Competition-driven selection on a native plant following a species invasion

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A Dissertation presented to the Graduate Faculty
of the University of Virginia in Candidacy for the Degree of
Doctor of Philosophy

Department of Biology

University of Virginia
May, 2014

ABSTRACT

Invasive plants often evolve rapidly in response to novel environments. We are only now beginning to explore whether native plants may also evolve in response to these novel competitors. Research suggests that at least some invaded native plant populations are capable of adaptive evolutionary responses. The underlying mechanisms driving this evolutionary response, however, remain largely unexplored. For example, our knowledge of how invasive plants may alter the strength and direction of natural selection on specific traits of native plants is limited. Here I examine the ecological and evolutionary influence of an invasive jewelweed, *Impatiens glandulifera*, on a native congener, *I. capensis*. I begin with a review of our current understanding of competition-driven plant evolution in the context of the character displacement literature. Then, using a series of greenhouse, manipulated field, and natural plant community studies, I explore how pollinator-mediated competition and vegetative competition with the invasive plant influence the reproductive success and selection regime experienced by the native congener.

The results show that pollinator sharing between the invasive and native jewelweeds was common and may affect selection on floral traits. When the invasive jewelweed was present, selection favored native jewelweed plants with shorter corolla heights. A follow-up study shows, however, that this result may not be consistent across years. While negative directional selection on corolla height was maintained in a complex community that included two additional competitors, it was no longer significant under pair-wise competition with the invasive jewelweed. In addition to potentially affecting phenotypic selection on floral traits, the invasive jewelweed also altered selection on vegetative traits in the native congener. When the invasive plant was present in both the greenhouse and the field, selection favored native jewelweed individuals investing less in rapid upward growth and more in branching and fruiting potential. Other factors, however, may limit the ability of native jewelweed populations to evolve in response to this altered selection pressure. Overall, this dissertation demonstrates that both pollinator-mediated and vegetative competition with invasive plants can significantly alter phenotypic selection in native plant communities.

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DEDICATION

I dedicate this dissertation to the many colleagues, friends, and family members who have helped me throughout this project. First, I thank my committee for creating a challenging and supportive environment for me to explore my research questions. My advisor, Deborah Roach, provided encouragement and much needed perspective. She was always ready to offer feedback, whether I stopped in her office or called her frantically from the field. I am grateful for the many ways she challenged me to improve as a scientist and a writer. My first reader, Laura Galloway, offered me a second home at UVA. Working with her and participating in her lab meetings greatly enriched my graduate experience. I am especially thankful for the clear and constructive feedback she gave me on all of my chapters. Henry Wilbur pushed me to think deeply about my experimental designs and to place my work within a broader historical context. From the start of graduate school, his encouragement inspired me to be a better scientist and natural historian. Dave Carr taught me biostatistics and then served as an invaluable resource whenever I encountered new statistical challenges. He also frequently asked thought-provoking questions that strengthened my experiments. Bob Cox stepped in just when I most needed to discuss the challenges inherent in selection analyses. I thank him for his immediate and thoughtful responses to my many questions.

I thank the many additional faculty members, post docs, and graduate students who contributed to this research by reading drafts or discussing experiments. They are: Janis Antonovics, Butch Brodie, Eric Nagy, T'ai Roulston, Doug Taylor, Vince Formica, Joel McGlothlin, Holly Prendeville, Malcolm Augat, Andrea Berardi, Ashley Dai, Catherine Debban, Francis Kilkenny, Karen Barnard-Kubow, Brian Sanderson, Brittany

Sutherland, Ray Watson, and Corlett Wood. I would especially like to thank my lab mates, Melissa Aikens, Danielle Racke, and Eric Yoshizuka.

I also thank colleagues from other institutions who introduced me to the *Impatiens* study system. Nava Tabak, Eric von Wettberg, Lynn Adler, and Nicole Soper Gordon helped me locate study sites and taught me how to care for *Impatiens* plants. When I was searching for the right study system, Bryan Connolly told me about the *Impatiens glandulifera* invasion and sent me my first batch of seeds.

I am indebted to the many homeowners and institutions who allowed me to study on their properties. I would particularly like to thank the staff of the University of Massachusetts Crop and Animal Research and Education Center for permitting me to use their resources for two summers. Kyle Bostrom, Keith Lilly, Shawn Walker, and Neal Woodard all kindly volunteered whenever I needed help mowing a field, fixing an irrigation system, or moving hundreds of plants into a barn before a hurricane hit. I am especially grateful to my UMass-Amherst host, Randy Prostak, who helped me with everything from setting up experiments to learning the local plants. He and Susan Prostak volunteered countless times to water plants when I was away.

The research presented here would not have been possible without the help of many undergraduates who enthusiastically worked alongside me in the greenhouse and the field. They include: Bridget Bailey, Nikki Forrester, Surbhi Gupta, Marcus Leibowitz, Michelle McCauley, Tim Park, Morgan Wagner, and Eric Wice. I am also very grateful to our greenhouse manager, Wendy Crannage, for caring for thousands of monstrous jewelweed plants over the past five years.

Finally, I thank my parents, Bruce and Kathie Beans, my brother, Christopher Beans, and my husband, Ravi Romel Sharma. My parents and brother always enthusiastically encourage my interests. Romel tirelessly supported my research goals, even when that meant long field seasons apart. When I couldn't finish my last experiment on my own, he worked alongside me in the field for three weeks. I thank all of my family for being a constant source of encouragement, humor, and love.

GENERAL INTRODUCTION

The success of invasive plants is often attributed to their ability to rapidly evolve in response to novel surroundings (Maron *et al*. 2004, Müller-Schärer *et al*. 2004, Montague *et al*. 2008, Kilkenny and Galloway 2013). Whether native plants may also evolve in response to novel competitors remains less well understood (Strauss *et al*. 2006). We know that invasive plants are often strong competitors for resources such as pollinator services, water, light, nutrients, and space (Martin 1999, Adams and Engelhardt 2009, Morales and Traveset 2009, Vilá *et al.* 2011). The intense competitive environment shaped by these species may result in altered natural selection on native plants (Leger and Espeland 2010). Invasive plants that are closely related to their native neighbors, and therefore share a similar niche space, may be especially likely to drive evolutionary change through competitive interactions.

Recent research suggests that native plants can, in fact, evolve in response to invasive competitors (Oduor 2013). The native plants *Lotus wrangelianus* (Lau 2006), *Sporobolus airoides* (Mealor and Hild 2007), *Elymus multisetus* (Rowe and Leger 2010), and *Pilea pumila* (Lankau 2013) have all been shown to evolve an increased ability to compete in invaded communities. More studies are needed, however, before we can answer whether native plant populations commonly respond to invasions through adaptive evolution. Furthermore, the mechanisms behind adaptive evolution in invaded communities remain largely unexplored. For example, there is currently a lack of studies that estimate how invasive competitors alter natural selection on specific traits.

The scarcity of studies exploring the evolutionary responses of native plants in response to invasive competitors may stem from a more general lack of focus on the evolutionary consequences of plant-plant interactions (Thorpe *et al*. 2011). Extensive research has been directed at understanding the ecological and evolutionary consequences of herbivores, light availability, climate, pollinator preferences, and a wide variety of other environmental stimuli (Agrawal 2005, Benitez-Vieyra *et al*. 2006, Montague *et al*. 2008, von Wettberg *et al*. 2008). Meanwhile, studies of plant-plant interactions have largely focused on the ecological consequences of competition without investigating the potential for subsequent evolutionary change (Thorpe *et al*. 2011). This gap in the literature may be a response to long-standing ecological theory that downplays the importance of plant-plant interactions in shaping communities in favor of abiotic or random processes (Gleason 1926, Hubbell 2005). Recent studies of competition-driven plant evolution, especially those in the invasion biology literature, suggest that this area of research deserves a closer look (Oduor 2013).

The role of plant competitors in shaping the evolution of floral morphology is one line of research into competition-driven plant evolution that has received relatively greater attention. Pollinator behavior can drive the evolution of floral shape (Fenster *et al*. 2004). Plant competitors may alter that behavior, or alter the consequences of existing behavior by increasing the likelihood of heterospecific pollen deposition (Mitchell *et al*. 2009). Studies exploring this topic in native plant communities have repeatedly found evidence that plants can alter natural selection on the floral form of their competitors (Fishman and Wyatt 1999, Caruso 2000, Smith and Rausher 2008, Wassink and Caruso 2013). Because invasive species often share the same pollinators as native species

(Morales and Traveset 2009), and may invade communities in large numbers, they may have the potential to greatly influence natural selection on the floral traits of native plants. This direction of research, however, has received little attention (Mitchell *et al.* 2009).

Our current understanding of how plants influence the evolution of competitor floral traits comes largely from studies of two-species systems (Smith and Rausher 2008, Wassink and Caruso 2013). Some argue, however, that the complexity of plant communities may inhibit competition-driven evolution (Connell 1980). According to this argument, an evolutionary response to competition requires that one species has constant interaction with another, such that the competitor imposes strong and continuous selection on the focal species. If another species is added, this new competitor may interrupt the frequency of contact between the initial two species, as well as impose selection on a given trait that may be opposite to that of the initial competitor, resulting in zero net selection. Some argue, however, that invasive plants may direct floral evolution in native plant even in these more complex communities because the often intense competitive effect of invasive species may overwhelm the influence of any other native plant competitors (Leger and Espeland 2010, Strauss 2014). To resolve this issue, we need empirical tests that tease apart the effect of one versus a community of competitors for pollination on the selection regime of a focal species (Mitchell *et al.* 2009).

In addition to altering the evolutionary trajectory of native plant floral traits, invasive plants may also influence the evolution of vegetative traits. A growing body of literature suggests that native plants may evolve to better compete vegetatively with invasive species (Lau 2006, Mealor and Hild 2007, Lankau 2013). The exact targets of natural selection that confer this greater competitive ability, however, are often unclear. In order to gain a deeper understanding of the process underlying competition-driven adaptive evolution in native plants, more studies are needed that directly test how an invasive species alters selection on specific vegetative traits such as growth rate and branching formation. When the traits studied are known to be heritable, these selection studies can also be used to predict the potential for future adaptive change in invaded communities.

This dissertation examines the ecological and evolutionary consequences of competition with an invasive plant. I focus primarily on the effect of the invasive jewelweed, *Impatiens glandulifera,* on the closely related native species, *Impatiens capensis* (spotted jewelweed), in the northeastern United States. Using a series of greenhouse, manipulated field, and natural plant community studies, I explore how pollinator-mediated competition and vegetative competition with the invasive jewelweed influence the reproductive success and selection regime experienced by the native congener.

The first chapter reviews our current understanding of competition-driven plant evolution in the context of the plant character displacement literature. Character displacement is the evolutionary process that occurs when competing species respond to selection to increase their mean difference in a trait associated with resource use or reproduction (Brown and Wilson 1956, Mayr 1970, Pfennig and Pfennig 2009). This process results in a divergence in phenotypes, which reduces the overlapping resource needs or reproductive interactions of the competing species and permits their coexistence. In this review, I address the paucity of character displacement examples in plants and then question whether plants meet the requirements for character displacement to occur. I then discuss the criteria for demonstrating character displacement and describe methods that have been successfully used to meet those criteria in the plant literature. Finally, I offer some tools that are especially amenable for testing character displacement in plants. While more empirical studies are needed, the few plant systems in which character displacement hypotheses have been rigorously tested suggest that character displacement, and therefore competition-driven evolution, may play an important role in shaping plant communities.

The second chapter examines the ecological and evolutionary consequences of pollinator-mediated competition between *I. capensis* and the invasive congener, *I. glandulifera*. I first use a pollinator choice experiment to test whether bumblebees show a preference for the native or invasive jewelweed, and whether they commonly move between the two species. I then use a hand pollination experiment to test whether pollen from the invasive jewelweed inhibits seed production in the native plant, and whether there is any evidence of hybridization. Finally, using a competition-for-pollination study and selection analysis (Lande and Arnold 1983), I test how the invasive jewelweed affects the seed production and selection regime of the native jewelweed. I show that, although bumblebees greatly prefer the invasive jewelweed, movement between the two species commonly occurs. Invasive pollen can inhibit seed production in *I. capensis*, but there is no evidence of hybridization. Most notably, I find that selection on *I. capensis* is altered by the presence of the invasive species. When the invasive jewelweed is present, selection favors *I. capensis* plants with shorter corolla heights. These results suggest that pollinator-mediated competition with invasive species has the potential to influence the evolutionary trajectories of native plant populations.

The third chapter builds on the findings from Chapter Two and tests how a more complex plant community alters selection on *I. capensis* floral traits. Using an experimental design proposed in the herbivory literature (Strauss *et al*. 2005), but not yet applied to studies of plant-plant interactions, I estimate selection on *I. capensis* floral traits when growing in pair-wise competition with three different competitor species and when in competition with all competitor species at once. I show that selection on *I. capensis* floral traits is dependent on the identity of competitors. Furthermore, selection is diffuse, meaning that selection on corolla height experienced under one of the pair-wise competition treatments is altered when more competitor species are added to the community (Strauss *et al*. 2005). Additionally, the overall intensity of selection is greatest in the more complex community. These results suggest that, rather than inhibiting selection as some have proposed (Connell 1980), increasing community complexity may at times increase the potential for competition-driven selection in plant populations.

The fourth chapter addresses the potential for invasive species to alter phenotypic selection on the vegetative traits of native plants. I first use a greenhouse experiment to test whether the fitness and selection regime of the native jewelweed differs when growing with its own species versus with the invasive jewelweed. I then use an invasive species removal experiment to test whether the results from the greenhouse experiment match what is occurring in a natural plant community. Finally, I use a survey of natural populations, which includes eight *I. capensis* populations growing with the invasive jewelweed and eight growing without it, to test whether the vegetative growth of the native jewelweed is altered in the presence of the invasive congener. In both the greenhouse and the field, I find that the invasive jewelweed alters phenotypic selection on *I. capensis* vegetative traits. Both studies show that when the invasive species is present, selection favors *I. capensis* individuals investing less in rapid upward growth and more in branching and fruiting potential. The survey of natural populations shows, however, that there has not been a consistent vegetative response to the invasion. This research suggests that invasive plants have the potential to greatly influence phenotypic selection on the vegetative traits of native plants. The ability of native plant populations to evolve in response to this altered selection pressure, however, may be limited by other factors.

Overall, this dissertation demonstrates that both pollinator-mediated and vegetative competition with invasive plants can result in altered natural selection in native plant communities. If native plants evolve in response to these altered selection pressures, the potential for coexistence with invasive plants seems possible. From a plant conservation standpoint, these results suggest that we may be able to predict which invaded plant communities will persist through adaptive evolution, and which will need remediation efforts to survive (Leger 2008). More generally, these results support the growing argument that competition plays a larger role in driving plant evolution than was previously accepted (Thorpe *et al*. 2011). Most importantly, this dissertation reveals that competition-driven phenotypic selection is a potentially important underlying mechanism at play as a novel species is integrated into an existing plant community.

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CHAPTER ONE:

The case for character displacement in plants

As published: Beans, C.M. 2014. The case for character displacement in plants. Ecology and Evolution. 4:852-865.

ABSTRACT

The evidence for character displacement as a widespread response to competition is now building. This progress is largely the result of the establishment of rigorous criteria for demonstrating character displacement in the animal literature. There are, however, relatively few well-supported examples of character displacement in plants. This review explores the potential for character displacement in plants by addressing the following questions: (1) Why aren't examples of character displacement in plants more common? (2) What are the requirements for character displacement to occur and how do plant populations meet those requirements? (3) What are the criteria for testing the pattern and process of character displacement and what methods can and have been used to address these criteria in the plant literature? (4) What are some additional approaches for studying character displacement in plants? While more research is needed, the few plant systems in which character displacement hypotheses have been rigorously tested suggest that character displacement may play a role in shaping plant communities. Plants are especially amenable to character displacement studies because of the experimental ease with which they can be used in common gardens, selection analyses, and breeding designs. A deeper investigation of character displacement in plants is critical for a more complete understanding of the ecological and evolutionary processes that permit the coexistence of plant species.

KEY WORDS: Ecological character displacement, niche differentiation, plant–plant interactions, reproductive character displacement

INTRODUCTION

Patterns of altered morphology in sympatric versus allopatric populations have long been described in the plant literature. *Pachycereus pringlei* grows taller where its range overlaps with other cacti species (Cody 1984), *Solanum grayi* blooms smaller when in contact with *Solanum lumholtzianum* (Whalen 1978), and *Arenaria uniflora* shifts from outcrossing to selfing where intermixed with *Arenaria glabra* (Fishman and Wyatt 1999). Character displacement, first defined in the animal literature, is frequently used to explain these patterns. Character displacement is the process whereby competing species respond to selection to increase their mean difference in a trait associated with resource use or reproduction (Brown and Wilson 1956, Mayr 1970, Pfennig and Pfennig 2009). The end result is a divergence in phenotypes, which reduces the overlapping resource needs or reproductive interactions of the species and permits their coexistence. This process of character displacement results in a pattern of competitors exhibiting greater trait divergence in regions of sympatry than in regions of allopatry (Brown and Wilson 1956).

Character displacement was first described in animals by Brown and Wilson (1956). Following this publication, both plant and animal biologists began documenting cases of character divergence in sympatric species and attributing these patterns to character displacement. Many of these early studies attracted controversy because they relied solely on patterns that could otherwise be explained by any number of factors, such as variation in resource availability between sympatric and allopatric populations, ecological sorting, or even chance (Mayr 1963, Grant 1975, Arthur 1982, den Boer 1986).

Despite the initial controversy, the evidence for character displacement as a widespread response to harmful competitive or reproductive interactions is now building (Losos 2000, Pfennig and Pfennig 2009). This progress is largely the result of the establishment of rigorous criteria for demonstrating character displacement (Grant 1994). These criteria include ruling out alternative hypotheses such as chance or ecological sorting, as well as establishing that the character divergence is driven by interspecific competitive or reproductive interactions (Taper and Case 1991).

Much progress has been made since the establishment of these criteria, but the support for character displacement still rests primarily on a limited number of animal species (i.e., *Plethodon* salamanders (Adams *et al*. 2007), sticklebacks (Pritchard and Schluter 2001), finches (Grant and Grant 2006), *Anolis* lizards (Losos and Spiller 1999), Mexican spadefoot toads (Martin and Pfennig 2011)). Although theoretical and experimental frameworks for studying character displacement in plants were developed decades ago (Levin 1970, Fowler and Antonovics 1981), there are still relatively few well-supported examples of plant character displacement (Schluter 2000a, Dayan and Simberloff 2005).

The goal of this review is to inspire a greater focus on this field by pointing to the lack of character displacement studies in plants and by offering tools – both new and old – for rigorously testing the character displacement hypotheses in plant systems. I will explore our current understanding of character displacement in plants by addressing the following questions: (1) Why aren’t examples of character displacement in plants more common? (2) Under what circumstances is character displacement likely to occur in plants? (3) What are the criteria for testing the pattern and process of character displacement and what methods can and have been used to address these criteria in the plant literature? (4) What are some additional approaches for studying character displacement in plants? A deeper investigation into character displacement in plants is critical for a more complete understanding of ecological and evolutionary forces that shape plant communities.

BACKGROUND

There are two forms of character displacement – ecological and reproductive. I define ecological character displacement here as the evolution of morphological, behavioral, physiological, or developmental trait divergence in one or more sympatric species in response to interspecific competition for limited resources (Brown and Wilson 1956, Hansen *et al*. 2000). I define reproductive character displacement as the evolution of divergence in any of these same traits in one or more sympatric species in response to competition for the resource of pollinators, or in response to the costs associated with sharing pollinators with another species (Pfennig and Pfennig 2009). These costs may include the loss of pollen to other species, the clogging of stigmas with heterospecific pollen, or the potential for unfit hybrid seed.

Reproductive character displacement may occur between distantly or closely related species. Distantly related species may evolve in response to competition for pollinators or the fitness costs associated with heterospecific pollen transfer. Closely related species capable of hybridization may undergo reinforcement, the evolution of traits that minimize costly mating or hybridization between recently diverged species (Hopkins 2013), which is defined here as one form of reproductive character displacement (Pfennig and Pfennig 2009). While intraspecific plant competition may also lead to character displacement and subsequent speciation, within-species interactions are beyond the scope of this review.

The line between reproductive and ecological character displacement is sometimes unclear. For example, a trait such as the timing of seed germination may impact the competitive ability of the seedling (ecological character displacement) as well as the flowering period (reproductive character displacement) (Armbruster 1986, Pfennig and Pfennig 2009).

Character displacement was first defined as a pattern of species trait divergence in regions of sympatry (Brown and Wilson 1956). Because this pattern could result from processes other than selection to reduce competitive or reproductive interactions, character displacement is now more commonly defined as the evolutionary process itself, rather than the resulting pattern (Pfennig and Pfennig 2009). This process of character displacement typically results in one of two patterns. The first is a shift in a trait mean where a species range overlaps with that of a competitor. The second is a pattern of overdispersion of trait means within an assemblage of ecologically similar species (Schluter 2000a).

Biologists have long sought to understand the relative importance of ecological, evolutionary, and stochastic forces in shaping plant communities (Cowles 1899, Clements 1916, Gleason 1926). The study of character displacement in plants has the potential to reveal whether competition-driven plant evolution plays an important role in this process. Both ecological and reproductive character displacements may promote the coexistence of species by enhancing niche differences and therefore reducing the magnitude of harmful interspecific interactions relative to intraspecific interactions (Hochkirch *et al*. 2007, Pfennig and Pfennig 2009). Ecological character displacement promotes species coexistence by reducing the competition for resources that might otherwise lead to competitive exclusion. Reproductive character displacement promotes species coexistence by reducing the competition for pollinators or costly interspecific reproductive interactions that might otherwise lead to low reproductive output and population decline described as “reproductive exclusion” (Pfennig and Pfennig 2009). The potential for a population to undergo character displacement therefore may mean the difference between survival in a new niche and local extinction. To date, however, we do not have enough examples of character displacement in plants to assess its relative role in structuring plant communities.

WHY AREN’T THERE MORE EXAMPLES OF CHARACTER DISPLACEMENT IN PLANTS?

Despite its potential relevance to our understanding of basic ecological and evolutionary processes, earlier reviews have uncovered relatively few studies of character displacement in plants (Levin 1970, Schluter 2000a, Dayan and Simberloff 2005). I surveyed the character displacement literature to assess whether there are still relatively few studies in plants. In June of 2012, I conducted a Google Scholar search for publications that contained the term character displacement in the title. The search returned 323 results. I updated this search in December of 2013. From these results, I selected peer-reviewed studies of character displacement involving two or more species where new data were presented. I only included studies where the explicit goal was to test for character displacement. For example, there are many studies that investigate resource partitioning among competitors. I did not select these studies unless the authors tested whether resource partitioning was achieved through evolved trait differences rather than some other ecological interaction such as plastic responses to competition or ecological sorting.

The results of this survey show that there are still few published studies of character displacement in plants (Fig. 1, Table A1). Since 1956 when the term was coined, there have been 150 animal studies and only 14 plant studies for which character displacement was an important enough focus of the research to be included in the title. While my literature search was not exhaustive, as studies of character displacement exist that do not include the term in the title, these results strongly indicate an overall trend toward more character displacement research in the animal versus plant literature. Why are there so few examples of character displacement in plants?

Language

The difference between the prevalence of character displacement studies in the animal versus plant literature may be an issue of language. Perhaps the plant literature describes the process of character displacement using different terms. To test this hypothesis, I compiled a list of alternate terms that may be used instead of character displacement to describe the same process. For ecological character displacement, I expected that the plant literature might also refer to this process as selection for evolution of niche partitioning or niche differentiation. For reproductive character displacement, I expected that the plant literature might also refer to this process as reinforcement, the Wallace effect, or natural selection for reproductive isolation. In June of 2012, I conducted an additional Google Scholar search for plant studies that included the alternate terms as follows: *plant AND selection AND intitle: "niche partitioning" OR intitle: "niche differentiation" OR intitle: "reproductive isolation" OR intitle: "reinforcement" OR intitle: "Wallace effect."* This search returned over 1000 results. I updated this search in December of 2013. Of these results, I selected only plant studies where the authors tested for trait divergence in sympatry by investigating competition-driven selection or adaptation for niche differentiation, niche partitioning, reproductive isolation, reinforcement, or the Wallace effect. There were many studies that suggest reproductive isolation as a by-product of natural selection in allopatry, but these studies were not included because they do not test whether reproductive isolation was itself under selection in sympatry. I was left with 11 papers that could be interpreted as studies of character displacement, only five of which used the term character displacement in the body of the manuscript (Table A2). Nine papers used the term reproductive isolation in the title and two used the term reinforcement. The other search terms did not return relevant results. After adding these 11 papers to the original search, there were a total of 25 plant studies of character displacement (Fig. 1). Twenty of these studies suggested that character displacement was a possible explanation for the observed pattern. Still, while this additional search nearly doubled the total number of plant studies, there remain far fewer studies of character displacement in plants than in animals. Differences in language therefore do not explain the lack of plant studies.

Theory

As noted in a recent review of plant interactions and evolution, the prevalence of theories that downplay the role of plant interactions in structuring plant communities may deter biologists from looking for an evolutionary response to these interactions (Thorpe *et al*. 2011). For example, the individualistic theory of plant distribution argues that plants are distributed according to their tolerances for different environmental conditions (Gleason 1926). Accordingly, a community of plant species is simply a grouping of species that share an affinity for the environmental conditions at a particular location. A species' place in this community is entirely independent of all other species in the community. Plant interactions therefore are unimportant, so there is no reason to predict they would drive evolutionary change. The neutral theory of plant community ecology also suggests that plant interactions are not critical to community assembly (Hubbell 2005). This theory argues that plant communities are constructed according to the processes of random speciation, random dispersal, and ecological drift. The random nature of plant community assembly under this theory ignores the competitive advantages and disadvantages of individual species. With all species treated as equal, there is no place for the competition that would otherwise drive an evolutionary response.

If these theories are correct, then character displacement may truly be a rare occurrence in plant communities. The lack of published studies of character displacement may reflect a bias toward only publishing positive results rather than a bias toward only studying character displacement in animals. If these theories are incorrect, however, as many studies suggest, their prevalence may be discouraging us from exploring a key component of species coexistence (Clements 1916, Odum 1971, Tilman 1981, Silvertown 2004, Wilsey *et al*. 2009, Thorpe *et al*. 2011).

Detection bias

There may be fewer studies of character displacement in plants simply because fewer biologists study plants. It is also possible that character displacement in plants may be more difficult to detect. Shifts in nitrogen form uptake, rooting depth, or style length are not nearly as obvious as, for example, a shift from a carnivorous to an omnivorous morph of spadefoot toad (Martin and Pfennig 2011). Ecological character displacement may be especially difficult to detect. Although there are at least a couple of plant studies suggestive of ecological character displacement, all studies uncovered by the literature search except one were of reproductive character displacement (Cody 1991, Veech *et al.* 2000). Reproductive traits are among the showiest plant traits and therefore may attract the attention of researchers more so than ecological traits. Ecological traits susceptible to character displacement could be physiological or developmental rather than morphological and therefore much more subtle. Yet these character shifts may be equally prevalent. Biologists have long documented that the intensity of competition is lower for plant populations with a history of coexistence with a competitor than for with those that are naïve to the competitor. (Turkington 1989, Shaw *et al.* 1995, Mealor and Hild 2007). But because the specific trait shifts responsible for the decreased intensity of competition are often not identified, these studies have largely remained separate from the character displacement literature.

UNDER WHAT CIRCUMSTANCES IS CHARACTER DISPLACEMENT LIKELY TO OCCUR IN PLANTS?

For character displacement to occur, a population must first meet the basic requirements for evolution in response to natural selection (Antonovics 1978). Then, character displacement is only likely to occur if the initial difference in trait means between the two competitors is intermediate (Schluter 2000b). If there is too little difference in trait means, there will be an initial slow response to selection, and competition may become severe enough that one species may drive the other to local extinction, whereas if the difference is too large, then selection will not be strong enough to encourage further divergence (Antonovics 1978, Taper and Case 1991, Schluter 2000b, Pfennig and Pfennig 2009). It seems likely that plant populations could meet the above requirements. There are, however, additional factors that encourage character displacement, which some suggest may not apply to plant communities (Connell 1978, Connell 1980, Keddy 1989).

Repeated contact

For character displacement to occur, competitors must have frequent contact with one another to maintain a constant force of selection (Pfennig and Pfennig 2009). This scenario is common in animal systems. For example, constant competition between similar species of sticklebacks has repeatedly resulted in character displacement in foraging depth and food choice (Pritchard and Schluter 2001). Connell argued that plant competition is unlikely to result in this same sort of niche differentiation because competition-driven evolution is dependent on the frequency with which two species come into contact and most plants are sympatric with a wide variety of competitors (Connell 1980). According to this argument, selection acting in many directions on multiple pairs of species would overwhelm selection driven by a single competitor.

There may, however, be exceptions that would allow for frequent contact between plant competitors. For example, in invaded plant communities, native species come into frequent contact with a single dominant invasive (Leger and Espeland 2010, Thorpe *et al.*

2011). Also, a plant population may be surrounded by a variety of species, but only compete with one for a specific resource. *Dalechampia* species only compete with congeners for pollination because only specialized bees are drawn to their unique resin-producing glands (Armbruster 1985, Armbruster 1986). Finally, although likely less common, multiple ecologically similar competitor species may all exert the same directional selection on a focal species. Under these circumstances, character traits in the focal species can evolve in response to the community of competitors (Cody 1991).

Available niche space

Some argue that character displacement does not occur in plants because all plant species depend on the same resources (sun, water, nutrients) and therefore cannot diverge in form or function to divide the available niche space (Connell 1978, Keddy 1989). Evidence now suggests, however, that this assumption may be incorrect.

Neighboring plant species have been shown to segregate according to microscale differences in habitat, thereby dividing up the available resource pool by specializing in different forms of resources (Fowler and Antonovics 1981). For example, differences in life history traits in sympatric *Acer* species were associated with a division of light resources in a Japanese deciduous forest (Tanaka *et al*. 2008). Additionally, species in plant communities ranging from European wet meadows to South African fynbos were shown to segregate according to fine-scale hydrological gradients (Araya *et al*. 2011). Plants can also divide niche space by preferentially taking up different forms of nutrients (Silvertown 2004, Miller *et al*. 2007). For example, the success of competitively superior plants in a diverse alpine dry meadow community was attributed to their ability to increase their uptake of nitrogen in the form of ammonium when competitors drew on the same resource pool (Ashton *et al*. 2010).

Finally, plants can divide niche space along multiple resource gradients at once (Tilman 1982, Vellend *et al*. 2000). The co-occurrence of five species of goldenrod (*Solidago*) was explained by the species' affinities for different combinations of soil acidity, clay content, and soil moisture, as well as by differences in life-history traits. For example, *Solidago altissima* and *Solidago gigantea* were most commonly associated with circumneutral soils, while the other goldenrod species preferred more acidic soils. Although *S. altissima* and *S. gigantea* shared a soil acidity niche, this niche space was further divided along a moisture gradient with *S. gigantea* associated with wetter soils (Abrahamson *et al*. 2005).

Potential for phenotypic plasticity

Character displacement is especially common among animal species that display phenotypic plasticity (Rice and Pfennig 2007). Character displacement may occur more readily in plastic species because plasticity permits survival among competitors long enough for selection to narrow the reaction norm of each species in opposite directions, or potentially produce a more fixed sympatric phenotype (Rice and Pfennig 2007).

Plants frequently respond plastically to competitors (van Kleunen and Fischer 2001, Callaway *et al*. 2003, Fan *et al*. 2008, Burns and Strauss 2012). When competing for light, the stoloniferous plant, *Trifolium repens*, altered branching number and length, petiole elongation, leaf mass, and specific leaf area (SLA) differently in response to pairwise competition with competitors of varying growth forms (Bittebiere *et al*. 2012). Similarly, the coastal shrubs, *Haplopappus ericoides* and *H. venetus* var. *sedoides*, responded to competition for water with the invasive succulent, *Carpobrotus edulis*, by developing deeper rooting systems (D’Antonio and Mahall 1991). The plastic nature of these responses could increase the likelihood of character displacement in these species.

WHAT ARE THE CRITERIA FOR TESTING THE PATTERN AND PROCESS OF CHARACTER DISPLACEMENT AND WHAT METHODS CAN AND HAVE BEEN USED TO ADDRESS THESE CRITERIA IN THE PLANT LITERATURE?

Early evidence for character displacement in plants and animals rested primarily on correlational studies, which demonstrated the patterns of competitor species diverging in phenotype in regions of sympatry. This correlational evidence alone was not convincing, as any number of alternative hypotheses might also explain the observed patterns (Grant 1975, Arthur 1982, den Boer 1986). In response to the early criticism of character displacement studies, a set of criteria for demonstrating that a pattern is the likely result of character displacement was established in the animal literature. The criteria below were compiled by Schluter and McPhail (1992) and were then elaborated on by Taper and Case (1991) and Pfennig and Pfennig (2012).

(1) The character displacement pattern was not formed by chance.

(2) Difference in the trait of interest between sympatric and allopatric populations is genetically based.

(3) Differences in character traits are the result of *in situ* evolution and not ecological sorting.

(4) A shift in the trait of interest is associated with a shift in resource acquisition or reproductive interactions.

(5) The strength of interspecific competition or reproductive interactions is positively

correlated with the degree of phenotypic similarity between species.

(6) Sympatric and allopatric sites have similar resource availability, which, in the case of reproductive character displacement, includes a similar diversity and abundance of pollinators.

The first three criteria rule out alternative hypotheses to *in situ* character divergence, and the last three address whether this character divergence is driven by interspecific interactions (Taper and Case 1991). While few studies meet all of these criteria, the growing number of studies in the animal literature that address four or more of them suggests that character displacement may be a widespread response to competition in animal communities (Schluter 2000a). In plants, the evidence is not yet as convincing. However, as described below, the studies that have attempted to address some of these criteria are strongly suggestive of character displacement.

Still, whether studying animals or plants, these criteria only require an exploration of existing patterns of character divergence. They do not investigate the process of character displacement itself. To prove character displacement is occurring, experiments must be designed to investigate the process of character displacement directly (Littlejohn and Loftus-Hills 1968, Losos 2000, Schluter 2000a, Stuart and Losos 2013). These experiments have rarely been attempted in either the animal or plant literature, but recent experimental studies in plants have found support for character displacement (Muchhala and Potts 2007, Hopkins *et al*. 2012).

Addressing established criteria for explaining character displacement patterns

Below I illustrate the six criteria that must be addressed in order to demonstrate that an existing pattern of character divergence is the result of character displacement.

For each criterion, I offer examples of plant studies that have satisfied it. To date, much of the evidence for character displacement in plants rests on the *Dalechampia*, *Burmeistera*, and *Phlox* systems (Table 1).

1. Character displacement pattern not formed by chance

Models that are equally applicable to plant and animal studies are now available to help distinguish between character displacement and chance patterns. For example, when multiple sympatric and allopatric populations exist for a pair of species, the differences in mean phenotype between competing species in sympatry can be tested against a null model that generates the differences in phenotype between pairs of allopatric communities of the two species sampled at random (Losos 2000). When multiple assemblages with overdispersed trait means exist, the average degree of dispersion of trait means for multiple communities of sympatric species can be compared against that of a null model where populations of species are randomized across communities (Schluter 2000a, Muchhala and Potts 2007). These models have been successfully used in plant systems to reject the alternative hypothesis that patterns of character divergence are caused by chance (Armbruster *et al*. 1994, Stone *et al*. 1998, Veech *et al*. 2000, Muchhala and Potts 2007). Additionally, direct experimental tests of the process of character displacement, which will be discussed in the following section, can also satisfy this criterion (Fishman and Wyatt 1999, Smith and Rausher 2008).

2. Difference in the trait is genetically based

A putative character displacement pattern may actually be caused by plastic responses to differences in environmental conditions or competitors between sites. To rule out this alternative hypothesis, the differences in phenotype between sites must be shown to have a genetic basis. The simplest method for testing for a genetic basis for trait differences between populations is a common garden experiment where individuals from one species taken from both sympatric and allopatric populations are grown in a common environment. If there is a genetic basis to the difference in trait means, then this same difference should be apparent when individuals from the two types of populations are grown together. It should be noted, however, that plasticity itself can be heritable and so can also potentially evolve in response to competition (Pfennig and Pfennig 2012). To test whether plasticity itself has been displaced, common garden experiments can be used to test for differences in reaction norms between individuals from sympatric and allopatric populations growing with and without the competitor. Reciprocal transplants between sympatric and allopatric sites can also be used to test for evolved plasticity.

As the names of the methods imply, plants make especially suitable subjects for common garden designs and reciprocal transplants. When testing for a genetic basis for trait differences, Armbruster (1985) grew both live rootstock and seeds from multiple populations of *Dalechampia scandens* in a common greenhouse environment. In the source populations, *D. scandens* appeared to diverge in resin gland size, gland-stigma distance, and anther-stigma distance in response to various combinations of sympatric congeners. Measurements of these reproductive traits for individuals in the greenhouse matched the measurements of individuals from their source populations, greatly strengthening the case for character displacement in this system.

3. Trait divergence is the result of in situ evolution

A putative character displacement pattern could be the result of ecological sorting as opposed to the *in situ* evolution of divergence in character. This alternative hypothesis can be rejected if the phenotypic range of a population in sympatry expands beyond what is found in any of the allopatric populations (Losos 2000, Schluter 2000a). Many cases of character displacement in plants show this pattern. For example, *Phlox drummondii* only has red corollas when in contact with *Phlox cuspidate* (Levin 1985), and *Opuntia echinocarpa* is shorter and narrower only when in contact with other cacti species (Cody 1991). This alternative hypothesis can also be rejected if trait means of a single species vary across communities in a pattern consistent with trait overdispersion (Schluter 2000a,b). This pattern is found in seed mass in *Pinus* assemblages (Veech *et al*. 2000) and timing of pollen release in *Acacia* assemblages (Stone *et al*. 1998).

One method for testing such a pattern is to generate null models that distinguish between ecological sorting and character displacement. When testing for character displacement in assemblages of *Burmeistera* species, Muchhala and Potts (2007) developed a null model for trait overdispersion caused by character displacement. The model randomly sorted species that occur in more than one assemblage across sites to generate a null model of what trait dispersion would look like if the species in each assemblage had evolved exsertion lengths of reproductive parts at random. Additionally, a phylogeny-based null model of expected trait dispersion of an assemblage of species was recently developed in the animal literature (Davies *et al*. 2012). This method could be equally applicable in plant systems.

4. A shift in the trait of interest is associated with a shift in resource acquisition or reproductive interactions

For character displacement to explain the difference in a trait between regions of sympatry and allopatry, the displaced trait must be linked to a shift in resource acquisition or reproductive interactions. Otherwise, the putative character displacement pattern could be explained by any number of other factors, including selection on traits that enhance the ability to compete for the same resources, rather than selection to rely upon a different set of resources (Aarssen 1983). By capturing pollinating bats, Muchhala (2008) demonstrated that the shift in exsertion length of reproductive parts of *Burmeistera* plants was associated with a shift in the location of pollen deposition on bat bodies. The body of a pollinating bat is a resource for plants, and this resource was divided in terms of the specific location of pollen placement. Similarly, by conducting pollinator observations, Whalen (1978) found that a shift from a large to small-flowered morph seen in numerous species of *Solanum* when sympatric with congeners corresponded with a shift in the size of visiting pollinators.

5. Strength of interspecific competition or reproductive interactions is positively correlated with the degree of phenotypic similarity between species

While the fourth criterion simply links a shift in a trait with a shift in resource acquisition or reproductive interactions, the fifth criterion addresses whether this shift actually reduces competition. If character displacement is a response to competition for limited resources, then individuals with similar phenotypes should interact more strongly. One method for addressing this criterion is to conduct common garden experiments where species pairs compete for a limited resource. One treatment would involve competition between similar phenotypes, while another treatment would involve competition between less similar phenotypes. For example, Muchhala and Potts (2007) used flight cage experiments with wild-caught bats to demonstrate that heterospecific pollen deposition between *Burmeistera* species pairs decreases as difference in exsertion length of reproductive parts between species increases. In cases where the diverged character results in spatial segregation, such as an evolved preference for shallower soil or deeper shade, the common garden design must include additional treatments that mimic this spatial heterogeneity. As discussed in more detail in the following section, common garden experiments can directly investigate the process of character displacement if the more similar phenotypes used in the experiment are drawn from allopatric populations and the more divergent phenotypes are drawn from sympatric populations (Martin and Harding 1981).

6. Sympatric and allopatric sites have similar resource availability

Differences in resource availability between sympatric and allopatric sites could explain the differences in phenotypes and reproductive compatibilities between sites. For example, reproductive isolation through flowering time and mating system differences between *Mimulus guttatus* and *Mimulus nasutus* in the western United States is likely a by-product of the local adaptation of *M. nasutus* in response to dry soil conditions, rather than a direct result of selection for reproductive isolation (Kiang and Hamrick 1978, Martin and Willis 2006).

It is nearly impossible to rule out the possibility that differences between sympatric and allopatric populations could be due to differences in resource availability between sites without experimentally manipulating the environment through reciprocal transplants or common garden designs. One exception may be large-scale comparative studies that separate adaptation in response to competitors from adaptation to local resources at a macro level by looking across many species pairs. A study of 41 sister-species pairs across three plant families in the Cape Floristic Region of South Africa found that shifts in pollination system follow adaptation to edaphic conditions only for sympatric sister species, suggesting that selection favors reproductive isolation in sympatry (van der Niet *et al*. 2006). If experimental designs or macro-level comparative studies are not possible, at a minimum, obvious differences in resources across sites, such as differences in water or light availability, differences in soil type, or differences in pollinator diversity or abundance, should be ruled out.

Experimentally testing the process of character displacement

Over a decade ago, Schluter (2000a,b) called for the direct experimental testing of character displacement hypotheses. He explained that if character displacement is the cause of an observed pattern, then experiments should test the process of character displacement by demonstrating that the intensity of competition declines in sympatric populations over time, and natural selection favors divergence in phenotypes among sympatric species. Testing these hypotheses offers the added benefit of also satisfying some or all of the six criteria for explaining observed patterns of character displacement. While Schluter has experimentally tested the process of character displacement in sticklebacks, few biologists in the animal or plant literature have followed his lead (Stuart and Losos 2013). The plant studies that have been conducted, however, demonstrate the experimental ease with which plants can be used in common gardens, selection analyses, and breeding designs to experimentally test character displacement hypotheses (Table 1). Plant biologists therefore have the opportunity to make a significant contribution to this new focus in character displacement research.

Testing whether the intensity of competition declines over time

If character displacement has occurred, the strength of competition between sympatric species should decline over time. One method for testing this hypothesis is to expose individuals from sympatric versus allopatric populations of a species to a competitor. If character displacement has occurred, then the intensity of competition should be greatest in the allopatric treatment. This competition experiment can be conducted in a greenhouse setting or in the field.

A field experiment was used to test whether *T. repens* had evolved in response to pair-wise competition with multiple grass species (Turkington 1989). Ramets of *T. repens* were collected from a field from patches dominated by each of three different species of grasses. After the ramets were divided and grown in a greenhouse, some *T. repens* plants from each divided ramet were planted back into the field in competition with the competitor grass from their source site, and some were planted into competition with each of the other competitor grass species. The strength of competition was weakest when *T. repens* grew with the competitor from its source site, indicating that *T. repens* had evolved to reduce competition with its neighboring grass. While the design of this experiment is appropriate for studies of character displacement, this experiment falls outside of the character displacement literature because the specific trait that diverged to diminish competition was not identified.

In a study where the displaced trait was clear, potted plants of both color morphs of *Phlox drummondii* were placed into a natural population of *P. cuspidata*, and then the proportion of seed resulting from hybridization with *P. cuspidata* for each color morph was estimated (Levin 1985). The allopatric morph produced 38% hybrid seed while the sympatric morph produced only 13%, indicating that the sympatric morph had likely evolved to reduce competition for conspecific pollination. More recent work on this system used common garden experiments and pollinator observations to reveal that the sympatric morph is favored in the presence of *P. cuspidata* because it has an allele conferring dark pigmentation. This intense pigmentation encourages pollinator constancy by enabling pollinators to distinguish between the two species, thereby limiting heterospecific pollen transfer (Hopkins and Rausher 2012).

An interesting extension of these previous studies would involve identifying communities with varying ages of sympatry and examining how the intensity of competition changes over time. This design could reveal the rate of character displacement and whether this rate is consistent across populations with similar histories of sympatry.

Natural selection should favor divergence in phenotypes among sympatric species

Character displacement is the result of natural selection favoring a divergence in phenotypes between species competing for resources. Using classic statistical techniques, there are a number of experimental designs that can test whether the presence of a competitor alters natural selection on a focal plant species (Lande and Arnold 1983, Fishman and Wyatt 1999, Caruso 2000). For example, Caruso (2000) studied populations of *Ipomopsis aggregata* growing both with and without *Castilleja linariaefolia*, a competitor for hummingbird pollination. She measured selection on floral traits in both population types and found that the presence of the competitor resulted in increased selection on corolla length.

One issue with comparing selection in sites of sympatry versus allopatry is that resource availability may differ between these sites, and differences in selection may therefore be the result of differences in resources rather than the presence or absence of the competitor. Smith and Rausher (2008) addressed this issue when testing for selection for reproductive character displacement in *Ipomoea hederacea*. Rather than compare selection in sites of sympatry versus allopatry, they planted seeds *of I. hederacea* in a checkerboard pattern with its congener and competitor for pollinators *Ipomoea purpurea*. They then alternated between allowing pollinator visits to both species and preventing pollinator visits to the competitor species by covering inflorescences with bridal veil. They measured natural selection on *I. hederacea* with and without the presence of the competitor while keeping the resource base constant. They found that the presence of the competitor results in selection for an increase in the clustering of anthers about the stigma, which favors selfing over the risk of outcrossing with the wrong species.

If character displacement has progressed to the point where the displaced species are no longer competing, then it will not be possible to pick up a signal of altered natural selection (Connell 1980). This issue can be dealt with in a number of ways in plants. First, if there are allopatric populations available, then the study should be conducted by comparing selection on the allopatric individuals with and without the competitor. Second, if no allopatric population is available, then breeding designs can be arranged to select for a range of phenotypes that overlaps more with the competitor phenotype, and this wider range of phenotypes can be used for the selection experiment. Finally, a recent study on character displacement in *Phlox drummondii* demonstrated that population genetic analyses can be used to uncover the signature of a past selective sweep in sympatric populations (Hopkins *et al*. 2012).

WHAT ARE SOME ADDITIONAL APPROACHES FOR STUDYING

CHARACTER DISPLACEMENT IN PLANTS?

Plant systems have proven to be especially amenable to the experimental designs required for testing character displacement hypotheses. In the following section, I explore some additional approaches that may be useful for uncovering examples of character displacement in plants.

Island systems

Some of the most well-supported examples of character displacement in the animal literature come from island systems such as the studies of Darwin's finches in the Galapagos (Grant and Grant 2006) and *Anolis* lizards in the Caribbean (Losos 2009). Islands offer the opportunity to test the repeatability of character displacement, as there may be many occurrences of sympatry and allopatry across an island chain. Island chains are equally suitable to the study of character displacement in plants (Cody 1984, Miyake and Inoue 2003). Furthermore, plants are sessile organisms often with highly structured populations. Even on the mainland, then, limited gene flow between plant populations may result in island-like patterns of species distributions ideal for the study of character displacement.

Species invasions

Species invasions offer the opportunity to capture the process of character displacement in action. When the time since the introduction of an invasive competitor is known across multiple communities, researchers can test for character displacement by investigating whether the strength of competition between a native and an invasive competitor declines as the time since invasion increases (Lankau *et al*. 2009). A greenhouse study using soil from source communities of varying stages of garlic mustard

(*Alliaria petiolata*) invasion showed that populations of *Pilea pumila*, a native annual from these same source communities, had adapted to the specific soil qualities present at each invasion stage (Lankau 2013). While the exact displaced character that permits coexistence is not clear, a previous study suggests that *P. pumila* populations that are experienced with *A. petiolata* have evolved to maintain their beneficial arbuscular mycorrhizal fungi connections even in the presence of allelochemicals released by the invader (Lankau 2012).

There are currently many examples of ecologically similar native and invasive species in direct competition with one another. The invasive jewelweed *Impatiens glandulifera* competes with the native congener *Impatiens capensis* in communities throughout New York, Massachusetts, and Maine (Tabak and von Wettberg 2008). Native and introduced subspecies of *Phragmites* are commonly found competing in the eastern United States (Meyerson *et al*. 2010). Native and invasive populations of the dandelion, *Taraxacum*, are found competing for pollinators throughout Japan (Kandori *et al*. 2009). All of these examples offer promising systems for the study of character displacement in plants.

A closer look

Perhaps the greatest challenge to the study of character displacement in plants is identifying which character traits are likely involved in competition for resources and therefore likely to be displaced. Studies of reproductive character displacement have successfully recognized shifts in visible traits such as flower color and style length. More cryptic reproductive traits such as floral scent, sugar and amino acid concentrations in nectar, pollen to ovule ratios, and subtle alterations in floral and inflorescence structure should also be investigated, as these traits may be equally susceptible to character displacement (Lindsey and Bell 1985).

Studies of ecological character displacement can also benefit from a closer look at the subtle traits involved in resource acquisition. Character displacement patterns have been noticed in clearly visible morphological traits such as growth form and seed mass, but more subtle morphological traits such as rooting structure and depth, specific leaf area, and petiole length may also be under selection for character divergence (Cody 1991, Veech *et al*. 2000). Furthermore, physiological traits such as the specific forms, ratios, and timing of nutrients absorbed should be explored (McKane *et al*. 1990, Ashton *et al*. 2010). Finally, a deeper investigation of developmental traits such as growth rate and life history may also provide examples of ecological character displacement in plants (Tanaka *et al*. 2008). Even once a diverged trait is identified, proving that it is solely responsible for the observed niche shift is challenging. The experimental designs presented here, however, should reveal whether the trait is at least partially responsible.

CONCLUSION

The existing studies of character displacement in plants do not yet provide irrefutable evidence that character displacement is a common response to interspecific competitive and reproductive interactions. They do, however, suggest that character displacement plays an important role in minimizing competition in at least some plant communities (Table 1). It is time to use these studies as a springboard to continue with rigorous testing of character displacement hypotheses in plants. The experimental designs for testing character displacement hypotheses are now readily available, and plant systems are especially amenable to these designs because of the experimental ease with which they can be used in common gardens, selection analyses, and breeding designs. Using these experimental approaches to test for ecological character displacement is especially critical as this area of research is mostly unexplored. By focusing greater attention on character displacement in plants, we have the potential to enhance our fundamental understanding of the ecological and evolutionary forces that shape plant communities.

ACKNOWLEDGMENTS

I thank members of the Ecology and Evolutionary Biology group at the University of Virginia for helpful discussion and comments, especially D.A. Roach, L.F. Galloway, H.M. Wilbur, D.R. Taylor, J. Antonovics, B. Sanderson, and all members of the Galloway laboratory meeting group. I also thank Y.E. Stuart, N. Muchhala, and J.C. Steven for generously offering constructive comments on earlier drafts of this manuscript. I thank two anonymous reviewers whose comments greatly improved the manuscript. Finally, I thank the Jefferson Scholars Foundation for financial support. This article was published in part thanks to funds provided by the University of Virginia Library Open Access Fund.

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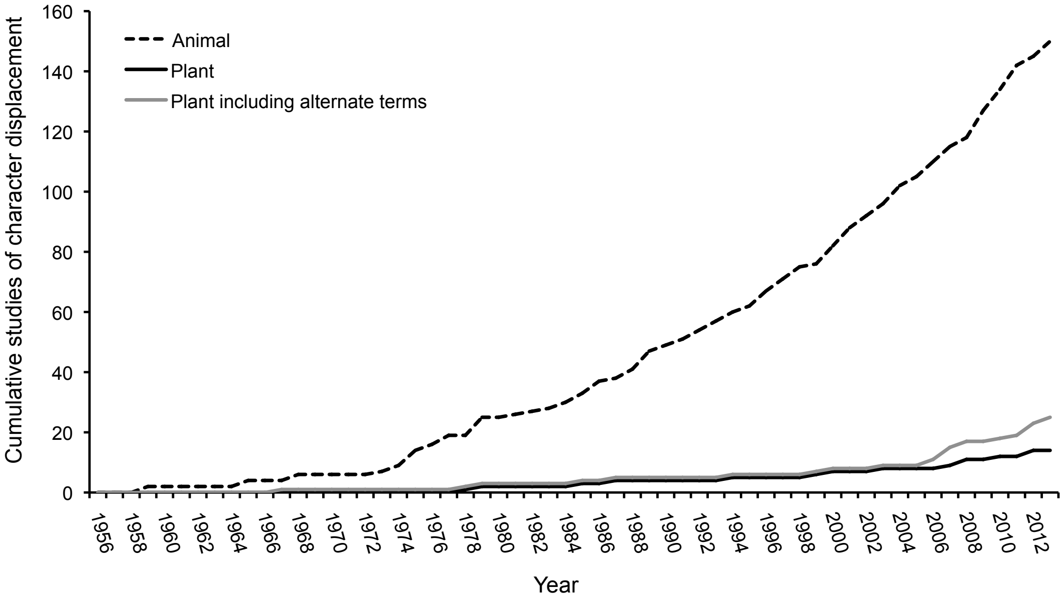
Table 1. Selected studies suggestive of character displacement in plants. Numbered columns mark whether studies were designed to address the criteria for testing the pattern of character displacement. Studies that experimentally test the process of character displacement often, as a byproduct of the experiment, satisfy some or all of the criteria for explaining the existing pattern, but only studies designed to address the specific criteria are marked.

System	**Studies**	**Trait(s) studied**	**Ecological (E) or Reproductive (R)**	**(1) Pattern is not formed by chance**	**(2) Trait differences are genetically based**	**(3) Trait differences result from *in situ* evolution**	**(4) Shift in trait is associated with shift in resource aquistion**	**(5) Similar phenotypes compete more strongly**	**(6) Sympatric and allopatric sites have same resources**	**Experimental test of CD process**	**Correlational evidence only**
Dalechampia species	Armbruster 1985; Armbruster 1986	Resin gland area, gland-stigma distance, anther-stigma distance	R	X	X	X	X				
Ipomopsis aggregata and *Castilleja linariaefolia*	Caruso 2000	Corolla length	R							X	
Pachycereus pringlei and other cacti species	Cody 1984	Growth form	E								X
Arenaria uniflora and *A. glabra*	Fishman and Wyatt 1999	Mating system (selfing versus outcrossing)	R				X			X	
Phlox drummondii and *P. cuspidata*	Levin 1985; Hopkins and Rausher 2011: Hopkins *et al*. 2012	Corolla color	R		X		X	X		X	
Burmeistera species	Muchhala and Potts 2007; Muchhala 2008;	Exsertion length of anthers and stigma	R	X		X	X	X		X	
Ipomea hederacea and *I. purpurea*	Smith and Rausher 2008	Clustering of anthers around stigma	R							X	
Pinus species	Veech *et al*. 2000	seed mass	E	X							
Solanum section *Androceras*	Whalen 1978	Floral size and phenology	R				X				

Figure 1. Cumulative studies of character displacement published from 1956 to December of 2013. The *Animal* and *Plant* categories include all studies that were published in each of these groups with the term "character displacement" in the title. The *Plant including alternate terms* category includes all studies testing for character displacement that used the term "character displacement" and/or alternate terms with similar meaning in the title.



Appendix A

Table A1. Animal and plant studies published from 1956 thorough December of 2013 with "character displacement" in the title. These studies are included in Figure 1.

Search date	Year	Authors	Animal (A) or Plant (P)	CD possible explanation for plant trait divergence?	Ecological or Reproductive Plant CD tested (E/R)
Jun-12	1978	Whalen	P	Y	R
Jun-12	1979	Fisher and Reimer	P	Y	R
Jun-12	1985	Levin	P	Y	R
Jun-12	1987	Murray *et al*.	P	N	R
Jun-12	1994	Armbruster *et al*.	P	Y	R
Jun-12	1999	Fishman and Wyatt	P	Y	R
Jun-12	2000	Hansen *et al*.	P	Y	R
Jun-12	2003	Miyake and Inoue	P	Y	R
Jun-12	2007	Muchhala and Potts	P	Y	R
Jun-12	2008a	Smith and Rausher	P	Y	R
Jun-12	2008b	Smith and Rausher	P	Y for selection but N for evolution	R
Jun-12	2010	Bendiksby *et al*.	P	Y	R
Jun-12	2012	Hopkins *et al*.	P	Y	R
Jun-12	2012	Roncal *et al*.	P	N	E and R
Jun-12	1959	Norrevang	A	N/A	N/A
Jun-12	1959	Ripley	A	N/A	N/A
Jun-12	1965	Parkes	A	N/A	N/A
Jun-12	1965	Taylor	A	N/A	N/A
Jun-12	1968	Ficken *et al*.	A	N/A	N/A
Jun-12	1968	Lloyd	A	N/A	N/A
Jun-12	1973	Ferguson	A	N/A	N/A
Jun-12	1974	Elliott and Kurczewski	A	N/A	N/A
Jun-12	1974	Huey	A	N/A	N/A
Jun-12	1975	Fenchel	A	N/A	N/A
Jun-12	1975	Fouquette	A	N/A	N/A
Jun-12	1975	Kellogg	A	N/A	N/A
Jun-12	1975	Loftus-Hills	A	N/A	N/A
Jun-12	1975	Waage	A	N/A	N/A
Jun-12	1976	Bell	A	N/A	N/A
Jun-12	1976	Husar	A	N/A	N/A
Jun-12	1977	McEachran and Martin	A	N/A	N/A
Jun-12	1977	Schindel and Gould	A	N/A	N/A
Jun-12	1977	Wasserman and Koepfer	A	N/A	N/A

Table A1. (cont.)

Search date	Year	Authors	Animal (A) or Plant (P)	CD possible explanation for plant trait divergence?	Ecological or Reproductive Plant CD tested (E/R)
Jun-12	1979	Case	A	N/A	N/A
Jun-12	1979	Dunham *et al*.	A	N/A	N/A
Jun-12	1979	Frier	A	N/A	N/A
Jun-12	1979	Jong	A	N/A	N/A
Jun-12	1979	Strong *et al*.	A	N/A	N/A
Jun-12	1979	Waage	A	N/A	N/A
Jun-12	1981	Tillier	A	N/A	N/A
Jun-12	1982	Angel	A	N/A	N/A
Jun-12	1983	Fjeldsa	A	N/A	N/A
Jun-12	1984	Crowder	A	N/A	N/A
Jun-12	1984	Suzuki	A	N/A	N/A
Jun-12	1985	Malmquist	A	N/A	N/A
Jun-12	1985	Markow	A	N/A	N/A
Jun-12	1985	Schluter *et al*.	A	N/A	N/A
Jun-12	1986	Alekseyev *et al*.	A	N/A	N/A
Jun-12	1986	Gallagher *et al*.	A	N/A	N/A
Jun-12	1986	Schluter	A	N/A	N/A
Jun-12	1986	Wallin	A	N/A	N/A
Jun-12	1987	Cherrill and James	A	N/A	N/A
Jun-12	1988	Butler	A	N/A	N/A
Jun-12	1988	Schluter	A	N/A	N/A
Jun-12	1989a	Dayan *et al*.	A	N/A	N/A
Jun-12	1989b	Dayan *et al*.	A	N/A	N/A
Jun-12	1988	Tsukagoshi	A	N/A	N/A
Jun-12	1989	Diamond *et al*.	A	N/A	N/A
Jun-12	1989	Grahame and Mill	A	N/A	N/A
Jun-12	1989	Thielcke	A	N/A	N/A
Jun-12	1989	Tidemann and Schodde	A	N/A	N/A
Jun-12	1990	Dayan *et al*.	A	N/A	N/A
Jun-12	1990	Losos	A	N/A	N/A
Jun-12	1991	Singh and Chatterjee	A	N/A	N/A
Jun-12	1991	Yom-Tov	A	N/A	N/A
Jun-12	1992	Dayan *et al*.	A	N/A	N/A
Jun-12	1992	Loftus-Hills and Littlejohn	A	N/A	N/A

Table A1. (cont.)

Search date	Year	Authors	Animal (A) or Plant (P)	CD possible explanation for plant trait divergence?	Ecological or Reproductive Plant CD tested (E/R)
Jun-12	1992	Schluter and McPhail	A	N/A	N/A
Jun-12	1993	Colwell	A	N/A	N/A
Jun-12	1993	Saloniemi	A	N/A	N/A
Jun-12	1993	Yom-Tov	A	N/A	N/A
Jun-12	1994	Dayan and Simberloff	A	N/A	N/A
Jun-12	1994	Gerhardt	A	N/A	N/A
Jun-12	1994	Stol	A	N/A	N/A
Jun-12	1995	Kawano	A	N/A	N/A
Jun-12	1995	Kieser	A	N/A	N/A
Jun-12	1996	Chiba	A	N/A	N/A
Jun-12	1996	Gingerich	A	N/A	N/A
Jun-12	1996	Giorni *et al*.	A	N/A	N/A
Jun-12	1996	Gorbushin	A	N/A	N/A
Jun-12	1996	Werdelin	A	N/A	N/A
Jun-12	1997	Fragoso and Rojas	A	N/A	N/A
Jun-12	1997	Jones	A	N/A	N/A
Jun-12	1997	Radtkey *et al*.	A	N/A	N/A
Jun-12	1997	Saetre *et al*.	A	N/A	N/A
Jun-12	1998	Hertel and Lehman	A	N/A	N/A
Jun-12	1998	Loy	A	N/A	N/A
Jun-12	1998	Poeser	A	N/A	N/A
Jun-12	1998	Rácz	A	N/A	N/A
Jun-12	1999	Chiba	A	N/A	N/A
Jun-12	2000	Adams and Rohlf	A	N/A	N/A
Jun-12	2000	Giannasi *et al*.	A	N/A	N/A
Jun-12	2000	Ilango	A	N/A	N/A
Jun-12	2000	Marshall and Cooley	A	N/A	N/A
Jun-12	2000	Pfennig and Murphy	A	N/A	N/A
Jun-12	2000	Woodman	A	N/A	N/A
Jun-12	2001	Cooley *et al*.	A	N/A	N/A
Jun-12	2001	Gabor and Ryan	A	N/A	N/A
Jun-12	2001	Gries *et al*.	A	N/A	N/A
Jun-12	2001	Leary	A	N/A	N/A
Jun-12	2001	Mikulová and Frynta	A	N/A	N/A

Table A1. (cont.)

Search date	Year	Authors	Animal (A) or Plant (P)	CD possible explanation for plant trait divergence?	Ecological or Reproductive Plant CD tested (E/R)
Jun-12	2001	Pritchard and Schluter	A	N/A	N/A
Jun-12	2002	Jaeger *et al*.	A	N/A	N/A
Jun-12	2002	Kawano	A	N/A	N/A
Jun-12	2002	Melville	A	N/A	N/A
Jun-12	2002	Wullschleger	A	N/A	N/A
Jun-12	2003	Geyer and Palumbi	A	N/A	N/A
Jun-12	2003	Hobel and Gerhardt	A	N/A	N/A
Jun-12	2003	Kawano	A	N/A	N/A
Jun-12	2003	Schluter	A	N/A	N/A
Jun-12	2004	Adams	A	N/A	N/A
Jun-12	2004	Albert and Schluter	A	N/A	N/A
Jun-12	2004	Izzo and Gray	A	N/A	N/A
Jun-12	2004	Marchinko *et al*.	A	N/A	N/A
Jun-12	2004	Swart and Adams	A	N/A	N/A
Jun-12	2004	Tynkkynen *et al*.	A	N/A	N/A
Jun-12	2005	Gabor *et al*.	A	N/A	N/A
Jun-12	2005	Gray *et al*.	A	N/A	N/A
Jun-12	2005	Smadja and Ganem	A	N/A	N/A
Jun-12	2006	Cooley *et al*.	A	N/A	N/A
Jun-12	2006	Gannon and Rácz	A	N/A	N/A
Jun-12	2006	Grant and Grant	A	N/A	N/A
Jun-12	2006	Pfennig *et al*.	A	N/A	N/A
Jun-12	2006	Weerd *et al*.	A	N/A	N/A
Jun-12	2007	Albert *et al*.	A	N/A	N/A
Jun-12	2007	Meiri *et al*.	A	N/A	N/A
Jun-12	2007	Montoya and Burns	A	N/A	N/A
Jun-12	2007	Mullen and Andres	A	N/A	N/A
Jun-12	2007	Russo *et al*.	A	N/A	N/A
Jun-12	2008	Higgie and Blows	A	N/A	N/A
Jun-12	2008	Rice and Pfennig	A	N/A	N/A
Jun-12	2008	Stewart	A	N/A	N/A
Jun-12	2009	Geyer and Lessios	A	N/A	N/A
Jun-12	2009	Jang *et al*.	A	N/A	N/A
Jun-12	2009	Johanet *et al*.	A	N/A	N/A

Table A1. (cont.)

Search date	Year	Authors	Animal (A) or Plant (P)	CD possible explanation for plant trait divergence?	Ecological or Reproductive Plant CD tested (E/R)
Jun-12	2009	Kameda *et al*.	A	N/A	N/A
Jun-12	2009	Kirschel	A	N/A	N/A
Jun-12	2009	Lemmon	A	N/A	N/A
Jun-12	2009	Marsteller *et al*.	A	N/A	N/A
Jun-12	2009	Pfennig and Martin	A	N/A	N/A
Jun-12	2009	Rice *et al*.	A	N/A	N/A
Jun-12	2010	Adams	A	N/A	N/A
Jun-12	2010a	Anderson and Grether	A	N/A	N/A
Jun-12	2010b	Anderson and Grether	A	N/A	N/A
Jun-12	2010	Pfennig and Martin	A	N/A	N/A
Jun-12	2010	Rando *et al*.	A	N/A	N/A
Jun-12	2010	Rice and Pfennig	A	N/A	N/A
Jun-12	2010	Seddon and Tobias	A	N/A	N/A
Jun-12	2011	Crampton *et al*.	A	N/A	N/A
Jun-12	2011	Martin and Pfennig	A	N/A	N/A
Jun-12	2011	McGraw *et al*.	A	N/A	N/A
Jun-12	2011	Meiri *et al*.	A	N/A	N/A
Jun-12	2011	Pfennig and Stewart	A	N/A	N/A
Jun-12	2011	Reifová *et al*.	A	N/A	N/A
Jun-12	2011	Thierry *et al*.	A	N/A	N/A
Jun-12	2011	Tyler and Leighton	A	N/A	N/A
Dec-13	2012	Davies *et al*.	A	N/A	N/A
Jun-12	2012	Soto	A	N/A	N/A
Jun-12	2012	Vallin *et al*.	A	N/A	N/A
Dec-13	2013	Bargielowski *et al*.	A	N/A	N/A
Dec-13	2013	Grava *et al*.	A	N/A	N/A
Dec-13	2013	Lambert *et al*.	A	N/A	N/A
Dec-13	2013	Park *et al*.	A	N/A	N/A
Dec-13	2013	Yamaguchi and Iwasa	A	N/A	N/A

Table A2. Additional plant character displacement studies published from 1956 through December 2013 that use an alternate term to describe character displacement in the title. These studies are included in Figure 1.

	Publication		Phrase Used in title:					Character Displacement		
Search Date	**Year**	**Author(s)**	**niche partitioning**	**niche differentiation**	**reproductive isolation**	**reinforcement**	**Wallace effect**	**CD term used?**	**CD possible?**	**Ecological or Reproductive? (E or R)**
Jun-12	1967	Levin and Kerster			X			Y	Y	R
Jun-12	2006	Kay			X			N	Y	R
Jun-12	2006	Niet *et al.*				X		Y	Y	R
Jun-12	2007	Martin *et al.*			X			N	Y	R
Jun-12	2007	Scopece *et al.*			X			N	Y	R
Jun-12	2007	Yang *et al.*			X			N	Y	R
Jun-12	2011	Nosrati *et al.*			X			N	N	R
Jun-12	2012	Hopkins and Rausher			X			N	Y	R
Jun-12	2012	Jewell *et al.*			X			Y	N	R
Dec-13	2013	Armbruster *et al.*			X			Y	Y	R
Dec-13	2013	Briscoe Runquist and Moeller				X		Y	Y	R

CHAPTER TWO:

An invasive plant alters pollinator-mediated selection on a native congener

ABSTRACT

Invasive plants may dominate native plant communities through strong vegetative competition. Recent studies suggest that they may also compete reproductively with native plants by reducing the quantity or quality of pollinator visits. While these studies reveal the ecological consequences of pollinator-mediated competition between invasive and native plants, the evolutionary outcomes of these interactions remain unexplored. I studied the ecological and evolutionary impact of pollinator-mediated competition with an invasive jewelweed, *Impatiens glandulifera*, on a co-occurring native congener, *I. capensis*. Using a pollinator choice experiment, a hand pollination experiment, and a selection analysis, I addressed the following questions: (1) Do native bumblebees show preference for the invasive or native jewelweed, and do they move between the two species? (2) Does invasive jewelweed pollen inhibit seed production in the native plant? (3) Does the invasive jewelweed alter phenotypic selection on the native plant's floral traits? I found that bumblebees prefer the invasive jewelweed. Invasive pollen inhibited seed production in the native plant, but pollinator constancy likely reduced the prevalence of heterospecific pollen deposition. The presence of the invasive jewelweed altered phenotypic selection on corolla height in the native plant. Invasive plants have the potential to alter phenotypic selection on floral traits in native plant populations. If native plants can evolve in response to this altered selection pressure, then the evolution of floral traits may play an important role in permitting long-term coexistence of native and invasive plants.

KEY WORDS: *Impatiens capensis*, *Impatiens glandulifera*, plant invasions, pollen

interference, pollinator constancy, pollinator-mediated selection

INTRODUCTION

Invasive plants have long been implicated in the decline of native plant populations (Vilà *et al*. 2011). While their ability to harm native plants through vegetative competition has received much attention (Gabor *et al*. 1996, Martin 1999, Gould and Gorchov 2000, Flory and Clay 2010, Ni *et al*. 2010, Hovick *et al*. 2011), there is growing evidence that invasive plants may also reduce the fitness of their native neighbors through competition for pollinator services (Chittka and Schürkens 2001, Brown *et al*. 2002, Flanagan *et al*. 2009, Matsumoto *et al*. 2010). Invasive plants may draw pollinators away from native plants, and subsequently reduce seed set in pollen-limited populations of native plants (Chittka and Schürkens 2001). Invasive plants may also reduce the quality of pollinator visits to native plants through heterospecific pollen deposition (Waser 1978a, Waser 1978b, Brown and Mitchell 2001, Mitchell *et al*. 2009, Matsumoto *et al*. 2010). Pollen from interspecific pollinator movements may inhibit seed production by reducing the stigmatic space available for conspecific pollen or by hindering conspecific pollen germination through allelopathy (Waser 1978b, Feinsinger 1987). Additionally, fitness of native plants may be reduced if heterospecific pollination results in the production of sterile hybrids (Mitchell *et al*. 2009).

Alternatively, invasive plants may facilitate pollinator visitation to native plants by drawing a greater abundance and diversity of pollinators to a plant community (Lopezaraiza-Mikel *et al*. 2007). Seed production in some native plants is unaffected by the pollen of their invasive neighbors, so it is possible for increased native plant visitation in invaded communities to result in increased seed production even when interspecific pollinator movements are common (Moragues and Traveset 2005, Tscheulin *et al*. 2009).

Whether pollinator-mediated interactions between invasive and native plants are facilitative or competitive, therefore, will depend on both pollinator preference and movement between species, and the effect of heterospecific pollen on native plants.

While the ecological effects of pollinator-mediated competition between invasive and native plants are now receiving attention, we have yet to explore the evolutionary outcomes of these interactions (Mitchell *et al*. 2009). The presence of an invasive competitor could create a strong selective force on the floral traits of a native plant. Character displacement studies of co-occurring native plant species suggest that the evolution of floral traits in response to competition for pollination is possible (Armbruster *et al*. 1994, Caruso 2000, Muchhala and Potts 2007). Additionally, recent studies have demonstrated that the vegetative traits of some native plants evolve rapidly in response to invasive competitors (Rowe and Ledger 2011, Lankau 2013, Oduor 2013).

Selection on native plant floral traits should be altered most dramatically by the presence of invasive plants with similar flowers because pollinator movement is most common between plants with similar floral shape or color (Morales and Traveset 2009, Gibson *et al*. 2012). Selection may favor a divergence in flower form if pollinator sharing results in reduced seed set in the native plant, or a convergence in flower form if pollinators prefer the invasive plant and invasive pollen has no effect on the native plant's seed set. Invasive pollen is especially likely to negatively affect seed production in closely related native plants because similarities in stigma and style morphology and chemistry may encourage heterospecific pollen germination on native stigmas (Ashman and Arceo-Gomez 2013).

I studied the ecological and evolutionary consequences of pollinator-mediated competition with an invasive jewelweed, *Impatiens glandulifera*, on a native congener, *I. capensis*, in the northeastern United States. Using a pollinator choice experiment, a hand pollination experiment, and a selection analysis, I addressed the following questions: (1) Do native pollinators show preference for the invasive or native jewelweed, and do they move between the two species? (2) Does invasive jewelweed pollen inhibit seed production in the native plant? (3) Does the invasive jewelweed alter phenotypic selection on the native plant's floral traits? The results of my study suggest that invasive competitors for pollination have the potential to alter phenotypic selection on floral traits in native plants.

METHODS

Study species

Impatiens capensis Meerb. (Balsaminaceae), spotted jewelweed, is an annual plant native to the United States and Canada. It germinates in early May and often reaches 1.5 meters in height by August. Flowers descend on pedicels from leaf axils and typically occur from late June through first frost. *Impatiens capensis* produces both open, chasmogamous flowers and closed, cleistogamous flowers. The chasmogamous flowers are zygomorphic with three petal-like sepals, and one upper and two lower petals. The posterior sepal is modified into a conical structure leading to a nectar-filled spur (Rust 1979). The flowers are orange with red spots on the lower petals. Although chasmogamous flowers are self-compatible, fertilization depends on pollinator visitation, as protandry prevents self-fertilization within each flower. The cleistogamous flowers are highly reduced in size and result in selfed seed. These flowers produce fewer seeds than the chasmogamous flowers, and their seeds have a lower rate of survival over winter (Mitchell-Olds and Waller 1985).

Impatiens glandulifera Royle, showy jewelweed, is native to the Himalayas, and in the late 1800's, it began spreading in the northeastern United States, where it now grows intermixed with *I. capensis* along roadsides and stream banks (Tabak and von Wettberg 2008). It germinates synchronously with *I. capensis* and can reach a height of nearly 3 meters. Unlike the native jewelweed, *I. glandulifera* produces only chasmogamous flowers, which range in color from pale pink to deep magenta. Although slightly larger, these flowers are shaped similarly to those of the native jewelweed.

Both species are visited by a diversity of pollinators including hummingbirds, sweat bees, honeybees, bumblebees, and hover flies. Pollinator assemblages, however, vary greatly by population (Travers *et al*. 2003). Bumblebees (*Bombus spp*), which are especially efficient pollinators of *Impatiens* species (Rust 1977), were the primary pollinators at my study sites. *Bombus vagans* was the most commonly observed species (C.M. Beans, University of Virginia, personal observation).

Pollinator choice experiment

Experimental Design—I designed a pollinator choice experiment to estimate bumblebee preference and constancy when offered flowers of the two *Impatiens* species. Pollinator preference is the preferential visitation of one plant species over another, while pollinator constancy is the preferential movement between flowers of the same species over movement across species (Flanagan *et al*. 2009). Pollinator preference may reduce seed production in the less desirable species when pollen is limited. Pollinator constancy may increase seed production by minimizing heterospecific pollen deposition.

I conducted pollinator observations at the Hitchcock Center for the Environment in Amherst, Massachusetts in an open field dominated by *I. capensis*. There are no naturalized *I. glandulifera* plants at this site. In late July, I placed potted *I. glandulifera* plants grown from seed from Petersham, Massachusetts into three 1X1m plots of naturally occurring *I. capensis*. Plots were spaced between three and nine meters apart and contained 8-10 *Impatiens* individuals, with an equal number of each species. I placed plants directly adjacent to one another with branches overlapping to mimic natural density. Over the course of the experiment, I continuously manipulated flower number within each plot to offer pollinators an equal number of open flowers from each species. I controlled flower number by either removing flowers from one species or rotating in different *I. glandulifera* individuals. The total flower number in plots ranged from 8 to 32 (mean=15.57, SE=0.59).

Pollinator observations—I observed bumblebee visits for seven consecutive days in July 2011. Each day, weather permitting, there were five observation periods between 9:50 am and 6:20 pm EDT. Within each period, I observed each plot for 20 minutes. The order of plot observations was randomized. I logged a total of 23 hours of pollinator observation.

During observation of a plot, a second observer and myself worked together by each tracking one bumblebee at a time. We recorded the order of all flower visits made by a bumblebee until it left the plot, and then began following the next bumblebee to enter. Movement from one open flower to another on the same plant was counted as a new visit to that species. If the same pollinator returned to a flower previously visited after visiting a different flower, this was also counted as a new visit to that species.

Data analysis—I used a replicated *G*-test of goodness-of-fit to test for pollinator preference (Ippolito *et al*. 2004). This analysis compares the actual number of visits to flowers of each species to the expected number of visits under the null hypothesis of equal visitations.

I tested for pollinator constancy using a replicated *G*-test of independence. This test compares the observed to the expected number of heterospecific and conspecific transitions between flowers, where the expected number is based on the frequency of visits to each species (Ippolito *et al*. 2004). For this analysis, I only included pollinator transitions by pollinators that visited both species at frequencies greater than 0.1 within a single foraging bout (Aldridge and Campbell 2007, Flanagan *et al*. 2009).

I calculated *G* statistics separately for each plot, as well as pooled across plots. This method allowed me to test for pollinator preference and constancy across all plots, as well as for differences in pollinator preference and constancy among plots (Flanagan *et al*. 2010). *G*-statistics were calculated in Excel (McDonald 2009).

Hand pollination experiment

Experimental design—I designed a hand pollination experiment to estimate how the heterospecific transitions observed in the choice experiment may influence seed production. I tested both the potential for pollen interference and for hybridization. In May 2012, I collected *I. capensis* seedlings from a natural population at Hampshire College Farm Center in Amherst, Massachusetts. *Impatiens glandulifera* does not grow at this site. I grew seedlings of *I. glandulifera* from seed collected in Petersham, Massachusetts the previous summer. I allowed seedlings of both species to grow to adult size in a mown field in 3-gallon pots at the University of Massachusetts Crop and Animal Research and Education Center in South Deerfield, Massachusetts.

In September 2012, I conducted two rounds of hand pollinations on 30 of the potted *I. capensis* individuals. Thirty-six additional potted *I. capensis* plants and 36 potted *I. glandulifera* plants served as pollen donors. For each round of the experiment, I haphazardly selected four male stage flowers per pollen recipient and secured a bridal veil bag over each flower with a fine wire. I then haphazardly assigned one bagged flower per plant to each of the following treatments by marking the wire with colored paint: (1) Conspecific pollen, (2) mixed pollen, (3) heterospecific pollen, or (4) no pollen. I surveyed flowers once a day for the following three days and performed hand pollinations on all flowers that had advanced to female stage. Because the stigma is exposed only after the androecia falls off, I did not need to emasculate flowers before hand pollinating.

I performed pollinations using "bee sticks"—commercially available freeze-dried honeybees (*Apis mellifera*) that I glued onto toothpicks. I used these bee sticks in order to best mimic the fitness effect of the pollinator transitions observed during the pollinator choice experiment. Although bumblebees were the primary pollinators of my *I. capensis* plants, honeybees foraging for nectar are equally efficient pollinators of this species (Young *et al*. 2007). I randomly assigned pollen donors to pollen recipients. I pollinated flowers in the conspecific pollination treatment with a bee stick that had visited a male stage *I. capensis* flower on each of two donor plants. I pollinated flowers in the mixed pollination treatment with bee sticks that had visited one *I. capensis* donor and one *I. glandulifera* donor. For this treatment, I alternated the order of visitation for each round so that each plant had one flower in the mixed pollen treatment that was pollinated with a bee stick that visited *I. capensis* first, and one with a bee stick that visited *I. glandulifera* first. Finally, I pollinated flowers in the heterospecific pollen treatment with bee sticks that had visited flowers on two separate *I. glandulifera* donor plants. Following pollination, I replaced the bridal veil bag to prevent further pollinator visits. For the no pollen control treatment, I removed and then replaced the bridal veil bag. After all flowers were pollinated in the first round, I began the second round of the experiment. After the bagged fruits ripened, I collected them, counted their seeds, and averaged the number of seeds produced per plant per treatment across the two rounds.

To estimate the number of pollen grains deposited during hand pollinations, I performed 4-5 test pollinations for each treatment and then immediately deposited the stigmas in microcentrifuge tubes and dried them at a constant temperature for 48 hours. I then estimated the pollen deposited on each of the stigmas with a hemacytometer. As expected in natural populations, the amount of pollen deposited by each bee stick varied. However, the average number of pollen grains (±SE) deposited in the conspecific (2,659±510), mixed (2,404±1,213), and heterospecific (3,644± 1,940) treatments far outnumbered the four to seven ovules available in an *I. capensis* ovary (Young 2008). Even in the mixed pollination treatments, therefore, there should have been sufficient conspecific pollen for maximum seed production.

Data Analysis—To test for heterospecific pollen interference and the potential for hybridization, I compared treatment effects using a mixed model ANOVA with average seed set as the dependent variable, hand pollination treatment as the independent variable, and plant as a random effect. I tested all pair-wise comparisons and adjusted *P* values using a Tukey adjustment for multiple comparisons. I performed analyses using PROC GLM in SAS v. 9.3 (SAS Institute 2013).

Selection Analysis

Experimental design—I designed an experiment to test whether the invasive jewelweed affects its native relative by reducing pollinator visits and seed production, and by altering phenotypic selection on floral traits. The plants used in this experiment were grown from the same populations as described above.

I conducted the experiment in late August and early September 2011 in a mown field at the University of Massachusetts Crop and Animal Research and Education Center in South Deerfield, Massachusetts. I randomly assigned *Impatiens capensis* and *I. glandulifera* individuals in 3 gallon pots to 2X2 meter plots representing two treatments: (1) *I. capensis* in intraspecific competition, (2) *I. capensis* in mixed competition with conspecifics and *I. glandulifera* in a 50:50 mixture. Each plot contained ten plants. The treatments were replicated across six spatial blocks with five meters between plots within a block and five meters between blocks. I repeated the experiment with random reassignment of plants to plots before the second trial.

I haphazardly selected two flowers per plant for floral measurements. Using calipers, I measured corolla height (the tip of the upper petal to the tip of the lower lip), corolla width (the widest horizontal point on the corolla tube), and corolla depth in millimeters (Fig. 1). On these same flowers I also measured spur angle using a protractor following Travers *et al*. (2003) (Fig. 1). I averaged floral trait values for each individual to represent each plant. I removed two plants from the analysis that were outliers for corolla height. The floral traits measured were not strongly correlated with one another (all *r* values <0.55). I also took floral measurements for all *I. glandulifera* individuals included in the experiment. Although *I. glandulifera* flowers are typically larger than those of *I. capensis*, the size distributions of each species for all traits measured were close enough to overlap or touch (Fig. 2).

I estimated female fitness during each trial by the average number of seeds per fruit on each individual. At the start of a trial, I placed a thin wire around the pedicel of up to 3 male phase flowers on each plant. After the flowers transitioned from male to female to fruit, I secured bridal veil bags over the fruits for seed capture. This method allowed me to ensure that the entire female phase passed while in the experimental formation.

I estimated male fitness during the first trial by recording bumblebee visitation to individual plants. Each plant received 40 minutes of pollinator observation spread out in ten-minute increments over a single day between 10:30 am and 5:40 pm EDT. I recorded the total number of bumblebee visits to each individual plant during an observation period. All observations were made within one week.

Data Analysis—I tested for the effects of competition treatment on seed production and on pollinator visits using mixed model ANOVAs with PROC GLM (SAS Institute 2013). I included block and treatment by block interactions as random effects, and designated block by treatment interaction as the error term for testing the significance of the treatment effect. I square-root transformed pollinator visits to meet ANOVA assumptions.

I estimated standardized linear selection gradients (ß) for floral traits in each treatment using two separate fitness components, average seeds per fruit and bumblebee visits (Lande and Arnold 1983). Within each treatment, I transformed fitness components to relative fitness and trait values to units of variance from a mean of zero. I then regressed each fitness component over the trait values in each treatment in a multiple regression. I included block in all regression models. Because pollinator visits were not normally distributed, I tested the significance of selection gradients for this fitness component using untransformed data in a generalized linear model that assumed a Poisson error distribution (Formica *et al*. 2011). The standard errors for selection gradients generated by this model were calculated using the formula outlined in McGlothlin *et al*. (2010).

I used analysis of covariance (ANCOVA) to test whether there were significant differences in selection between treatments for each fitness component. The models included treatment, all floral traits, block, all treatment by trait interactions, and the treatment by block interaction as independent variables. For the pollinator visits fitness component, I again used a model that assumed a Poisson error distribution. I performed all selection analyses in PROC GLIMMIX (SAS Institute 2013).

RESULTS

Pollinator choice experiment

Bumblebees preferred *I. glandulifera* at a ratio of over 4 to 1, with *I. glandulifera* flowers receiving 1,568 visits and *I. capensis* flowers receiving only 364 (G_P=808.52, df=1, P<0.0001). There was no significant difference in preference across plots (G_H=5.26, df=2, P=0.07). I commonly observed pollinator movement between species. Out of 294 bumblebees that made one or more transition, 142 visited both species.

Bumblebees did, however, exhibit constancy. Individual bumblebees were more likely to transition between flowers within species than across species (G_P=18.21, df=3, P<0.001). There were 469 transitions between *I. glandulifera* flowers compared to an expectation of 443.1. There were 101 transitions between *I. capensis* flowers compared to an expectation of 74.2. Interestingly, when bumblebees did move between species, they made the exact same number of transitions from *I. capensis* to *I. glandulifera* as vice versa, with each transition type receiving 155 visits compared to an expectation of 181.3. There was no difference in pollinator constancy across plots (G_H=0.27, df=6, P>0.99).

Hand pollination experiment

Heterospecific pollen interfered with *I. capensis* seed production. Flowers that received mixed pollen produced on average 42% fewer seeds than flowers that received conspecific pollen (Tukey adjusted P=0.001, Fig. 3). The heterospecific pollen treatment produced the same number of seeds as the no pollen treatment, so there was no evidence for hybridization (Tukey adjusted P=0.99, Fig. 3).

Selection analysis

There was no difference in *I. capensis* seed production or pollinator visitation between treatments. *Impatiens capensis* plants in intraspecific and mixed competition treatments averaged 2.90 (95% CI: 2.60-3.21, n=92) and 2.91 (95% CI: 2.47-3.34, n=48) seeds per fruit, respectively ($F_{(1,11)}$<0.001, P=0.97). Bumblebee visits for these treatments averaged 8.98 (95% CI: 7.56-10.55, n=49) and 7.11 (95% CI: 5.46-8.98, n=30) per individual *I. capensis* plant, respectively ($F_{(1,5)}$=0.88, P=0.39). As in the pollinator choice experiment, bumblebees preferred the invasive over the native jewelweed. In the mixed competition treatment, invasive jewelweed individuals received on average 15.07 visits

(95% CI: 11.72-18.84, n=30), over twice as many as native jewelweed individuals received ($F_{(1,5)}$=7.03, P<0.05).

There were significant differences in phenotypic selection on *I. capensis* floral traits between treatments (Table 1). With average seed set per fruit as the fitness measure, there was no selection on floral traits in the intraspecific competition treatment. There was, however, strong negative selection on corolla height in the mixed competition treatment, and selection on this trait differed significantly between treatments. With pollinator visitation as the fitness measure, there was positive selection on corolla depth and height in the intraspecific competition treatment. In the mixed competition treatment there was again strong negative selection on corolla height, and this selection gradient differed significantly between treatments. There was also positive selection on corolla depth, but selection on this trait did not differ between treatments.

DISCUSSION

The ecological and evolutionary consequences of reproductive interactions between invasive and native plants depend first on the extent that species share the same pollinators (Gibson *et al*. 2012). The pollinator choice experiment demonstrated that the native and invasive jewelweed species share bumblebee visitors. Given that bumblebees at my study site had never encountered *Impatiens glandulifera* before, I might have expected them to prefer the familiar native plant. The results, however, showed that native bumblebees strongly prefer the invasive jewelweed. This result agrees with studies in Europe that showed that generalist native pollinators readily visited introduced *I. glandulifera* plants (Chittka and Schürkens 2001, Lopezaraiza-Mikel *et al*. 2007, Thijs *et* *al*. 2012). Whether pollinator preference for the invasive jewelweed results in reduced seed production in the native plant depends on the degree of pollen limitation in native jewelweed communities, as well as on the influence of the invasive jewelweed on overall pollinator diversity and abundance. Although bumblebees commonly moved between jewelweed species, they were more likely to transition to flowers within than between species. Pollinator constancy, therefore, may somewhat limit the deposition of heterospecific pollen in invaded communities.

The hand pollination experiment suggests that when pollinators do move between jewelweed species, there can be major fitness consequences for the native plant. It is possible that *I. glandulifera* pollen may reduce seed set in the native jewelweed through clogging of the stigmatic surface. *Impatiens glandulifera* pollen germinates readily on many surfaces, sometimes even within the androecium or on the bodies of pollinators (Titze 2000). These foreign pollen tubes, therefore, may result in less space available for conspecific pollen germination. In another hand pollination study, heterospecific pollen from *Impatiens pallida*, a native co-occurring congener, was shown to inhibit seed production in *I. capensis* by germinating and clogging the stigmatic surface and style (Randall and Hilu 1990). As in my study, there was no evidence for hybridization between jewelweed species (Randall and Hilu 1990).

I found no difference in seed production or pollinator visits between *I. capensis* plants in intraspecific versus mixed competition treatments. This result is somewhat surprising given the evidence for the detrimental effect of heterospecific pollen and for the overwhelming preference of bumblebees for the invasive jewelweed. Unlike in my study, two European studies found that *I. glandulifera* drew pollinators away from native plants (Chittka and Schürkens 2001, Thijs *et al*. 2012). Still, another European study found that *I. glandulifera* facilitated native plant pollinator visitation (Lopezaraiza-Mikel *et al*. 2007). It is possible that in my study, like this latter study, the invasive jewelweed may have drawn more individual bumblebees to the mixed competition treatment plots, which may have compensated for the tendency of bumblebees to prefer the invasive over the native jewelweed (Flanagan *et al*. 2010). Additionally, pollinator constancy may have limited heterospecific pollen deposition in the mixed competition treatment.

Although I found no differences in fitness between the competition treatments, there were significant differences in selection. In the mixed competition treatment, corolla height was under strong negative selection both when seed set and when pollinator visits served as the fitness measure. These results suggest that in the presence of *I. glandulifera*, there is selection for *I. capensis* to diverge in floral form from the invasive jewelweed, as has been found in studies of character displacement of floral traits when there is a cost to sharing pollinators with a neighboring species (Caruso 2000, Smith and Rausher 2008). A shorter corolla may encourage pollinator constancy by setting flowers apart from *I. glandulifera* flowers that have taller corollas.

Given that *I. capensis* and *I. glandulifera* distributions of corolla depth, corolla width and spur angle partially overlap, I expected selection to also favor driving these traits apart in order to further encourage pollinator constancy. Selection on these traits, however, did not differ across treatments. Because these traits are less showy, I suspect they may play a more minor role than corolla height in attracting pollinators. Furthermore, while corolla shape is generally considered important to the mechanical fit of a pollinator and flower, a previous study that manipulated *I. capensis* corolla depth and width found that these traits had no influence on bumblebee pollen transport success (Wilson 1995).

For the selection analysis, I chose to keep the total number of plants in the competition treatments constant in order to present pollinators with similar display sizes across treatments, as well as to mimic natural populations where total jewelweed abundance appears relatively constant as *I. glandulifera* displaces the native plant (C.M. Beans personal observation). Because of this experimental design, however, I cannot be certain that differences in selection between treatments are the direct result of *I. glandulifera* presence, rather than the result of a change in intraspecific competitor abundance (Snaydon 1991). It seems unlikely, however, that a reduction in intraspecific competition would result in stronger selection on floral traits.

Impatiens capensis populations have repeatedly been shown to evolve at a micro-environmental scale to a variety of environment stimuli including light and water availability (Dudley and Schmitt 1995, Heschel *et al*. 2002). It seems likely, then, that they may also evolve in response to the altered selection pressure imposed by *I. glandulifera*. For this evolutionary response to occur, however, the selected floral traits must be heritable. A repeatability analysis, which offers an upper limit estimate of trait heritability (Falconer and Mackay 1996), showed that corolla height, depth, width, and spur angle were all highly repeatable (repeatability score > 0.5) in a natural population in Maine (C.M. Beans and B. Bailey, University of Virginia, unpublished data). The spur angle repeatability agrees with previous work that found a broad-sense heritability for spur angle in *I. capensis* of 0.64 (Travers *et al*. 2003). Investigations of narrow sense heritability in other taxa have commonly found corolla size dimensions to be heritable traits (Mitchell and Shaw 1993, Campbell 1996, Kulbaba and Worley 2008, Gomez *et al*. 2009). My results, combined with these studies in other taxa, suggest that *I. capensis* corolla height is likely to be heritable, and thus may have the potential to evolve in response to the invasive jewelweed.

My study suggests that invasive plants may alter phenotypic selection on floral traits in native plant populations. More studies are needed, however, before we can determine whether invasive plants commonly alter floral selection on their neighbors, and under what conditions altered selection is likely to arise. For example, it is unknown whether my results would remain the same in a more complex plant community where multiple plant competitors may impose conflicting selection on *I. capensis* floral traits (Connell 1980). We also need to investigate whether and under what conditions and time scales native plants may evolve in response to the altered pollinator-mediated selection imposed by invasive species.

Exploring these topics further will enable us to discern whether native plants are likely to evolve and persist as invasive plants aggressively compete with them for pollination. Finally, these studies will provide a unique window into plant community assembly (Strauss *et al*. 2006, Sargent and Ackerly 2008, Thorpe *et al*. 2011). By studying native plants as they respond to invasive competitors, we can learn which factors lead to competitive exclusion, and which factors permit adaptive floral trait evolution that enables long-term coexistence. My study is a first step towards understanding the evolutionary consequences of pollinator-mediated interactions between invasive and native plants.

ACKNOWLEDGMENTS

I thank undergraduate field assistants, E. Wice, N. Forrester, T. Park, and M. Wagner. I thank K.A. Bostrom, N.C. Woodard, S. Walker, and K. Lilly of the University of Massachusetts Crop and Animal Research and Education Center for providing field space and significant support. I especially thank my UMass, Amherst host, R.G. Prostak, without whose help this project would not have been possible. I also thank the Hitchcock Center for the Environment for providing field space. N.L. Soper Gordon collected *I. capensis* seedlings. R.L. Malfi provided bumblebee identification. L.F. Galloway, D.E. Carr, R.M. Cox, H.M. Wilbur, and all members of the Galloway and Roach labs offered guidance throughout this project. This research was funded by the Jefferson Scholars Foundation, the University of Virginia Society of Fellows, a Sigma Xi Grant-in-Aid of Research, and a Harrison Undergraduate Research Award to M. Wagner.

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Table 1. Directional selection gradients (β±SE) on *Impatiens capensis* floral traits when in intraspecific and mixed species competition treatments. The interaction demonstrates the significance of the difference between selection gradients in the two treatments. Significant selection gradients and interactions are shown in bold.

		Competition Treatment		**Interaction**		
		Intraspecific	**Mixed**			
Fitness measure	**Floral trait**	**(β±SE)**	**(β±SE)**	***F***	***P***	**df**
Average seed set	Corolla depth	-0.01 ± 0.06	0.10 ± 0.10	0.73	0.40	1, 108
	Corolla height	0.03 ± 0.07	**-0.28 ± 0.11***	**5.69**	**0.02**	**1, 108**
	Corolla width	0.07 ± 0.06	0.12 ± 0.10	0.19	0.67	1, 108
	Spur angle	0.04 ± 0.06	0.11 ± 0.09	0.38	0.54	1, 108
Number of pollinator visits	Corolla depth	**0.11 ± 0.05***	**0.15 ± 0.06***	0.16	0.70	1, 59
	Corolla height	**0.17 ± 0.06***	**-0.12 ± 0.03***	**5.50**	**0.02**	**1, 59**
	Corolla width	-0.04 ± 0.03	0.15 ± 0.11	3.73	0.06	1, 59
	Spur angle	0.08 ± 0.04	0.23 ± 0.12	1.15	0.29	1, 59

******P***<0.05**

Figure 1. Depiction of floral traits measured on *I. capensis* flowers from (A) top view, (B) frontal view, and (C) lateral view.

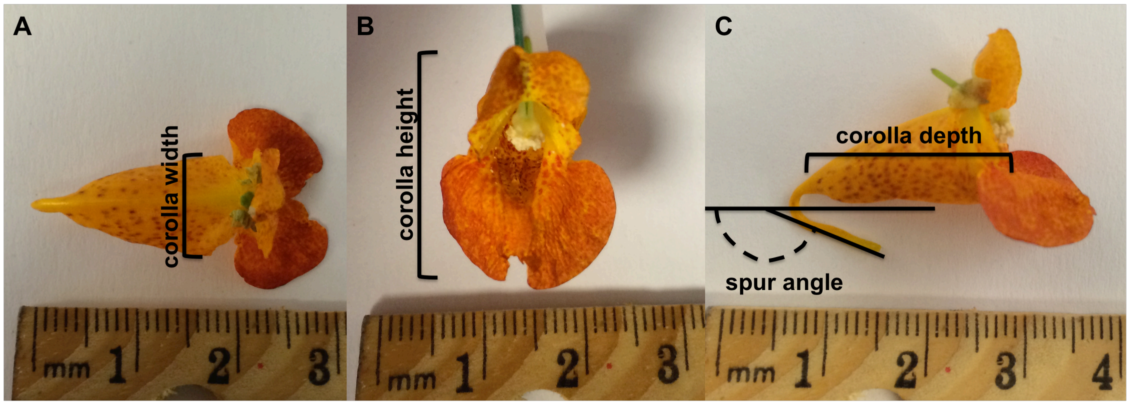


Figure 2. Floral trait distributions by species for *Impatiens* plants included in floral selection analysis (*I. capensis* n=86, *I. glandulifera* n=90).

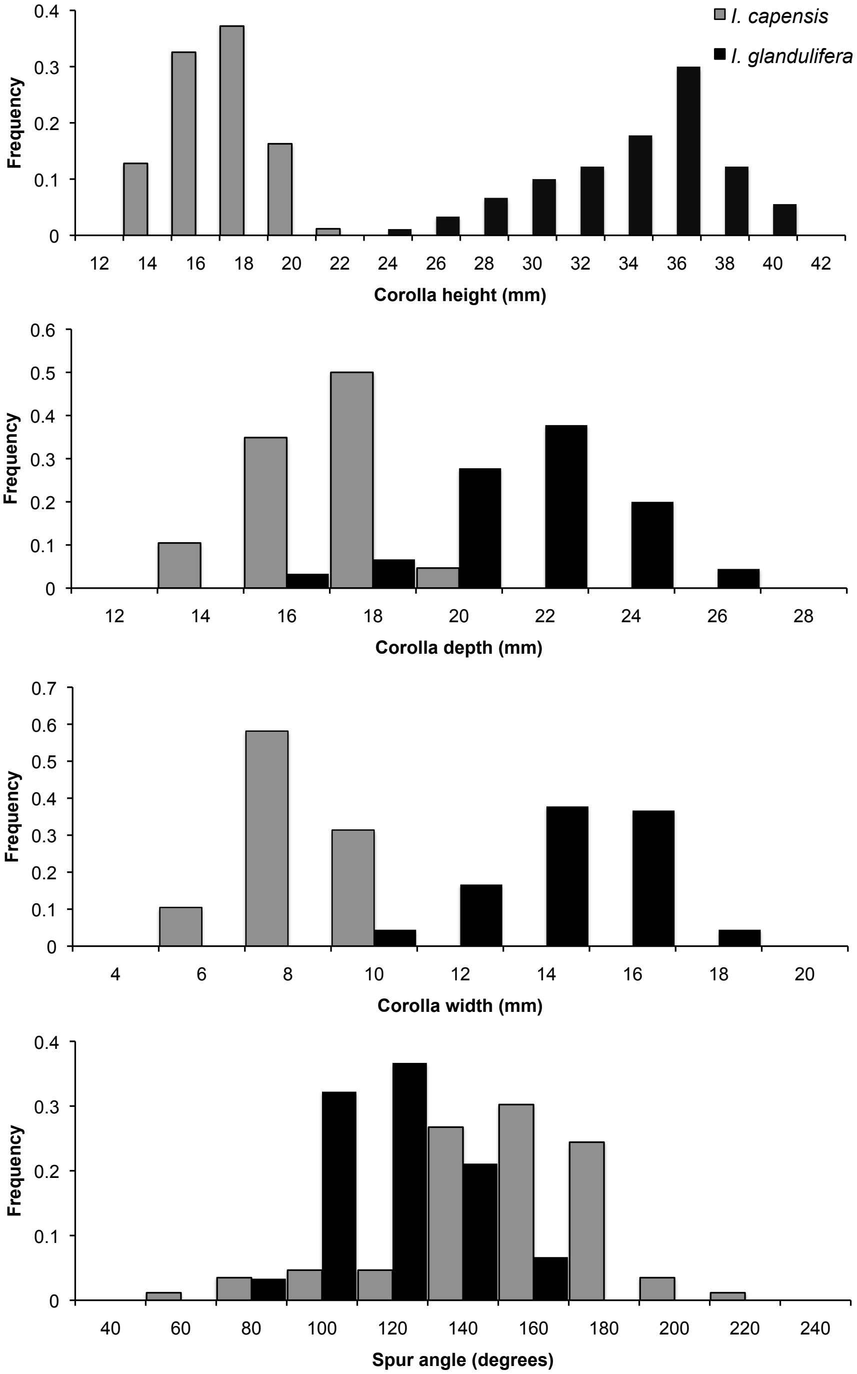
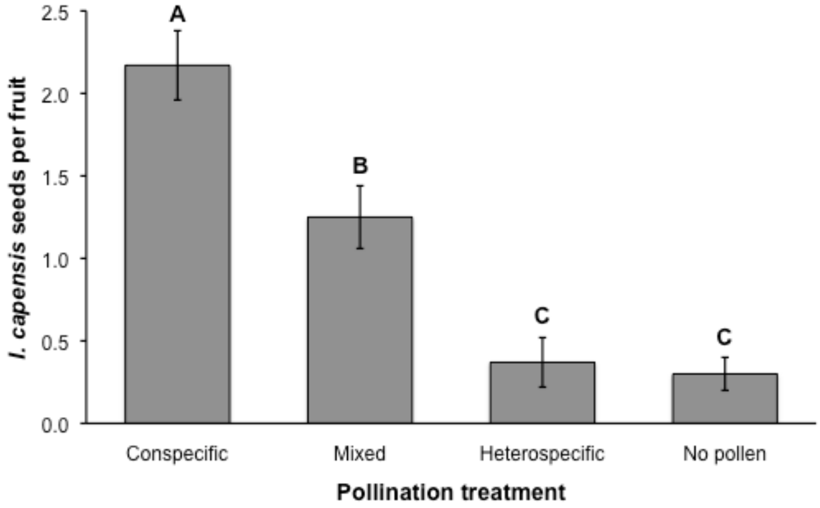


Figure 3. Mean (±SE) *I. capensis* seeds per fruit resulting from hand pollination treatments. Treatments with different letters are significantly different (Tukey adjustment for multiple comparisons, $P<0.05$).



CHAPTER THREE:

Pollinator-mediated selection in a complex plant community: Deconstructing diffuse selection into pair-wise parts

ABSTRACT

We know from studies of diffuse competition that the effect of pair-wise inter-specific plant competition is often altered dramatically as additional species are added to a community. The evolutionary consequences of these complex competitive interactions are less well understood. Experimental designs for testing this question have been proposed in the herbivory literature, but have not yet been applied to plant-plant interactions. I tested how phenotypic selection on *Impatiens capensis* floral traits was altered under pollinator-mediated competition with three different pair-wise competitors versus a more complex community of competitors. This species addition experiment allowed me to answer the following questions: (1) Does the identity of competitors affect the seed production of *I. capensis* individuals? (2) Does phenotypic selection on *I. capensis* floral traits depend on the identity of competing species? (3) Is selection on *I. capensis* floral traits diffuse?

I found that although there were no differences in reproductive success across treatments, selection on floral traits was dependent on the competitor community. Most notably, selection was diffuse. Phenotypic selection on corolla height in one pair-wise competition treatment was significantly altered by the addition of more competitor species. In the more complex plant community, the direction of selection was altered and the magnitude increased. These results suggest that pollinator-mediated plant competition may have the potential to alter evolutionary trajectories even in complex plant communities where multiple competitors exert differing selection pressures on a focal species.

KEY WORDS: competition-driven evolution, diffuse selection, *Impatiens capensis*, pollinator-mediated competition, selection analysis

INTRODUCTION

Ecologists have long studied the effects of inter-specific competition on plant abundance and distributions (Clements 1916, Tilman 1982, Keddy and Weiher 1999). Many plant competition studies have focused, not only on pair-wise competition between individual species, but also on diffuse competition, where a focal species competes with multiple competitor species at once (MacArthur 1972). These experiments have demonstrated that the effect of pair-wise inter-specific plant competition is often altered dramatically as additional species are added to a community (Fowler 1981, Callaway and Pennings 2000, Metlen *et al*. 2013).

The evolutionary consequences of these complex competitive interactions have received less attention (Thorpe *et al*. 2011). We know that competition from a single competitor species can alter phenotypic selection on a focal plant species (Fishman and Wyatt 1999, Caruso 2000, Smith and Rausher 2008, Wassink and Caruso 2013). Whether this altered selection pressure may be maintained or further altered in a more complex community, however, is less well understood (Mitchell *et al*. 2009). If different competitors impose opposite selection pressures on the same trait, or on positively correlated traits, then the total selection experienced by the focal species could be zero. For this reason, it has been argued that plants cannot evolve in response to competition in complex communities (Connell 1980). Others argue, however, that if one competitor imposes much stronger selection than others, or if competitors impose selection on different, uncorrelated traits, then an evolutionary response may still be possible (Leger and Espeland 2010, Beans 2014, Strauss 2014). Additionally, competitors may act together to select on the same trait in the same direction.

There is now a call for more studies to test how selection imposed through pair-wise interactions between species is maintained or altered in more complex communities (Strauss *et al*. 2005, Mitchell *et al*. 2009). Experimental designs have been proposed in the herbivory literature to test both whether selection is dependent on the identity of competitor species and whether selection is diffuse (Strauss *et al*. 2005). Selection is considered diffuse when adding more community members changes its strength or direction (Strauss *et al*. 2005). Recent studies have investigated how selection on plants is altered by pair-wise versus multi-species interactions with herbivores, pollinators, and single plant competitors (Iwao and Rausher 1997, Juenger and Bergelson 1998, Lau 2008, Lankau and Strauss 2008, Sahli and Conner 2011). To my knowledge, no study has investigated the effect of a single plant competitor species versus multiple plant competitor species in driving phenotypic selection on a focal plant.

Here I test how phenotypic selection on floral traits is altered under pollinator-mediated competition with a single plant competitor versus a more complex community of competitors. We already know from two-species studies that pollinator-mediated competition can result in altered selection on floral traits (Fishman and Wyatt 1999, Caruso 2000, Smith and Rausher 2008, Wassink and Caruso 2013). For example, selection under pair-wise competition may favor a shift in floral traits that encourages greater visitation or reduced heterospecific pollen deposition (Wassink and Caruso 2013, Chapter 2).

I used a species addition experiment where a focal species, *Impatiens capensis* (spotted jewelweed), grew alone, in pair-wise competition with each of three different competitor species, and in diffuse competition with all three competitor species at once. I then tested the following questions: (1) Does the identity of competitors affect the seed production of *I. capensis* individuals? (2) Does phenotypic selection on *I. capensis* floral traits depend on the identity of competing species? (3) Is selection on *I. capensis* floral traits diffuse?

METHODS

Study species

Impatiens capensis Meerb. (spotted jewelweed), the focal species in this study, is an annual plant in the Balsaminaceae. Native to the United States and Canada, it is commonly found in damp areas along roadsides and streambeds. Its yellow-orange flowers with red-orange spots attract a broad range of pollinators including bumblebees and hummingbirds. The flowers have a wide conical shape that narrows into a nectar-filled tube called a spur (Rust 1979). While spurs typically bend downward, they vary in angle across individuals. These showy, chasmogamous flowers are open pollinated and protandrous, with the flower opening in a male stage and progressing to female stage only after the androecia falls off and exposes the stigma. *Impatiens capensis* plants also produce closed cleistogamous flowers that are highly reduced in size and result in selfed seed. Selfing results in lower seed production and lower survival of overwintering seeds (Mitchell-Olds and Waller 1985).

Impatiens glandulifera Royle*,* a jewelweed native to the Himalayas, is currently spreading and now grows completely intermixed with the native jewelweed in plant communities across the northeastern United States. Its flowers range in color from pale pink to magenta. The two *Impatiens* species have similar floral shapes and attract the same generalist pollinators. A previous study found that, while bumblebees prefer the invasive *Impatiens*, they commonly move between the two species (Chapter 2).

In addition to the invasive jewelweed, I also included *Monarda didyma* L. (Lamiacea) and *Lobelia siphilitica* L. (Campanulaceae), as competitors in this study. I chose these plants because they overlap in flowering time with the *Impatiens* species, grow in the same New England streamside environments, and attract the same bumblebee pollinators. *Monarda didyma*, like the *Impatiens* species, also attracts hummingbird pollinators (Whitten 1981). My expectation was that these species should, therefore, compete strongly for pollination with *I. capensis*. Additionally, by including these competitors, I created a pollinator environment with a diversity of floral color and form. *Monarda didyma* has bright red flowers with narrow corolla tubes that are arranged in tight terminal clusters. *Lobelia siphilitica* has light blue flowers with wider corolla tubes that are arranged along dense terminal racemes. I predicted that differences in the floral traits of competitors across treatments would lead to differences in pollinator behavior, which would ultimately result in selection on the focal species varying across treatments.

Experimental design

I conducted a species addition experiment, in summer 2012, by arranging potted plants in an open field at the University of Massachusetts Crop and Animal Research and Education Center in South Deerfield, Massachusetts. I collected the focal species, *I. capensis*, in early spring 2012 at the cotyledon stage from a wild population approximately 15 kilometers from my study site. I immediately planted seedlings into 4-inch square pots in Fafard 3B growing medium and cared for them in a greenhouse. I grew the invasive competitor, *I. glandulifera*, from seed collected the previous fall. I cold stratified the seeds for 2.5 months. I then planted them in MetroMix 200 in trays with 3X3X4.5cm wells in early May to coincide with germination of wild populations. In late May, I transplanted them into the same growing conditions as used for the native jewelweed. In late June, I transplanted all jewelweed plants into 3-gallon pots in Fafard 3B potting medium. At this time I also purchased individuals of *Lobelia siphilitica* from Van Berkum Nursery in Deerfield, New Hampshire, and *Monarda didyma* from Nasami Farm in Whately, Massachusetts. I transplanted these plants from 2-quart to 3-gallon pots in Fafard 3B. I grouped all potted plants by species and cared for them in the field.

In mid August, when all species were flowering, I collected floral measurements on two haphazardly selected flowers per *I. capensis* plant. I measured corolla height (the tip of the upper petal to the tip of the lower lip), corolla width (the widest horizontal point on the corolla tube), and corolla depth in millimeters using calipers. I also measured spur angle using a protractor following Travers *et al*. (2003). I then averaged trait values for each plant. One plant was removed from the analysis because it was an extreme outlier for spur angle.

After collecting floral measurements for each *I. capensis* individual, I randomly assigned plants to 2X2 meter plots representing the following five competition treatments:

(1) Conspecific competition only—Four *I. capensis* plants

(2) Pair-wise competition with *I. glandulifera*—Four *I. capensis* plants plus four *I. glandulifera* plants

(3) Pair-wise competition with *L. siphilitica*—Four *I. capensis* plants plus four *L. siphilitica* plants

(4) Pair-wise competition with *M. didyma*—Four *I. capensis* plants plus four *M. didyma* plants

(5) All-competitors—Four *I. capensis* plants plus four of each of the competitor species

I arranged treatments into blocks with each treatment occurring once in each block. I placed the four *I. capensis* plants in each plot in a diamond formation approximately 70 cm apart. I placed competitor plants directly adjacent to *I. capensis* plants. There were five meters between each treatment within a block and five meters between each block. I repeated this experiment four times in the month of August with the 1st, 2nd, 3rd, and 4th trials including 4, 5, 6, and 6 blocks, respectively, for a total of 84 focal plants per treatment. I randomly reassigned plants to positions before each trial.

On the first day of a trail, I marked two to three haphazardly selected male-stage flowers per plant with a metal wire. I allowed plants to remain in the experimental formation until all marked flowers transitioned from male to female stage to fruit (~3-4 days). Once they transitioned to the fruiting stage, I covered fruits with bags made of bridal veil so I could capture seeds upon ripening. I then calculated the average number of seeds produced by each plant during each trial.

Statistical analysis

Fitness effects— I tested for the effects of competition treatment on the average seed set per fruit using mixed model ANOVAs with PROC GLM (SAS Institute 2013). I included block and treatment by block interactions as random effects. Because *I. capensis* individuals within a plot were not independent of one another, I designated the block by treatment interaction as the error term for significance testing.

Selection analysis—I first estimated standardized linear selection gradients (ß) for floral traits in each treatment (Lande and Arnold 1983). Using average seed set per fruit as a fitness measure, I regressed relative fitness within each treatment over trait values that were transformed to a mean of zero and standard deviation of one. I also included block as a random variable in all regression models.

To test whether selection on *I. capensis* floral traits is dependent on the identity of competing species, I used an analysis of covariance (ANCOVA) that included all pair-wise competition treatments and the all-competitors treatment (Strauss *et al*. 2005). The analysis included average seed set per fruit as the dependent variable and treatment, all floral traits, block, all treatment by trait interactions, and the treatment by block interaction as independent variables.

Because the identity of competitors affected selection, I was then able to test whether selection in the all-competitors treatment was diffuse by conducting three additional ANCOVAs (Strauss *et al*. 2005). These ANCOVAs tested for differences in selection between each pair-wise competition treatment and the all-competitors treatment. A significant result for this analysis would suggest, not only that selection on floral traits is dependent on the identity of a competitor species, but also that selection on a trait in the presence of one competitor is altered in a more complex community.

RESULTS

Fitness effects

The average seed set per fruit for *Impatiens capensis* individuals was nearly constant across all treatments (Figure 1). The variance in this fitness measure was also very similar across treatments.

Selection analysis

Although there were no differences in reproductive success across treatments, there were some differences in selection on *I. capensis* floral traits (Table 1). In the conspecific competition treatment, there was marginally significant positive selection on spur angle, meaning that selection favored individuals with spurs angled more tightly under the corolla. In the all-competitor treatment, there was marginally significant positive selection on corolla depth. There was also strong negative selection on corolla height. I did not detect significant selection gradients in any of the pair-wise competition treatments.

When comparing selection across all treatments with one or more competitors, I found that the identity of the competitor was marginally significant in determining selection on corolla height (Table 2). By contrasting each pair-wise competition treatment with the all-competitors treatment, I found that this difference largely stemmed from a significant difference in selection on corolla height between the *L. siphilitica* pair-wise competition treatment and the all-competitors treatment (Table 3). In the *L. siphilitica* pair-wise competition treatment there was positive, but non-significant selection on corolla height (ß=0.10±0.08, *P*=0.23). In the all-competitors treatment, however, there was strong negative selection on this trait (ß=-0.19±0.07, *P*=0.01). This difference suggests that selection in the all-competitors treatment is diffuse, as selection in the presence of *L. siphilitica* is significantly altered when more competitor species are added to the community.

DISCUSSION

Despite finding no differences in reproductive success between treatments, I did find differences in selection. Most notably, I detected stronger selection on floral traits when the focal species competed with a range of competitors than with any single competitor species. I also found that selection was diffuse. Pair-wise selection on corolla height experienced under competition with *L. siphilitica* was altered when additional competitors were added to the community. These results suggest that selection has the potential to be intensified in complex plant communities, rather than being cancelled out by opposing individual selection pressures. In these communities, plants may evolve in response to the combination of competitors, rather than to any individual competitor species. If floral traits under selection are heritable, an evolutionary response to this competition-driven selection is possible.

The lack of fitness differences across treatments is surprising given the variation in both competitor identity and abundance. I expected that *I. glandulifera* in particular would reduce seed production in the native plant because previous studies suggest that invasive plants sharing similar floral morphologies with native plants have the greatest potential to negatively affect native seed production (Morales and Travaset 2009). It is not uncommon, however, for community members to alter selection on a focal species without altering overall fitness (Caruso 2001, Strauss *et al*. 2005, Parachnowitsch and Kessler 2010, Wassink and Caruso 2013).

In a previous experiment, I found that *I. capensis* individuals competing with *I. glandulifera* experienced selection for decreased corolla height (Chapter 2). In the present study, which was conducted in the same field only one year later, I did not detect any selection on floral traits in the *I. glandulifera* competition treatment. Pollinator behavior may have been altered in the present study by the greater diversity of plant species and the differences in overall plant number between treatments. In the previous study, only plots with *Impatiens* species were present and every treatment contained ten total plants. In the present study, there were two additional species present and plant number across treatments ranged from four to sixteen. Because the overall plot display size was greatest in the all-competitors treatment, pollinators may have been preferentially drawn to these plots. This issue could be addressed by offering pollinators one treatment at a time (Smith and Rausher 2008), or by adding additional treatments that vary species frequency while maintaining total plant number (Brown *et al*. 2002). These options, however, have time and space requirements that are often not feasible for studies of diffuse competition, which include many treatments due to multiple pair-wise interactions.

Pollinator behavior has often been linked to differences in selection on floral traits (Levin 1985, Muchhala and Potts 2007, Chapter 2). While pollinator behavior likely also explains the altered selection between treatments in the present study, it is unclear why selection on corolla height would be positive (although non-significant) in the *L. siphilitica* treatment, but negative in the all-competitor treatment. One explanation may be that flowers with smaller corollas attracted fewer pollinators and, therefore, had a reduced risk of receiving heterospecific pollen in the all-competitor treatment. Additional research tracking individual pollinator movements is necessary to fully understand the mechanism behind this altered selection pressure.

While the experimental design presented in this study has long been utilized in the herbivory literature (Iwao and Rausher 1997), my study is the first to apply it to understanding competition-driven selection in plant communities. We can trace this gap in the plant literature to the long-standing dominance of ecological theory that downplays the importance of plant-plant interactions in shaping communities in favor of abiotic or random processes (Gleason 1926, Hubbell 2005). Despite the prevalence of these theories, there is currently a growing interest in understanding the evolutionary consequences of plant-plant interactions (Thorpe *et al*. 2011, Lankau 2012, Wassink and Caruso 2013). My study adds to this field of research by dissecting phenotypic selection in a complex community into its pair-wise parts. In doing so, I found that competition can, in fact, drive phenotypic selection in the presence of multiple plant competitors.

ACKNOWLEDGMENTS

I thank my undergraduate field assistant M. Wagner for her hard work and dedication to this project. I thank K.A. Bostrom, N.C. Woodard, S. Walker, and K. Lilly of the University of Massachusetts Crop and Animal Research and Education Center for providing field space and significant support. I especially thank my UMass, Amherst host, R.G. Prostak, without whose help this project would not have been possible. L.F. Galloway, D.E. Carr, R.M. Cox, H.M. Wilbur, and all members of the Galloway and Roach labs offered guidance throughout this project. This research was funded by the

Jefferson Scholars Foundation, the University of Virginia Society of Fellows, a Sigma Xi Grant-in-Aid of Research, and a Harrison Undergraduate Research Award to M. Wagner.

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Table 1. Directional selection gradients (β±SE) on *I. capensis* floral traits under each competition treatment. Significant and marginally significant selection gradients are shown in bold.

	Competition Treatment				
Trait	**Conspecifics only**	***I. glandulifera***	***L. siphilitica***	***M. didyma***	**All competitor species**
Corolla depth	-0.04 (±0.07)	0.11 (±0.07)	0.03 (±0.09)	0.08 (±0.09)	**0.14 (±0.08)***
Corolla height	0.003 (±0.09)	-0.02 (±0.08)	0.10 (±0.08)	-0.10 (±0.10)	**-0.19 (±0.07)****
Corolla width	-0.08 (±0.09)	-0.09 (±0.08)	-0.10 (±0.09)	0.06 (±0.12)	0.01 (±0.09)
Spur angle	**0.11 (±0.06)***	0.03 (±0.07)	-0.05 (±0.07)	0.02 (±0.07)	-0.07 (±0.06)

*$P<0.1$

**$P<0.05$

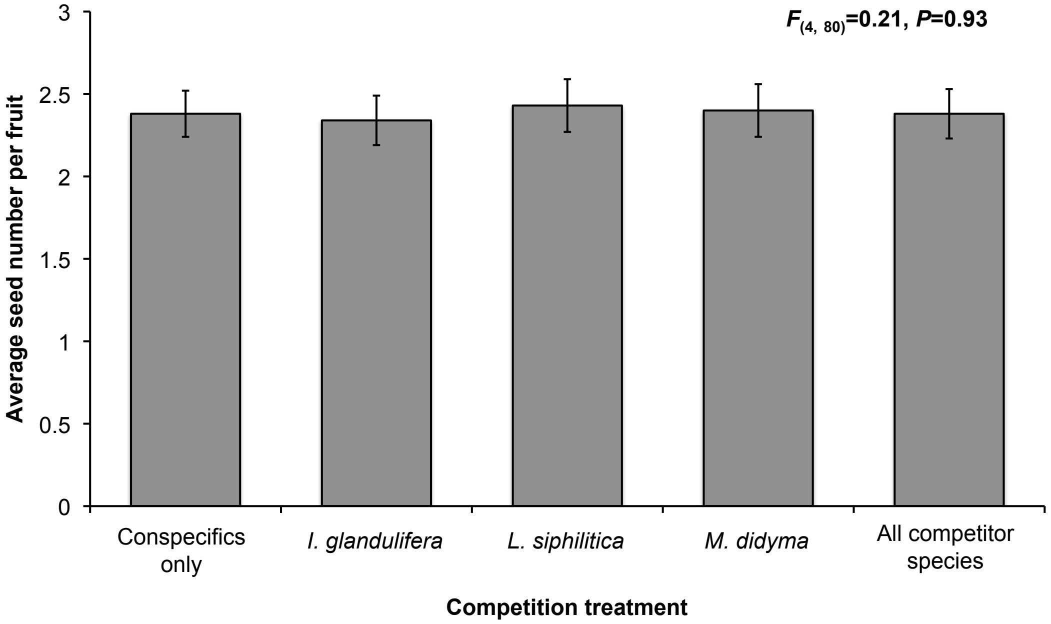
Table 2. Results of ANCOVA testing for the effect of competitor identity on selection on each floral trait. The analysis included all pair-wise competition treatments and the all-competitors treatment.

	Interaction (df = 3, 162)	
Trait	***F***	***P***
Corolla depth	0.45	0.72
Corolla height	2.47	**0.06**
Corolla width	0.51	0.67
Spur angle	0.54	0.66

Table 3. Results of ANCOVA testing whether selection on *I. capensis* floral traits is diffuse by contrasting selection in each pair-wise competition treatment with the all-competitor treatment.

	Interaction					
	I. glandulifera **vs. All competitors** (df=1, 82)		***L. siphilitica*** **vs. All competitors** (df=1, 86)		***M. didyma*** **vs. All competitors** (df=1, 84)	
Trait	***F***	***P***	***F***	***P***	***F***	***P***
Corolla depth	0.33	0.56	1.10	0.30	0.24	0.63
Corolla height	2.19	0.14	**6.77**	**0.01**	0.61	0.44
Corolla width	0.63	0.43	0.74	0.39	0.04	0.84
Spur angle	1.58	0.21	0.12	0.73	1.12	0.29

Figure 1. Average seed number per fruit produced by *I. capensis* individuals in each competition treatment.



CHAPTER FOUR:

An invasive plant alters selection on the vegetative growth of a native congener

ABSTRACT

The evolution of invasive plants in response to novel environmental challenges is well documented. We are only now, however, beginning to explore whether native plants may also evolve in response to novel competitors. I tested whether an invasive jewelweed, *Impatiens glandulifera*, altered phenotypic selection on the vegetative traits of a closely related native congener, *I. capensis*.

Using a greenhouse competition experiment, an invasive species removal experiment, and a survey of natural populations, I asked the following questions: (1) Does the presence of the invasive jewelweed alter the fitness of native jewelweed populations? (2) Does the native jewelweed respond plastically to the invasive competitor? (3) Does the invasive jewelweed alter phenotypic selection on vegetative traits of the native congener? (4) Does the invasive jewelweed affect the vegetative growth of the native congener similarly across multiple natural plant communities?

The greenhouse and field results showed that when the invasive jewelweed is present, phenotypic selection favors native jewelweed individuals investing less in rapid upward growth and more in branching and fruiting potential through the production of internodes. The survey of natural populations suggested, however, that there has not been a consistent evolutionary or plastic response to the invasion. Overall, this research demonstrates that invasive plants have the potential to greatly alter natural selection on native competitors. The ability of native plant populations to increase their chances of survival by evolving in response to this altered selection pressure, however, may be limited by other influences.

KEYWORDS: competition-driven evolution, invasive plants, *Impatiens capensis*, *Impatiens glandulifera*, phenotypic selection

INTRODUCTION

While the ability of invasive plants to evolve in response to novel landscapes is well documented (Maron *et al*. 2004, Müller-Schärer *et al*. 2004, Montague *et al*. 2008, Kilkenny and Galloway 2013), we are only now beginning to explore whether native plants may also evolve in response to novel competitors (Strauss *et al*. 2006). Invasive plants reduce the biodiversity of native plant communities through strong vegetative competition, often outcompeting native plants for sunlight and water, and overwhelming native communities through high reproductive output (Martin 1999, Adams and Engelhardt 2009, Vilá *et al.* 2011). The intense competitive environment shaped by these species may create a strong selective force for adaptive evolution in native plants (Leger and Espeland 2010).

Whether native plant populations can evolve in response to invasive competitors may determine whether they persist or are competitively excluded from invaded communities. Understanding the underlying evolutionary mechanisms at play in invaded communities can aid conservationists in predicting which plant populations will survive an invasion and which require remediation efforts (Rowe and Leger 2011). From a basic science perspective, understanding whether native plants evolve in response to invasive competitors can offer valuable insight into the role of competition-driven evolution in shaping plant communities (Strauss *et al*. 2006). Although the importance of competition to plant evolution has frequently been downplayed (Gleason 1926, Hubbell 2005), there is currently a resurgence in support for its role as a central force in driving adaptive plant evolution (Thorpe *et al.* 2011, Beans 2014). The study of species invasions can make an important contribution to this field of research by allowing us to watch ecological and evolutionary processes unfold as a novel species is integrated into a plant community.

Some studies already suggest that adaptive evolution in response to invasive competitors is possible (Lau 2006, Mealor and Hild 2007, Leger and Espeland 2010, Oduor 2013). Seedlings of the native grass, *Elymus multisetus,* grown from seeds from communities invaded by the invasive grass, *Bromus tectorum*, grew larger when competing with the invasive than those from uninvaded areas (Rowe and Leger 2011). Increased competitive ability was correlated with a greater root to shoot ratio. Similarly, seedlings of the native herb, *Pilea pumila*, grown from seed from areas invaded by the invasive biennial, *Alliaria petiolata*, were more successful competitors than those from uninvaded communities (Lankau 2012). This greater success was correlated with an increased ability to maintain mycorrhizal colonization.

I build on these previous studies by testing whether an invasive jewelweed, *Impatiens glandulifera*, alters phenotypic selection on the vegetative traits of a closely related native congener, *I. capensis*. I expected that the invasive jewelweed had the potential to direct evolutionary change in the native plant because the two species frequently grow intermixed and share a similar niche space (Tabak and von Wettberg 2008). Additionally, *I. capensis* has previously been shown to evolve at a micro-environmental scale to changes in light availability (Dudley and Schmitt 1995). Because the invasive jewelweed nearly always grows taller than the native congener, I predicted that the native jewelweed would experience selection on its vegetative response to shading, much as it has already been shown to experience altered selection on shading response under a dense forest canopy (Dudley and Schmitt 1995).

Competition-driven phenotypic selection has been estimated for native plants competing with other native plants (Caruso 2000, Smith and Rausher 2008a). My study shows how these same techniques can be used to explore the potential for future evolutionary change in invaded communities. Using a greenhouse competition experiment, an invasive species removal experiment, and a survey of natural populations, I asked the following questions: (1) Does the presence of the invasive jewelweed alter the fitness of native jewelweed populations? (2) Does the native jewelweed respond plastically to the invasive competitor? (3) Does the invasive jewelweed alter phenotypic selection on vegetative traits of the native congener? (4) Does the invasive jewelweed affect the vegetative growth of the native congener similarly across multiple natural plant communities?

METHODS

Study Species

Impatiens capensis Meerb. (Balsaminaceae), spotted jewelweed, is an annual plant native to much of the United States and Canada. It germinates in early May and continues to grow until first frost, often reaching a height of up to 1.5 meters. Flowering from late June onward, it produces fruits from both open, chasmogamous flowers and closed, cleistogamous flowers. *Impatiens capensis* senses the shift in red to far-red radiation that occurs when sun shines through a competitor and can respond with what is known as the “shade avoidance syndrome” (Dudley and Schmitt 1995). This response is characterized, in part, by greater investment in elongation through the extension of internodes at the cost of branching and fruiting (Smith and Whitelam 1997). *Impatiens* *capensis* populations under dense woodland shade evolve in response to strong selection to suppress this elongation response, as rapid upward growth will never result in outgrowing the forest canopy (Dudley and Schmitt 1995).

Native to the Himalayas, *Impatiens glandulifera* Royle was introduced to the northeastern United States in the late 1800's, and now grows intermixed with *I. capensis* along roadsides, stream banks, and woods edges (Tabak and Wettberg 2008). The invasive jewelweed germinates synchronously with the native congener, but because it can reach a height of nearly 3 meters, it often towers over the native jewelweed by the end of the growing season.

Both jewelweed species experience strong intraspecific vegetative competition. This competition is especially intense early in the growing season when jewelweed populations go through a self-thinning process where all but the fastest growers become suppressed by shading or experience early senescence. The early-season density of native jewelweed seedlings can reach well over 2,000 individuals per square meter (Schmitt *et al*. 1987). The addition of the fast-growing invasive competitor may make the thinning process even more competitive for native jewelweed seedlings.

Greenhouse Competition Study

Experimental design

I conducted a greenhouse competition study to test whether *I. glandulifera* alters phenotypic selection on *I. capensis* in a controlled environment. In mid-September 2011, I bulk collected seed from an *I. capensis* population uninvaded by *I. glandulifera* at the Hitchcock Center for the Environment in Amherst, Massachusetts. On the same day, I bulk collected seed from an *I. glandulifera* population about 30 km away in Petersham, Massachusetts. In early October, I placed the *I. capensis* seeds in cold stratification at 4˚C on moistened filter paper in petri dishes. Because *I. glandulifera* seeds require a shorter cold stratification period, I waited until mid-November to place them in the same conditions.

Seeds of both species began germinating in cold stratification in mid-January 2012. In late January, I planted the seeds into trays with 3X3X4.5cm wells in Metro Mix 200 potting medium and placed them in the greenhouse at the University of Virginia. One week later, while seedlings were at the cotyledon stage, I transplanted them into 10cm-square by 34.5cm-deep pots filled with Fafard 3B potting medium in the following three treatments with 85 replicates each:

(1) No competition—a focal *I. capensis* seedling in the center of the pot without competitors.

(2) Conspecific competition—a focal *I. capensis* seedling surrounded by four conspecifics.

(3) Invasive competition—a focal *I. capensis* seedling surrounded by four *I. glandulifera* seedlings.

I randomly assigned pots to 17 blocks across 3 benches. In each block, I placed five replicates of each treatment together, with the order of treatments alternating across blocks. I spaced blocks 20cm apart and treatments within blocks 15cm apart. Within each competition treatment, I spaced pot sides 10 cm apart with the corners of adjacent pots touching. In the no competition treatment, I spaced pot sides 10 cm apart with corners 8 cm apart. To minimize edge effects, I placed a wire around each competition treatment group in each block to restrict plants from growing into the open space between treatment groups.

I measured the height of focal plants at the date of transplanting into the treatments and then once a week for the duration of the experiment. I measured the length of the hypocotyl and first internodes of focal plants in late March after their elongation was complete. In mid-April, as plants across all treatments were just beginning to show signs of senescence, I estimated the total number of fruits produced by each focal plant by counting the number of buds, flowers, fruits, and pedicels with dehisced fruit (Dudley and Schmitt 1996). I also counted the total number of internodes. I then harvested the focal plants, dried them at a constant temperature, and weighed them to obtain above-ground biomass.

Statistical Analysis

Fitness effects—I compared the effects of competition treatment on *I. capensis* total fruit production, final height and above-ground biomass using mixed model ANOVAs with PROC GLM (SAS Institute 2013). I included block and treatment by block interactions as random effects. Because individual plants within the same treatment and block were not independent of one another, I designated the block by treatment interaction as the error term for testing the significance of the treatment effect. I used independent contrasts for pair-wise comparisons of treatments and corrected *P* values using a Tukey adjustment. I logarithmically transformed fruit and above-ground biomass to improve normality.

Plastic responses to competition—To find whether *I. capensis* vegetative traits responded plastically to the presence of competition with conspecifics and with *I. glandulifera*, I tested whether they differed across treatments using mixed model ANOVAs in PROC GLM (SAS Institute 2013). The vegetative traits included early growth rate, hypocotyl length, first internode length, and total internode number. Early growth rate was estimated as the average gain in height (cm) per day over the first quarter of the growing season. As in the analysis of fitness effects, I included block and treatment by block interactions as random effects and designated block by treatment interaction as the error term for significance testing. I used independent contrasts for pair-wise comparisons of treatments with Tukey-adjusted *P* values.

Selection Analysis—To understand how competition with *I. glandulifera* alters selection on the *I. capensis* vegetative traits included in the above analysis, I estimated standardized linear selection gradients (ß) for each trait in each of the two competition treatments (Lande and Arnold 1983). Within each treatment, I used relative fruit production as the fitness measure for each individual and regressed this value over vegetative trait values standardized to a mean of zero and standard deviation of one. I also included block in each multiple regression. Because total fruit set was not normally distributed, I tested the significance of selection gradients using a generalized linear model that assumed a Poisson error distribution with untransformed data (Formica *et al.* 2011). I calculated the standard errors for selection gradients using the formula outlined in McGlothlin *et al*. 2010.

To test whether selection gradients differed significantly between treatments, I used an analysis of covariance (ANCOVA) with untransformed data. The model included fruit set as the dependent variable and treatment, all vegetative traits, block, all treatment by trait interactions, and the treatment by block interaction as the independent variables.

This model also assumed a Poisson error distribution. I used PROC GLIMMIX for all selection analyses (SAS Institute, 2013).

Invasive Species Removal Study

Experimental design

To test whether *I. glandulifera* alters phenotypic selection on *I. capensis* vegetative traits in a natural plant community, I designed an invasive species removal experiment in a community where the two jewelweeds grow intermixed. In early May 2013, while the jewelweeds were at the cotyledon stage, I set up 30, 0.5X1m, blocks along a damp 55m ditch in Camden, Maine. I placed blocks in areas densely covered with a mixture of *I. capensis* and *I. glandulifera* seedlings (mean initial number of *Impatiens* seedlings per block: 302.20, SE: 26.32). Blocks were no less than one meter apart. I divided each block into 2, 0.5X0.5m, plots and randomly assigned one plot to the *I. glandulifera* removal treatment and the other to the control. I removed all *I. glandulifera* seedlings from the removal treatment plot by cutting the seedlings at the stem base with scissors. In each plot, I marked up to ten *I. capensis* seedlings by inserting a numbered swizzle stick in the ground near the base of the plant and wrapping a wire loosely around the stick and plant. To minimize edge effects, I only marked seedlings that were ten centimeters or more from the edge of the plot. While I initially marked 550 plants, I was only able to gather data throughout the entire growing season on 22 blocks. I was left with a total of 394 plants.

I tracked marked plants for the duration of the growing season. At the start of the experiment, I measured plant height. I continued to measure plant height once a month through September. Each month, I also recorded the survival of each marked plant and estimated the total number of fruits produced up to that point by counting the total number of pedicels, including those with buds, flowers, fruit, or dehisced fruit. At the August and September censuses, I measured the height of the hypocotyl and first internode, and counted the total number of internodes. In early September, I harvested all surviving plants. I dried them at a constant temperature and then estimated their above-ground biomass.

Statistical Analysis

Fitness effects—I tested for fitness differences in total fruit production, height, and above-ground biomass between the removal and control treatments using mixed model ANOVAs in PROC GLM (SAS Institute 2013). As in the greenhouse experiment, I included block and treatment by block interactions as random effects and designated the block by treatment interaction as the error term for significance testing. The fruit and height models included all plants that were included in the following selection analysis. The above-ground biomass model included only plants that survived to the September census. I logarithmically transformed all fitness components to improve normality.

I also compared survival to harvest between treatments using PROC LOGISTIC with survival as the dependent variable and treatment, block, and the treatment by block interaction as independent variables (SAS Institute 2013).

Plastic responses to competition—I estimated plastic responses to the different competition treatments using the same statistical techniques as in the greenhouse study. To improve normality, I square-root transformed early growth rate and hypocotyl length, and log10-transformed internode number.

Selection Analysis—I compared phenotypic selection on vegetative traits in the removal and control treatments using the same statistical techniques that I used in the greenhouse selection analysis. Here, however, fruit number was used as the fitness measure. I included all plants that survived to the August census. Early-season growth rate was again estimated as the average gain in height (cm) per day over the first quarter of the growing season. Because fruit numbers were not normally distributed, I tested the significance of selection gradients and the significant difference between selection gradients across treatments using models that assumed a Poisson error distribution (Formica *et al.* 2011).

I also measured selection in each treatment with models that used the estimated total seed number as the fitness measure. I estimated seed number for each plant by multiplying the total number of chasmogamous and cleistogamous fruits by the average number of seeds produced by each fruit type in the population. Because this alternative fitness measure resulted in similar selection gradients as the first, I do not present the results of this analysis here.

Natural Populations Survey

Experimental design

I conducted a survey of the vegetative traits of *I. capensis* populations growing with and without the invasive jewelweed to test whether the invasive affects the vegetative growth of the native congener similarly across multiple natural plant communities. In late August and early September, towards the end of the *Impatiens* growing season, I visited 16 *I. capensis* populations across coastal Maine in the northeastern United States (Table 1). Eight populations grew intermixed with *I.*

glandulifera and eight grew without the invasive plant. Whenever possible, I selected pairs of populations growing with and without the invasive plant in the same town in order to maintain the same geographic spread of the population types. These paired populations were spaced a minimum of 30m apart. In each population, I laid 50m of transect and sampled the *I. capensis* plant closest to each meter mark. When populations were narrower than 50m, I ran multiple parallel transects no closer than 3m apart. I sampled up to 50 plants per population for a total of 723 plants. For each sample, I measured plant height, the length of the hypocotyl and first internode, and counted the total number of internodes. I also estimated total fruit production by counting the total number of pedicels.

Statistical analysis

I tested for differences in total fruit number and vegetative traits between populations of *I. capensis* growing with and without *I. glandulifera* using PROC GLIMMIX with models that included invasion status as a fixed effect, and population as a random effect. Because micro-site environmental conditions varied greatly even within paired populations, I did not treat these pairs as blocks in the analysis. To improve normality, I logarithmically transformed fruit number and square-root transformed height and hypocotyl length. I removed one extreme outlier for fruit number from the analysis.

RESULTS

Greenhouse Competition Study

Fitness effects

The competition treatments significantly affected the fitness of focal *Impatiens capensis* plants. Total fruit production was reduced by over fifty percent in both the conspecific and invasive competition treatments compared to the no competition treatment (no competition vs. conspecific: $F_{(1,16)}$=72.99, P<0.0001; no competition vs. invasive competition: $F_{(1,16)}$=91.43, P<0.0001; Figure 1). There was, however, no significant difference in fruit production between the conspecific and invasive competition treatments ($F_{(1,16)}$=0.92, P=0.60; Figure 1). Conspecific competitors reduced plant height relative to the no competition treatment ($F_{(1,16)}$=23.00, P<0.0001), and invasive competitors further reduced plant height relative to the conspecific competition treatment ($F_{(1,16)}$=14.65, P=0.001; Figure 1). Above-ground biomass was similarly reduced by competition with conspecifics relative to the no competition treatment ($F_{(1,16)}$=104.19, P<0.0001) and further reduced by competition with the invasive jewelweed relative to the conspecific competition treatment ($F_{(1,16)}$=17.23, P=0.0001; Figure 1).

Plastic responses to competition

The vegetative traits of the native jewelweed did not show a strong plastic response to the invasive competitor (Table 2). Internode number, however, was reduced by the invasive competitor ($F_{(1, 16)}$ =16.59, P=0.0002).

Selection analysis

Although there was no difference in fruit production between the two treatments with competitors, the presence of the invasive jewelweed altered selection on a number of *I. capensis* vegetative traits (Table 3). Under competition with conspecifics, selection favored *I. capensis* individuals with faster early growth rates. Under competition with the invasive jewelweed, however, there was no significant selection on this trait, and the selection gradients differed significantly between treatments (Table 3). When competing with conspecifics, there was also positive selection on the length of the first internode, but this trait had no effect on fruit production under invasive competition. The selection gradients on first internode length differed significantly between treatments (Table 3). *Impatiens capensis* plants in both competition treatments experienced positive selection on the number of internodes. Selection on this trait, however, was over twice as strong when plants competed with the invasive jewelweed, with the selection gradients on this trait also differing significantly between treatments (Table 3). An additional selection analysis that included height as a covariate did not change the pattern of altered selection on early growth rate or internode number (data not shown).

Invasive Species Removal Study

Fitness effects

The presence of the invasive competitor reduced the fitness of the native jewelweed. Both total fruit production ($F_{(1,21)}$=9.81, P<0.01) and final height ($F_{(1,21)}$=8.39, P<0.01) were reduced in the control treatment (Figure 2). Above-ground biomass was also reduced in the control treatment, but this fitness measure did not differ significantly between treatments ($F_{(1,18)}$=1.64, P=0.22; Figure 2). Survival to harvest was significantly reduced from 47.76% in the removal treatment to 32.0% in the control treatment (χ^2=7.99, P=0.005). For both treatments, most mortality occurred in the second half of the growing season (removal treatment survival to July: 89.76%, control treatment survival to July: 89.16%).

Plastic responses to competition

Impatiens capensis plants in the control treatment demonstrated some plastic responses to the invasive competitor (Table 2). The presence of the invasive resulted in increased early growth rate ($F_{(1, 21)}$=5.69, P=0.03) and decreased internode number ($F_{(1, 21)}$=20.37, P=0.0002).

Selection analysis

The presence of the invasive jewelweed altered selection on the native jewelweed (Table 3). As in the greenhouse experiment, plants growing without the invasive in the removal treatment experienced significant positive selection on early growth rate, while those competing with the invasive did not experience selection on this trait. Selection on early growth rate differed significantly between treatments (Table 3). Also as in the greenhouse study, the magnitude of selection on internode number was greatest when plants competed with *I. glandulifera*. There was not, however, a significant difference in selection between treatments for this trait (Table 3). Unlike the greenhouse study where only the conspecific competition treatment produced positive selection on first internode length, plants growing both with and without the invasive competitor experienced positive selection on this trait (Table 3). An additional selection analysis that included height as a covariate did not change the pattern of altered selection on early growth rate or internode number (data not shown).

Natural Populations Survey

Impatiens capensis populations growing with and without the invasive competitor did not differ in fruit production (mean with the invasive=22.42(95%CL:16.28-30.88), mean without the invasive=29.37(95%CL:21.33-40.45), $F_{(1, 706)}$=1.34, P=0.25) or in late season height (mean with the invasive=80.28(95%CL:69.56-91.78), mean without the invasive=71.57(95%CL:61.46-82.45), $F_{(1, 707)}$=1.27, P=0.26). I also detected no difference in vegetative traits across population types (Table 2).

DISCUSSION

Invasive plants have long been known to reduce the fitness of native plant populations (Vilá *et al*. 2011). A previous study showed that *Impatiens glandulifera* reduced seed production in native plants through competition for pollinators (Chittka and Schürkens 2001). The results of my invasive species removal experiment show that *I. glandulifera* also has the potential to reduce fruit production in a native plant through vegetative competition. In the greenhouse, however, there was no difference in fruit production between competition treatments. This lack of treatment response may be the result of the more protective environment of the greenhouse relative to the field. Plants in both greenhouse competition treatments experienced zero mortality and produced about six times as many fruits as plants in the field study.

In both the greenhouse and field, when the invasive jewelweed was present, phenotypic selection favored native jewelweed individuals investing less in rapid upward growth through a fast early growth rate and more in the production of internodes. Fruit production and branching, which offers the potential for even greater fruit production, both occur at internodes. These results match my initial hypothesis that, in the presence of the taller invasive species, selection would favor a reduction in the shade avoidance response, much as it does for *I. capensis* plants growing under a thick forest canopy (Dudley and Schmitt 1995). *Impatiens capensis* populations adapted to sunny locations elongate their first internodes in response to competitors, while those adapted to forest canopies suppress this response (Dudley and Schmitt 1995). In the controlled conditions of the greenhouse, I also found strong positive selection for elongation of the first internode under intraspecific competition, but no selection on this trait under shading from the invasive species. In the field, where conditions were less controlled, there was overall positive selection on elongation of the first internode in both treatments.

The greenhouse and field experimental designs were similar in that they both enabled me to measure selection on *I. capensis* in the presence and absence of the invasive jewelweed. One important difference between these studies, however, was that the greenhouse study maintained overall plant density between treatments while the field study did not. Density is often an important factor in determining the ecological consequences of plant competition (Goldberg *et al*. 2001, Treberg and Turkington 2010) and may influence selection on vegetative traits (Dudley and Schmitt 1996). Despite not controlling for density in the field, however, the field results matched the greenhouse study. The presence or absence of the invasive species, therefore, was more important than the density differences between treatments.

The results of my study are different from those of a study that tested whether selection on *I. capensis* vegetative traits differed when under competition with a diverse array of heterospecifics versus when all heterospecifics were removed (McGoey and Stinchcombe 2009). This study found, for example, that there was positive selection on early growth rate in both the heterospecific competition and the removal treatments, while I found positive selection on this trait only under intraspecific competition. The fact that the results of my study differ from this previous study suggests that the strength and direction of competition-driven phenotypic selection on plants likely depends on the specific identity of the competitor species.

Though previous studies of native plant populations have detected evolved responses to species invasions (Lau 2006, Mealor and Hild 2007, Leger and Espeland 2010, Oduor 2013), my study suggests that invaded native plant populations experiencing strong competition-driven selection may at times fail to respond through adaptive evolution. The survey of natural populations showed no differences in vegetative traits of *I. capensis* populations growing with and without the invasive competitor. If an evolutionary response had occurred, I would have expected to see trait differences across population types in the same direction as the differences in selection in the presence and absence of the invasive competitor.

There are many reasons why native plant populations may fail to evolve in response to species invasions. First, insufficient genetic variation may limit an evolutionary response. Second, evolution may be constrained by trait correlations (Smith and Rausher 2008b). I believe that neither of these explanations is likely for *I. capensis* populations because extensive research has shown that *I. capensis* vegetative traits often evolve in response to environmental stimuli at a micro-environmental scale (Dudley and Schmitt 1996, Heschel *et al*. 2002, von Wettberg and Schmitt 2005). Another explanation is that an invasion is too recent for a response to have occurred. I also believe this explanation is unlikely because most of the populations surveyed likely had fifty or more generations to respond to the species invasion (Tabak 2005). One final explanation is that evolved and plastic responses to other environmental variables overwhelm any response to a single plant competitor. For example, populations of the native plant *Lotus* *wrangelianus* evolved in response to the invasive competitor *Medicago polymorpha*, but this evolutionary response could only be detected when plants were protected from an invasive herbivore (Lau 2006). *Impatiens capensis* populations may each respond to variables such as water availability and canopy cover that vary greatly across the range and could limit an evolutionary response to the invasive competitor. A greenhouse experiment using seed from invaded and uninvaded populations of *I. capensis* is currently underway to test whether an evolutionary response is detectable in a more controlled environment.

Overall, this research demonstrates that invasive plants have the potential to greatly alter natural selection on native competitors. The fact that the same results were found in the greenhouse and the field suggest that invasive plant competitors may influence selection on native plants relatively constantly across different densities and populations. The ability of native plant populations to increase their chances of survival by evolving in response to this altered selection pressure, however, may be limited by other influences. Even when altered natural selection indicates the potential for an adaptive evolutionary response, the future of native plant populations may remain in jeopardy.

ACKNOWLEDGMENTS

I thank B. Bailey, S. Gupta, M. McCauley, and R.R. Sharma for significant greenhouse and field assistance. I thank the homeowners who offered use of their properties, especially R. Elms, K. Hall, J. Hall, S. Howland, and A. Tikkanen. I thank the Galloway Lab reading group for constructive comments on an earlier version of this manuscript. L.F. Galloway, D.E. Carr, R.M. Cox, and H.M. Wilbur offered guidance throughout this project. This research was supported by the Jefferson Scholars Foundation, the American Society of Naturalists, a NSF Doctoral Dissertation Improvement Grant (DEB-1311453), and a Harrison Undergraduate Research Award to B. Bailey.

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Table 1. Sample locations for natural populations survey. Populations are labeled by township name. All towns are located in the state of Maine, in the northeastern United States.

Population	Invasion Status	Survey Date	Latitude	Longitude
Calais 1	not invaded	8/28/13	N 45.18397	W 67.28895
Calais 2	invaded	8/28/13	N 45.18372	W 67.28991
Bass Harbor 1	not invaded	8/27/13	N 44.23833	W 68.34621
Bass Harbor 2	invaded	8/27/13	N 44.23753	W 68.34627
Vinylhaven 1	not invaded	8/29/13	N 44.05037	W 68.84184
Vinylhaven 2	invaded	8/29/13	N 44.04639	W 68.85041
Northport	not invaded	9/8/13	N 44.33888	W 68.95581
Camden 1	invaded	8/21/13	N 44.25060	W 69.03266
Camden 2	not invaded	8/21/13	N 44.25037	W 69.03275
Camden 3	not invaded	8/20/13	N 44.20988	W 69.05898
Camden 4	invaded	8/20/13	N 44.21216	W 69.06034
Rockland	invaded	9/8/13	N 44.17526	W 69.09361
Warren	not invaded	8/22/13	N 44.07866	W 69.22408
Friendship	invaded	8/22/13	N 44.00760	W 69.29150
Boothbay Harbor 1	invaded	8/26/13	N 43.86353	W 69.61668
Boothbay Harbor 2	not invaded	8/26/13	N 43.86120	W 69.62141

Table 2. Means with 95% confidence limits for vegetative traits in greenhouse competition study, field invasive species removal study, and natural populations survey. Within each study, treatment effects with different letters are significantly different (*P*<0.05).

Study	**Competition treatment**	**Early growth rate (cm/day)**	**Hypocotyl (cm)**	**First internode (cm)**	**Internode number**
Greenhouse (df=1, 16)	conspecific	0.65 (0.63-0.68)^{A}	6.07 (5.86-6.28)^{A}	12.04 (11.46-12.62)^{A}	11.14 (10.68-11.59)^{A}
	invasive	0.65 (0.63-0.67)^{A}	5.90 (5.69-6.11)^{A}	12.15 (11.58-12.73)^{A}	9.81 (9.36-10.26)^{B}
	none	0.59 (0.57-0.62)^{B}	5.58 (5.36-5.79)^{B}	9.17 (8.59-9.75)^{B}	13.82 (13.37-14.28)^{C}
Field (df=1, 21)	removal	0.61 (0.58-0.62)^{A}	5.15 (4.88-5.38)^{A}	12.42 (11.92-12.92)^{A}	10.47 (9.55-11.22)^{A}
	control	0.67 (0.65-0.71)^{B}	5.20 (4.93-5.48)^{A}	13.15 (12.65-13.65)^{A}	7.94 (7.41-8.71)^{B}
Natural populations survey (df=1, 706)	uninvaded	*	4.80 (4.24-5.38)^{A}	11.23 (9.20-13.26)^{A}	15.24 (13.77-16.70)^{A}
	invaded	*	5.15 (4.57-5.76)^{A}	13.43 (11.40-15.46)^{A}	13.27 (11.80-14.73)^{A}

Table 3. Greenhouse and field studies directional selection gradients (β±SE) on *Impatiens capensis* vegetative traits in the presence and absence of *I. glandulifera*. The interaction shows whether selection gradients significantly differed between treatments. Significant selection gradients and interactions are shown in bold.

		I. glandulifera **presence**		**Interaction**		
		-	**+**			
Study	**Trait**	**(β±SE)**	**(β±SE)**	***F***	***P***	**df**
Greenhouse	Early growth rate	**0.09 (±0.02)**	0.003 (±0.002)	**4.38**	**0.04**	1, 122
	Hypocotyl length	**-0.04 (±0.02)**	-0.01 (±0.03)	0.76	0.39	1, 122
	Internode 1 length	**0.15 (±0.02)**	0.04 (±0.02)	**8.35**	**0.005**	1, 122
	Number of internodes	**0.14 (±0.01)**	**0.36 (±0.02)**	**82.74**	**<0.0001**	1, 122
Field	Early growth rate	**0.15 (±0.03)**	-0.09 (±0.11)	**3.93**	**0.049**	1, 222
	Hypocotyl length	-0.07 (±0.07)	-0.10 (±0.12)	0.09	0.76	1, 222
	Internode 1 length	**0.07 (±0.03)**	**0.22 (±0.10)**	0.17	0.68	1, 222
	Number of internodes	**0.79 (±0.03)**	**1.04 (±0.05)**	0.88	0.35	1, 222

******P***<0.05**

Figure 1. Means of fitness effects (±95% confidence limits) by treatment in greenhouse competition study. Treatments with different letters are significantly different (Tukey adjustment for multiple comparisons: $P<0.05$).

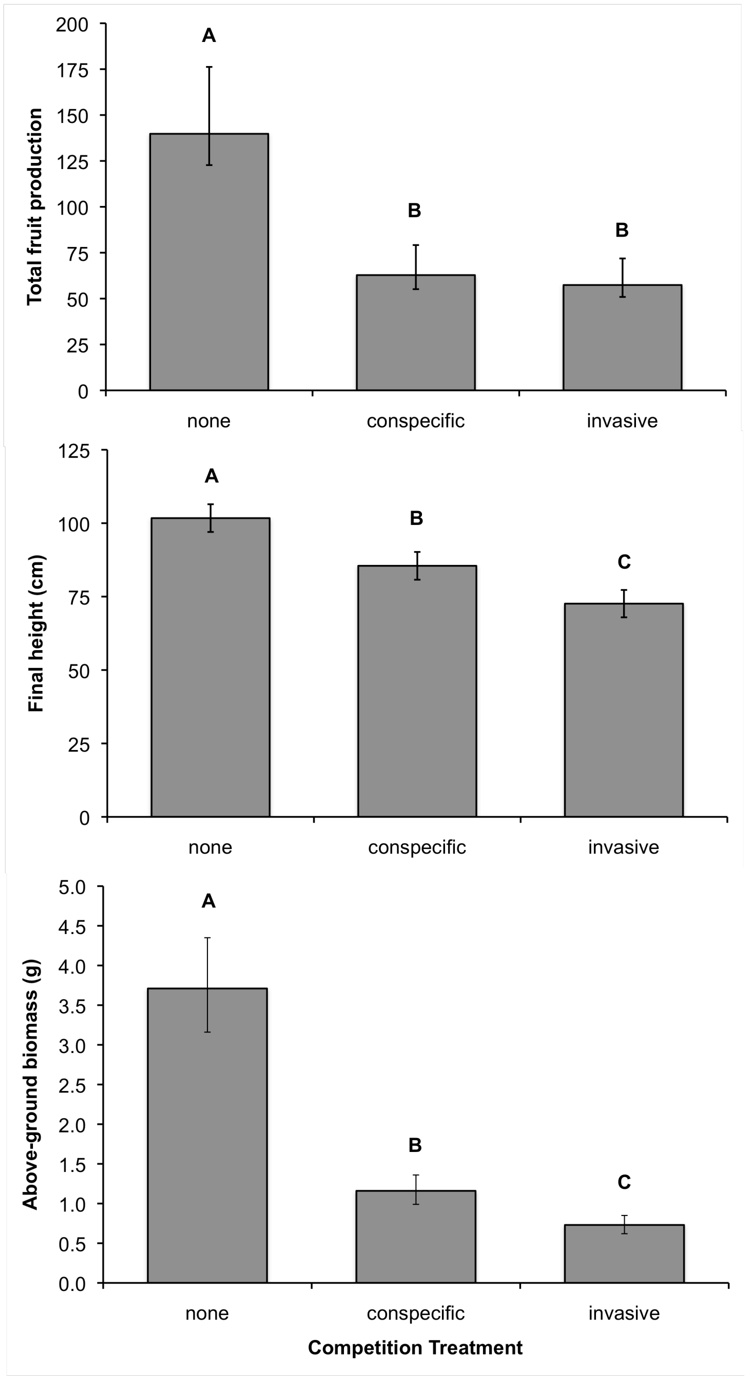

Figure 2. Means of fitness effects (±95% confidence limits) by treatment in field competition study. Treatments with different letters are significantly different (*P*<0.05).