatments*

Removal of marsh vegetation had significant effects on a host of plant phenotypes, including fitness. Plants in the removal treatment, compared to the interspecific competition treatment, differed significantly in several phenotypes: they were shorter early in the season (May and June), taller later in the season (August), reached taller maximum heights, had shorter first internodes, and more total internodes (Table 1-2). Several of these differences support the hypothesis that plants growing in interspecific competition elongate earlier, and the greater number of internodes suggests accelerated developmental rates. In contrast to expectations based on the shade avoidance syndrome, plants in the interspecific competition treatment flowered later than those in the removal treatment (Table 1-2).

My finding that plants in the removal treatment were shorter early in the season and taller late in the season suggests temporal shifts in growth, and two analyses support this view. First, when heights were examined over the entire season with a repeated measures ANOVA, there was a significant interaction between treatment and time (p=0.024). Second, when I tested for treatment effects on early and late growth rate (cm/day growth between the first and last two censuses, respectively), I found that plants in the removal treatments grew significantly slower early in the season (Table 1-2). Plants in the removal treatment had higher late season growth rates, but the difference was not significant (Table 1-2).
Table 1-1: Light parameters in May and August for the removal and interspecific competition blocks.

<table>
<thead>
<tr>
<th></th>
<th>Removal</th>
<th>Interspecific Competition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R:FR Above</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>1.079 ± 0.021</td>
<td>0.952 ± 0.052</td>
</tr>
<tr>
<td>August</td>
<td>0.978 ± 0.054</td>
<td>0.907 ± 0.070</td>
</tr>
<tr>
<td><strong>R:FR Below</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>0.630 ± 0.051</td>
<td>0.444 ± 0.052</td>
</tr>
<tr>
<td>August</td>
<td>0.579 ± 0.06</td>
<td>0.247 ± 0.033</td>
</tr>
<tr>
<td><strong>PAR Above</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>1554 ± 84</td>
<td>1204 ± 114</td>
</tr>
<tr>
<td>August</td>
<td>989 ± 108</td>
<td>400 ± 112</td>
</tr>
<tr>
<td><strong>PAR Below</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>158 ± 20</td>
<td>97 ± 35</td>
</tr>
<tr>
<td>August</td>
<td>426 ± 78</td>
<td>120 ±35</td>
</tr>
</tbody>
</table>
Table 1-2. Mean phenotypic traits (± 1 s.e.) for plants from removal and interspecific competition treatments, along with F-statistics for the treatment effect.

<table>
<thead>
<tr>
<th>Trait (units)</th>
<th>Removal</th>
<th>Interspecific Competition</th>
<th>F_{1, 20}</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>May Height (cm) ***</td>
<td>6.1 ± 0.091</td>
<td>8.3 ± 0.015</td>
<td>35.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>June Height (cm) ***</td>
<td>20.5 ± 0.34</td>
<td>26.8 ± 0.52</td>
<td>34.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>July Height (cm)</td>
<td>62.5 ± 1.13</td>
<td>56.7 ± 1.23</td>
<td>0.93</td>
<td>0.35</td>
</tr>
<tr>
<td>August Height (cm) **</td>
<td>82.8 ± 1.59</td>
<td>67.8 ± 1.60</td>
<td>9.82</td>
<td>0.0052</td>
</tr>
<tr>
<td>September Height (cm)</td>
<td>94.8 ± 1.52</td>
<td>90.7 ± 1.70</td>
<td>0.01</td>
<td>0.9157</td>
</tr>
<tr>
<td>Maximum Height (cm) **</td>
<td>69.9 ± 1.94</td>
<td>56.4 ± 1.73</td>
<td>8.99</td>
<td>0.0071</td>
</tr>
<tr>
<td>Flowering time (days)*</td>
<td>61.0 ± 0.2</td>
<td>61.9 ± 0.27</td>
<td>5.36</td>
<td>0.0313</td>
</tr>
<tr>
<td>Hypocotyl length (cm)</td>
<td>8.5 ± 0.17</td>
<td>8.8 ± 0.19</td>
<td>2.52</td>
<td>0.1279</td>
</tr>
<tr>
<td>First Internode length (cm) **</td>
<td>8.0 ± 0.14</td>
<td>9.4 ± 0.18</td>
<td>8.34</td>
<td>0.0091</td>
</tr>
<tr>
<td>Total number of internodes **</td>
<td>11.9 ± 0.175</td>
<td>9.8 ± 0.177</td>
<td>15.33</td>
<td>0.0009</td>
</tr>
<tr>
<td>Early growth rate (cm/day)***</td>
<td>0.47 ± 0.011</td>
<td>0.62 ± 0.012</td>
<td>23.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Late growth rate (cm/day)**</td>
<td>0.65 ± 0.037</td>
<td>0.35 ± 0.035</td>
<td>19.09</td>
<td>0.0003</td>
</tr>
<tr>
<td>Absolute fitness (seeds)**</td>
<td>43.5 ± 3.2</td>
<td>21.6 ± 2.0</td>
<td>12.14</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

* p<0.05
** p<0.01
*** p<0.001

Values in bold are significant.
Figure 1-1: Mean fruit and seed production of *Impatiens capensis* in the marsh experiment and natural, monoculture populations.

(A) Mean cleistogamous and chasmogamous fruit production (± 1 s.e.) in the two marsh treatments (removal: open bars; interspecific competition: grey bars). The difference between means is highly significant according to ANOVA (Table 1-2). For purposes of illustration, the mean fruit production (± 1 s.e.) of plants growing in the four monoculture populations. Note the separate y-axis for monoculture populations on the right, using the same scale. No formal comparison was made between monoculture and marsh treatments.

(B) Total seed production in the two marsh treatments, and four monoculture populations, as in (A).
Plants in the removal treatment also set significantly more seeds, having an absolute fitness of approximately twice that of plants in the interspecific competition treatment (Figure 1-1b). The differences in average seed number per plant between the two treatments appears to be driven by plants in the removal treatment producing significantly more of both types of fruits, rather than their relative production (chasmogamous and cleistogamous, Figure 1-1a). Per capita fruit and seed production in the marsh also exceeded that of the monoculture populations (Figure 1-1a and 1-1b). While these data do not preclude the possibility of dispersal from monocultures to the marsh, they do indicate that the marsh is not poor or stressful habitat that necessarily leads to low reproductive success.

**Fitness effects and directional natural selection**

In the interspecific competition treatment, there was a significant negative relationship between total plant density (both intraspecific and interspecific competitors) and *I. capensis* fitness \( (r=-0.472, p=0.031, \text{Fig. 1-2b}) \). There were also negative correlations between fitness and total conspecifics \( (r=-0.516, p=0.017) \), as well as fitness and total heterospecifics \( (r=-0.271, p=0.23) \), although only the former correlation was significant. In removal plots (which contain only *I. capensis*), there was a negative correlation between number of *I. capensis* and fitness (see Fig. 1-2a), but it was only marginally significant \( (r=-0.375, p=0.094) \). Light quantity (PAR) and quality (R:FR) also had significant effects on fitness. There were positive correlations between fitness and R:FR \( (r=0.462, p=0.035) \), as well as fitness and PAR \( (r=0.545, p=0.011) \) for the interspecific competition plots. For removal plots, only PAR \( (0.326, p=0.015) \) was positively correlated with fitness.
Of the traits I expected \textit{a priori} to be under selection, maximum height and early growth were significant terms in the ANCOVA (see Table 1-3). The interaction between treatment and hypocotyl length, treatment and maximum height, and treatment and late growth rate were the only interaction terms that were significant. Taken together, these data suggest early growth was under selection in the marsh experiment, but that the strength of selection did not differ across treatments. In contrast, hypocotyl length, maximum height, and late growth showed evidence of being under variable selection between the two treatments. Comparison of the ANCOVA with the model selection analyses (i.e., Tables 1-3 –vs- 1-4), suggests that the significant trait*treatment interactions were driven by traits being under significant selection in one treatment but not the other (hypocotyl and maximum height, which were not retained in the removal treatment model), or variation in the strength of selection (late growth).

Directional selection varied between treatments (Table 1-4). For example, one notable difference between the two marsh treatments was directional selection for higher late growth rates in the interspecific competition treatment but not in the removal treatment. There were similarities as well; maximum height was under significant positive directional selection in both competition treatments in the marsh. Total internode number was not a significant predictor of relative fitness in either treatment.
Figure 1-2: The effects of intraspecific and total plant density on *Impatiens capensis* fitness.

(A) The effect of intraspecific density on *I. capensis* fitness in the removal treatment. Plotted points are mean *I. capensis* density per plot and mean seed production per plot.

(B) The effects of intraspecific density (open circles, dashed line) and total density (closed circles, solid line) on *I. capensis* fitness in the interspecific competition treatment. Plotted points are mean densities per plot and mean seed production per plot.
Table 1-3: Results of ANCOVA for relative fitness. Traits were standardized to a mean of 1 prior to analysis. The overall model had 596 error degrees of freedom.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0.52</td>
<td>0.219</td>
<td>0.64</td>
</tr>
<tr>
<td>Block</td>
<td>8.46</td>
<td>3.58</td>
<td>0.059</td>
</tr>
<tr>
<td>Block x Treatment</td>
<td>0.98</td>
<td>0.41</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Maximum Height</strong>*</td>
<td>274.46</td>
<td>116.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Days until flowering</td>
<td>0.004</td>
<td>0.0017</td>
<td>0.97</td>
</tr>
<tr>
<td>Hypocotyl Length</td>
<td>8.1</td>
<td>3.43</td>
<td>0.064</td>
</tr>
<tr>
<td>First internode length</td>
<td>4.79</td>
<td>2.03</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Early growth</strong>*</td>
<td>13.76</td>
<td>5.83</td>
<td>0.016</td>
</tr>
<tr>
<td>Late growth</td>
<td>4.58</td>
<td>1.94</td>
<td>0.16</td>
</tr>
<tr>
<td>Treatment x maximum height **</td>
<td>23.27</td>
<td>9.86</td>
<td>0.0017</td>
</tr>
<tr>
<td>Treatment x days</td>
<td>0.17</td>
<td>0.078</td>
<td>0.79</td>
</tr>
<tr>
<td>Treatment x hypocotyl length *</td>
<td>12.82</td>
<td>5.43</td>
<td>0.020</td>
</tr>
<tr>
<td>Treatment x first internode length</td>
<td>0.16</td>
<td>0.066</td>
<td>0.80</td>
</tr>
<tr>
<td>Treatment x Early growth</td>
<td>6.22</td>
<td>2.64</td>
<td>0.11</td>
</tr>
<tr>
<td>Treatment x Late growth **</td>
<td>17.34</td>
<td>7.35</td>
<td>0.0069</td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01  
*** p<0.001  
Values in bold are significant
Table 1-4: Directional phenotypic selection gradients ($\beta$, 95% CL) for interspecific competition, removal treatments, as determined by model selection using AIC for each treatment. Traits were standardized to a mean of 1 prior to analysis. P-values are from standard parametric hypothesis tests, while confidence intervals are from bootstrapping the residuals of the multiple regression. Traits which are denoted as ‘NA’ were not included in the model selected by AIC.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Removal $\beta$ (95% C.L.)</th>
<th>Interspecific Competition $\beta$ (95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early growth</td>
<td>NA</td>
<td>0.04398 (-0.288, 0.438)</td>
</tr>
<tr>
<td>First Internode Length</td>
<td>-0.8605 ** (-1.578, -0.28)</td>
<td>-0.476 * (-0.869, -0.127)</td>
</tr>
<tr>
<td>Height in May</td>
<td>-0.789 * (-1.622, 0.038)</td>
<td>-0.443 * (-0.980, -0.071)</td>
</tr>
<tr>
<td>Hypocotyl</td>
<td>NA</td>
<td>0.310 (-0.0095, 0.797)</td>
</tr>
<tr>
<td>Late growth</td>
<td>NA</td>
<td>0.2023 * (0.0439, 0.396)</td>
</tr>
<tr>
<td>Maximum Height</td>
<td>2.697 *** (2.15, 3.42)</td>
<td>1.879 *** (1.39, 2.55)</td>
</tr>
<tr>
<td>Total Internodes</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01  
*** p<0.001

For values in bold, the confidence intervals do not cross zero.
Non-linear selection and Canonical Analysis of $\gamma$

In both the interspecific competition and removal treatments there was significant nonlinear selection on maximum height (Table 1-5a, 1-5b). Inspection of partial regression plots suggested that maximum height was under curvilinear selection, rather than true disruptive selection with an intermediate fitness minima. The non-linearity in this relationship suggests that there were accelerating fitness benefits of larger size—*i.e.*, plants at the top of the height distribution received more fitness benefit per unit of height than those in the middle or bottom of the height distribution. In the removal treatment, there was significant stabilizing selection on late growth, indicating that intermediate late growth rates had higher fitness than faster or slower late growth rates. For the interspecific competition treatment, the point estimate of selection on late growth was also negative, although non-significant.

I detected several significant correlational selection gradients in each treatment (Table 1-5a, 1-5b). Inspection of individual coefficients suggests that in the interspecific competition treatment, positive correlations among phenology, size, and growth traits were favored (hypocotyl length, days to flowering, maximum height, late growth), while in the removal treatment, negative correlations between growth rates (early and late) and size components (hypocotyl length and maximum height) were favored. However, visualizing correlational selection for multiple traits is difficult (Phillips and Arnold 1989; Blows 2007a), and inspection of a handful of coefficients of a matrix can be misleading (cf. Walsh 2007).

Canonical analysis of the $\gamma$ matrices for the interspecific competition and removal treatments revealed that the overall pattern of non-linear selection was disruptive. In the
removal treatment, the first axis ($m_1$) explained 98% of the variation (Table 1-6a) and indicated significant disruptive selection. Inspection of the eigenvectors of this axis revealed strong contributions from days to flower and first internode length (eigenvectors of table 1-6a). The second and third most important axes ($m_2$, $m_7$) each explained less than 1% of the variation in the curvature of the fitness surface. The $m_2$ axis appeared to be driven by opposing contributions from maximum height on the one hand, and hypocotyl length and height in May on the other hand. The $m_7$ axis, which is indicative of stabilizing selection on the synthetic trait (negative coefficient), is statistically significant but explains a low percentage of the curvature in the fitness surface.

In the interspecific competition treatment, the first axis ($m_1$) explained only 89% of the variation in the non-linear fitness surface, and also appeared to be driven strongly by days to first flowering, with additional contributions from hypocotyl length (Table 1-6b). In contrast to the removal treatment, the 2$^{nd}$ and 3$^{rd}$ most important axes ($m_2$, $m_7$) explained appreciable variation ($\approx 5\%$). The $m_2$ axis was driven by contributions from maximum height, hypocotyl length, and early growth (Table 1-6b). Similar to the removal treatment, the $m_7$ axis is subject to stabilizing selection; however, in this case it appears to reflect a larger percentage of the variation in the curvature of the fitness surface ($\approx 4.5\%$). The $m_7$ axis appears to be largely a function of positive contributions from late growth, hypocotyl length, and first internode length, and opposing contributions from height in May and days to flower.

Comparison of the traditional analysis of the $\gamma$ matrix with the canonical analysis reveals several findings. First, in the traditional analysis, only four or five of the original selection gradients were significant (out of 28 estimated for each treatment). In contrast,
in the canonical analysis, six of the seven canonical gradients are significant in each
treatment. The differences in significance are likely due to increased power as a result of
fewer parameters in the model. Comparison of the quadratic selection gradients (diagonal
of Table 1-5a, 1-5b) with the eigenvalues of Table 1-6 suggests differences in the
strength of stabilizing/disruptive selection. Using the traditional method, the median $\gamma_{ii}$
was 1.08 in the removal treatment and 0.526 in the interspecific competition treatment;
the median $\lambda_i$’s from the canonical analysis were 3.039 and 0.9, respectively. These
figures suggest that there is more curvature in relative fitness along the axes described by
the synthetic traits than along the axes described by the original traits (Blows & Brooks
evidence of selection that had not previously been present, but instead re-allotates it to
different coefficients (i.e., among 7 $\lambda_i$’s instead of among 21 $\gamma_{ij}$’s and 7 $\gamma_{ii}$’s).

The sub-space similarity method allows an estimation of the similarity between
the two estimated $\gamma$ matrices. I find that the sum of the eigenvalues of $S$ equaled 2.03 (out
of a maximum of 3). These data suggest that the two non-linear fitness surfaces, as
described by their first 3 PC’$s$, share similar, but not identical geometric orientations.
Table 1-5: Stabilizing, disruptive and correlational phenotypic selection gradients (γ, CI) for interspecific competition and removal treatments. Traits were standardized to a mean of 1 prior to analysis. P-values are from standard parametric hypothesis tests, while confidence intervals are from bootstrapping the residuals of the multiple regression.

### a) Removal Treatment

<table>
<thead>
<tr>
<th></th>
<th>Early growth</th>
<th>Late growth</th>
<th>Height in May</th>
<th>Hypocotyl Length</th>
<th>First Internode Length</th>
<th>Days to Flower</th>
<th>Maximum Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early growth</td>
<td>-0.237</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-3.44, 5.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late growth</td>
<td><strong>-1.44</strong>*</td>
<td><strong>-0.277</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-2.41, -0.313)</td>
<td>(-0.5108, 0.1012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height in May</td>
<td>0.197</td>
<td>0.621</td>
<td>0.0718</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-3.58, 3.17)</td>
<td>(-0.522, 1.70)</td>
<td>(-3.74, 4.88)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocotyl length</td>
<td>2.198</td>
<td>0.149</td>
<td>-0.348</td>
<td>-1.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-0.782, 4.83)</td>
<td>(-0.669, 1.08)</td>
<td>(-2.72, 1.99)</td>
<td>(-2.76, 1.04)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Internode Length</td>
<td>-0.529</td>
<td>0.621</td>
<td>-1.23</td>
<td>1.02</td>
<td>-1.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-3.94, 2.20)</td>
<td>(-0.361, 1.39)</td>
<td>(-4.63, 2.48)</td>
<td>(-1.42, 3.56)</td>
<td>(-5.08, 3.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days to Flower</td>
<td>0.169</td>
<td>0.400</td>
<td>-3.62</td>
<td>-2.36</td>
<td>8.10</td>
<td>57.0</td>
<td>3.16*</td>
</tr>
<tr>
<td></td>
<td>(-10.2, 137)</td>
<td>(-3.88, 5.11)</td>
<td>(-18.2, 9.14)</td>
<td>(-13.3, 8.73)</td>
<td>(-6.49, 24.4)</td>
<td>(-7.12, 78)</td>
<td>(0.184, 7.18)</td>
</tr>
<tr>
<td>Maximum Height</td>
<td>0.837</td>
<td><strong>0.762</strong>*</td>
<td>-1.66</td>
<td><strong>-2.95</strong>*</td>
<td>-0.68</td>
<td>-2.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-3.15, 3.77)</td>
<td>(0.306, 1.65)</td>
<td>(-5.04, 1.67)</td>
<td>(-5.80, -0.417)</td>
<td>(-3.22, 2.09)</td>
<td>(-14.8, 8.1)</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01  
*** p<0.001

For values in bold, the confidence intervals do not cross zero
b) Interspecific Competition

<table>
<thead>
<tr>
<th></th>
<th>Early growth</th>
<th>Late growth</th>
<th>Height in May</th>
<th>Hypocotyl Length</th>
<th>First Internode Length</th>
<th>Days to Flower</th>
<th>Maximum Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early growth</td>
<td>0.817</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late growth</td>
<td>0.00976</td>
<td>-0.145</td>
<td>-0.440</td>
<td>-0.440</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height in May</td>
<td>-0.286</td>
<td>0.337</td>
<td>0.526</td>
<td>-0.013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocotyl length</td>
<td>-0.119</td>
<td>-0.0925</td>
<td>0.0848</td>
<td>-0.0520</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Internode Length</td>
<td>-0.677</td>
<td>-0.246</td>
<td>0.311</td>
<td>-0.124</td>
<td>-0.395</td>
<td>3.71**</td>
<td>1.36**</td>
</tr>
<tr>
<td>Days to Flower</td>
<td>-1.20</td>
<td>-0.167</td>
<td>-0.0697</td>
<td>3.71* (0.957, 8.23)</td>
<td>1.07 (-2.42, 6.79)</td>
<td>9.29 (-19.0, 36.8)</td>
<td></td>
</tr>
<tr>
<td>Maximum Height</td>
<td>0.232</td>
<td>0.480*</td>
<td>-1.065</td>
<td>1.36**</td>
<td>0.140</td>
<td>-0.921</td>
<td>1.28* (0.422, 3.02)</td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01  
*** p<0.001

For values in bold, the confidence intervals do not cross zero
Table 1-6. Canonical analysis of the $\gamma$ matrices, including the eigenvalues ($\lambda_i \pm 1$ s.e.) and the eigenvectors of the canonical axes ($m_i$). Significance of the eigenvalues was estimated with a quadratic regression containing synthetic traits estimated from the eigenvectors and the original traits. (A) Removal treatment. (B) IC treatment. * Indicates $P < 0.01$, $\Theta$ Indicates $P = 0.0584$.

### (A) Removal treatment

<table>
<thead>
<tr>
<th>$m_i$</th>
<th>$\lambda_i$</th>
<th>%</th>
<th>Early growth</th>
<th>Late growth</th>
<th>Height in May</th>
<th>Hypocotyl Length</th>
<th>First Internode Length</th>
<th>Days to Flower</th>
<th>Maximum Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58.5582 ± 20.56*</td>
<td>98.13</td>
<td>-0.001</td>
<td>0.007</td>
<td>-0.062</td>
<td>-0.034</td>
<td>0.135</td>
<td>0.987</td>
<td>-0.048</td>
</tr>
<tr>
<td>2</td>
<td>5.1301 ± 0.52*</td>
<td>0.75</td>
<td>-0.071</td>
<td>0.093</td>
<td>-0.239</td>
<td>-0.436</td>
<td>-0.073</td>
<td>0.021</td>
<td>0.856</td>
</tr>
<tr>
<td>3</td>
<td>1.9130 ± 0.48*</td>
<td>0.10</td>
<td>0.715</td>
<td>-0.473</td>
<td>-0.330</td>
<td>0.344</td>
<td>-0.003</td>
<td>0.005</td>
<td>0.194</td>
</tr>
<tr>
<td>4</td>
<td>0.3326 ± 0.4</td>
<td>&lt;0.001</td>
<td>-0.225</td>
<td>0.380</td>
<td>-0.649</td>
<td>0.375</td>
<td>0.483</td>
<td>-0.097</td>
<td>-0.007</td>
</tr>
<tr>
<td>5</td>
<td>-0.3985 ± 0.16*</td>
<td>&lt;0.001</td>
<td>0.447</td>
<td>0.672</td>
<td>0.448</td>
<td>0.289</td>
<td>0.067</td>
<td>0.036</td>
<td>0.241</td>
</tr>
<tr>
<td>6</td>
<td>-3.0394 ± 0.72*</td>
<td>0.26</td>
<td>-0.069</td>
<td>-0.350</td>
<td>0.405</td>
<td>-0.049</td>
<td>0.814</td>
<td>-0.076</td>
<td>0.193</td>
</tr>
<tr>
<td>7</td>
<td>-5.0882 ± 0.88*</td>
<td>0.74</td>
<td>0.478</td>
<td>0.221</td>
<td>-0.207</td>
<td>-0.681</td>
<td>0.275</td>
<td>-0.093</td>
<td>-0.363</td>
</tr>
</tbody>
</table>

### (B) Interspecific competition treatment

<table>
<thead>
<tr>
<th>$m_i$</th>
<th>$\lambda_i$</th>
<th>%</th>
<th>Early growth</th>
<th>Late growth</th>
<th>Height in May</th>
<th>Hypocotyl Length</th>
<th>First Internode Length</th>
<th>Days to Flower</th>
<th>Maximum Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.8468 ± 3.4*</td>
<td>89.26</td>
<td>-0.123</td>
<td>-0.021</td>
<td>0.007</td>
<td>0.313</td>
<td>0.093</td>
<td>0.936</td>
<td>-0.049</td>
</tr>
<tr>
<td>2</td>
<td>2.5740 ± 0.22*</td>
<td>5.03</td>
<td>0.208</td>
<td>0.087</td>
<td>-0.428</td>
<td>0.346</td>
<td>-0.088</td>
<td>-0.032</td>
<td>0.799</td>
</tr>
<tr>
<td>3</td>
<td>0.9001 ± 0.48$\Theta$</td>
<td>0.61</td>
<td>0.832</td>
<td>-0.126</td>
<td>-0.166</td>
<td>-0.009</td>
<td>-0.376</td>
<td>0.131</td>
<td>-0.324</td>
</tr>
<tr>
<td>4</td>
<td>0.4493 ± 0.14*</td>
<td>0.15</td>
<td>0.239</td>
<td>0.559</td>
<td>0.731</td>
<td>0.241</td>
<td>-0.123</td>
<td>-0.022</td>
<td>0.150</td>
</tr>
<tr>
<td>5</td>
<td>-0.3176 ± 0.10*</td>
<td>0.08</td>
<td>-0.259</td>
<td>0.701</td>
<td>-0.415</td>
<td>-0.207</td>
<td>-0.430</td>
<td>0.087</td>
<td>-0.185</td>
</tr>
<tr>
<td>6</td>
<td>-0.6939 ± 0.22*</td>
<td>0.37</td>
<td>0.353</td>
<td>0.380</td>
<td>-0.162</td>
<td>-0.362</td>
<td>0.750</td>
<td>0.104</td>
<td>0.023</td>
</tr>
<tr>
<td>7</td>
<td>-2.4377 ± 0.48*</td>
<td>4.51</td>
<td>-0.013</td>
<td>0.167</td>
<td>-0.238</td>
<td>0.742</td>
<td>0.282</td>
<td>-0.296</td>
<td>-0.444</td>
</tr>
</tbody>
</table>
Discussion

Changes in light resources due to competition can have direct effects on plant fitness and plasticity. My experiment had three major findings. First, I found that heterospecific competitors affected light resources and cues (i.e., PAR and R:FR), and shade avoidance phenotypes. Second, censuses of per capita reproductive output in marsh and monoculture populations suggest that marsh habitats are not inherently poor habitats that lead to low reproductive success. Third, the presence or absence of heterospecifics significantly altered natural selection on traits expected to be involved in shade avoidance responses. I discuss these findings below, along with the potential caveats that apply to my results, in the context of their implications for the evolution of phenotypic plasticity.

*Phenotypic differences and Light Resources*

My results demonstrate that numerous plant traits, including total reproductive output, are affected by the presence of heterospecifics. The direction and timing of these effects can be explained by changes in light quantity and quality. By inducing plastic changes in shade avoidance phenotypes that are subject to natural selection (see below), heterospecifics have the potential to alter the evolution of the shade avoidance syndrome. For example, early in the season, R:FR ratios were lower in the removal treatment (Table 1-1), which would signal the presence of competitors to the focal *I. capensis*; early in the season, the average height in interspecific competition treatment was greater than the removal treatment. Later in the season, both PAR and R:FR decreased in the removal treatment (due to shading from conspecifics and heterospecifics in the surrounding marsh), and at this time the height trends had reversed. In essence, my removal treatment delayed the onset of the R:FR signal until later in the season when plants had been
growing in higher PAR for two months (Table 1-1). These higher PAR levels are indicative of greater light resources, which may have allowed the removal plot plants to elongate late in the season, whereas the plants growing with heterospecifics were likely too energy limited to continue responding to low R:FR. The resulting differences in early and late growth rates (Table 1-2) were themselves subject to variable natural selection (Tables 1-3 and 1-4).

My results are consistent with other studies that indicate that the presence of heterospecific competitors can affect the evolution of shade avoidance traits by modifying their effectiveness, expression, or timing. For instance, Weinig (2000) found that when *Abutilon theophrasti* plants were grown under weeds, they lacked the carbon resources necessary to elongate, despite low R:FR cues. Other studies have found that the presence of heterospecific leaf litter can alter selection on early elongation traits in *I. capensis* (Stinchcombe and Schmitt 2006). Collectively, these studies suggest that heterospecific plant species, by altering light quality and quantity, and the timing of these cues of competition, may substantially affect the evolution of shade avoidance.

*Are Marshes Poor Habitat?*

We predicted that *I. capensis* would have lower reproductive output under competition in the marsh than in the removal treatments, but that plants in both of these treatments would have lower reproductive success than plants from monoculture populations. Instead, plants in both marsh treatments had greater average per capita reproductive output than plants from monoculture blocks. This demonstrates that the marsh represents suitable habitat for *I. capensis*, despite intense interspecific competition. While subsidy from monoculture habitats may occur (due to *I. capensis’* floating,
ballistically dispersed seeds), my results suggest that marsh habitats are qualitatively different, but not necessarily poorer habitats, than monoculture sites. It is important to note that the average initial density of *I. capensis* in the monoculture (213.5 pl /m$^2$) populations was significantly greater than in the marsh (44.2 pl / m$^2$). These densities, which differ in the opposite direction than one would predict based on my data on total seed production—marsh plants have an approximately 6-fold advantage in seed production, but have nearly 5-fold lower initial densities—suggests that marsh populations must experience higher seed mortality (perhaps due to differences in seed quality: McCall *et al*. 1991), a longer seed bank, or some combination of the two. Winsor (1983) noted that *I. capensis* dominates some habitats due to early germination and growth under colder temperatures, and the formation of dense canopies that shade interspecific competitors. The lack of monocultures in the marsh habitats despite high seed production suggests that these life history strategies and features are ineffective in the marsh habitat or against marsh competitors.

*Directional Selection*

Directional selection on shade avoidance traits changed depending on the presence of heterospecifics. The most obvious difference between selection models was the inclusion of growth rates and hypocotyl length in the interspecific competition model. These data suggest that under interspecific competition growth rates became important for determining fitness. Hypocotyl length was likely under positive selection in the interspecific competition treatment because very early growth would allow *I. capensis* to remain taller than competitors early in the season. Similar results for hypocotyl length
have been described for *I. capensis* germinating under forest leaf litter (Stinchcombe and Schmitt 2006).

My use of naturally occurring plants may have led to biased estimates of phenotypic selection due to environmentally induced covariances between traits and fitness (Rausher 1992; Stinchcombe *et al.* 2002). I attempted to minimize this influence by randomly selecting blocks in the marsh, randomly allocating treatments to each half of the block, and accounting for block effects in my statistical models. While the initial densities of *I. capensis* did not differ between treatments, my treatment itself could have produced environmentally induced covariances. For example, in the removal treatment, plants would have received more light, and as a consequence reached larger size and had higher fitness, potentially leading to an inflated estimate of the true relationship between size and fitness. Environmental covariances induced by the treatment itself are, however, less likely to explain selection on novel traits and trait combinations in the interspecific competition treatment—these sections of the marsh were left unaltered and simply observed, so the treatment itself was unlikely to alter environmental conditions.

Available evidence suggests environmentally induced covariances between traits and fitness mainly bias the magnitude of selection gradients and not the sign (Stinchcombe *et al.* 2002). Many of the selection gradients in the removal treatment (which should be more susceptible to environmentally induced covariances caused by the treatment itself) show larger selection gradients than the control (see Tables 1-4, 1-5, especially for maximum height, first internode length), and may be biased estimates. While my data are consistent with this type of bias, it is also possible that environmental covariances could have reversed the sign of individual selection gradients (either across
the experiment, or how selection was occurring within individual plots) or had the effect of masking selection and leading me to conclude it was absent. Ultimately, addressing any of these possibilities, however, requires transplanting quantitative genetic families into the marsh to perform genotypic selection analysis (Stinchcombe et al. 2002).

Non-linear selection

Maximum height was under both positive directional and curvilinear selection in both the treatments (see Tables 1-4, 1-5). In the removal treatment, a greater height would allow an I. capensis plant more access to light than its neighbours, giving it a competitive edge. In the interspecific competition treatment, increased height would allow plants to emerge from as much vegetation as possible. Comparison of the $\gamma$ matrices suggests a handful of differences in non-linear selection (see Tables 1-5a,b). In the removal treatment, there was saturating curvilinear selection on late growth ($i.e.$, the fitness benefits of late growth leveled off) and there was negative correlational selection on early and late growth; neither of these were significant in interspecific competition. Opposite combinations of early and late growth being favoured suggest that plants that grow slowly throughout season are at a disadvantage (they are at the bottom of the size hierarchy), as are plants that grow quickly the entire season—possibly because later in the season, selection favors allocation to reproduction or branching rather than vertical growth.

The canonical analysis facilitated an overall assessment of the mode of non-linear selection in the experiment, and how similar it was between treatments. These analyses revealed that approximately 95 to 99% of the variation in the $\gamma$ matrix was associated with disruptive selection (axes $m_{1-2}$ in each treatment). Partial residual blocks of relative
fitness against the synthetic variables suggested true disruptive selection on axes m₁ and m₂ in each treatment, with a hint of stabilizing selection on axis m₇ in each treatment. Overall, the contribution of stabilizing selection on the synthetic traits appears to be weak. Sub-space similarity methods suggested that the two γ matrices shared similar, but not identical orientations in multivariate space.

Using canonical analysis, I found more evidence for statistically significant non-linear selection on the synthetic traits than I did on the original traits, and the median values of the selection gradients (γ₁i – vs – λᵢ) were larger for synthetic traits. Both of these patterns have been previously demonstrated (Blows & Brooks 2003; also see Blows 2007a, 2007b). While it is an algebraic necessity that at least one λᵢ will be larger than a γᵢi (provided that there is any correlational selection; Kruuk & Garant 2007), I found selection on the canonical traits to be appreciably stronger than on the original traits. These data suggest that combinations of traits were under stronger non-linear selection in the experiment than the individual traits themselves. Put another way, there is more curvature in the fitness surface along the axes described by the synthetic traits than along the axes described by the original traits. This result is notable for shade avoidance traits, for which I have ample prior evidence of the phenotypic traits under selection, and for which phenotypic differences can be both quite apparent and dramatic (Table 1-2). It also counters the hypothesis that canonical analysis is unlikely to be revealing in field studies (Preziosi and Harris 2007). As emphasized by Blows (2007), if combinations of traits are under selection, methods such as canonical analysis that can identify those trait combinations will be necessary.
Conclusions

My study indicates that shade avoidance traits can be under very different types of selection depending on the level and type of competition, which could lead to the evolution of increased plasticity. This is contingent on gene flow between patches in different competitive habitats, which is known to occur in I. capensis (von Wettberg et al. 2008). My results suggest that shade avoidance responses may facilitate the coexistence of I. capensis with dominant heterospecific competitors, by providing for flexible elongation timing based on the R:FR light cue.
Chapter 3: Population differentiation in shade avoidance phenotypes and plasticity in response to density and R:FR greenhouse treatments

Summary

*Impatiens capensis* populations are found in habitats with variable intraspecific competition, interspecific competitor identity and density, and canopy cover. These different characteristics of patches could select for different phenotypes, and for various levels of sensitivity to the R:FR cue.

I collected seeds from two *I. capensis* patches, one was a monoculture with variable shading from trees, and the second was in marsh habitat, where *Impatiens* grow with several heterospecific competitors. After one generation in the greenhouse under common conditions, seeds derived from these families were planted in a 2x2 experiment that manipulated both density (high and low) and the R:FR ratio of light (ambient and reduced). I measured and compared morphological and reproductive traits as well as natural selection in the four different light and density treatment combinations.

The two populations differed in their average hypocotyl lengths, and there was also a marginally significant difference in flowering time. There was no evidence of significant differences between populations in plasticity, although there was significant variation among families. My analyses indicated that selection acted on both the elevation and slope of reaction norms and depended on treatment. In addition, I demonstrated that the frequency of a competitive environment would affect selection on plasticity. This experiment demonstrates the importance of the competitive context for the evolution of shade avoidance reaction norms.
Introduction

Phenotypic plasticity, the ability to change phenotypic traits based on environmental cues, is thought to be an important factor in allowing species to persist in a wide range of habitats (Williams et al. 1995; Griffith and Sultan 2006). For plants, which are immobile, plastic reactions are a particularly significant means of responding to their surroundings (Neff et al. 2000; Donohue 2003; Gurevitch et al. 2006). An important example is shade avoidance syndrome, a suite of responses induced by decreases in the red to far-red ratio (R:FR) of light. Plants sense the presence of surrounding vegetation through changes in R:FR and respond with stem elongation, and sometimes, accelerated flowering time (Donohue et al. 2001; Franklin 2008).

Theoretical models have demonstrated the importance of the relative frequency of different environments for the evolution of phenotypic plasticity (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992). The environment a plant experiences is largely influenced by both its conspecific and heterospecific competitors (Dudley and Schmitt 1996), both of which vary greatly in density. Plants typically grow in a range of environments, and experience different competitor identities, plant densities, and light characteristics. Therefore, populations may experience selection for both different sensitivities to the R:FR cue and different means in their shade avoidance traits, or both. The slope of a reaction norm (plasticity) is often positively correlated with its elevation or mean phenotype (Nussey et al. 2007). As a consequence, directional selection to increase phenotypic plasticity (e.g., sensitivity to the R:FR cue) in common or frequently occurring competitive environments could lead to a correlated response in mean phenotypes, and vice versa.
Chapter Three

Theory also predicts that environmental variability will promote the evolution of plasticity (Van Tienderen 1991) and there is empirical support for this hypothesis in both animal and plant species (Galloway 1994; Gianoli 2004; Lind and Johnasson 2007). Species interactions, such as competition, are inherently variable in both space and time (Agrawal 2001). Predictions from theory, therefore, make contrasting predictions about how the diverse competitive environments experienced by plants will influence the evolution of the shade avoidance syndrome. On the one hand, if competitive environments are relatively stable through space and time, persistent directional selection on shade avoidance phenotypes and/or their plasticities may lead to the evolution of increased plasticity and sensitivity to R:FR cues. On the other hand, the inherent variability of competition in both space and time, especially in heterogeneous habitats, may lead to the evolution of increased plasticity, even in the absence of stable and persistent competitors. Empirical data discriminating between these possibilities remains lacking.

The annual plant, *Impatiens capensis* grows in both monoculture populations that are stable from year to year where it is an understorey habitat dominant (Winsor 1983), and in marsh environments with many different heterospecifics, which have diverse growth forms, resource requirements, and micro-habitat distributions. However, it remains unknown whether *Impatiens* from the more variable, interspecific competition dominated marsh environment or stable monoculture habitats are more plastic. Marsh-derived plants may be more sensitive to the R:FR cue so they can elongate early when growing with heterospecifics, respond to shading if it occurs late in the season, and avoid elongating if they are in low density. Continued shading by heterospecifics could induce
earlier flowering time. Or, since early mortality is likely more prevalent in the marsh, there may be selection for canalized earlier flowering time (Donohue et al. 2000a).

Alternatively, if the shade avoidance syndrome is more effective in monocultures, than there may be selection for greater plasticity to the R:FR cue for plants growing in intraspecific competition. Elongation in the monocultures could be more effective since the competitors are other *Impatiens* plants, and so it is possible for an individual *I. capensis* to remain the tallest understory plant. In addition, there may be selection for early elongation, since the height hierarchy is established quickly for plants, with the dominant plants gaining an energy advantage.

Here I compare the shade avoidance traits, and plastic reactions of *Impatiens* lines derived from populations that grow in different competitive contexts. To determine if lines and populations differ in their plasticity, I tested the response of *Impatiens* plants (descended from different populations) to different levels of light quality (R:FR) and intraspecific density. Plastic responses are not necessarily adaptive (Cook and Johnson 1968), and with this experiment, I also examined the effects of plasticity to competitive cues on fitness in the various treatments. Specifically, I asked the following questions: (1) Do populations differ in the slope and elevation of their reaction norms for elongation and flowering time plasticity? (2) Is there variation among *Impatiens* lines for sensitivity to competitive cues? (3) What traits are important for fitness in the density and light treatments?
Methods

Study species and Experimental Design

*Impatiens capensis* (jewelweed, touch-me-not) is a common North American annual found in forested areas and wetlands (Schemske 1978; Kelly 1997; Huber et al. 2004; Steets et al. 2006, 2007). Jewelweed has a mixed mating system and produces mostly self-fertilizing cleistogamous flowers (Waller 1980; Schmitt 1993; Kelly 1997). Larger individuals also produce chasmogamous flowers, which are showy and highly out-crossing (Schmitt 1993; Kelly 1997). *Impatiens capensis* grows in heterogeneous light environments (Schmitt 1993), in monocultures and with other plant species.

To examine the effects of cues from both intra-specific competitors (that could conceivably be over-topped) and interspecific competitors (that could not— *e.g.*, tree canopies), I used full-sib families developed from seeds collected at the University of Toronto Koffler Scientific reserve. Seeds were originally collected from two (sub-)populations of *Impatiens capensis* at the Koffler Scientific Reserve in September 2007. One *Impatiens* patch was a monoculture growing between a road and a pond with variable shading from trees. The second group of *Impatiens* was growing in a marsh with several different species including cattails (*Typha latifolia*), marsh marigold (*Caltha palustris*), stinging nettle (*Urtica dioica*) and horsetails (*Equisetum fluviatile*).

Field collected seeds were stored in microplate wells filled with distilled water and refrigerated for 4.5 months. Once germination began, seeds were planted into 4-inch pots in the greenhouse. These seedlings were grown until they too flowered and produced seeds, which were collected again and stored in distilled water. Seed collection was from cleistogamous fruits. Growing the *Impatiens* from the two source populations in the
greenhouse for a generation not only provided many seeds per family, but also reduced the contribution of direct environmental and maternal environmental effects on experimental plants.

In September 2008, I planted replicate seeds from each family in cone-tainers and placed them in a two by two design in the greenhouse, where *Impatiens* density and light quality (the red to far-red ratio of light) were manipulated. There were five blocks (see Figure 2-1), and every block had one plot for each density and R:FR combination (total of 20 plots). I used red absorbing filters (Rosco Filters # 4430, Jack Frost, Mississauga) to reduce the R:FR ratio, which simulated dominant competitors that cannot be overtopped, such as those found in the marsh environment. Clear plastic sheets over the control light treatment (ambient R:FR) reduced PAR light to similar levels as the reduced red light treatment (148 μmol²s⁻¹ vs 160 μmol²s⁻¹), but did not affect the R:FR ratio of light. Both kinds of sheets were held above the trays by wooden posts and were surrounded by aluminum foil, to reflect back the light and minimize edge effects. Cone trays were designated as either high (530 plants/m²) or low (260 plants/m²) density.

Once the seedlings emerged in October 2008, I assessed the height of all plants. Subsequently, each month I measured a subset of plants to estimate growth rates in the light and density treatments. Beginning in November, plants were monitored to determine the first flowering dates. In January, I ended the experiment and measured hypocotyl and first internode lengths, total number of internodes, final height and total reproductive structures (pedicels, flowers and fruits) for all plants at harvest. Since chasmogamous flowers could not have been pollinated in the greenhouse, the total number of cleistogamous fruits produced over each plant’s lifetime was my proxy for fitness.
Figure 2-1: Experimental design in the greenhouse
Data Analysis

I used mixed-model ANOVAs to compare average traits, including early height, final height, flowering time, hypocotyl length and first internode lengths, produced in the different light quality and density treatments, source populations, and full-sib families. In these mixed models, population, R:FR, density, and the R:FR* density interaction were fixed effects, while block, block*density and block*R:FR, and family (nested within population) were random effects. Because of the complicated structure of my experimental design and statistical models, I used the Kenward-Rogers approximation to determine the appropriate degrees of freedom for hypothesis testing of fixed effects (Schaalje et al. 2001). To determine if family nested in population was significant, I used a likelihood ratio test by comparing the -2 log likelihoods of models with and without this term, which is $\chi^2$ distributed with 1 df. Importantly, when this term is significant when tested with a 1-tailed test (because a variance component cannot be less than zero), there is variation in responses among full-sib families.

To compare the relative plasticities of the lines collected from the monoculture and the marsh populations, I estimated plasticity for both elongation and flowering time. For elongation, the shade avoidance traits of interest are height and hypocotyl length, and for flowering time, I used the elapsed time between germination and first flowering. I defined plasticity as the difference in phenotype between experimental treatments for a given family. There were four different types of plastic reactions. Two were reactions to density differences, one in response to density changes in ambient R:FR, and a second in response to density when growing in reduced R:FR. Similarly, there were two reaction norms in response to R:FR, one at each of low and high density.
To examine which traits and treatments had significant effects on fitness, I used an ANCOVA model including shade avoidance traits, density, R:FR and the interactions between density, R:FR, and their interactions with the traits. In these models, I used average phenotypes for each *Impatiens* full-sib family in each experimental treatment. I refer to this as the ‘Fitness model’. The fitness model describes natural selection on individual traits within the experiment as a whole (*i.e.*, all treatments), and whether there is heterogeneity in the strength of selection on these traits between treatments.

To test which traits were important for fitness in the different R:FR and density treatments, I used selection analyses (Lande and Arnold 1983; Rausher 1992; Griffith and Sultan 2006). For each of the four density and R:FR combinations, I used a model that included shade avoidance traits and the lines’ plasticity to the competitive cues (hypocotyl length, flowering time and final height, along with their corresponding plastic reactions to both density and the R:FR ratio of light). For example, the model for high density and ambient R:FR, included the average hypocotyl length, flowering time and final height in that treatment, along with the average plasticity to density changes (in the ambient R:FR treatment) and to R:FR changes (at high density). A significant regression coefficient for a particular trait indicates directional selection on that trait within the particular experimental treatment, while a significant negative parameter estimate for plasticity in these models indicates a cost of plasticity (van Tienderen 1991).

The models above assume that the frequencies of the competitive environments are equal. To examine the impact that varying environmental frequencies would have on the effect of plasticity on fitness, I ran an additional analysis. I used a model including plasticity in the three traits (hypocotyl length, flowering time and final height) as
explanatory variables and mean fitness, across density or R:FR treatments, as the response variables. For example, the model for plasticity in response to density in ambient R:FR included the difference between the two density treatments (in ambient R:FR) in hypocotyl lengths, flowering time and final height as the predictor variables, and mean fitness across the two density treatments in ambient R:FR. I then analyzed the reduced R:FR treatment using plasticities to density and mean fitness across density treatments calculated in the same way, but utilizing only data from the reduced R:FR treatments. To evaluate the sensitivity of my results to the assumption that density environments occur at an equal frequency, I also estimated mean fitness across density environments when the high density treatment was at frequencies of 0.25 and 0.75.

**Results**

*Early mortality*

The *Impatiens capensis* plants in this study suffered from a spider mite outbreak soon after they were planted, leading to high early mortality rates (38%, dropped from N=920 to N=570), and fewer plants surviving long enough to be measured in the experiment. It is probable that this high early mortality impeded my ability to detect differences between the populations, and to get the full range of responses to the treatments that I would have seen otherwise.

*Light Treatment*

Since I conducted the experiment in the fall, and *Impatiens capensis* are summer annuals, I needed to supplement the sunlight reaching the plants with artificial lighting. However, light measurements taken during the experiment revealed that the greenhouse
lights had a higher than normal R:FR ratio (ambient light R:FR ratio 2.2, R:FR reduction treatment ratio of 1.5, versus the ratio for sunlight of~1.1-1.3 (Schmitt and Wulff 1993; Dudley and Schmitt 1996; Franklin 2008)). This effectively undermined my light treatments—the R:FR reduction treatment resembled full sun, while the ambient R:FR treatment had artificially high levels of R light that likely precluded or at least reduced shade avoidance responses.

Population Differentiation, Treatment Effects, and Full-Sib Family Variation

Mixed model analyses were used to compare traits produced in different density and light treatments, and for plants from different populations and families. I included block, block*R:FR and block*density as random effects, but the maximum likelihood estimate for these variance components was zero in most of the models, and as such I do not present or discuss block effects.

Despite the reduced sample sizes, I found differences between source populations in shade avoidance traits, significant effects of density on traits, and differences in what characteristics affected fitness depending on the R:FR and density treatments. The analyses comparing traits produced by plants derived from the two populations revealed two interesting differences. Population had a significant effect on hypocotyl length, with plants from the pond population having longer hypocotyls (See Figure 2-4, Table 2-2, p=0.0084) and a marginally significant effect on flowering time, with plants from the marsh population flowered slightly earlier on average (see Table 2-2, p=0.0642).

Density was a significant factor for early height (plants in high density were 0.36 cm taller on average, p=0.033), first internode lengths (high density plants had 0.10 cm longer first internodes, p<0.0001) and flowering time (plants in high density flowered an
average of 2.9 days earlier, p=0.021) (see Table 2-1). Light quality was never a
significant factor, although the interaction of R:FR and density was marginally significant
in the model for hypocotyl length (p=0.054). This was initially surprising, but can be
explained by the inflated R:FR values caused by the artificial greenhouse lighting.

In all cases, line nested in population was significant as a random effect.
Importantly, this demonstrates broad-sense genetic variation between full sib families in
their phenotypes. I also detected family*density and family*R:FR interactions for
hypocotyl lengths and flowering time, and family*R:FR*density interactions for first
internode lengths and flowering time. These latter interactions indicate broad sense
genetic variation for plasticity to density, R:FR, and R:FR-density combinations among
the full sib families used in my experiment. Despite the presence of genetic variation for
plasticity, I did not see significant differences in the average plasticity of the two source
population groups (P > 0.059 for all comparisons).

In my fitness ANCOVA model, light quality had a marginal effect on fitness
(p=0.051), with plants having higher fitness in the ambient R:FR treatment. Significant
interaction terms included height*density (p=0.050), height*R:FR (p=0.034) and average
internode length*density (p=0.047). Height was positively related to fitness in both
density environments, but this relationship was stronger under high density (r=0.54,
p<0.0001 in high density and r=0.42, p<0.0001 in low density, the same pattern was true
for average internode length (r=0.52, p<0.0001 versus 0.41, p<0.0001)). Similarly, height
was also positively correlated with fitness in both light quality treatments, however there
was a stronger relationship in the reduced R:FR treatment (r=0.65, p<0.0001 in reduced
R:FR compared to r=0.35, p<0.0001 in ambient R:FR).
Table 2-1: Least Square Means (± 1 s.e.) of traits for the Marsh and Pond populations using mixed model.

<table>
<thead>
<tr>
<th>Trait (units)</th>
<th>Marsh</th>
<th>Pond</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Height (cm)</td>
<td>8.41 ± 0.602</td>
<td>8.400 ± 0.470</td>
<td>&lt;0.01</td>
<td>0.989</td>
</tr>
<tr>
<td><strong>Hypocotyl Length (cm)</strong></td>
<td>1.55 ± 0.13</td>
<td>1.90 ± 0.10</td>
<td>7.64</td>
<td>0.008</td>
</tr>
<tr>
<td>First Internode Length (cm)</td>
<td>2.63 ± 0.14</td>
<td>2.71 ± 0.11</td>
<td>0.29</td>
<td>0.593</td>
</tr>
<tr>
<td>Final Height (cm)</td>
<td>25.25 ± 1.25</td>
<td>25.41 ± 0.99</td>
<td>0.02</td>
<td>0.900</td>
</tr>
<tr>
<td><strong>Flowering Time (days)</strong></td>
<td>66.35 ± 1.7</td>
<td>69.22 ± 1.4</td>
<td>3.64</td>
<td>0.064</td>
</tr>
<tr>
<td>Cleistogamous Fruits</td>
<td>30.73 ± 2.3</td>
<td>30.46 ± 1.8</td>
<td>0.01</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Values in bold are significant.
Table 2-2: Results of mixed models examining effects of density and light treatments on relevant shade avoidance traits. Line (nested within population), block, block x density and block x light were random effects. Degrees of freedom were estimated in SAS following methods developed by Kenward and Roger.

<table>
<thead>
<tr>
<th>Source</th>
<th>Early Height</th>
<th>Hypocotyl</th>
<th>First Internode Length</th>
<th>Height</th>
<th>Days to Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>R:FR</td>
<td>F(_{1,2.56})=0.16 P=0.718</td>
<td>F(_{1,402})=1.34 P=0.248</td>
<td>F(_{1,2.56})=0.83 P=0.439</td>
<td>F(_{1,9.9})=1.20 P=0.299</td>
<td>F(_{1,418})=0.42 P=0.517</td>
</tr>
<tr>
<td>Density</td>
<td>F(_{1,29.1})=5.02 P=0.0328</td>
<td>F(_{1,1})=1.81 P=0.407</td>
<td>F(_{1,402})=28.51 P=&lt;0.0001</td>
<td>F(_{1,9.85})=3.93 P=0.076</td>
<td>F(_{1,422})=5.40 P=0.0206</td>
</tr>
<tr>
<td>R:FR x Density</td>
<td>F(_{1,2.28})=0.05 P=0.841</td>
<td>F(_{1,404})=3.74 P=0.0537</td>
<td>F(_{1,400})=0.01 P=0.919</td>
<td>F(_{1,9.26})=1.39 P=0.267</td>
<td>F(_{1,423})=0.05 P=0.816</td>
</tr>
<tr>
<td>Population</td>
<td>F(_{1,43.1})=0.00 P=0.989</td>
<td>F(_{1,417})=7.64 P=0.0084</td>
<td>F(_{1,35.2})=0.29 P=0.593</td>
<td>F(_{1,43.2})=0.02 P=0.901</td>
<td>F(_{1,37.1})=3.64 P=0.0642</td>
</tr>
<tr>
<td>R:FR x Population</td>
<td>F(_{1,52.4})=1.62 P=0.204</td>
<td>F(_{1,403})=1.03 P=0.311</td>
<td>F(_{1,398})=0.02 P=0.898</td>
<td>F(_{1,404})=1.88 P=0.171</td>
<td>F(_{1,419})=0.70 P=0.403</td>
</tr>
<tr>
<td>Density x Population</td>
<td>F(_{1,525})=0.00 P=0.965</td>
<td>F(_{1,406})=0.03 P=0.854</td>
<td>F(_{1,402})=0.49 P=0.484</td>
<td>F(_{1,407})=2.28 P=0.132</td>
<td>F(_{1,421})=0.02 P=0.890</td>
</tr>
</tbody>
</table>

Values in bold are significant
### Figure 2-2: Directional selection on the means of shade avoidance traits and their associated plasticities. Only statistically significant (p<0.05) values are shown.

<table>
<thead>
<tr>
<th>Density</th>
<th>Light quality (R:FR)</th>
<th>Ambient</th>
<th>Reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Height</td>
<td>Height</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Flowering time</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Plasticity to density</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Flowering time</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasticity to density</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only statistically significant (p<0.05) values are shown.
Figure 2-3: The effect of changing the frequency of the high density environment on selection on plasticity in height.
Selection

Selection models in the different treatment combinations revealed that selection on trait means and plasticity was contingent on the light and density conditions (see Figure 2-2). Height was under significant positive directional selection in all treatments \((p<0.05)\) except for the low density, ambient R:FR treatment. There was selection for earlier flowering in the low density, ambient R:FR treatment \((p=0.013)\). There was also a significant cost to plasticity in flowering time in response to density in this treatment \((p=0.023)\). In contrast, sensitivity to density in flowering time responses was advantageous in the high density, reduced R:FR treatment \((p=0.037)\). Using models with different environment frequencies did affect the relative benefits of being plastic. As seen in Figure 2-3, as the high density environment becomes more frequent, selection on plasticity to density becomes stronger (more positive).

Discussion

The competitive environment experienced by an *Impatiens capensis* plant has effects on the development of shade avoidance traits and selection on those traits (McGoey and Stinchcombe 2009). Populations growing with various competitor types and densities, may experience different patterns of selection on both the slope and elevation of a given reaction norm, leading to different average values of a given phenotype and varying sensitivity to the R:FR cue. With this greenhouse experiment, I demonstrated that there are population differences in shade avoidance traits. In addition, within populations there is variation among full sib families in plastic responses to density and R:FR treatments. Plasticity to density had significant effects on fitness, and
these effects were dependent on the frequency of high density environments. Below I discuss the differentiation between the two populations, the effects of my treatments on shade avoidance phenotypes and selection within the treatments, and the importance of habitat frequency for the evolution of plasticity.

*Population Differentiation*

There were a handful of significant trait differences between populations, suggesting that there are genetic differences between the two groups. The ‘Pond’ plants had significantly longer hypocotyl lengths than the ‘Marsh’ plants (see Fig. 2-4a). These results from a common garden study are also consistent with field observations of plants in the original source populations (see Fig. 2-4b). Plants in the ‘Pond’ location emerge in dense intraspecific competition early in the season. Having consistently long hypocotyls makes sense in these circumstances, where it is important to quickly out-compete the surrounding conspecifics for light resources. In contrast, the *I. capensis* plants growing in the marsh environment grow with variable densities of both conspecifics and heterospecifics. In addition, they emerge before many of their interspecific competitors. Data from the field experiment showed that plants in the marsh (interspecific competition treatment) experienced positive correlational selection on hypocotyl lengths and flowering time (McGoey and Stinchcombe 2009).

The population differences in flowering time could be due to a number of factors, including pollinators, mortality and light conditions in the competitive contexts, as well as stochastic forces such as drift. Since I have only one representative population for each of the two competitive contexts, I cannot determine if it is indeed distinctive competitive conditions that led to differences in shade avoidance traits produced.
Figure 2-4: Average hypocotyl lengths

A) Average hypocotyl lengths for the marsh (M) and pond (P) populations in the greenhouse. The data were divided by density treatment, since R:FR did not have a significant effect, I did not separate by R:FR treatment. Standard error bars are shown.

B) Average hypocotyl lengths for the marsh (M) and pond (P) populations in the field. These data were collected in the summer of 2008. Standard error bars are shown.
I did not find any evidence that the two populations differed in their sensitivity to competitive cues. There was a lot of variation among families for plasticity, however, demonstrating that there was sufficient genetic variation to enable a response to selection. I had hypothesized that the marsh population might show greater plasticity because of the variability that characterizes the marsh habitat. Alternatively, the marsh *Impatiens* may be under selection to be less plastic, if the shade avoidance response is ineffective. I may have missed real differences in plasticity because my sample sizes were too small. In contrast, both habitats may select for sensitivity to shading cues for different reasons. In the marsh, where both intraspecific and heterospecific densities are variable, being able to elongate and adjust flowering times would allow *Impatiens* to respond to a variety of competitors. Likewise, density varies in the monoculture plots, as does shading from the canopy, and sensitivity, particularly early in the season, may be advantageous. Elongating early would give a plant a relative advantage over the surrounding *Impatiens*. Data from the field experiment suggested that both the pond and marsh habitats induce elongation, but that the timing of these responses differed (see Appendix 1). Larger sample sizes from a wider range of habitats would be required to fully examine any differentiation in plasticity caused by competitive context.

*Density and Light Effects*

The differences seen in traits among treatments were primarily due to the density treatment. My light treatment appears to have had little effect on the traits produced. At first glance this might appear surprising, given the differences in the R:FR ratio between my high and reduced R:FR treatments (see Figure 2-5). However, despite the differences between the two types of light treatments, the R:FR ratios seen in the reduced R:FR
treatments were not as low as values that I have recorded in the field. I believe this is because lighting in the greenhouse was characterized by a high R:FR ratio, and that the levels in the reduced treatment were not depressed enough to elicit a shade avoidance response. Although the light treatments did not appear to have an effect on the average traits produced, they did have an influence on how traits affected fitness. For example in ambient R:FR and low density, there was selection for earlier flowering time, which was not true in low density and reduced R:FR. The effects of light quality differences can also be seen in the significant interaction in terms in my fitness model between light quality and shade avoidance traits.

Density had the expected effect on *Impatiens capensis*, causing the plants to elongate early in their development. Plants growing in high density also began flowering significantly earlier (p=0.027). Some plant species accelerate reproduction when elongation fails to lead to an increase in the R:FR ratio (Franklin 2008), and this may explain the density dependent flowering times observed here. Plants in both populations may be selected to reproduce before competition significantly reduces their energy resources and they face early mortality.

The significant interactions seen in my ANCOVA fitness model demonstrate that final height, and the size of internodes both explain variation in fitness when the effect of the density and R:FR treatments are taken into consideration. The consequences of elongation would depend on the competitive context. The results of my fitness model, and my selection analyses, demonstrate that the importance of vertical size depend on the light and competitive conditions. Selection on flowering time plasticity was also dependent on treatment. In the high density, reduced R:FR treatment, there was selection
for increased plasticity to density. In this treatment, there was the greatest signal of competition (reduced R:FR). The opposite was true in the low density, ambient R:FR treatment, where there was little signal of competition, and flowering time plasticity was disadvantageous.

The relative frequencies of different environments are theoretically important for the evolution of plasticity (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992). My models demonstrate that the composition of a landscape of competitive habitats would have effects on the relative advantage of plasticity. As seen in figure 2-3, a greater frequency of high density habitats, would make elongation plasticity more beneficial.

This study demonstrates the immense variation in Impatiens responses to different density conditions. To better assess how competitive context affects selection both on shade avoidance traits and plasticity, larger sample sizes are required from more source populations. I have shown that Impatiens derived from two competitive contexts differ in average shade avoidance traits. In addition, my study reinforces the concept that fitness effects of plasticity are dependent on the environment. Linking empirical information to theoretical predictions, I showed that the relative advantage of plasticity depends on the frequency of a given environment. Elucidating the impacts that ecological conditions have on the fitness effects of plasticity is critical to our understanding of its evolution.
Figure 2-5: R:FR ratio in the ambient and reduced R:FR treatments. Measurements were taken both above and below the level of the *Impatiens* plants. Standard error bars are shown.
Chapter 4:  
Conclusions and Synthesis

The goal of my thesis was to determine the impact of the competitive context experienced by *Impatiens* plants on the evolution and expression of shade avoidance syndrome. Species interactions such as competition are expected to be important both for the ecology and evolution of plants. Since plants are immobile, their competitors largely structure the ecological context they experience (Dudley and Schmitt 1996). Likewise, since they cannot respond behaviorally, or through moving to a new habitat, adaptive plastic responses are critical for adjusting to their environment (Schlichting 1986; Donohue 2003). Past studies had established that the *Impatiens capensis* shade avoidance responses were adaptive in intraspecific competition (Dudley and Schmitt 1996; Donohue *et al.* 2000a). However, the diverse marsh and wetland habitats where *Impatiens* grow in variable levels of interspecific competition had not been similarly investigated. I conducted two experiments to examine the effects of competitive context on shade avoidance syndrome in *I. capensis*.

My thesis has three main conclusions. First, both conspecifics and heterospecific competitors induce shade avoidance responses, and the shade avoidance phenotypes produced depend on the level and type of competition that an *Impatiens* plant experiences. Next, the marsh does not represent inherently poor habitat for *Impatiens* compared to the typical monoculture habitats. Lastly, that selection on shade avoidance traits does depend on the competitive context. All of these findings have important implications for the evolutionary ecology of plasticity in *I. capensis*.  

68
Shade avoidance traits

The phenotypes produced by Impatiens are known to depend on the R:FR ratio of light (Dudley and Schmitt 1996). My thesis experiments demonstrate that canopy cover, heterospecific, and conspecific competitors, all uniquely affect light parameters. The differences in both R:FR and PAR are relevant for shade avoidance traits. Plants growing in low PAR and R:FR conditions will have less energy resources available to them and would have more to gain from shade avoidance through elongation. At the same time there may be fitness consequences to growing in these low light quality and quantity conditions.

In my field experiment, the timing of elongation was different when heterospecific competitors were present, as was flowering time (Table 1-2). Since the marsh plots were randomly placed, the differences in elongation timing between the interspecific competition and removal plots revealed the significant plasticity utilized by Impatiens capensis. The significant differences in the greenhouse treatments also demonstrate the sensitivity of Impatiens to light cues. The ability of Impatiens to adjust their phenotypes is likely vital to their ability to grow in such diverse habitats. There is growing interest in the ecological consequences of phenotypic plasticity (Callaway et al. 2003; Miner et al. 2005). By elongating early when growing with heterospecifics, I. capensis plants could exploit light resources before being completely shaded by more dominant competitors. Thus plasticity would act to mitigate the stress of competition and facilitate coexistence between Impatiens and a diverse array of other plants in the marsh.

The shade avoidance response could allow both a wider range of habitable communities for Impatiens, and greater community diversity, by facilitating the
coexistence of *Impatiens* with other, more dominant, species. I believe this topic warrants further investigation. The question of how biodiversity persists is central to the field of evolutionary ecology. Studies that manipulate the ability of plants to respond to competitors (*i.e.* how plastic they are) and examine the consequences for species coexistence could help us to understand how plasticity influences plant interactions and diversity.

**Reproductive output in the marsh habitat**

Based on the observations that *Impatiens* plants were found at much lower densities in the marsh and grew with more dominant competitors, I had assumed that the marsh habitat was of poor quality, when compared to those experienced by the monoculture populations. Consequently, an initially surprising result was the dramatic differences in absolute reproductive output between the observational plots and the two marsh treatments. Both removal and interspecific competition plots had a greater average reproductive output than plants from observational plots (Figure 1-1). This demonstrates that the marsh is not just a sink for seeds from elsewhere on the reserve, but rather represents suitable habitat for *Impatiens*, despite the intense interspecific competition.

There are several possible explanations for why plants in observational plots did relatively poorly, which are not mutually exclusive. Firstly, those plants growing in observational plots did not face the dominant heterospecifics found in the marsh, but they did grow with a greater total number of competitors (in this case, intraspecific competitors). *Impatiens capensis* are known to experience density-dependent mortality (*Schmitt et al.* 1987), therefore these high *Impatiens* densities may have led to self-
thinning in these plots. There was much higher early mortality in the observational plots when compared to the marsh (the plants that died early typically had an absolute fitness of zero, since they had yet to flower and produce fruits). A second factor is that stochastic forces inherent to the observational plots (e.g., flooding, falling branches) and absent from the marsh plots, which affected survival, and therefore fitness. While these risks might be rare in the absolute sense, the relative risk of them differs qualitatively between the habitats. Past studies have also documented *Impatiens* deaths due to flooding (Lively *et al*. 1995).

A likely important factor is the vegetation growing above observational plots. All the observational plots experienced some shading from trees, and the percentage of canopy cover was negatively correlated with fitness (r=-0.44, p=0.055). The plants in observational plots received the lowest amount of photosynthetically active light, which was positively associated with fitness (r=0.53, p<0.01).

Finally, because my study was observational in nature, there could be unmeasured abiotic factors (such as nutrients) or biotic factors (pathogens), which were more benign in the marsh and more stressful in observational plots. While formally a possibility, it remains difficult to reconcile the observation of dense monocultures of *Impatiens* with the hypothesis that these populations are somehow lacking critical nutrients. I believe that the explanations described above (density dependent mortality, stochastic forces, shading from canopy trees) are more likely.

The number, and type of flowers produced by marsh and observational plants had a direct effect of fitness. Marsh plants produced far more chasmogamous flowers, and far more pollinators were observed in the marsh. The chasmogamous flowers of *I. capensis*
can be pollinated by several different bee species and hummingbirds (Steets et al. 2007). Bees were constantly seen pollinating *I. capensis* in the marsh, but rarely in the observational plots (B. McGoe, pers. obs.). I saw several hummingbirds over the course of the summer in the marsh, but none in the observational plots. Thus, there were more chasmogamous flowers, which were presumably pollinated more frequently in the marsh. Since chasmogamous fruits produce a greater number of seeds (3.475 –vs- 1.2), the difference in seed production was further exaggerated.

The observation that *Impatiens capensis* growing in the marsh reproduce so successfully is very relevant for the evolution of plasticity. Theoretical models have suggested that the relative fitness of populations can have significant impacts on the evolution of plasticity (Via and Lande 1985; Gomulkiewicz and Kirkpatrick, 1992). I did not investigate the population structure at my field site, and I cannot comment on the amount of gene flow between different *Impatiens* habitats. However, the vast production of seeds, that could be dispersed secondarily to other habitats, make selection on shade avoidance traits in the marsh significant to the evolution of plasticity in the entire Koffler Reserve population.

**Selection depends on competitive context**

My thesis demonstrates that the patterns of natural selection on shade avoidance traits depend on the environmental context where *I. capensis* plants are growing. This is supported by both the field and greenhouse experiments. In the field experiment, there were differences in directional nonlinear and correlational selection on traits depending on the presence or absence of heterospecific competitors. In the greenhouse experiment,
the two source groups had significantly different hypocotyl lengths and flowering times. Since they were growing in a common environment, and I had reduced maternal effects, this demonstrates genetic differences between plants from a monoculture and marsh habitat. The differences were qualitatively consistent with observations from the field experiment and are suggestive of historically divergent natural selection on shade avoidance traits. However, my experiments cannot rule out other evolutionary forces, nor can I be sure that the competitive setting is primarily responsible for the differences. Variable selection on traits is extremely important for the evolution of plasticity. If the two different environments select for different phenotypes, and there is gene flow between them, then this could indirectly select for increased plasticity (Bradshaw 1965; Kingsolver and Huey 1998). A reciprocal transplant study of lines derived from plants in different competitive contexts could help determine if *Impatiens* have adapted to be best able to respond to their own competitive environment. In addition, it would be interesting to quantify gene flow between *Impatiens* patches in an environment such as at the Kofler Scientific Reserve where different kinds of habitat are found in close proximity to one another.

**Additional future directions for research**

Plastic traits are often considered individually, without acknowledgement of the complex effects of ecological and environmental factors. The scarcity of empirical evidence for costs and limits on plasticity has led to the hypothesis that organisms are limited in their responses because they must react to many interactions simultaneously (Valladares *et al.* 2007). For example, damage from herbivory may limit a plant’s ability
to respond plastically to other variables (Valladares et al. 2007). Plant populations may face strong competition and herbivores at the same time; in fact high-density plant populations can lead to an increased population of herbivores (Cipollini 2004). Indeed, a positive correlation between intraspecific competition and herbivory has been found in *Impatiens capensis* populations (Steets et al. 2006). Theory predicts an inherent trade-off between competing through increased vegetative growth and defending against herbivory (Herms and Mattson 1992). These observations suggest that herbivory may act as an ecological limit, preventing perfect plastic responses to competition. While conducting my field experiment, I noticed significant herbivory on *I. capensis* plants growing in the marsh. I am interested in knowing the effects that herbivory has on the ability of *Impatiens* to sense and respond to competitors.

Another observation made during my field experiment was the large discrepancy between the numbers of pollinators found in the marsh compared to the monoculture populations. The huge numbers of bees seen in the marsh may have been influenced by an ongoing *Lythrum salicaria* experiment (Colautti and Barrett, unpublished). Regardless, I think examining the effects of pollinator availability on the mating system of *Impatiens* would be interesting. Steets and colleagues (2006, 2007) have found that both competition and herbivory affect the mating system in *I. capensis*. Examining how competitive context affects pollinator numbers, and in turn how pollinators affect mating system would be a worthwhile synthesis of different areas of research.
Final Thoughts

The implications of plasticity for the evolution and ecology of species are increasingly being appreciated. Plasticity is universal among species, and can occur in a wide array of traits in response to a vast number of cues. Plasticity provides a fascinating opportunity to study the reciprocal effects that the evolution and ecology of a species have on one another. With this thesis, I have demonstrated that competitive context has significant impacts on the shade avoidance syndrome in Impatiens capensis. This is an example of ecological factors acting as a selective agent on reaction norms. A growing synthesis between the fields of ecology and evolution will serve to further both. The study of phenotypic plasticity is an excellent realm for studying evolutionary ecology.
References Cited


Appendix One

Table A-1. Mean phenotypic traits (± 1 s.e.) for experimental and observational plants. (A) Plants from removal and interspecific competition treatments, along with F-statistics for the treatment effect. (B) Plants from the observational plots, along with with F-statistics for the plot-effect. O1 is the pond population from which seeds for the greenhouse experiment were collected.

<table>
<thead>
<tr>
<th>A. Trait (units)</th>
<th>Removal</th>
<th>Interspecific Competition</th>
<th>F_{1, 20}</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>May Height (cm) ***</td>
<td>6.1 ± 0.091</td>
<td>8.3 ± 0.015</td>
<td>35.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>June Height (cm) ***</td>
<td>20.5 ± 0.34</td>
<td>26.8 ± 0.52</td>
<td>34.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>July Height (cm)</td>
<td>62.5 ± 1.13</td>
<td>56.7 ± 1.23</td>
<td>0.93</td>
<td>0.35</td>
</tr>
<tr>
<td>August Height (cm) **</td>
<td>82.8 ± 1.59</td>
<td>67.8 ± 1.60</td>
<td>9.82</td>
<td>0.0052</td>
</tr>
<tr>
<td>September Height (cm)</td>
<td>94.8 ± 1.52</td>
<td>90.7 ± 1.70</td>
<td>0.01</td>
<td>0.9157</td>
</tr>
<tr>
<td>Maximum Height (cm) **</td>
<td>69.9 ± 1.94</td>
<td>56.4 ± 1.73</td>
<td>8.99</td>
<td>0.0071</td>
</tr>
<tr>
<td>Flowering time (days)*</td>
<td>61.0 ± 0.2</td>
<td>61.9 ± 0.27</td>
<td>5.36</td>
<td>0.0313</td>
</tr>
<tr>
<td>Hypocotyl length (cm)</td>
<td>8.5 ± 0.17</td>
<td>8.8 ± 0.19</td>
<td>2.52</td>
<td>0.1279</td>
</tr>
<tr>
<td>First Internode length (cm) **</td>
<td>8.0 ± 0.14</td>
<td>9.4 ± 0.18</td>
<td>8.34</td>
<td>0.0091</td>
</tr>
<tr>
<td>Total number of internodes **</td>
<td>11.9 ± 0.175</td>
<td>9.8 ± 0.177</td>
<td>15.33</td>
<td>0.0009</td>
</tr>
<tr>
<td>Early growth rate (cm/day)***</td>
<td>0.47 ± 0.011</td>
<td>0.62 ± 0.012</td>
<td>23.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Late growth rate (cm/day)***</td>
<td>0.65 ± 0.037</td>
<td>0.35 ± 0.035</td>
<td>19.09</td>
<td>0.0003</td>
</tr>
<tr>
<td>Absolute fitness (seeds)**</td>
<td>43.5 ± 3.2</td>
<td>21.6 ± 2.0</td>
<td>12.14</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Trait (units)</th>
<th>O1</th>
<th>O2</th>
<th>O3</th>
<th>O4</th>
<th>F_{796}</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>May Height (cm) ***</td>
<td>10.45 ± 0.23</td>
<td>10.85 ± 0.24</td>
<td>13.47 ± 0.33</td>
<td>11.56 ± 0.24</td>
<td>25.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>June Height (cm) ***</td>
<td>35.92 ± 0.76</td>
<td>46.37 ± 1.00</td>
<td>48.78 ± 1.16</td>
<td>29.55 ± 0.69</td>
<td>81.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>July Height (cm) ***</td>
<td>51.30 ± 1.19</td>
<td>61.84 ± 1.68</td>
<td>65.00 ± 1.55</td>
<td>37.80 ± 1.03</td>
<td>53.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>August Height (cm) ***</td>
<td>53.23 ± 1.50</td>
<td>66.51 ± 2.14</td>
<td>71.22 ± 1.59</td>
<td>40.89 ± 1.15</td>
<td>35.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>September Height (cm) ***</td>
<td>69.88 ± 1.34</td>
<td>80.42 ± 2.12</td>
<td>145.4 ^</td>
<td>50.91 ± 0.99</td>
<td>14.91</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maximum Height (cm) ***</td>
<td>50.93 ± 1.53</td>
<td>50.92 ± 2.03</td>
<td>47.66 ± 2.07</td>
<td>36.36 ± 1.22</td>
<td>15.60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypocotyl length (cm) ***</td>
<td>18.37 ± 0.48</td>
<td>9.03 ± 0.40</td>
<td>13.70 ± 0.51</td>
<td>6.58 ± 0.24</td>
<td>105.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>First Internode length (cm) ***</td>
<td>12.74 ± 0.41</td>
<td>16.76 ± 0.40</td>
<td>18.71 ± 0.50</td>
<td>11.79 ± 0.28</td>
<td>29.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total number of internodes ***</td>
<td>5.25 ± 0.14</td>
<td>8.71 ± 0.25</td>
<td>7.65 ± 0.20</td>
<td>6.65 ± 0.17</td>
<td>31.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Early growth rate ***</td>
<td>0.85 ± 0.023</td>
<td>1.18 ± 0.032</td>
<td>1.16 ± 0.034</td>
<td>0.60 ± 0.020</td>
<td>85.36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Late growth rate</td>
<td>0.049 ± 0.030</td>
<td>0.13 ± 0.027</td>
<td>0.071 ± 0.025</td>
<td>0.072 ± 0.018</td>
<td>1.15</td>
<td>0.33</td>
</tr>
<tr>
<td>Absolute fitness ***</td>
<td>9.45 ± 0.82</td>
<td>8.65 ± 1.36</td>
<td>3.05 ± 0.52</td>
<td>5.91 ± 0.55</td>
<td>10.96</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

^ Only one individual was still alive in O3 in September
Figure A-1: Average heights through time for the two marsh treatments (removal and interspecific competition) and natural, monoculture plots (observational plots).