ABSTRACT

Title of Dissertation: FACTORS THAT INFLUENCE THE MATING SYSTEM IN NATIVE AND NON-NATIVE POPULATIONS OF THE POTENTIALLY INVASIVE PLANT, *MIMULUS GUTTATUS*

Jason A. Berg, Doctor of Philosophy, 2018

Dissertation directed by: Adjunct Associate Professor, Elizabeth A. Zimmer; Associate Professor, Nathan G. Swenson, Department of Biology

The mating system of a colonizing plant population will influence establishment success in a new region, as well as the propensity to invade locations beyond the initial point of introduction. In mixed-mating plant species, defined as those that are capable of both self-fertilization and outcrossing, the mating system of nascent populations introduced to regions outside of the native distribution is often free to evolve. While theories exist that attempt to model the relationships among colonization, spread, and mating system, few studies have examined this dynamic in nature between native, naturalized, and invasive populations of a species.

My dissertation addresses several questions pertaining to the evolution of mating system in the context of an invasive plant species, namely *Mimulus guttatus* (common monkeyflower). I use molecular approaches and crossing experiments to determine the

importance of selfing and outcrossing in several native, naturalized, and invasive populations of *M. guttatus*. I first use data from highly variable molecular markers designed for *M. guttatus* to assess outcrossing rates, inbreeding coefficients, and inbreeding depression in nature in the native and non-native populations. This demonstrates the role of selfing and outcrossing, as well as the fitness consequences of each, in non-native populations compared to native populations in their natural setting. Next, I use the same molecular markers to examine population structure within and among the *M. guttatus* populations to determine genetic diversity and relationships between the native and non-native populations. The results from this chapter demonstrate how mating system dictates the amount of genetic diversity in the populations and allows for inferences as to the native sources for naturalized and invasive populations. Finally, I conduct a greenhouse crossing experiment to experimentally determine the fitness consequences of selfing and outcrossing, as well as to examine plasticity in fitness traits in native and non-native populations of *M. guttatus*. I conclude that native and invasive *M. guttatus* populations generally are characterized by greater genetic variation and express higher levels of inbreeding depression compared to the two naturalized populations. Also, the non-native naturalized and invasive populations express greater plasticity for fitness compared to native populations.

Throughout, I explore the role of mating system in invasion success and underscore that different establishment pathways are possible in an invasive plant species. Therefore, these studies contribute to the scholarship on evolution in invasive plants.

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by

Jason A. Berg

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Advisory Committee:
Associate Professor Nathan G. Swenson, Chair
Adjunct Associate Professor Elizabeth A. Zimmer
Associate Professor Daniel S. Gruner
Professor Maile C. Neel
Assistant Professor Katherine Tully

Dedication

I dedicate this dissertation to my wife, Chelsea.

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1 INTRODUCTION

The influence of introduced plants and animals on native biodiversity and ecosystem processes has been of interest to ecologists for nearly 200 years. In *The Origin of Species* (1859), Darwin opines on the ubiquity of non-native species and the apparent inability of natives to compete: "...for in all countries, the natives have been so far conquered by naturalised productions, that they have allowed foreigners to take firm possession of the land. And as foreigners have thus everywhere beaten some of the natives, we may safely conclude that the natives might have been modified with advantage, so as to have better resisted such intruders." In addition to the superior competitive ability of some non-native species, Darwin was also profoundly interested in the evolution of mating systems in plants generally, as well as their phenotypic response to different environments (Darwin 1876; West-Eberhardt 2005). These topics were notably juxtaposed in the mid-20th century to explain the disparate colonization success between self-compatible plant species and dioecious species, or monoecious species that demonstrate self-incompatibility (Baker 1955; Stebbins 1957; Carlquist 1965; Baker 1967).

Today, ecologists are still interested in how selfing, outcrossing, and the interaction between genotype and environment influence invasive plant populations, and have begun using genetic approaches and rigorous computational analysis to elucidate the mechanisms that facilitate establishment and spread. Contemporary questions in invasion ecology focus on how selfing and outcrossing may either facilitate or impede establishment following introduction to non-native regions (Barrett et al. 2008; Eckert et al. 2010; Pannell 2015), the genetic structure within and among native and non-native populations (Lee 2002; Lachmuth et al. 2010; Handley et al. 2011; Fitzpatrick et al.

2012), and the differential fitness benefits afforded to non-native populations via selfing, outcrossing, and plasticity (Murren et al. 2009; Verhoeven et al. 2011; Davidson et al. 2011; Keller et al. 2014; Rius & Darling 2014). Therefore, the major goals of this research were to: 1) determine whether differences in mating system exist between native and non-native populations by estimating outcrossing rates and inbreeding coefficients in the potentially invasive model plant species, *Mimulus guttatus*; 2) examine population structure within and among several native, naturalized, and invasive populations of *M. guttatus*; 3) examine differences in inbreeding depression, heterosis, and phenotypic plasticity for fitness traits among several native, naturalized, and invasive *M. guttatus* populations. Below, I provide an overview of our current knowledge regarding mating system evolution and phenotypic plasticity in the context of invasive plant species.

Mating system evolution and invasive plants

In the mid-20th century, ecologists began to scrutinize the relationship between a plant species' mating system and its ability to colonize territory outside of its native range. H.G. Baker theorized that self-compatible plant species should be over-represented on oceanic islands compared to self-incompatible species following long-distance dispersal (Baker 1955). His reasoning was born from the fact that selfing results in reproductive assurance, and a single individual has the ability to produce seed in the absence of cross-compatible mates and/or appropriate pollinator species. This theory, dubbed "Baker's Law" (Stebbins 1957), was met with some contention as other investigators pointed out that a fair number of species that were purportedly unable to self-fertilize existed on insular islands such as Hawaii (Carlquist 1966 *a*, *b*). This rebuttal to Baker's Law was

based on the argument that outcrossing is an essential component when colonizing distant locations because it produces the genetic variation required to respond to novel selection pressures in the recipient environment (Williamson & Fitter 1996; Genton et al. 2005). Considering that self-fertilization reduces heterozygosity by 50% each generation (Charlesworth & Charlesworth 1987; Carr & Dudash 2003) and can reduce fitness via inbreeding depression, critics of Baker's Law suggested that the genetic advantages of outcrossing during establishment exceed the benefits from selfing, namely reproductive assurance in the absence of mates or pollinators. Additionally, this genetic advantage also outweighs the downside of requiring at least two cross-compatible propagules to establish contemporaneously and within close proximity to one another (Carlquist 1966 *a*).

In 1967, Baker submitted further defense for his theory by proposing means by which genera and species commonly known for their self-incompatibility in their native mainland range (due to mating strategies like dioecy, herkogamy, dichogamy, or S-allelic interactions) could evolve self-compatibility following long-distance dispersal (Baker 1967). Theoretically, he suggested, the physiological shift from a hermaphroditic system showing self-incompatibility to one capable of self-fertilization was much more probable than the reciprocal transition, i.e. from selfing to self-incompatibility. This claim is supported by several examples in nature, including *Armeria maritima*, a species that has appeared to transition from the heteromorphic self-incompatible mating system it is known for in its native Europe to a self-compatible mating system following its introduction to North America (Baker 1967). Other examples of this process represent evolution from heterostyly (a form of self-incompatibility via herkogamy) in the species' native lands to homostyly and self-compatibility on islands (*Turnera ulmifolia*, Barrett &

Shore 1987; *Eichornia paniculata*, Barrett et al. 1989). These types of evolutionary shifts following long-distance dispersal may not be uncommon, as comparative studies often show a higher frequency of self-compatible species compared to self-incompatible species on islands and lend credence to Baker's Law (McMullen 1987; Webb & Kelly 1993; but see Carr et al. 1986).

While comparative studies that focus on the shift from self-incompatibility in native populations to self-compatibility in non-native locations provide a glimpse into mating strategy evolution in invasive species (Elam et al. 2007; Petanidou et al. 2012; Costa et al. 2017), there are other approaches. Alternatively, outcrossing rates can be estimated in situ and compared between native and non-native populations of mixed-mating species (i.e. individuals capable of producing both selfed and outcrossed progeny, demonstrating intermediate outcrossing rates; Winn et al. 2011). Molecular data derived from highly variable markers such as microsatellites can be subjected to a progeny array analysis to estimate outcrossing rates from populations (Ritland 2002; Jarne & David 2008). Because the molecular data is derived from plant tissue collected in the field, progeny arrays provide outcrossing rate estimates of populations in the wild (van Kleunen et al. 2007; Petanidou et al. 2012). This approach can be used to address Baker's Law by comparing outcrossing rates between native and invasive populations to determine if non-natives self-fertilize more frequently than natives. To broaden the scope of inference further and thus allow ecologists to make conclusions about the importance of selfing during different stages of invasion, studies should also include naturalized populations that have established in non-native regions but have not yet become invasive (Richardson et al. 2000; Richardson et al. 2012).

The fitness consequences of selfing and outcrossing in non-native regions can be further conceptualized by performing experimental crosses and measuring inbreeding depression and heterosis, either in the field, a common garden, or the greenhouse, (Frankham 2005; Murren et al. 2009; Verhoeven et al. 2011; Keller et al. 2014). Studies have found that while the increased genetic diversity produced by outcrossing relative to selfing can improve a non-native plant population's chances of adapting in a recipient range, many invasive mixed-mating populations have succeeded despite experiencing a genetic bottleneck following introduction (Dlugosch & Parker 2008). It has been suggested that small populations that rely on selfing can purge the deleterious recessive alleles responsible for inbreeding depression and then continue to self, unfettered by the deleterious fitness consequences known as inbreeding depression (Dudash et al. 1997; Dudash & Carr 1998; Byers & Waller 1999). This may explain how incipient plant populations that have undergone a genetic bottleneck can rely on selfing, benefit from reproductive assurance, and persist in a new location.

This dissertation is comprised of three chapters that follow this introductory chapter. First, I examined data from highly variable microsatellite markers to compare outcrossing rates for populations in native, naturalized, and invasive regions of the mixed-mating plant species, *Mimulus guttatus* (Chapter 2), and also to assess genetic structure within and among regions containing *M. guttatus* (Chapter 3). Next, I conducted an associated crossing program in the greenhouse to examine inbreeding depression, heterosis, and outbreeding depression within the three *M. guttatus* regions (Chapter 4). Together, results from these experiments elucidate how the mating system may or may not differ in non-

native regions of a potentially invasive plant species, and how the fitness consequences of selfing and outcrossing may be driving mating system evolution.

Plasticity for fitness traits in invasive plants

I have discussed how mixed-mating plant populations introduced to non-native regions may fluctuate between higher selfing or outcrossing rates as an adaptive response to the effects of a recent bottleneck and/or novel selection pressures. However, the evolution of a plant population's mating system may not be the only mechanism available to cope with a population's maladaptation in a novel environment. Phenotypic plasticity (i.e. the change in phenotypic expression of a genotype in response to environmental conditions) provides a population with the flexibility to alter mean phenotypes as the environment changes (Schlichting 1986). In the context of invasion, plasticity could result in a buffering effect when a non-native population would otherwise experience a loss in fitness following introduction to a region characterized by stressful environmental conditions (Davidson et al. 2011; Godoy et al. 2011; Lande 2015). Plasticity is a trait, subject to evolution by natural selection and expressed to various degrees by populations if genetic variation for plasticity exists in the species (Schlichting 1986; Pigliucci 2001; Murren et al. 2015). The question as to whether invasive populations or species express greater plasticity compared to their native counterparts (or naturalized exotics that have not become invasive) has received considerable attention in recent years, with studies reporting mixed conclusions that likely depend on environmental and phylogenetic context (Davidson et al. 2011; Godoy et al. 2011; Palacio-Lopez & Gianoli 2011).

Much like the role of mating system evolution in invasion by introduced plants, many of the fundamental questions regarding phenotypic plasticity were proposed fifty years ago by Baker in his contemplative study of common traits in weeds (Baker 1965). He described two aspects of fitness traits in response to environmental variation that could facilitate invasion by "weedy" plants: 1) a "general purpose genotype" that is robust and maintains fitness across a range of environments including stressful conditions ("fitness homeostasis"; Hoffman & Parsons 1991; Rejmanek 2000); 2) a tendency to opportunistically increase fitness by exploiting favorable environments (Sultan 2001; Paolacci et al. 2018). By conceptualizing the role of plasticity in the colonization and spread of weeds in this way, Baker suggests that plasticity for fitness traits must be considered separately from plasticity for structural or physiological traits (Sultan 2001; Richards et al. 2006; Davidson et al. 2011). However, the two are inextricably related, as I will discuss below.

Richards et al. (2006) elaborated on the synergy between plasticity in fitness and non-fitness traits by introducing the "Jack-of-all-trades" scenario, where the ability to maintain fitness in stressful environments is produced by plasticity in underlying, morphological or physiological traits. This situation is illustrated by a "flat" reaction norm of a fitness trait across an environmental gradient of increasingly stressful conditions. There are several examples of invasive plants demonstrating fitness maintenance in response to environmental variation. For example, one study found that an annual invasive thistle (*Centaurea solstitialis*) demonstrated the Jack-of-all-trades pattern for number of inflorescences in response to different canopy gap sizes (Gerlach & Rice 2003).

A second scenario proposes by Richards et al. (2006) is based on Baker's opportunistic genotype and dubbed the "Master-of-some" scenario. The Master-of-some more effectively exploits favorable environments (e.g. a disturbed location that results in an increase in available resources) compared to natives. Many studies have demonstrated the exceptional ability of invasive plants to utilize resources more effectively than natives (Leishman & Thomson 2005; Funk & Vitousek 2007; Alexander et al. 2014). A recent study based on a greenhouse experiment showed that an invasive grass, *Lolium perenne*, showed similar fitness as co-occurring grass species in sterile soil, but responded with significantly greater fitness following nutrient additions (Broadbent et al. 2018).

For this research, I conducted an experiment in the greenhouse to examine fitness traits in native and non-native *M. guttatus* individuals in response to optimal and stressful watering conditions. My goals were to determine whether non-native individuals (i.e. from the naturalized and invasive regions) will be able to maintain fitness across two experimental treatments for more traits than native individuals, or if non-native individuals demonstrate a greater increase in fitness compared to native individuals. The results from this experiment will add to what is known about plasticity and its role in allowing non-native *M. guttatus* individuals to thrive in optimal and stressful environments, or if plasticity for fitness is ubiquitous in *M. guttatus* and not dependent on region of origin.

2 PROGENY ARRAY ANALYSIS TO ESTIMATE OUTCROSSING RATES, INBREEDING COEFFICIENTS, AND INBREEDING DEPRESSION AMONG NATIVE, NATURALIZED, AND INVASIVE POPULATIONS OF *MIMULUS GUTTATUS* [PHRYMACEAE]

Introduction

In hermaphroditic plants, self-compatibility is common and populations of the same species can vary substantially in the degree to which they rely on outcrossing (Goodwillie et al. 2005). In native early-successional species (i.e. "weedy" species) or non-native plant species, outcrossing can provide a selective advantage by increasing the standing genetic variation required by populations to adapt to novel environmental conditions through admixture and recombination (Sax et al. 2007; Catford et al. 2009). However, high outcrossing rates (t) may be unattainable in some cases due to issues concerning demography, collectively known as Allee effects (Allee 1951). These effects include population bottlenecks upon colonization, lack of pollinators, and dispersal limitations inherent to sedentary life forms (Baker 1967; Kolar & Lodge 2001). In cases like these, uniparental modes of reproduction such as self-fertilization ("selfing") or clonal propagation may be more selectively advantageous.

Given that the adaptive advantages of both selfing and outcrossing are dependent on demographic and stochastic factors, classic studies, both theoretical and empirical from Lande and Schemske, concluded that the evolution towards complete outcrossing ($t = 1$) or complete selfing ($t = 0$) are two alternative stable states for plant mating systems (Lande & Schemske 1985; Schemske & Lande 1985). They argued that populations demonstrating intermediate outcrossing rates ($0.2 < t < 0.8$), known as a "mixed mating"

strategy, are simply examples of a transitional state toward complete selfing or outcrossing. However, more recent studies have suggested that mixed-mating is an alternative stable state (Goodwillie et al. 2005; Jarne & Auld 2006; Winn et al. 2011). These studies suggest that relaxation of certain selective forces acting against the evolution of selfing can result in a stable mixed-mating strategy. It is during the early stages of colonization and establishment that the trajectory towards one of these three mating system strategies, complete selfing, complete outcrossing, or mixed-mating, begins.

Selfing can provide reproductive assurance when mates or pollinators are scarce (Barrett 2002; Moeller & Geber 2005). Also, alleles that cause selfing experience a 50% transmission advantage over alleles promoting outcrossing, assuming no pollen discounting (Fisher 1941). Selfing individuals can pass on alleles in three ways: through ovules and pollen in self matings, and through pollen in outcross matings. Outcrossing individuals can pass on alleles in only two ways, via either their ovules or their pollen (Jain 1976; Jarne & Charlesworth 1993). Due to the strong selective advantage provided by reproductive assurance and the transmission advantage associated with selfing, it has evolved in many independent lineages from ancestral outcrossing mating systems (Stebbins 1974; Wright et al. 2013). However, there is ample evidence suggesting that obligate selfing is uncommon among seed plants (Barrett & Eckert 1990; Holsinger 1988; Vogler & Kalisz 2001), and that there are selective forces opposing self-fertilization.

The strongest selective force preventing the transition from outcrossing to complete selfing is inbreeding depression, the decrease in fitness in progeny produced by selfing relative to that produced by outcrossing (Lande & Schemske 1985; Charlesworth and

Charlesworth 1987; Dudash 1990). Inbreeding depression occurs because selfing increases homozygosity by 50% each generation, allowing deleterious recessive alleles to be expressed at higher frequencies compared to outcrossing populations with greater heterozygosity (Charlesworth & Charlesworth 1987; Dudash et al. 1997; Roff 2002). While inbreeding depression is likely the primary selective agent that prohibits evolution of plant populations toward complete selfing, studies have suggested that the effects of inbreeding depression can be mitigated over time as deleterious recessive alleles are purged from the population by selection (Hedrick 1994; Dudash & Carr 1998; Byers & Waller 1999).

A second selective force combating the transition to selfing is pollen discounting (i.e. a decrease in pollen available for outcrossing due to self-fertilization), which offsets the transmission advantage afforded to selfers (Holsinger & Thomson 1994; Fishman 2000). If pollen discounting is negligible and a population has persisted long enough to purge its genetic load, there will be minimal selection against an allele that promotes selfing. Theoretically, if selfed progeny are at least half as fit as outcrossed progeny (i.e. inbreeding depression is below 0.5), then an allele that causes increased selfing can invade a population due to its transmission advantage (Fisher 1941; Jarne & Charlesworth 1993).

Given the importance of mating system on population genetic structure, pollinator syndromes, and dispersal, an immense literature has developed investigating the evolution of mating systems in hermaphroditic plants (Dudash 1990; Fenster & Ritland 1994; Barrett & Harder 1996; Goodwillie et al. 2006). Central to this discussion is the theory presented over 60 years ago, known as Baker's Law (Baker 1955; Stebbins 1957).

This theory suggested that self-compatible species should be more successful colonizers following long-distance dispersal compared to obligate outcrossing species, in part because the former would need only one individual to establish a naturalized population (Baker 1967; Kolar and Lodge 2001). Since Baker's initial observation, empirical evidence has been collected that both support and contradict his theory (*for review see* Pannell & Barrett 1998; Randle et al. 2009; Cheptou 2012). The influence of mating system on colonizing success is likely context dependent, and many areas of inquiry remain as to how selfing and outcrossing may facilitate establishment by introduced plant species.

Invasive plant populations provide opportunities to investigate questions related to evolutionary processes, including transitions in mating system following introduction to regions outside of the native distribution (Williamson & Fitter 1996; Sax et al. 2007; Lockwood et al. 2013). Understanding the mating system of invasive populations is important because the rates of selfing and outcrossing will influence population size, dispersal, and genetic structure of populations in the new environment (Kinlan & Hastings 2005). For example, in cases where only a single or few individuals have become established, Allee effects can threaten persistence in a new location (Allendorf & Lundquist 2003; Prentis et al. 2008). However, these factors can be offset by high rates of selfing, clonal propagation, or a combination of the two (Baker 1955; Charlesworth & Charlesworth 1987; Rambuda & Johnson 2004). Alternatively, multiple introductions of plants from the same or different source populations can result in an establishment pathway that ameliorates the demographic and genetic constraints associated with an initially small colonizing cohort (Facon et al. 2008; Lombaert et al. 2010). Multiple

introductions can result in larger population sizes and greater outcrossing rates, which in turn can generate the genetic diversity required to deal with novel selection pressures in the recipient location (Genton et al. 2005; Dlugosch & Parker 2008; Wilson et al. 2009; Estoup & Guillemaud 2010).

Despite the importance of mating system on establishment and invasion success of non-native plant species, we are aware of no studies that have compared *in situ* outcrossing rates between plant populations that have become invasive to populations of the same species that have remained relatively benign and localized following introduction into areas outside of the species' native distribution. These latter populations, often described as *naturalized* populations, are able to sustain population size without further influx of outside propagules, but have not spread beyond the point of introduction (Richardson et al. 2000; Aiko et al. 2010).

The aim of this study was to investigate differences in mating system between native and non-native populations of the mixed-mating plant, *Mimulus guttatus* (Phrymaceae), and how self-fertilization and outcrossing may play a role in the successful colonization and subsequent spread of introduced populations. This experimental design allowed us to examine the mating system *in situ* in populations that fall along the invasion spectrum: native populations that have been evolving in their environments for millennia; naturalized populations that have established but have not spread; and invasive populations that have dispersed aggressively beyond the point of initial introduction (Richardson et al. 2000). Studies of plant invasions that include comparisons between naturalized and invasive populations are uncommon because the former are often cryptic and benign in the environment, and thus difficult to identify in the field (*but see* Muth &

Pigliucci 2006). In this study, we include two naturalized *M. guttatus* populations to examine how the mating system of these seemingly benign populations compare to those that are considered harmful and invasive.

We tested the following hypotheses: 1) Outcrossing rates and mean inbreeding coefficients of maternal individuals differ among populations in three regions: native, naturalized, and invasive. If non-native populations of *M. guttatus* adhere to Baker's Law, we predict that outcrossing rates will be lower, and inbreeding coefficients higher, in naturalized and invasive populations compared to native populations; 2) Inbreeding depression differs between populations in the three regions. We predict that levels of inbreeding depression that populations experience in their natural environment (as opposed to experimentally in a common garden or greenhouse environment) will be lower in populations that have higher selfing rates (i.e. those that rely on selfing) because they may have purged their genetic load.

Methods

Study species and sample locations

Mimulus guttatus (Phyrmaceae; $2n = 2x = 28$), or common monkeyflower, is a mixed-mating species native to the west coast of North America, found from Mexico to Alaska (Dudash et al. 1997; Carr et al. 1997; Kelly & Arathi 2003; Lowry et al. 2008; Wu et al. 2007). In its native range, *M. guttatus* populations can be found with either an annual or perennial life history, and this depends on water availability (van Kleunen & Fischer 2008; Lowry et al. 2008). For this study, we only sampled from perennial native populations because all known non-native populations are perennial. The eight native

populations sampled include three from Alaska (AKS1, AKS2, AKA), one from Washington (WA), three from Oregon (OR02, OR04, and OR06), and one from California (BB1). Coordinates for all sampled populations can be found in Table 1.

In the United Kingdom (UK), *M. guttatus* is considered a harmful invasive that was intentionally introduced as a horticultural species approximately 200 years ago (Truscott et al. 2008; van Kleunen & Fischer 2008). Three populations from the UK were sampled: BRA, DBL, and HOU. There are also a few isolated populations in eastern North America that have become naturalized and have shown no detectable spread beyond their current locations (Murren et al. 2009). Little is known of the evolutionary history of these naturalized populations, but the two included in this study, in Fly Creek, NY (FC) and Springfield, New Brunswick, Canada (NBS), are thought to have established at least 50 years ago (Murren et al. 2009). Conversions from selfing to an outcrossing mating system are common within the *Mimulus* genus (Fenster & Ritland 1994) and it has been shown that transitions in mating system in *M. guttatus* are likely controlled by polygenic inheritance (Fenster et al.1995). *Mimulus guttatus* is pollinated by bees and other insects, suggesting limited gene flow via pollen dispersal (van Kleunen & Johnson 2007); however, the seeds are small and can potentially be dispersed long distances by water and possibly wind (Grant 1924; van Kleunen & Fischer 2008).

Native populations of *M. guttatus* exhibit wide variation in outcrossing rates (Ritland & Ganders 1987; Dudash & Carr 1998). The mode of self-fertilization in *M. guttatus* is largely *competing selfing*, which occurs concurrently with outcrossing, as opposed to prior or delayed selfing (Lloyd & Schoen 1992; Leclerc-Potvin & Ritland 1994). To our knowledge, neither outcrossing rates nor inbreeding coefficients in naturalized and

invasive populations have been estimated in the *M. guttatus* complex. In addition to its mixed-mating strategy for reproduction, *M. guttatus* is also capable of forming clones via asexual reproduction, i.e. fragmentation and stolons (Grant 1924; Vickery 1959; Truscott et al. 2006). *Mimulus guttatus* has become a model system in studies of evolutionary and population genetics because of its broad phenotypic and genetic diversity (Dudash et al. 2005; Wu et al. 2007).

Genotyping for progeny array analysis

To conduct a progeny array analysis to estimate outcrossing rates (*t*), inbreeding coefficients (*F*), and relative fitness of selfed progeny for *M. guttatus* populations in the native, naturalized, and invasive ranges, we conducted fieldwork in 2012 and 2013 (Table 1). Our sampling of native populations along the west coast of North America covered a large latitudinal range (Table 1). The route to survey eight native perennial populations was based on records obtained from colleagues (B. Blackman & D. Lowry, *pers. comm.*) and local contacts. In the naturalized region on the east coast of North America, the two populations sampled were previously studied by Murren *et al.* (2009) and located in Fly Creek, New York and Springfield, New Brunswick, Canada. For each of the 10 native and naturalized populations, we randomly sampled seed and leaf tissue from 30-50 maternal families that were > 1 m apart to increase the chances of sampling multiple genotypes. Leaf tissue represented the maternal genome for maternal families used in the progeny array, and was immediately stored in silica gel upon collection until DNA extraction was performed. The seed collected in the field was from the same plants that leaf tissue was collected from, and thus represents the progeny in the progeny arrays.

For the three *M. guttatus* populations in the invasive region in the UK, we did not collect leaf tissue, but instead obtained field-collected seed from Mario Vallejo-Marin at the University of Stirling in Scotland. Therefore, maternal genotypes for the three UK populations had to be inferred by the software BORICE (Koelling et al. 2012), which can provide joint estimates of *t* and *F* from data sets that incorporate progeny arrays from maternal families that require statistical inference of missing maternal genotypes. Some families from native and naturalized populations were also missing maternal genotypes that required inference by BORICE (Table 1). Seed representing progeny for all maternal families was grown in the University of Maryland (UMD) greenhouse and when seedlings were ~ 6 cm tall, leaf tissue was collected from eight progeny/maternal family and stored in silica gel. Thirty maternal families per population were randomly sampled (13 populations x 30 maternal families x 8 progeny), but fewer families were used in the progeny array analysis due to poor quality DNA in some families (Table 1).

To genotype the 13 populations included in the progeny arrays, we used 11 codominant markers (Table 2). These included five microsatellite loci previously used to genotype North American and British *M. guttatus* populations (Kelly & Willis 1998; Vallejo-Marin & Lye 2013), and six markers revealing length polymorphisms in introns of single-copy nuclear genes in *M. guttatus* (Fishman & Willis 2005; Lowry et al. 2008, Vallejo-Marin and Lye 2013). These intron length polymorphisms, or MgSTS (*Mimulus guttatus* sequence-tagged sites) were found to be suitable for genotyping based on a selection strategy of Vallejo-Marin *et al.* (2011). These markers are variable in samples of *M. guttatus*, its close relative, the tetraploid *M. luteus*, and the triploid hybrid produced

by them, *M. x robertsii* Silverside, and are suitable for multiplexing (Vallejo-Marin & Lye 2013).

To extract DNA from field-collected maternal leaf tissue (all populations except the three from the invasive region) and from leaf tissue collected from greenhouse-grown progeny originating from field-collected seed (all populations), a modified CTAB protocol (Doyle & Doyle 1990) was employed on an AutoGenprep 965/960 instrument (AutoGen, Holliston, MA, USA) using the Plant DNA Extraction Kit AGP965/960, following the manufacturer's protocol. DNAs were amplified for the 11 loci in sets of two multiplexed reactions using a 2 x Qiagen Type-It Microsatellite PCR kit (Qiagen, California, USA) containing 2 μM of each of the fluorescent forward primers labeled with either FAM or HEX dyes, 2 μM of each reverse primer and 5-50 ng of template DNA. DNA amplification consisted of a denaturing step of 5 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 55 °C for 180 s and 72 °C for 30 s and a final elongation step of 30 min at 60 °C. We examined success of the PCR amplifications in a 1.5% agarose 1x sodium hydroxide-boric acid buffer electrophoresis gel (Brody & Kern 2004). PCR products were diluted in nuclease-free water (dilutions ranged from 1:10 to 1:50), and one μL of each dilution was added to 9 μL of HiDi formamide and 1 μL ROX molecular weight standards (DeWoody et al. 2004). Samples were heated to 95 °C for six minutes, cooled to 4 °C for six minutes, and loaded onto an ABI 3730xl automated capillary sequencer with a 50 cm, 96 channel array containing POP-7 polymer for fragment analysis at the Laboratories of Analytical Biology (LAB) of the Smithsonian National Museum of Natural History.

We performed allele binning and analyzed raw peak sizes from fluorescent fragment profiles using GeneMapper v5.0 software (Applied Biosystems), which allows calling of multiple peaks per locus. Maternal or progeny DNAs that failed to amplify were run a second time. Those that failed following the second run of PCR were left out of the analysis. A random sample of 10% of individuals that were successfully genotyped was then re-assayed and re-scored to check consistency.

Bayesian estimation of t and F using BORICE

To calculate a joint estimation of the population outcrossing rate (*t*) and mean inbreeding coefficient of maternal individuals (*F*), we used the Bayesian approach implemented in the program BORICE (Bayesian Outcrossing Rate and Inbreeding Coefficient Estimation; Koelling et al. 2012). BORICE was designed to provide unbiased estimates of *t* and *F* from progeny arrays with a low number of progeny sampled from each family (≤ 8) and missing maternal genotypes. BORICE calculates the likelihood for maternal families using data from progeny arrays representing each population, regardless of whether the maternal genotype from the family is present. The likelihood for family *k*, l_k, is:

$$lk \quad = \Pr[M_k] \prod_{i=1}^{n_k} (tP_{out}\,[A_{ik}|M_k] + (1-t)P_{in}[A_{ik}|M_k]) \qquad \text{(Eqn. 1)}$$

where M_k is the vector of genotypes for maternal individual *k*; A_{ik} is the vector of genotypes for progeny *i* of maternal individual *k*; and n_k is the number of individuals in family *k*. The probability of M_k depends on population allele frequency and the latent (i.e. unobserved) variable, C_k, which is the inbreeding history of each maternal individual. In

BORICE, C_k values equal the number of generations of selfing in the ancestry of a maternal individual and are used to determine the inbreeding coefficients for maternal individuals (either absent or present) in the progeny arrays. The relationship between C_k and F is:

$$F = 1 - \left(\frac{1}{2}\right)^{C_k} \quad \text{(Eqn 2)}$$

For example, when $C_k = 0$, then the individual is outbred and $F = 0$. When $C_k = 1$, the individual is considered a selfed progeny of a maternal individual with $C_k = 0$, and therefore $F = ½$. When $C_k = 2$, the individual is a selfed progeny of a maternal individual with $C_k = 1$ and $F = ¾$, and so on. The C_k values are integers ranging from 0 to 6. Values larger than 6 results in a negligible difference in F compared to $C_k = 6$, and therefore BORICE pools these individuals with those with a $C_k = 6$. With F, the maternal genotype probability at a given locus x is dependent on allele frequencies and given by standard formulas (Hartl and Clark 1989):

$$Prob[A_iA_j] = 2(1-F)q_{xi}q_{xj} \text{ for } A_i \neq A_j \text{(heterozygote)} \quad \text{(Eqn 3a) or}$$

$$Prob[A_iA_i] = (1-F)q_{xi}^2 + Fq_{xi} \text{ for homozygotes} \quad \text{(Eqn 3b)}$$

where q_{xi} is the allele frequency at locus x. $\Pr[M_k]$, $P_{out}[A_{ik}|M_k]$, and $P_{in}[A_{ik}|M_k]$ are products over loci, and loci are assumed to be unlinked.

To estimate the parameters included in the likelihood function, BORICE uses Markov Chain Monte Carlo with a Metropolis-Hastings algorithm (Metropolis et al. 1953). The outcrossing rate t and the allele frequencies are standard parameters estimated from the observed data, while C_k and all unknown maternal genotypes are latent variables. BORICE assumes a uniform prior density for both t and the allele frequencies, and an iteration of the chain has four stages: 1) Propose and accept/reject adjustment to t;

2) Propose and accept/reject adjustment to q_{xi} for each locus *x* in series; 3) Propose and accept/reject new value of C_k with each maternal plant *k* considered in series; 4) Propose and accept/reject a new genotype for a random locus of maternal genotype M_k within each family *k* considered in series.

The proposed value *t'* for each iteration of the chain is the current value *t* summed with a small increment adjustment. The default range of incremental adjustment in BORICE is uniform between -0.05 and 0.05. The proposal ratio (R) is:

$$R = \frac{Prob[Data|t']}{Prob[Data|t]} \quad \text{(Eqn 4)}$$

If R > 1, then the proposed adjustment is accepted. If R < 1, a uniform random number *u* is drawn and if *u* < R the adjustment then *t'* is accepted.

For allele frequencies, a score is updated and tracked, y_{xi}, corresponding to each allele *i* at each locus *x*. Updates to y_{xi} are made using the same methods as for updates to *t*, and is based on previous work on proportion variables in phylogenetics (Lewis et al. 2010):

$$R = \frac{Prob[Data|y'_{xi}]}{Prob[Data|y_{xi}]}\left(e^{y_{xi}-y'_{xi}}\right) \quad \text{(Eqn 5)}$$

For the latent variables (i.e. C_k values and missing maternal genotypes), proposed values are sampled probabilistically based on current values of t and allele frequencies. The proposed value of C_k of maternal plant k is sampled from a geometric distribution: Prob[C'_k = 0] = *t*, Prob[C'_k = 1] = (1-*t*)*t*, Prob[C'_k = 2] = (1-*t*)^2*t*, Prob[C'_k = 3] = (1-*t*)^3*t*,… Prob[C'_k=6] = $1 - \sum_{i=0}^{5} \text{Prob}[C'k = i]$. The proposal ratio for C'_k values is family specific likelihood (i.e. changes to C_k affect only one family):

$$R = \frac{Prob[C'_k|M_k]}{Prob[C_k|M_k]} \quad \text{(Eqn 6)}$$

Imputed maternal genotypes are sampled from the probability distribution derived from current allele frequencies and C_k values (Eqn 3). The proposal ratio, like that of C_k is family specific and given by:

$$R = \prod_{i=1}^{n_k} \left(\frac{tP_{out}[A_{ik}|M'_k] + (1-t)P_{in}[A_{ik}|M'_k]}{tP_{out}[A_{ik}|M_k] + (1-t)P_{in}[A_{ik}|M_{k]}}\right) \quad \text{(Eqn 7)}$$

Prior to analysis, 10 impossible genotypes for loci AAT230, AAT278, and MgSTS84 were reported by BORICE (i.e. genotypes in progeny that do not contain at least one maternal allele). Therefore, we followed advice by Koelling et al. (2012) and allowed null alleles in the model to calculate family likelihoods, l_k.

We used a chain of 100,000 steps with a burn-in of 10,000 steps. Once the posterior *t* and *F* were estimated, we calculated Ritland's (1990) estimator for relative fitness of selfed progeny (ω) to characterize inbreeding depression (1- ω) in each population. Assuming *F* is constant across generations, relative fitness is found using:

$$\omega = 2 \times t \times F/[(1-t)(1-F)] \quad \text{(Eqn 8)}$$

and inbreeding depression is 1-ω. Because inbreeding depression is dependent only on *t* and *F*, understanding different outcomes regarding the selection against selfed individuals is straightforward. For example, if both *t* and *F* in a population are low, inbreeding depression will be relatively high. This occurs because while many selfed progeny are produced (low *t*), few survive to adulthood (low *F* among maternal individuals). Alternatively, if *t* is low and *F* is high, the measure of inbreeding depression will be low because the high number of offspring produced by selfing is surviving and represented in the next maternal individuals, as evidenced by the high *F*. Lastly, when outcrossing rates are high, the estimate of inbreeding depression will generally be low

regardless of the magnitude of *F*, because few selfed progeny are produced for selection to act upon.

Statistical analysis

We used Welch's t-tests, which do not require the assumption of equal variances between unpaired samples, to examine pairwise differences between *M. guttatus* regions (native, naturalized, and invasive) regarding *t*, *F*, and inbreeding depression (1- ω).

Results

All markers were polymorphic, with between three and 26 alleles (Table 2). There was evidence for the presence of null alleles in the form of 'impossible genotypes' reported by BORICE. A total of 10 impossible genotypes for loci AAT230, AAT278, and MgSTS84 were reported in five populations (AKA, OR02, BB1, FC, & HOU). Therefore, while null alleles were allowed in the final analysis of all populations, a random sample of three populations (AKA, OR04, & HOU) was run without allowing for null alleles and the results for *t* and *F* were not statistically different from the models that allowed for nulls (t-test, $P > 0.05$).

Estimates of outcrossing rates *t* in the invasive region of *M. guttatus* were similar, on average, to those in the native region (Fig. 2A; native mean = 0.80 (SE ± 0.03), $n = 8$ populations; invasive mean = 0.78 (SE ± 0.11), $n = 3$ populations; t score = 0.15, $P = 0.90$). However, one population from the invasive region (HOU) had the lowest *t* recorded among all populations sampled, $t = 0.58$. In contrast, the mean *t* for the two naturalized populations was significantly lower than that in the native region (naturalized mean = 0.63 (SE ± 0.01), $n = 2$ populations; t score = 3.63, $P < 0.01$). The mean estimate

of *t* in the naturalized region was also lower than the invasive region, but not significantly so at the $P = 0.05$ level (t score = 1.11, $P = 0.35$).

The mean estimate of *F* was also similar among the native and invasive regions (Fig. 2B; native mean = 0.07 (SE ± 0.01); invasive mean = 0.05 (SE ± 0.03); t score = 0.52, $P = 0.62$). The mean *F* in the two naturalized populations was higher than *F* estimates in the native and invasive regions (naturalized mean = 0.13 (SE ± 0.06); vs. native, t score = 2.03, $P = 0.08$; vs. invasive, t score = 3.10, $P = 0.12$).

Inbreeding depression ($1 - \omega$, where ω = the relative fitness of progeny produced by selfing) was greatest in the invasive region, significantly greater than that found in the native region (Fig. 2C; native mean = 0.44 (SE ± 0.04); invasive mean = 0.66 (SE ± 0.04); t score = 2.87, $P = 0.02$). The mean level of inbreeding depression in the naturalized population was intermediate between native and invasive levels, but there was a large amount of variance between the two naturalized populations, FC and NBS (naturalized mean = 0.49 (SE ± 0.26); vs. native, t score = 0.31, $P = 0.76$; vs. invasive, t score = 0.88, $P = 0.44$).

Discussion

The evolution of mating systems in colonizing plants has been a primary focus in ecology for decades and is likely to be case dependent, with demographic and stochastic variables playing consequential roles in determining the extent of selfing in incipient populations (Baker 1967; Cheptou 2012; Pannell et al. 2015). This is the first study to compare *in situ* outcrossing rates (*t*) and mean inbreeding coefficients (*F*) of maternal individuals between native populations of *M. guttatus* and two separate categories of non-native populations, naturalized and invasive. Our data revealed that non-native *M.guttatus*

populations do not uniformly adhere to either strict outcrossing or selfing. Naturalized populations in eastern North America, as well as one population in the invasive UK region, demonstrated higher selfing rates and levels of inbreeding compared to native populations. The remaining two populations in the invasive region had relatively high outcrossing rates, similar to native populations. Below we discuss scenarios that may explain the estimates of *t* and *F* in non-native populations, and how different levels of inbreeding depression calculated from these estimates may be used to predict the maintenance of current mating systems in these populations.

How do outcrossing rates in non-native M. guttatus populations compare to native populations?

Our prediction that outcrossing rates would be significantly lower, and mean inbreeding coefficients of maternal individuals higher, in non-native populations compared to native populations from the west coast of North America was partially supported by the relatively low *t* and high *F* in the naturalized populations located in eastern North America, FC and NBS. However, the average *t* and *F* for populations in the invasive region (United Kingdom) were nearly identical to that in the native region. The range of outcrossing rates among the eight native *M. guttatus* populations sampled for this research, 0.70-0.91 (Table 3), was similar to outcrossing rates found in past studies (0.41-0.76, Ritland & Ganders 1987; 0.68-0.80, Dudash & Ritland 1991). Inbreeding coefficients in the native region were generally low, ranging from 0.03-0.11 (Table 3). The estimates of *t* and *F* for two of the three populations from the invasive region fell

within the ranges of *t* and *F* for the native region (BRA: $t = 0.81$, $F = 0.03$; DBL: $t = 0.95$, $F = 0.01$).

It has been widely documented that invasive *M. guttatus* populations in the UK are the unintended consequence of the horticulture trade between the US and Europe (Preston 2002; Truscott et al. 2008; van Kleunen & Fischer 2008; Puzey & Vallejo-Marin 2014). By repeatedly introducing propagules to non-native regions (intentionally as a horticultural species, in the case of *M. guttatus*), the likelihood of forming natural populations with greater densities and genotypic diversity increases (Kalisz et al. 2004; Eppley & Pannell 2007; Friedman & Barrett 2008). A positive correlation between outcrossing rates and plant density has frequently been shown in mixed-mating seed plants (Herlihy & Eckert 2004; Brunet & Sweet 2006). For example, a study using experimental plots that differed in plant density found that pollinator visitation and outcrossing rates in the congener *Mimulus ringens* increased with density (Karron et al. 1995). For this study, we only assessed approximate population sizes at each of the 13 locations. An interesting follow-up study would critically survey the effective population size in both native and non-native populations to examine possible correlations between population density and outcrossing rates.

Mimulus guttatus belongs to a large group of other invasive plants that were introduced into Europe as ornamentals through the horticulture trade (Reichard & White 2001; Bastlová & Kvēt 2002; Mack 2003; Thuiller et al. 2005). Traits that are associated with fitness and show a positive relationship with higher outcrossing rates, such as floral size/number and biomass, are often enhanced in these ornamental plants and increase the likelihood that escaped plants will establish (Mack 2000; Kowarik 2003; Karron et al.

2004). A recent study compared floral traits, along with sexual and vegetative reproduction, between invasive versus native populations of perennial *M. guttatus* and found no significant differences (van Kleunen & Fischer 2008). Alternatively, Murren et al. (2009) found evidence for increased floral size in non-native *M. guttatus* populations compared to natives. This result is supported by a companion greenhouse study to the research discussed here, where we found increased floral size in invasive populations compared to native populations (Berg 2018). Because floral size traits have been associated with outcrossing rates in *M. guttatus* in general (Ritland & Ritland 1989; Dole 1992; Fishman et al. 2002; Wu et al. 2007), more research is required to evaluate the role of enhanced sex allocation in the mating systems of non-native populations.

While the BRA and DBL populations in the invasive region appear to depend on outcrossing for successful colonization, the naturalized M. guttatus populations (FC & NBS) and one UK population (HOU) rely more on selfing to persist. In the naturalized populations, the lower outcrossing rates can be explained by the lack of genotypic diversity in each population. The proportion of unique multilocus genotypes (MLGs) and observed heterozygosity in the FC and NBS progeny arrays were much lower compared to the 11 native and invasive populations, indicating that clonal propagation and fixation of alleles were important characteristics of naturalized *M. guttatus* populations. In FC and NBS, the proportion of unique MLGs of the total number of individuals sampled, maternal and progeny, was only 31% and 11%, respectively. These proportions are low compared to an average 82% (SD = 0.13) across the remaining 11 populations in the study.

The lack of genotypic and genetic diversity in the progeny arrays of naturalized populations (observed heterozygosity in FC was 0.02, and effectively zero in NBS; Table 3) results in an inability to distinguish whether any particular offspring was the result of selfing, because the progeny genotypes are identical to the maternal genotype, or rather of outcrossing, because many of the maternal genotypes are identical to one another. In these cases, the Bayesian posterior probability of *t* will approach 0.5 because half of the offspring will be deemed the product of a selfing event, and the other half of the product of outcrossing between parents that share the same genotype. Evidence for a lack of confidence in determining whether progeny were produced via selfing or outcrossing in the two naturalized populations can be seen in the high variability surrounding the mean posterior estimate of *t* in FC and NBS (Table 3; Figure 1A).

The relatively low genetic diversity, and subsequent outcrossing rates, in the naturalized populations may be the result of a recent bottleneck (Tsutsui et al. 2000; Frankham 2005; Prentis et al. 2008) or a dominating role of genetic drift over new mutations (Crooks & Soulé 1999; Eckert et al. 1996) and made it difficult to distinguish selfing events from outcrossing. However, the relatively low outcrossing rate in the invasive HOU population ($t = 0.58$) can be attributed with more confidence to actual selfing events. This may be due at least in part to greater genotypic and genetic structure among maternal individuals within this population. Nearly all maternal individuals (93%) had a unique MLG, making exclusion of paternity via an outcrossing event more straightforward compared to the naturalized populations. The contrast in outcrossing rates between the HOU population and the other two populations in the invasive UK region (BRA & DBL) is interesting and could be associated with any number of factors,

including smaller effective population size in the HOU population or differences in environmental factors (Schemske & Lande 1985; Ellstrand & Elam 1993; Van Treuren et al. 1993). Another possibility is that HOU is the result of a "bridgehead effect", a phenomenon in which the source of a non-native population is not from the native region, but rather from a distant non-native population (Lombaert et al. 2010). Genetic data from a companion study (Berg 2018) indicates that the HOU population is closely related to the BRA population, and therefore could be evidence of the latter providing the source propagules for the former. There are few empirical examples specifying bridgehead effects as the pathway to invasion for non-native plant populations, and the HOU population could serve as an interesting model to investigate this phenomenon.

Inbreeding depression in native and non-native M. guttatus populations

Six of the thirteen *M. guttatus* populations demonstrated levels of inbreeding depression below 0.5, the theoretical threshold where an allele causing selfing should be able to spread through an outcrossing population (Ritland 1990). Contrary to our prediction, however, the levels of inbreeding depression were not lower in naturalized and invasive populations compared to native populations. To the contrary, five of the six populations with inbreeding depression below a level of 0.5 were from the native region (AKS1, AKS2, WA, OR02, & BB1) and only one from the naturalized region, NBS. Levels of *in situ* inbreeding depression for a population are measured by using *t* as an estimate of the proportion of selfed progeny produced, and mean maternal *F* as an estimate of the number of those selfed individuals that survive to adulthood (Eqn. 8; Ritland 1990). When the level of inbreeding depression is below the 0.5 threshold, this indicates selection against selfing in an outcrossing population is soft enough to allow a certain

proportion of progeny produced by selfing to survive to adulthood. For populations with high outcrossing rates ($t \geq 0.8$), this required proportion, reflected in the value of *F*, is low. For example, populations with outcrossing rates near 0.8, such as AKS2 and WA, require an inbreeding coefficient of only 0.06 or greater to remain below the inbreeding depression threshold of 0.5. As the outcrossing rate increases and approaches 0.9 (e.g. AKS2 and OR02), the value of *F* required to remain below the threshold decreases further to approximately 0.3.

How can the relatively low levels of inbreeding depression in populations with high outcrossing rates be explained? The expression of inbreeding depression can occur at different life history stages in plants (Dudash 1990, Husband & Schemske 1997; Angeloni et al. 2011). In the four native populations with $t \geq 0.8$, mean *F* was high enough to result in levels of inbreeding depression below 0.5. However, selection against selfed native progeny could be most intense during the production of gametes and/or of zygotic viability. This "early-onset" inbreeding depression would not be detected using our approach, and may be restricting native populations from demonstrating even higher values of *F*. Inbreeding depression can be intense in early life history stages and purged from later stages (Husband & Schemske 1997; Dudash & Carr 1998), particularly when negative density-dependent interactions occur in populations (Mitchell-Olds & Waller 1985). Results from studies focusing on *M. guttatus* have concluded that inbreeding depression occurs with regard to pollen viability (Willis 1993), and pollen and ovule production (Carr & Dudash 1995; Carr et al. 1997; Dudash & Carr 1998). There is also evidence of purging in *M. guttatus*, although with variation among maternal families, for various life history traits (Dudash et al. 1997). An ongoing companion greenhouse study

has been investigating the magnitude of inbreeding depression at later life history stages among these same populations, and may add to what is known about the timing of inbreeding depression in native, naturalized, and invasive populations of *M. guttatus* (Berg 2018).

The only non-native population that fell below the inbreeding depression threshold of 0.5 was the clonal naturalized population, NBS. The NBS population consisted of only a few unique multilocus genotypes (MLGs; Table 3). Also, all loci were monomorphic for a single allele except for locus AAT225, which had a second, rare allele (frequency of allele "97" = 0.99, frequency of allele "103" = 0.01). It is difficult to know whether the genetic diversity described in NBS is the result of an origin in which a single or few genotypes were introduced and through subsequent selfing and vegetative propagation resulted in a genotypically homogenized monoculture at that location. It is also possible that multiple introductions occurred at this location, and through selection, drift (or a combination of the two), and concurrent high rates of selfing, resulted in the genetic pattern we see today.

There has been much discussion about the ability of invasive plant populations to purge their genetic load and escape the detrimental effects of inbreeding depression (Dudash & Carr 1998; Byers & Waller 1999; Dudash & Fenster 2000; Frankham 2005). A meta-analysis on purging of genetic load in plant populations found that fewer than half of the studies comparing populations and species found significant evidence of purging (14 of 34 populations) and that purging of the genetic load was a highly variable force within populations (Byers & Waller 1999). We have concurrently completed a companion study to investigate whether the magnitude of inbreeding depression

expressed in a variety of traits, is greater in non-native *M. guttatu*s populations compared to native populations (Berg 2018).

Conclusion

The proliferation of invasive plant species across the globe provides researchers with opportunities to address long-standing questions in the fields of ecology and evolution. While the proposal presented by Baker's Law (Baker 1955; Baker 1967), that selfing is the optimal mating system strategy to facilitate colonization compared to outcrossing, has been supported by empirical studies (Pannell & Barrett 1998; Cheptou 2012), other work has contradicted the theory. In fact, mating system evolution in colonizing and non-native plants is likely case-dependent and we have provided evidence for both high rates of outcrossing and high rates of selfing in non-native populations of a single species, *M. guttatus*. High outcrossing rates in non-native plant populations can occur because many individuals are introduced multiple times, increasing populations' sizes and genetic variation within and among populations. In today's world of high-speed globalization and increased trade among distant countries, the requirement of selfing to provide reproductive assurance in newly-formed non-native plant populations may be rendered a moot point due to the ubiquitous transport of plants, intentionally or not, by humans across regional and global boundaries.

Table 2.1 Location and approximate population size of 8 *Mimulus guttatus* populations from the native region, two from the naturalized region, and three from the invasive region used for progeny array analyses. Each progeny array consisted of randomly chosen families in a population, and each family may or may not have been represented by a maternal individual along with eight.progeny. If a family had an absent maternal individual, it was inferred from the progeny genotypes and allele frequencies by BORICE (Koelling et al. 2012).

Native region populations	**Code**	**Latitude**	**Longitude**	**# of families used in progeny array (%with inferred maternal genotypes)**	**Approximate # individuals in population**
Seward, AK (1)	AKS1	60.07021	-149.28102	19 (21%)	> 1000
Seward, AK (2)	AKS2	60.12142	-149.25660	20 (25%)	>200
Anchorage, AK	AKA	63.33945	-148.49102	11 (27%)	>1000
Shelton, WA	WA	47.23089	-123.08862	20 (15%)	>1000
Oswald State Park, OR	OR06	45.45797	-123.58105	21 (5%)	>1000
Haceta Head, OR	OR04	44.08163	-124.07605	20 (30%)	>500
Humbug Mt. State Park, OR	OR02	42.43072	-124.27879	8 (12.5%)	>500
Bodega Bay, CA	BB1	38.31701	-123.07117	20 (20%)	>1000
Naturalized region					
Fly Creek, NY	FC	42.44391	-74.58212	16 (44%)	>1000
Springfield, New Brunswick, Canada	NBS	45.41486	-65.49202	16 (6%)	>500
Invasive region					
Brampton, Norfolk, UK	BRA	52.7681	-1.27985	19 (100%)	>100
Dunblane, Perthshire, UK	DBL	56.18861	-3.96608	8 (100%)	>300
Houghton Lodge, Hampton, UK	HOU	51.09699	-1.5084	20 (100%)	>100

Table 2.2 Diversity of eleven molecular markers used in the study, across 13 native and non-native populations of *M. guttatus*. AAT markers are microsatellites, and MgSTS are intron-based polymorphic markers designed for *Mimulus guttatus* (Kelly & Willis 1998).

Locus	Approx. size range (bp)	Total no. of alleles	H_o	H_e
AAT217	177-195	6	0.12	0.67
AAT225	113-127	14	0.07	0.50
AAT230	179-210	26	0.32	0.88
AAT240	94-106	10	0.18	0.76
AAT278	127-135	3	0.04	0.12
MgSTS84	209-231	10	0.21	0.66
MgSTS234	272-321	12	0.22	0.69
MgSTS321	290-303	12	0.15	0.68
MgSTS430	238-269	13	0.25	0.65
MgSTS681	338-366	8	0.31	0.73
MgSTS685	242-251	15	0.23	0.84
Average		11.73	0.19	0.65
SD		5.92	0.03	0.06

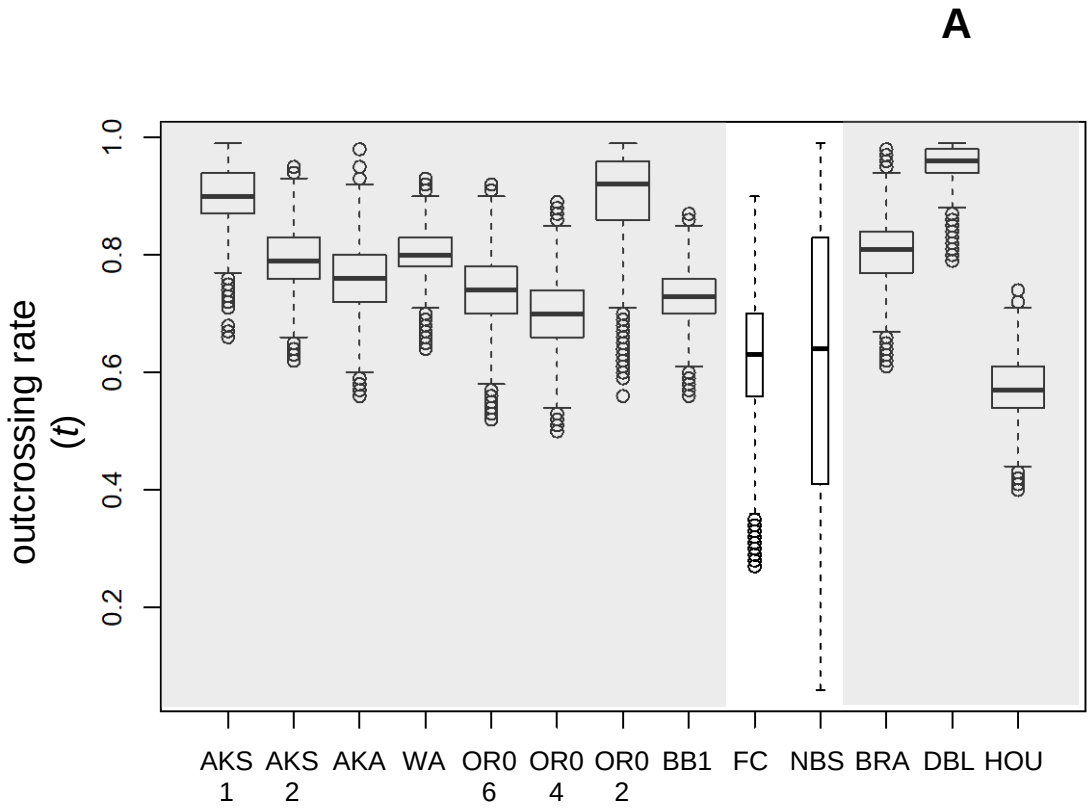
Table 2.3 Outcrossing rates, inbreeding coefficients, and inbreeding depression in native and non-native populations of *M. guttatus*. *N* number of individuals in progeny array (number of families in parentheses), maternal and progeny; the unique MLGs is the number of *N* that represent a unique multilocus genotype in the progeny array; *P* number of polymorphic loci in each population; *Na* average number of alleles per locus; H_o observed heterozygosity; H_e unbiased heterozygosity were calculated using GENALEX (Peakall & Smouse 2006); *t* outcrossing rate; *F* inbreeding coefficient calculated using BORICE (Koelling et al. 2012). Percent missing genotypes, across maternal individuals and progeny, for each population. BORICE infers missing maternal genotypes, and ignores missing genotypes in progeny; *Inbreeding depression* 1- {(2 x t x F)/[(1 - t)(1 – F)]} (Ritland 1990).

Native

Population	*N* *(# foms)*	Unique MLGs	*P*	N_a *(range)*	H_o*(SE)*	H_e*(SE)*	*t* *(95% credible interval)*	*F* *(95% credible interval)*	% missing data	Inbreeding depression (1-ω)
AKS1	177 (20)	118	9	2.73 (1-5)	0.17 (0.06)	0.18 (0.06)	0.91 (0.79, 0.99)	0.03 (0.00, 0.09)	12.2	0.37
AKS2	173 (20)	134	10	3.18 (1-4)	0.20 (0.06)	0.30 (0.08)	0.80 (0.70, 0.89)	0.06 (0.00, 0.12)	16.4	0.49
AKA	96 (11)	88	9	2.27 (1-3)	0.25 (0.06)	0.33 (0.07)	0.77 (0.65, 0.87)	0.06 (0.00, 0.18)	11.6	0.57
WA	176 (20)	169	10	1.94 (1-6)	0.24 (0.07)	0.37 (0.08)	0.81 (0.73, 0.88)	0.08 (0.03, 0.15)	15.2	0.26
OR06	188 (21)	135	9	2.46 (1-4)	0.16 (0.05)	0.25 (0.07)	0.74 (0.62, 0.85)	0.07 (0.00, 0.14)	17.2	0.57
OR04	174 (20)	122	9	2.64 (1-6)	0.17 (0.05)	0.26 (0.07)	0.70 (0.59, 0.81)	0.09 (0.03, 0.17)	14.9	0.54
OR02	71 (8)	47	7	2.18 (1-4)	0.15 (0.06)	0.15 (0.06)	0.90 (0.73, 1.00)	0.03 (0.00, 0.16)	12.0	0.44

BB1	176 (20)	167	9	3.70 (1-7)	0.26 (0.06)	0.36 (0.08)	0.74 (0.65, 0.82)	0.11 (0.03, 0.19)	9.0	0.30
Naturalized										
FC	136 (16)	42	6	1.70 (1-3)	0.02 (0.01)	0.16 (0.06)	0.63 (0.38, 0.82)	0.07 (0.00,0.17)	18.9	0.74
NBS	143 (16)	16	1	1.00 (1-2)	0.003 (0.002)	0.003 (0.002)	0.62 (0.14, 0.98)	0.19 (0.00, 0.67)	17.3	0.23
Invasive										
BRA	166 (19)	143	11	2.91 (0.25)	0.27 (0.06)	0.30 (0.06)	0.81 (0.71, 0.90)	0.03 (0.00, 0.08)	18.6	0.74
DBL	70 (8)	70	10	4.09 (1-8)	0.40 (0.07)	0.44 (0.07)	0.95 (0.89, 1.00)	0.01 (0.00, 0.06)	3.4	0.62
HOU	174 (20)	144	10	3.46 (1-6)	0.21 (0.06)	0.31 (0.07)	0.58 (0.49, 0.67)	0.12 (0.04, 0.21)	20.1	0.62

Figure 2.1 Median and quartiles of posterior distributions for outcrossing rates (A) and inbreeding coefficients (B) calculated by BORICE (Koelling et al. 2012). The posterior distributions reflect eight native (gray box on left), two naturalized (white center box), and three invasive populations (gray box on right). Width of bars indicates relative sample size for each region. Whiskers show values outside of the 25th and 75th quartiles of the posterior distribution, and circles represent more extreme values.



B

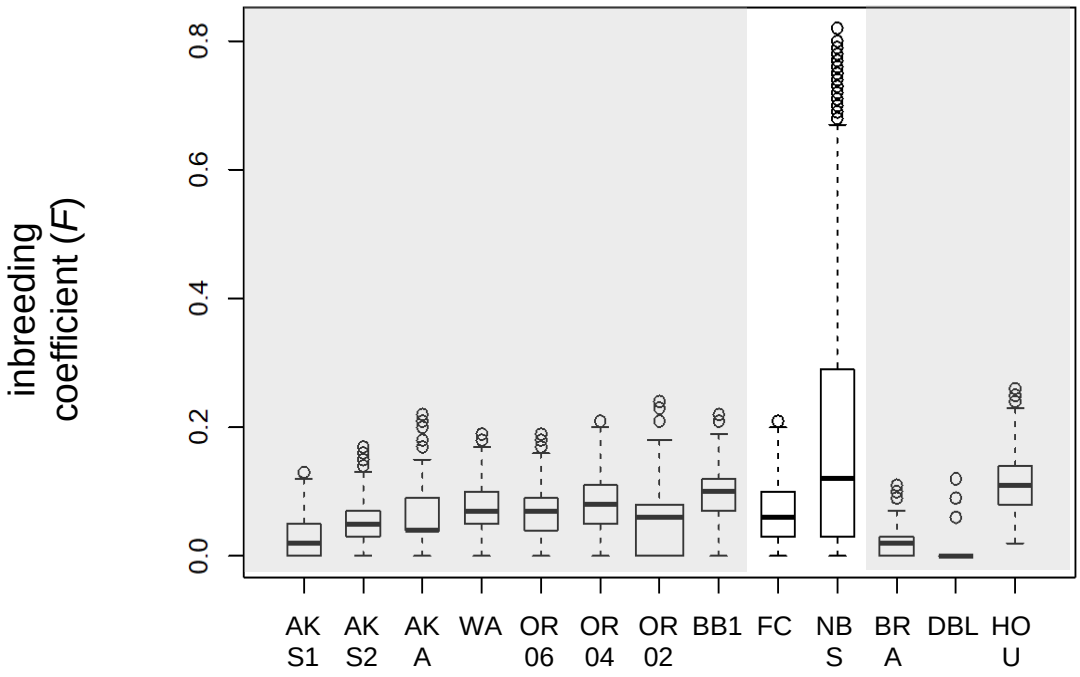
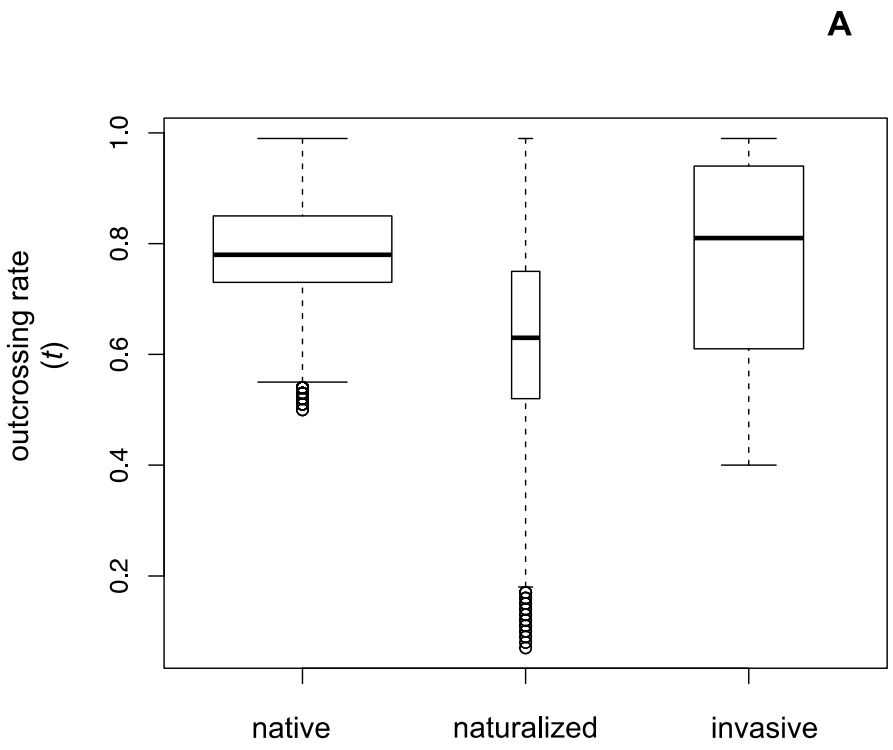

inbreeding coefficient (F)
0.0
0.2
0.4
0.6
0.8
AK S1
AK S2
AK A
WA
OR 06
OR 04
OR 02
BB1
FC
NB S
BR A
DBL
HO U

Figure 2.2 Outcrossing rates *t* (A) and inbreeding coefficients *F* (B) for three regions. Bars are medians of eight native, two naturalized, and three invasive populations.



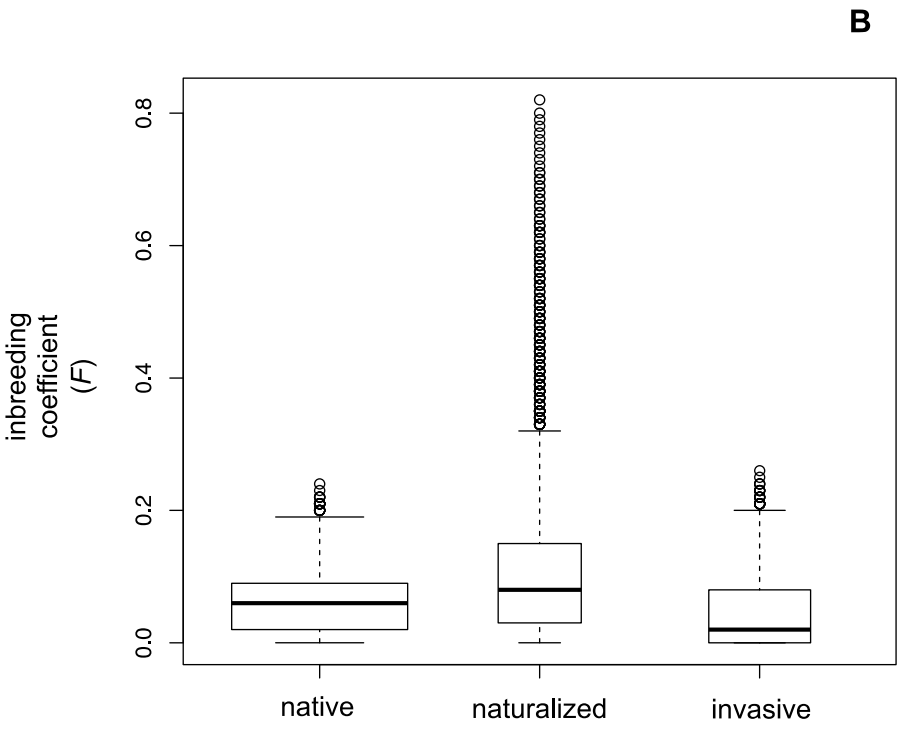
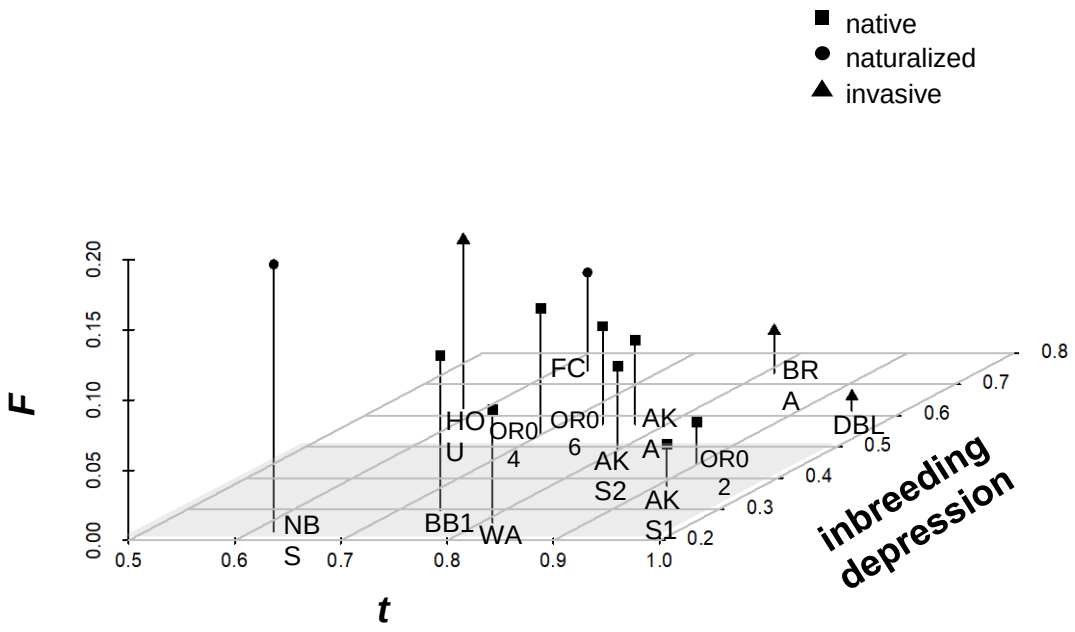

B
inbreeding coefficient (F)
0.0
0.2
0.4
0.6
0.8
native
naturalized
invasive

Figure 2.3 Relationship between *t*, *F* and inbreeding depression for 13 *M. guttatus* populations. Gray area illustrates portion of graph where inbreeding depression < 0.5. Theoretically, when inbreeding depression is lower than 0.5, a selfing allele should be able to increase in frequency in a population (Fisher 1941).



3 GENETIC DIVERSITY AND POPULATION STRUCTURE AMONG NATIVE, NATURALIZED, AND INVASIVE POPULATIONS OF COMMON YELLOW MONKEYFLOWER, *MIMULUS GUTTATUS* [PHRYMACEAE]

Introduction

Establishment by non-native plant species is a relatively rare occurrence, and the probability of successfully colonizing a novel location outside of the species' native range depends on many variables. Factors that can restrict the establishment and spread of introduced plant populations include low genetic diversity and Allee effects associated with founder events, insufficient propagule pressure in the form of a single or few introductions, maladaptation to novel environmental pressures, or some combination of these (Williamson and Fitter 1996; Richardson et al. 2000; Lee 2002; Lockwood et al. 2013). However, when a nascent plant population is able to overcome the influence of a novel suite of environmental pressures, the formation of establishment pathways can lead to a stage in the invasion process called naturalization.

The term "naturalization", while used extensively in the invasion biology literature, has become a source of confusion due to the myriad definitions applied to it. We use the term naturalization following the definition of Richardson et al. (2000), as a category of non-native plant populations that maintain sufficient population size by sexual reproduction or asexual vegetative proliferation, so the probability of extinction due to environmental stochasticity is low. Naturalization is often considered an intermediate stage prior to a population becoming invasive, representing a lag phase of slow population growth as it deals with deficiencies inherent to a novel population's

demographics or to maladaptation (Frappier et al. 2004; Aikio et al. 2010; Murren et al. 2009; Richardson & Pyšek 2012). The naturalization stage is considered in many theoretical models of invasion a critical point in determining whether a non-native population goes extinct, remains cryptic and benign, or alternatively adapts and spreads aggressively into new locations (Catford et al. 2009; Richardson & Rejmánek 2011). Despite the importance of the naturalization stage in characterizing the progression from casual colonization to impactful invasion in predictive models, few empirical studies include genetic diversity data from naturalized populations to compare to native and invasive populations (Pyšek et al. 2008). This gap in our understanding is largely due to the difficulty in locating and recognizing naturalized populations prior to their becoming invasive (Aikio et al. 2010).

The important transition from naturalization to invasion is often dictated by the genetic constitution of the plant population, which is in turn governed by the mode of reproduction and mating system of the plant species in question (García-Ramos & Rodríguez 2002; Kinlan & Hastings 2005). Invasive plant species display extensive variation with regard to the importance of sexual vs. asexual reproduction, and the degree to which sexual reproduction relies on outcrossing (i.e. mating between unrelated individuals) vs. self-fertilization (Barrett et al. 2008). The mode of reproduction determines establishment and invasion potential because it influences population genetic parameters such as the amount of additive genetic variation, effective population size, and partitioning of genetic diversity within and among populations (Barrett 1998; Vogler & Kalisz 2001; Eckert et al. 2010).

Invasion processes have resulted in transitions to higher rates of self-fertilization in mixed-mating plants (e.g. selfing and asexual reproduction) in the introduced range (Barrett et al. 2008; Barrett 2011; Clements & Ditommaso 2011). The study of self-fertilization and clonality and their role in facilitating colonization of non-native locations, goes back decades to "Baker's Law" (Baker 1955; Stebbins 1957). Baker suggested that self-compatible species should theoretically be more successful colonizers following long-distance dispersal compared to obligate outcrossing species, in part because the former would need only one individual to establish a naturalized population (Kolar and Lodge 2001). Asexual reproduction can also contribute to an introduced plant population's establishment and persistence in heterogenic environments, such as riparian ecosystems or roadside seeps (Cushman & Gaffney 2010) that often results in a single or few genotypes expanding in an area, evidenced by the successful aquatic plant invader, the water hyacinth, *Eichhornia crassipes* (Zhang et al. 2010).

While a single introduction, followed by some form of uniparental reproduction, has been shown to be a successful strategy to become naturalized, a more common scenario appears to be by multiple introductions of plant propagules followed by at least occasional outcrossing (Dlugosch & Parker 2008; Wilson et al. 2009). When outcrossing is the primary mode of reproduction, additive genetic variation within the population increases compared to populations that rely on selfing that results in a 50% decrease in heterozygosity after each generation (Charlesworth & Charlesworth 1987; Carr & Dudash 2003). By enhancing genetic variation, outcrossing enables an incipient population to respond and adapt more quickly to the changes in environmental conditions common during invasion (Lynch and Walsh 1998; Charlesworth 2003; Barrett et al.

2008). Outcrossing can also result in interspecific hybridization among closely related species that have recently come into contact, and examples of allopolyploid species such as *Tragopogon mirus* (Soltis et al. 2004), *Senecio cambrensis* (Abbott & Lowe 2004), and a *Spartina* hybrid cross between the *S. foliosa* and *S. alterniflora* (Ayers et al. 2004; Ainouche et al. 2009) have been described. Hybridization among sister taxa, whether they share a common ploidy level or not (e.g. diploid x tetraploid = triploid hybrid; Vallejo-Marin & Lye 2013), may stimulate invasiveness through heterosis or recombination (Baack & Rieseberg 2007).

In this study, we use molecular data to examine the genetic diversity and structure among a range of native and non-native populations of diverse origins and residence times. Specifically, we compare two naturalized eastern North American populations of the mixed-mating plant species, *Mimulus guttatus* D.C. (Phrymaceae), a nearby naturalized population comprised of a heretofore-undescribed hybrid *Mimulus* taxon, three non-native *M. guttatus* populations in the United Kingdom, where the species is considered invasive, and native populations that occur across a large span of the species' home range in western North America. Our goal was to address the following questions: 1) How does genetic and genotypic diversity in non-native populations (i.e. naturalized and invasive populations) compare to diversity in native populations? We predict that populations in the invasive region will have similar levels of genetic diversity as do native populations due to the species' history of multiple introductions as an ornamental plant (Truscott et al. 2008); 2) Which native location is most likely the source for non-native *M. guttatus* populations? We predicted that non-native *M. guttatus* populations in the UK are derived from populations on the northern edge of the native distribution based

on prior evidence (Puzey & Vallejo-Marin 2014). There has been no prior investigation regarding the source region for naturalized populations on the east coast of North America, and we aimed to shed light on the origin of these non-native populations.

Methods

Study species

Mimulus guttatus ($2n = 2x = 28$), or common monkeyflower, is an herbaceous species native to the west coast of North America, found from Mexico to Alaska (Dudash et al. 1997; Carr et al. 1997; Kelly & Arathi 2003; Lowry et al. 2008; Wu et al. 2007). In the United Kingdom (UK), *M. guttatus* is considered a harmful invasive that was intentionally introduced as a horticultural species approximately 200 years ago (Truscott et al. 2008; van Kleunen & Fischer 2008). Recently, naturalized populations in New York State and New Brunswick, Canada, have received attention (Murren et al. 2009). Little is known of the evolutionary history of these naturalized populations, but they are thought to have established at least 50 years ago. Native populations of *M. guttatus* are described as mixed-mating and exhibit wide variation in outcrossing rates (Ritland & Ganders 1987; Dudash & Carr 1998). To our knowledge, outcrossing rates in naturalized and invasive populations have not been estimated. *Mimulus guttatus* is also capable of asexual reproduction via fragmentation and stolons (Grant 1924; Vickery 1959; Truscott et al. 2006). *Mimulus guttatus* has become a model system in studies of ecological and evolutionary genomics because of its broad phenotypic and genetic diversity (Dudash et al. 2005; Wu et al. 2008). In its native range, *M. guttatus* populations can be found as either annuals or perennials, and this difference in life history depends on water availability (van Kleunen 2007; Lowry et al. 2008; Dudash & Murren unpubl. data). For

this study, we only sampled from perennial native populations because all known non-native populations are perennial.

Sampling sites

To compare genetic diversity and population structure among *M. guttatus* populations in the native, naturalized, and invasive ranges, we conducted fieldwork in 2012 and 2013 (Table 4). Our sampling of native populations along the west coast of North America covered a large latitudinal transect (~ 5150 km) from Point Reyes, CA to Seward, AK. The route to survey 11 native perennial populations was based on records obtained from colleagues (B. Blackman & D. Lowry, pers. comm.) and local contacts. In the naturalized region on the east coast of North America, two of the three populations sampled were previously studied by Murren *et al.* (2009) and located in Fly Creek, New York and Springfield, New Brunswick, Canada. Local botanists provided the location of a third population, near Bass River, New Brunswick (NBBR). Initially, the NBBR population was thought to be comprised of *M. guttatus* individuals. However, following assessment of genotyping data, chromosome counts, and morphological traits (e.g. low pollen viability and reduced seed set in the greenhouse), it was apparent that the NBBR population was comprised of polyploid hybrid *Mimulus* individuals. These individuals likely represent a triploid ($2n = 3x = 42$-46; Berg unpublished data) with *M. guttatus* constituting at least one of the parental taxa. It has been shown that *M. guttatus* readily hybridizes with closely related *Mimulus* species to form allopolyploids (Clausen et al. 1950; Vickery 1978; Vallejo-Marin 2012), including the triploid hybrid *M. x robertsii* Silverside ($2n = 3x = 44$-46) formed by *M. guttatus* and a South American species, *M. luteus*, which is also found throughout the UK (Vallejo-Marin & Lye 2013). The second

parent taxon of the NBBR hybrid population is likely a tetraploid *Mimulus* species, but its identification was beyond the scope of this study. Here, we utilize the NBBR population in the surveys of genetic diversity, but do not include it in the structure analyses due to the difficulties that arise in combining polyploid and diploid data in these assessments.

For each of the 14 native and naturalized populations, we randomly sampled fruits and leaf tissue from 30-50 individual plants that were > 1 m apart to increase the chances of sampling multiple genotypes. Leaf tissue was immediately stored in silica gel until DNA extraction. For the three *M. guttatus* populations in the invasive region in the UK, we obtained field-collected seed from a colleague, Mario Vallejo-Marin, at the University of Stirling in Scotland. This seed was then grown at the University of Maryland (UMD) greenhouse and when seedlings were ~ 6 cm tall, leaf tissue was collected from 20 individuals per population and stored in silica gel. Following DNA extraction and genotyping (see below) the sample size was reduced to 14 individuals for each of the UK populations due to poor quality DNA in some samples.

Genetic markers

To genotype the 17 populations from the native, naturalized, and invasive regions, we used 12 codominant markers (Table 5) including six microsatellite loci previously used to genotype North American and British *Mimulus* populations (Kelly & Willis 1998; Vallejo-Marin & Lye 2013), and six markers revealing length polymorphisms in introns of single-copy nuclear genes in *M. guttatus*, *M. x robertsii*, and *M. luteus* (Fishman & Willis 2005; Lowry et al. 2008, Vallejo-Marin & Lye 2013). These intron length polymorphisms, or MgSTS (*Mimulus guttatus* sequence-tagged sites) are suitable for

genotyping based on a selection strategy of Vallejo-Marin *et al.* (2011). These markers have been shown to be variable in samples of *M. guttatus,* its close relative, the tetraploid *M. luteus,* and the triploid hybrid produced by them, *M. x robertsii* Silverside and are suitable for multiplexing (Vallejo-Marin & Lye 2013).

DNA extraction PCR amplification

To extract DNA from leaf tissue, a modified CTAB protocol (Doyle & Doyle 1990) was employed on an AutoGenprep 965/960 instrument (AutoGen, Holliston, MA, USA) using the Plant DNA Extraction Kit AGP965/960, following the manufacturer's protocol. DNAs were amplified for the 12 loci in sets of two multiplexed reactions using a 2 x Qiagen Type-It Microsatellite PCR kit (Qiagen, California, USA), 2 μM of each of the fluorescent forward primers labeled with either FAM or HEX dyes and 2 μM of each reverse primer and 5-50 ng of template DNA. PCR cycles consisted of a denaturing step of 5 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 55 °C for 180 s and 72 °C for 30 s and a final elongation step of 60 °C for 30 min. We examined success of the PCR amplifications in a 1.5% agarose 1x sodium hydroxide-boric acid buffer electrophoresis gel (Brody & Kern 2004). PCR products were diluted in nuclease-free water (dilutions ranged from 1:10 to 1:50), and one μL of each dilution was added to 9 μL of HiDi formamide with 1 μL ROX standard (DeWoody *et al.* 2004). Samples were heated to 95 °C for six minutes, cooled to 4 °C for six minutes, and loaded onto an ABI 3730xl automated capillary sequencer with a 50 cm, 96 channel array containing POP-7 polymer for fragment analysis at the Laboratories of Analytical Biology (LAB) of the Smithsonian National Museum of Natural History.

Genetic analyses

We performed allele binning and analyzed raw peak sizes from fluorescent fragment profiles using GeneMapper v5.0 software (Applied Biosystems, Foster City, CA, USA)), that allows calling of multiple peaks per locus. A random sample of 10% of individuals was re-assayed and re-scored to check consistency. For the polyploid individuals at the NBBR site, determining conventional genetic diversity parameters based on allele frequency (e.g. expected heterozygosity) is problematic because of the difficulty in identifying alleles in partial heterozygotes. Therefore, to assess allelic diversity in each population and between regions, we calculated the following statistics (Sampson & Byrne 2012): the total number of alleles across all loci (*A*); the average number of alleles per locus in each population (*A'*); the average number of alleles per locus in an individual (*H'*); the proportion of observed heterozygotes, averaged over all loci (H_O); and the number of private alleles (*P*). Welch's two sample t-tests were used to test for significant differences between regions for these statistics (the NBBR population containing polyploid individuals was excluded from t-tests). For the diploid *M. guttatus* populations, we used GenAlEx 6.5 to calculate expected heterozygosity and deviations from Hardy-Weinberg equilibrium (HWE). To determine pairwise genetic differences between individuals within each population, we used the method developed for microsatellite data by Bruvo *et al.* (2004) in the R package *poppr* (Kamvar *et al.* 2014). The distance measure of Bruvo *et al.* (2004) is similar to band-sharing indices and is appropriate for relative distance comparison among intraspecific individuals of different ploidy levels, and takes into account stepwise mutational processes.

We used *poppr* for multilocus genotype (MLG) assignment, to determine expected

proportion of MLGs from the total number of individuals sampled (*R*) using a rarefaction method to account for sample size (Hurlbert 1971), and to calculate the complement of Simpson's diversity index *D* (Simpson 1949).

Population genetic structure

We used four complementary analyses to investigate population structure among the 16 *M. guttatus* populations in the native, naturalized, and invasive regions: 1) a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.* 1992); 2) a discriminant analysis of principal components (DAPC); 3) clustering of the 16 *M. guttatus* populations based on Nei's genetic distance (Nei 1978) visualized using a dendrogram; and 4) Mantel tests to examine the correlation between geographic and genetic distance in order to detect cases of isolation by distance. The putative polyploid *Mimulus* hybrid population from the naturalized region (NBBR population) was left out of the analyses of population structure because it was the only polyploid population found. This omission allowed us to focus on the structure among the remaining diploid *M. guttatus* populations.

First, we conducted a hierarchical AMOVA in *poppr* to estimate variance and distribution of diversity within and among regions, populations and individuals within populations. The significance of variance components calculated for all levels was tested with 1000 permutations.

The second analysis, a discriminant analysis of principal components (DAPC), is a multivariate method to identify clusters comprised of genetically similar individuals (Jombart et al. 2010). DAPC uses principle components derived from principle components analysis (PCA) as variables to optimize between group variation and

minimize within group variation in order to separate individuals into pre-defined groups (Jombart et al. 2010). The method uses a *k*-means clustering algorithm to analyze any number of potential clusters (*k*'s) in a sequential manner. The optimal *k* should correspond with the lowest Bayesian Information Criterion (BIC) score. DAPC has been suggested as an alternative to other Bayesian clustering methods such as STRUCTURE (Pritchard *et al.* 2000) because it does not require a population genetic model to identify clusters. Therefore, DAPCs are suitable for analyzing complex genetic data sets such as those that may not adhere to the assumption of random mating within populations (e.g. clonality or self-fertilization). The DAPC analysis was conducted in the R package *adegenet* 1.3-1 (Jombart & Ahmed 2011). Prior to DAPC analysis, we used the *adegenet* function *clonecorrect* to account for clonality in the data set. Next, to find the optimal number of clusters, we used *k*-means clustering of principal components using the function *find.clusters* in *adegenet*. The function *xvalDapc* was used to cross-validate the number of principal components used in the analysis.

To complement the DAPC analysis and help resolve structure among the 16 *M. guttatus* populations, we constructed a dendrogram based on Nei's genetic distance (Nei 1978). Data were bootstrapped in the R package *poppr* using the *aboot* function from a sample of 1000 bootstrapped trees. The function *clonecorrect* was applied to the data prior to bootstrapping to account for clonality within populations.

Finally, to examine the relationship between geographic location and genetic differentiation and the possible existence of isolation by distance (IBD), three separate Mantel tests were performed with the *ade4* package in R, using the function *mantel.rtest*. The output of each of the three tests was based on a Monte Carlo method using 1000

replicates. The first test included all 16 *M. guttatus* populations from the three regions, the second test included the 11 native *M. guttatus* populations only, and the third test included the 5 non-native populations from the naturalized and invasive regions. The measure of pairwise genetic distance between populations used was Jost's *D*, which measures the fraction of allelic variation among populations (Jost 2008). Jost's *D* will equal unity at complete differentiation, and zero with no differentiation between populations. Geographic distance between each pair of populations was recorded as the shortest distance between populations and measured in kilometers. The function *clonecorrect* was applied to the data prior to each of the Mantel tests.

Results

Genetic and genotypic diversity within populations and between regions

Individual genotypes consisted of either one or two peaks per locus in the 16 *M. guttatus* populations, and ranged from one to three peaks in the putative polyploid hybrid population NBBR. Markers deviating from Hardy-Weinberg equilibrium (HWE) were found in each of the 11 native and three invasive *M. guttatus* populations. The two naturalized *M. guttatus* populations, NBS (New Brunswick, Canada) and FC (New York), were monomorphic for eleven and seven loci, respectively (the polyploid NBBR population was left out of structure analyses). All loci that were not monomorphic in these populations deviated from HWE. The total number of alleles amplified per locus (*A'*) ranged from 6 at locus AAT267 to 23 at locus AAT230, when both the *M. guttatus* and the putative polyploid taxa were considered (Table 5). In total, 100 different alleles (*A*) were amplified in the 16 *M. guttatus* populations and 32 alleles in the putative polyploid (NBBR) population for the 12 microsatellite and intron-length polymorphic

markers. Of the 32 alleles from the polyploid hybrid population, six (19%) were found exclusively in that population.

Levels of intrapopulation genetic diversity (*A, A', H', H_O, P*; Table 6) in the native and invasive regions were generally higher than those in the naturalized region, but not significantly so (Welch's two-sample t-tests; $P > 0.05$). The total number of alleles (*A*; Table 6) in the native and invasive regions (28.27±4.24 and 30.67±7.67, respectively) was nearly twice that found in the two naturalized *M. guttatus* populations, NBS and FC (15.50±3.54). The largest number of alleles was found in the putative polyploid NBBR population in the naturalized region (32), while the fewest was found in the naturalized *M. guttatus* population NBS (13; Table 6). Populations in the native and invasive regions also averaged more alleles per locus (*A'*; Table 6) than those in the naturalized region (native: 2.35±0.34; invasive: 2.55±0.64; naturalized: 1.30±0.28; $P < 0.06$ for both comparisons, naturalized vs. native and naturalized vs. invasive). The putative polyploid NBBR population had the greatest number of alleles per locus (2.7). Average observed heterozygosity in the native and invasive region was similar (0.22±0.05 and 0.26±0.06, respectively; Table 6) and twice that of the two naturalized *M. guttatus* populations (0.12±0.16), while heterozygosity in the NBBR population was relatively high (0.49), as expected for a population consisting of polyploid individuals (Husband & Schemske 1997). The naturalized FC population had the highest number of private alleles (five) among non-native *M. guttatus* populations. The other naturalized population, NBS, had zero private alleles. The populations from the invasive region averaged 2.3 private alleles (SD+0.58 ; Table 6).

The mean pairwise genetic difference between individuals (*H'*; Table 6) in each population was lowest, on average, in the naturalized region (0.024±0.005), greater within native populations (0.163±0.009), and greatest in the invasive region (0.225±0.018). Mean pairwise genetic distance in the NBBR population was the lowest of all populations and similar to distances in the other two naturalized populations (0.012). The mean number of unique multilocus genotypes (MLGs; Table 6) was 17.9±4.5 in the native populations vs. 14.0±0 in populations in the invasive region and 4.5±3.54 in naturalized populations. Only six MLGs represented the 26 NBBR individuals sampled. The mean proportion of MLGs among the number of individuals sampled, following rarefaction to account for differences in sample size (*R*; Table 6), was 1.0 in the invasive region (all sampled individuals within populations represented a unique MLG), 0.97±0.07 in the native region, and 0.27±0.14 in the naturalized region. The proportion of MLGs in the NBBR population (0.28) was similar to the two *M. guttatus* naturalized populations. There was very little difference between the native and invasive regions' mean complement of Simpson's diversity (*D*; Table 6) indices (0.94±0.01 and 0.93±0, respectively), and both indices were more than twice that found in the naturalized region (0.46±0.03) and nearly threefold larger than the *D* calculated for NBBR (0.34).

Population genetic structure

An AMOVA (Table 7) with a three level hierarchy (three regions, populations, and individuals) indicated that 46.33% of diversity was maintained across populations and 37.71% among individuals within populations ($\Phi_{\text{Population, Region}} = 0.55$, $P < 0.001$;

$\Phi_{Individuals, Total} = 0.62$, $P < 0.001$; Table 7), while only 15.97% of diversity was maintained among regions ($\Phi_{Region, Total} = 0.16$, $P < 0.001$).

The discriminant analysis of principal components (DAPC) showed that the k-means clustering separated the data set (248 MLGs representing 310 diploid *M. guttatus* individuals from 16 populations) into 16 clusters (Fig. 4), indicating that each sampled population was genetically distinct from one another. To address the possibility of overfitting in the DAPC, which can occur if too many principal components are withheld in the model (Jombart et al. 2010), we ran the analysis several times using a range of principal components from 5-50. Each time, the optimal number of clusters was 16 based on the Bayesian information criterion.

When plotted along the first two principle components used for the DAPC, the 16 populations sorted into four distinct groups (Fig. 5). The first group consisted of four of the 11 native populations, and included the three Alaskan populations and the Washington State population. The second group included the remaining seven native populations, specifically the five Oregon populations and the two California populations. The third group consisted of the three populations from the invasive region in the UK, along with the naturalized population from Springfield, New Brunswick (NBS). The fourth group consisted of a single population, from Fly Creek, New York (FC) in the naturalized region on the east coast of North America.

A dendrogram based on Nei's genetic distance (Nei 1978) provided complementary evidence of the genetic structure among the 16 *M. guttatus* populations (Fig. 6). Each of the 1000 trees sampled for bootstrapping showed that the naturalized FC population was genetically distinct from the other 15 populations, which corresponded to this

population's distinct placement determined by the principle components (Fig. 5). Also, the dendrogram showed 62% support for a clade consisting of the three populations from the invasive region in the UK and the *M. guttatus* population from Springfield, New Brunswick in the naturalized region (NBS). The three native populations from Alaska, along with the population from Washington state formed a clade within the larger grouping of native populations (72% support). This AK/WA clade was also shown as being the most closely related native group to the invasive UK/NBS clade; however, support for this relationship was low (28.5 %).

Three Mantel tests were performed to determine the correlation between pairwise genetic distance and geographic distance (r_m; Table 8): the first test included all 16 *M. guttatus* populations and showed a positive correlation between genetic and geographic distance ($r_m = 0.33$; $P < 0.01$), which indicates a classic pattern of isolation by distance. The second test included only the 11 native populations, and also showed a positive correlation ($r_m = 0.36$; $P < 0.01$). The third test included the five non-native populations (excluding the putative polyploid NBBR population) and, contrary to the previous tests, showed little correlation between genetic and geographic distance ($r_m = 0.29$; $P > 0.1$).

Discussion

Understanding the role of genetic variation in plant invasions is a primary focus in modern ecology, and reconciling the paradox that exists when plant populations with low genetic diversity are able to establish in non-native locations is a goal for researchers interested in managing these populations (Lee 2002; Dlugosch & Parker 2008; Moran & Alexander 2014). To shed light on this issue our study examined the genetic diversity and structure of naturalized populations of a potentially invasive plant species, *Mimulus*

guttatus, and compared this diversity to populations in the native and invasive regions. Using molecular data from microsatellite and intron-length polymorphic markers, our study revealed several major findings: 1) The naturalized *M. guttatus* populations in eastern North America had low genetic diversity compared to populations in the native and invasive regions, so the occurrence of only a few multilocus genotypes in naturalized populations suggests a population bottleneck and persistence due to a selfing/asexual reproduction; 2) We found some evidence for the northern edge of the native distribution representing the source location for populations in the invasive region, as well as some data suggesting that an invasive UK population could be at least one source for a naturalized population on the east coast of North America; 3) One naturalized population in New Brunswick, Canada, was identified as a polyploid species of *Mimulus*. Overall, we have provided evidence for multiple pathways of establishment for the non-native populations of *M. guttatus*. Below we discuss our results in more detail and consider their relevance to broader questions in invasion genetics.

Genetic and genotypic diversity in native, naturalized, and invasive regions

Our comparison of genetic diversity revealed that in the two naturalized *M. guttatus* populations, NBS and FC, diversity was substantially lower, on average, than that found in populations located in the native region. While these differences were not statistically significant at the $P = 0.05$ level (due in part to the low level of detectable genetic variability in the naturalized region), we found that the average total number of alleles, number of alleles per locus, and observed heterozygosity in native populations were nearly twice that found in the two naturalized populations (Table 6). Genetic diversity in

the NBS (Springfield, New Brunswick, Canada) was effectively non-existent, with 11 homozygous loci and only one locus (MgSTS84) segregating for two alleles. This lack of genetic diversity, coupled with the presence of only two multilocus genotypes (MLGs) in the 30 individuals sampled (i.e. low genotypic diversity), indicates that the NBS population is the product of a colonizing cohort of *M. guttatus* individuals that were subjected to founder effects following introduction. This population could be the result of a single introduction of a few propagules, followed by a reliance on asexual reproduction and/or self-mating to persist. Alternatively, the NBS population could have resulted from multiple introductions that were subjected to environmental filters that allowed only a few genotypes to establish.

There are examples of invasive plants that have become invasive while maintaining low genetic diversity relative to native populations; however, most of these examples reproduce apomictically or rely solely on some form of uniparental reproduction (Dlugosch & Parker 2008; Bakker et al. 2009; Fennel et al. 2010; Roux et al. 2011). Examples of mixed-mating species becoming invasive despite low genetic diversity, as we found in the NBS population, are much less common. The results of Hagenblad et al. (2015) are one such example, in which invasive populations of the mixed-mating species *Impatiens glandulifera* in Europe had much lower genetic diversity than native populations from India despite being introduced multiple times. The researchers concluded that phenotypic plasticity, a characteristic expressed in many introduced plant populations and thought to influence invasion success (Davidson et al. 2011; Murren & Dudash 2012), might have played a large role in allowing populations with depauperate genetic diversity to establish and spread in novel environments far from the native region

(Hagenblad et al. 2015). Murren and Dudash (2012) have also found evidence for increased expression of phenotypic plasticity in certain architectural traits in *M. guttatus* when grown in field sites in non-native locations. Perhaps the two MLGs in the NBS population that we uncovered in this study were selected for certain adaptive plastic traits that suited them well following introduction into the remote location in Springfield, New Brunswick, Canada. Given the low genetic variation and genotypic diversity in the NBS population, a logical next step would be to examine these individuals for their capacity to express plasticity in novel, stressful environments. A companion greenhouse study aimed to shed light on the role of phenotypic plasticity in response to abiotic conditions that naturally varies among native and non-native populations (Berg doctoral dissertation).

The current status of the NBS population has been categorized here as naturalized and not invasive because it consists of only a few hundred individuals restricted to a small area of approximately 1500 square feet. Like many introduced plant populations, we cannot be sure of the introduction history of the NBS population. Knowing the potential for *M. guttatus* to become invasive, it remains to be seen if the NBS population will spread by overcoming barriers restricting it to its current location. By monitoring this naturalized population over the coming years, we could learn much about the importance of environmental, demographic, and genetic factors in restricting *M. guttatus* from rapid population growth.

The second naturalized *M. guttatus* population in this study, FC (Fly Creek, New York), was also relatively deficient in some measures of genetic diversity relative to native populations but overall its observed heterozygosity was similar to the average heterozygosity found in the native populations. The FC population may have a similar

introduction history as the NBS population, but it is unlikely they originated from the same source population (see discussion of source populations below). Methods designed to detect a recent reduction in population size based on the principle of excess heterozygosity using microsatellites or other molecular markers (Cornuet & Luikart 1996; Beaumont 1999; Garza & Williamson 2001) typically require larger sample sizes than the seven MLGs representing FC in this study in order to obtain robust statistical results (a total of 21 FC individuals were genotyped in the study and resulted in only seven unique multilocus genotypes; see Table 6). Therefore, based on our findings, further sampling is warranted to determine whether the naturalized *M. guttatus* population located in Fly Creek, New York may be the product of a recent bottleneck.

In the three populations from the invasive region in the UK, genetic and genotypic diversity was similar to the native populations, supporting the evidence of multiple introductions in this region and suggesting that outcrossing is the prominent mode of reproduction. *Mimulus guttatus* was introduced into the UK repeatedly as a horticultural species (Truscott et al. 2008; van Kleunen and Fischer 2008), and this intentional, repeated introduction of propagules has likely manifested in high genotypic diversity. Each of the successfully sampled individuals in the three populations, BRA, DBL, and HOU (n = 14 successful samples from an original total of 20 individuals for each invasive population) represented a unique MLG. While few morphological, environmental, or demographic variables can be considered as universal facilitators across all plant invasions, propagule pressure has been found to be a common denominator explaining nearly all successful invasions for which there are historical records of introduction (Colautti et al. 2006). Based on the scope of our study, we cannot

say definitively that the greater genotypic diversity found in *M. guttatus* populations from the invasive region compared to the naturalized region provides an ample explanation as to why some *M. guttatus* populations become invasive while others do not. However, our results can be used in combination with future environmental comparisons and evaluations of residence times (Pyšek et al. 2009) to develop a clearer picture of what factors may promote invasion in *M. guttatus*.

Sources for non-native populations and population structure

Our complementary analyses of population genetic structure of the 16 diploid *M. guttatus* populations suggest that there was little gene flow among populations within or between the three regions. An AMOVA showed that more variation in genetic diversity was maintained between populations than between regions (46.33% of the variance vs. 15.97%, respectively; Table 7), and the DAPC clustered genotypes into 16 distinct groups (Fig. 4). Despite these results demonstrating definitive population groupings, there was evidence for admixture between the two Seward, Alaska populations (AKS1 & AKS2), between the Washington (WA) and AKS2, between Oregon populations, and between the two California populations (BB1 & PR). Taken with the results of the Mantel test that included only the native populations, we can conclude isolation by distance is resulting in differentiation among native populations but admixture may occur between neighboring sites. However, this isolation by distance interpretation is contradicted by admixture data between the WA and AKS2 sites, which are separated by more than 2,000 km. Our leading hypothesis is that the urban WA population originated from propagules from a source near the remote Alaska sites, on the northern edge of *M.*

guttatus' northern distribution. Genetic variation in the WA population is relatively high, and the occurrence of four private alleles leads us to believe that the population may be the product of multiple introductions.

We found no strong association that would identify any of the 11 native *M. guttatus* populations as a source for any non-native populations in our study, although there was slight evidence for the native Alaskan group being the most closely related to the group comprised of the three invasive UK populations and the NBS population (Fig. 6). This would support a prior study that identified the northern edge of the native distribution as the potential source for introduced populations in the UK. Using genome resequencing data, Puzey and Vallejo-Marin (2014) found that several non-native *M. guttatus* populations in the UK (including the DBL and HOU populations sampled for this study) are derived from a region in the North Pacific, specifically in the Queen Charlotte Islands in British Columbia, Canada. Future "Next-Gen" sequencing may provide greater resolution of the geographic source region compared to traditional SSR marker techniques regarding introduction histories and establishment pathways, and recent methods for analysis such as approximate Bayesian computation can allow the evaluation of different invasion scenarios (Cristescue 2015).

As mentioned above, we found some support for the inclusion of the naturalized NBS population within the clade formed by the UK populations in the invasive region (Fig. 6, bootstrap value = 52%). That NBS was more closely related to the UK populations than the other east coast population, FC, was also supported by the Mantel test that included only the five non-native *M. guttatus* populations. Had NBS been less differentiated from its closest neighbor on the east coast compared to the populations in Europe, this test

would have shown a correlation between genetic and geographic distance; however, this was not the case. This result is interesting because it may reveal that the NBS population originated from a source in the invasive region in Europe, rather than from a source in the native region of western North America. This scenario would represent what is known as a bridgehead effect, which occurs when non-native populations become invasive and subsequently serve as the source for nascent populations in remote new territories (Lombaert et al. 2010). Theoretically, populations that have become invasive have already been subjected to selective filters in environments outside of the native region, and thus should be well adapted to colonizing new locations. Thus, if certain genotypes are more successful colonizers, either because they express traits that allow for persistence during founder effects (e.g. low inbreeding depression, exploitation of resources following disturbance) or they fit Baker's description of the "general-purpose genotype" by being more phenotypically plastic than other genotypes (Baker 1974), then the bridgehead effect could act as an efficient process for choosing adaptive colonizers that can leapfrog into other territories. It is plausible that the source population for the NBS population in New Brunswick, Canada is located in Europe, as the two continents maintain a robust trade in horticultural products. From 2013 to 2015, the EU exported over $9 billion in horticultural products to the US alone (USDA 2016). It is possible that the founding propagules that colonized the NBS site arrived from Europe prior to the enforcement of current efforts such as the USDA Plant Protection and Quarantine program, enacted to restrict the import of potentially invasive plant species.

The naturalized Fly Creek (FC) population located in New York demonstrated high differentiation from the other populations, completely isolated from other groups by the

first two principal components (Fig. 5) and received 100% bootstrap support as its own clade on the dendrogram (Fig. 6). There are several possible explanations for such high differentiation in naturalized populations, including founder effects combined with genetic drift (Bossdorf et al. 2005; Roman & Darling 2007). Low genetic variation in naturalized plants will limit evolution and restrict the species' progression to the invasion stage (Müller-Schärer et al. 2004) unless the species harbors variation for plasticity (Davidson et al. 2011).

The occurrence of a Mimulus *hybrid in the naturalized region*

While conducting this study, we identified the presence of a heretofore-unknown *Mimulus* hybrid species in eastern North America. Chromosome counts conducted revealed between 44 – 46 chromosomes (Berg *unpubl. data*), greater than the 28 chromosomes typically found in the diploid *M. guttatus* (Vickery 1995) but lower than the 56 chromosomes expected in a tetraploid. The North American *M. guttatus* is known to form mostly-sterile triploid hybrids in the UK with closely related sister taxa from geographically disparate regions, namely the tetraploid South American species *M. luteus* (2n = 4x = 60-62) and *M. cupreus* (2n = 4x = 62) (Stace 2010). Perhaps most notable is the triploid hybrid *M. x robertsii* (2n = 3x = 44-46; Silverside 1990) formed by *M. guttatus* and *M. luteus*, which escaped cultivation in the late 19th century and has established several naturalized populations in the UK (Preston 2002; Vallejo-Marin 2012). The chromosome counts of the NBBR individuals were similar to those found in UK *M. robertsii* individuals (Vallejo-Marin 2012). Also, pollen viability in the greenhouse was low (Berg, personal observation), as might be expected from a triploid

species. However, we cannot infer that the NBBR individuals represent a newly-found population of *M. x robertsii* in North America without further genome-wide analyses. On the east coast of North America, neither *M. cupreus* or *M. luteus* has been recorded. The other yellow-flowered species that has been found is *M. moschatus*, a non-native escape from garden plots (Pennel 1935). *Mimulus moschatus* is a tetraploid (2n = 4x = 32), which makes it a candidate as the second parental taxon with *M. guttatus* that could produce the putative triploid population NBBR. However, the results from chromosome counts revealed higher counts than would be expected from a *M. guttatus x M. moschatus* hybrid, and they have also been reported as being incompatible (Vallejo-Marin 2012). Because most triploid *Mimulus* hybrids in the UK have been found to be largely sterile (Vallejo-Marin 2012; Vallejo-Marin and Lye 2013), the NBBR population may represent a pathway to establishment in the naturalized region directed by uniparental asexual reproduction. Its sterility and the fact that few potential parent *Mimulus* species occur on the east coast of North America to propagate more hybrids means that additional colonization by this hybrid would have to come from emigrants from the present population or future escapees. More research is required to definitively identify this population/species and its ploidy level before we can make accurate assessments concerning its potential to progress from a naturalized population to one that may begin to spread and become invasive on the east coast of North America.

Conclusion

Our study of *M. guttatus* populations from native, naturalized, and invasive regions demonstrates that naturalized populations in eastern North America have low genetic and genotypic variation compared to native populations on the west coast of North America.

It is likely that these two naturalized populations experienced founder effects and rely on uniparental reproduction, asexual reproduction and/or selfing, to persist. A third naturalized population in New Brunswick, Canada was identified as a polyploid *Mimulus* species, and may demonstrate interspecific hybridization as a successful pathway to establishment in remote novel areas. Populations in the invasive region in the UK have similar genetic and genotypic diversity as the native populations, an expected result because of their historical record of multiple introductions. The invasive region may have also served as the source population for the naturalized population, NBS, providing an example of the bridgehead effect. More work is required to determine whether naturalized populations are restricted from becoming invasive because they lack genetic variation, or because they are limited by environmental factors. By continuing to monitor these naturalized populations, we can learn much about the invasion process while controlling their potential spread.

Table 3.1 Location and approximate population size of 11 *Mimulus guttatus* populations from the native region, two from the naturalized region, and three from the invasive region) used for genetic analysis. A third naturalized population included in the genetic and genotypic diversity analyses was a putative polyploid *Mimulus* hybrid population (NBBR).

Native region populations	Code	Latitude	Longitude	Approximate # individuals in population
Seward, AK (1)	AKS1	60.07021	-149.28102	> 1000
Seward, AK (2)	AKS2	60.12142	-149.25660	>200
Anchorage, AK	AKA	63.33945	-148.49102	>1000
Shelton, WA	WA	47.23089	-123.08862	>1000
Oswald State Park, OR	OR06	45.45797	-123.58105	>1000
Cloverdale, OR	OR05	45.14347	-123.58188	>100
Haceta Head, OR	OR04	44.08163	-124.07605	>500
Otter Point, OR	OR03	42.27994	-124.25329	>1000
Humbug Mt. State Park, OR	OR02	42.43072	-124.27879	>500
Bodega Bay, CA	BB1	38.31701	-123.07117	>1000
Point Reyes, CA	PR	37.997989	-122.995067	>1000
Naturalized region				
Bass River, New Brunswick, Canada (polyploid hybrid)	NBBR	46.32904	-65.06621	>500
Fly Creek, NY	FC	42.44391	-74.58212	>1000
Springfield, New Brunswick, Canada	NBS	45.41486	-65.49202	>500
Invasive region				
Brampton, Norfolk, UK	BRA	52.7681	-1.27985	>100
Dunblane, Perthshire, UK	DBL	56.18861	-3.96608	>300
Houghton Lodge, Hampton, UK	HOU	51.09699	-1.5084	>100

Table 3.2 Number of alleles and observed heterozygosity for 16 *M. guttatus* populations and one polyploid *Mimulus* hybrid population (NBBR) at six microsatellite loci (*AAT*) and six intron-based length polymorphism markers (*MgSTS*). Forward primers for the first six markers on the list (AAT217-AAT278) were labeled with FAM dye, and the remaining six markers (MgSTS84-MgSTS685) had forward primers labeled with HEX dye. Number of individuals analyzed per taxon (number of populations): *M. guttatus*: 310 (16); triploid *Mimulus* hybrid population: 26 (1); For each locus, only individuals amplifying for at least one allele were used in calculations of the parameters shown.

Locus	Approx. size range (bp)	*M. guttatus*		polyploid hybrid		*Both taxa*
		Total no. of alleles	H_O	*Total no. of alleles*	H_O	*Total no. of alleles*
AAT217	177-195	6	0.129	4	1.00	10
AAT225	113-127	9	0.103	2	0	11
AAT230	179-210	21	0.252	2	1.00	23
AAT240	94-106	5	0.100	3	1.00	8
AAT267	117-131	4	0.139	2	0	6
AAT278	127-135	4	0.090	3	0.96	7
MgSTS84	209-231	8	0.213	2	0	10
MgSTS234	272-321	6	0.190	2	0	8
MgSTS321	290-303	9	0.313	4	0	13
MgSTS430	238-269	9	0.277	3	0.96	12
MgSTS681	338-366	10	0.332	3	0.92	13
MgSTS685	242-251	9	0.359	2	0	11
Average		8.33	0.223	2.667	0.487	11.0
SD		4.52	0.091	0.778	0.509	4.411
Total		100		32		132

Table 3.3 Measures of genotypic and genetic diversity of 16 *M. guttatus* populations and 1 polyploid *Mimulus* hybrid population (NBBR) sampled from three regions, native (western North America), naturalized (eastern North America), and invasive (United Kingdom). *N* number of sampled individuals, *G* number of multilocus genotypes, *R* expected proportion of multilocus genotypes from total number of sampled individuals using rarefaction to account for sample size, *D* complement of Simpson's Index of diversity , *A* total number of alleles seen over all loci, A' average number of alleles per locus in the population, *H'* average number of alleles per locus per individual, H_O proportion of individuals with heterozygous genotype (averaged over loci), *P* number of private alleles. Pairwise genetic distance based on method developed by Bruvo *et al.* (2004).

Native region										Mean Pairwise difference
Population	*N*	*G*	*R*	*D*	*A*	*A'*	*H'*	H_O	*P*	(±s.d.)
AKS1	18	17	0.97	0.94	25	2.1	1.19	0.20	0	0.164
AKS2	16	16	1.0	0.94	28	2.3	1.24	0.25	2	0.171
AKA	22	20	0.95	0.95	24	2.0	1.18	0.18	1	0.115
WA	20	20	1.0	0.95	36	3.0	1.27	0.28	4	0.209
OR06	27	24	0.94	0.95	24	2.0	1.15	0.17	0	0.109
OR05	12	12	1.0	0.92	22	1.8	1.16	0.16	2	0.167
OR04	15	15	1.0	0.93	29	2.4	1.18	0.17	6	0.171
OR03	13	13	1.0	0.92	31	2.6	1.24	0.24	3	0.202
OR02	28	27	0.98	0.96	30	2.5	1.15	0.16	3	0.146
BB1	17	17	1.0	0.94	33	2.75	1.29	0.30	2	0.186
PR	29	16	0.78	0.92	29	2.4	1.26	0.26	2	0.158

Mean ± SD	19.7	17.9	0.97	0.94	28.27	2.35	1.21	0.22	2.27	0.163
	±6.0	±4.5	±0.07	±0.01	±4.24	±0.34	±0.05	±0.05	±1.74	SE=±0.009
Total Native region	217	197			311				25	

Naturalized region

Population	*N*	*G*	*R*	*D*	*A*	*A'*	*H'*	H_O	*P*	Mean Pairwise difference (±s.d.)
NBBR	26	6	0.28	0.34	32	2.7	1.64	0.49	6	0.012
FC	21	7	0.37	0.48	18	1.5	1.30	0.23	5	0.028
NBS	30	2	0.17	0.44	13	1.1	1.00	0	0	0.019
Mean ± SD (FC&NBS only)	25 ±6.4	4.50 ±3.54	0.27 ±0.14	0.46 ±0.03	15.5 ±3.54	1.30 ±0.28	1.15 ±0.21	0.12 ±0.16	2.50 ±3.54	0.024 SE=±0.00
Total Naturalized region	77	15			63				5	

Invasive region

Population	*N*	*G*	*R*	*D*	*A*	*A'*	*H'*	H_O	*P*	Mean Pairwise difference (±s.d.)
BRA	14	14	1.0	0.93	24	2.0	1.32	0.32	2	0.189
DBL	14	14	1.0	0.93	39	3.25	1.25	0.25	3	0.249
HOU	14	14	1.0	0.93	29	2.4	1.20	0.20	2	0.236

Mean ± SD	14 ±0	14 ±0	1.0 ±0	0.93 ±0	30.67 ±7.64	2.55 ±0.64	1.26 ±0.06	0.26 ±0.06	2.33 ±0.58	0.225 SE=±0.02
Total Invasive region	42	42			92				7	

Table 3.4 Analysis of molecular variance (AMOVA) for 16 *M. guttatus* populations and 1 polyploid *Mimulus* hybrid population, following correction for identical MLGs (clone correction) within each population. Asterisks indicate significant (P < 0.001) structure at a given hierarchical level.

Grouping (16 *M. guttatus* populations)	Source of variation	d.f.	Variance components	Variance (%)
Regions/Populations/Individuals	Among regions	2	1.38*	15.97
	Among populations within regions	13	4.01*	46.33
	Within populations	232	3.26*	37.71
	Total	247	8.65	100.00

Table 3.5 Three separate Mantel tests to test for isolation by distance among a) all 16 *M. guttatus* populations, native, naturalized, and invasive; b) the 11 native populations only; c) the five naturalized and invasive populations only (the polyploid NBBR population was excluded from Mantel tests). Jost's D was used as the genetic distance. A significant correlation (r_m) indicates isolation by distance. An asterisk indicates a significant result at the $P < 0.01$ level.

Mantel test	r_m
a) 16 native and non-native populations	0.33*
b) 11 native populations	0.36*
c) five non-native populations	0.29

Figure 3.1 Cluster plot of DAPC for 248 multilocus genotypes (MLGs) from 16 diploid *M. guttatus* populations. Illustrates the probability of membership for each MLG to 16 genetic clusters identified in the analysis (*k*=16). Populations AKA through PR are from the native region; FC and NBS are from the naturalized region; BRA, DBL, and HOU are from the invasive region. Population codes can be found in Table 4.

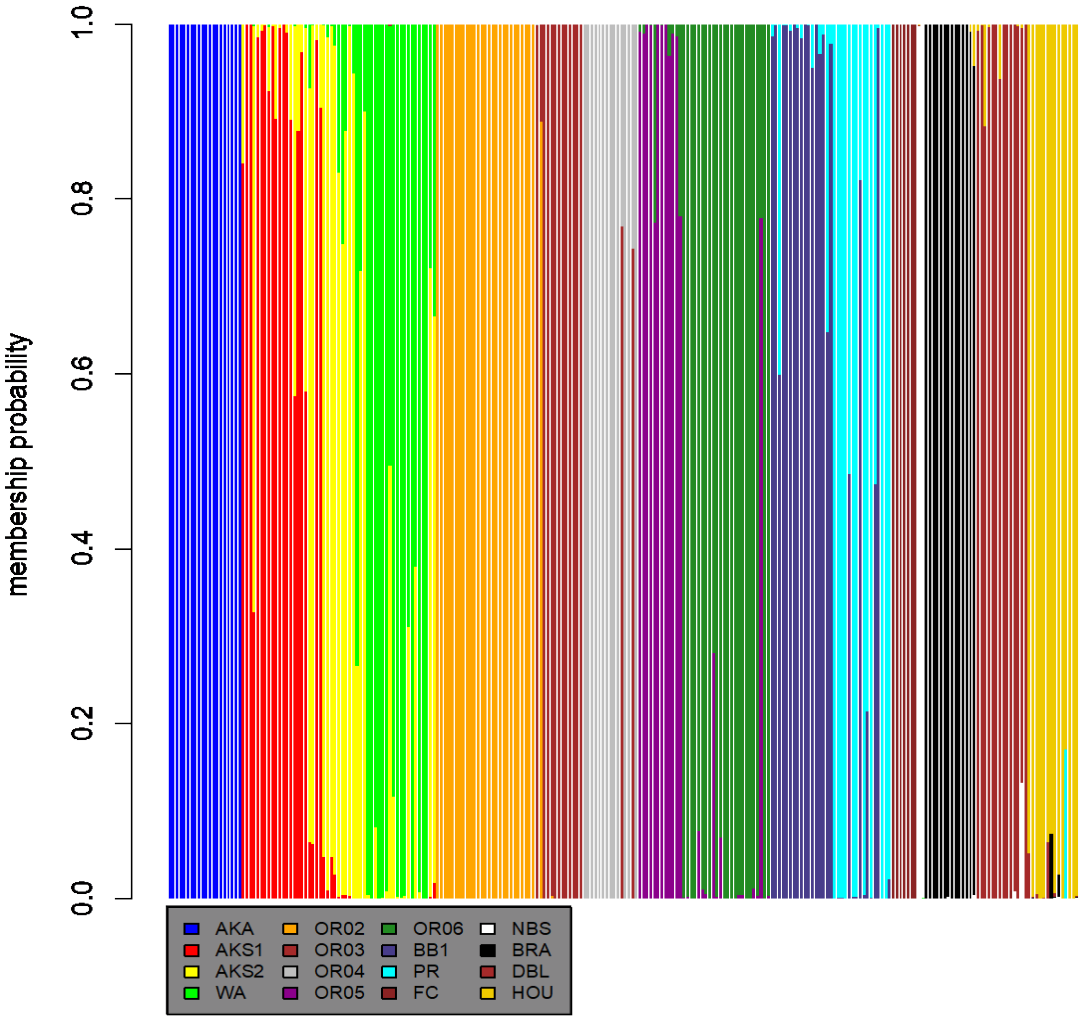


Figure 3.2 Scatterplot of DAPC of the first two principal components discriminating 16 diploid *M. guttatus* populations. Points are 248 sampled multilocus genotypes. Lines and shapes represent population membership. The optimal number of clusters (k) identified in the DAPC analysis was 16, matching the number of sampled populations (11 from the native region, 2 from the naturalized region, and 3 from the invasive region; population codes are found in Table 4).

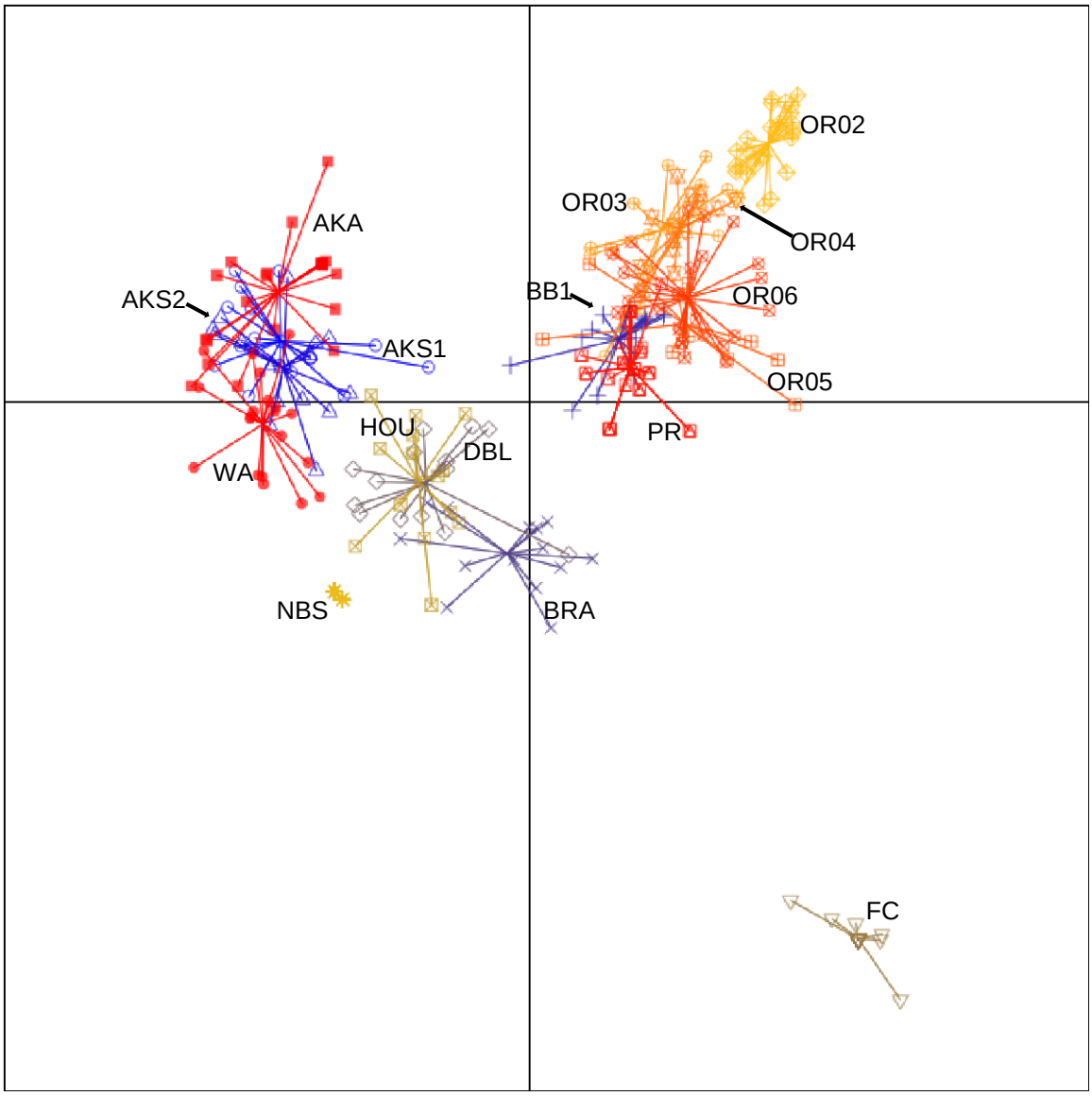
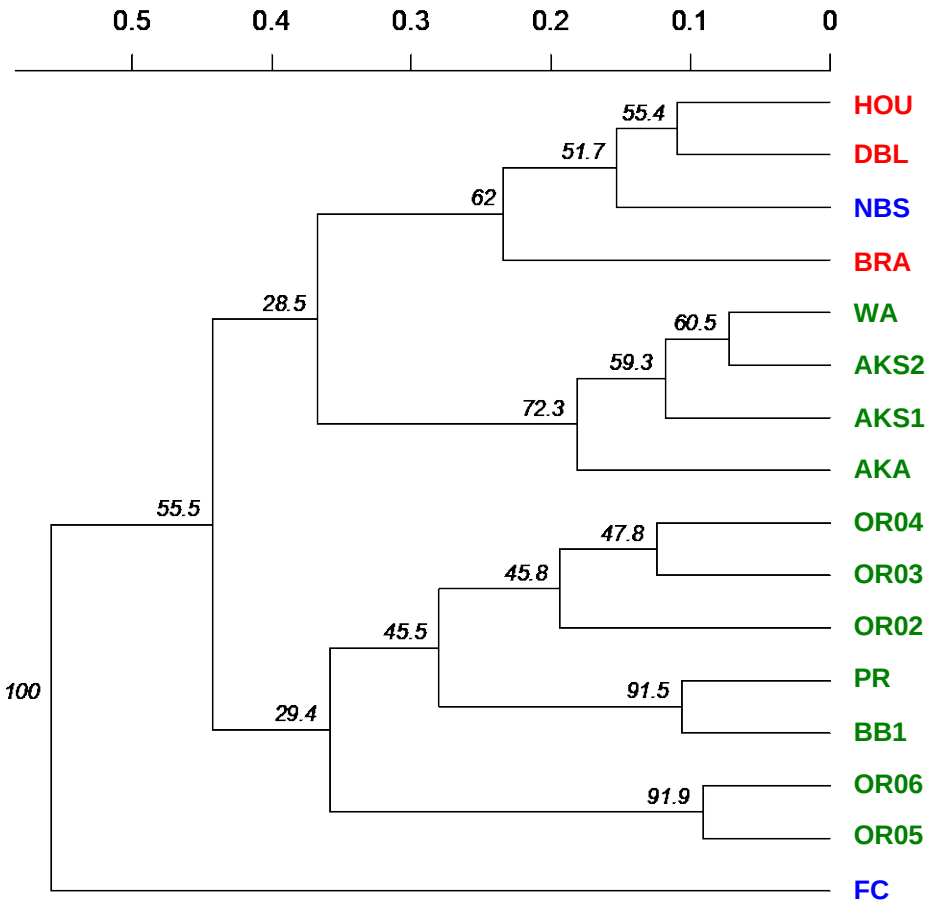


Figure 3.3 Dendrogram based on Nei's genetic distance (Nei 1978) detailing genetic structure among the 16 diploid *M. guttatus* populations. Populations in red are from the invasive region in the UK; blue from the naturalized region on the east coast of North America; and green are native populations from the west coast of North America. Scale bar shows relative genetic difference between populations, and numbers at nodes are bootstrap values for a sampling of 1000 trees.



4 SELFING, HETEROSIS, AND PLASTICITY FOR FITNESS MAY FACILITATE ESTABLISHMENT SUCCESS IN NON-NATIVE POPULATIONS OF COMMON MONKEYFLOWER, *MIMULUS GUTTATUS* [PHRYMACEAE]

Introduction

Establishment and spread of non-native plant species is, while a serious threat to native biodiversity, a relatively rare occurrence. Most introduced individuals likely fail to establish because of factors associated with colonization, such as lack of compatible mates or native pollinators (Williamson and Fitter 1996; Richardson et al. 2000; Lee 2002; Lockwood et al. 2013). Understanding the mechanisms that promote invasion success by a relatively small fraction of introduced plant populations that have a disproportionately large negative affect on natural ecosystems is a primary goal in invasion ecology. Once non-native propagules are transported to a novel region, preadaptation to the recipient location may promote naturalization (i.e. a population that has become established and reproducing without further influx of migrants, but has not become invasive by spreading beyond the point of initial introduction; Richardson et al. 2000) and subsequent invasion throughout the landscape (Schlaepfer et al. 2010; Petitpierre et al. 2012). However, sufficient genetic diversity that allows for evolutionary shifts in morphological traits, and the ability to mitigate the stress of novel environmental pressures also play important roles in populations becoming invasive (Lee 2002; Müller-Schärer et al. 2004). For example, the genetic consequences of a non-native plant population's mating system (Daehler 1999; Bailey & McCauley 2006) or the expression

of phenotypic plasticity in a new location (Sexton et al. 2002; Parker et al. 2003; Murren & Dudash 2012) may affect invasion success.

Self-compatible species may be more successful colonizers following long-distance dispersal compared to obligate outcrossing species, in part because the former would need only one individual to establish a naturalized population (Baker 1955; Stebbins 1957; Pannell & Barrett 1998). Self-compatibility negates any reliance on unrelated mates or the presence of native pollinators and provides reproductive assurance that can be adaptive when colonizing or invading a new landscape (Baker 1967; Kolar & Lodge 2001; van Kleunen et al. 2007). However, a major trade-off that counters the adaptive benefits provided by reproductive assurance through selfing, and a topic requiring more examination in the field of invasion ecology, is inbreeding depression (Verhoeven et al. 2011; Mullarkey et al. 2013). The fitness consequences of inbreeding depression have been attributed to two possible genetic models, both of which are based upon the fact that selfing increases homozygosity by 50% each generation and erodes advantages that may be associated with the heterozygous state (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Roff 2002; Carr & Dudash 2003). The “partial dominance” model states that recessive or partially recessive deleterious mutations (i.e. the genetic load of a population) are expressed at greater frequencies following selfing compared to outcrossing. The “overdominance” model assumes that heterozygotes have greater fitness compared to homozygous individuals and are reduced in the population following repeated generations of selfing. While inbreeding depression is likely the primary selective agent that prohibits evolution of plant populations toward complete selfing (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Byers & Waller 1999;

but see Holsinger 1988), studies have suggested that the effects of inbreeding depression can be mitigated over time as the deleterious recessive alleles responsible for the decrease in fitness are purged from the population through repeated generations of selfing (Hedrick 1994; Dudash & Carr 1998; Byers & Waller 1999). Successful purging of the genetic load would reduce the differences in fitness between selfed and outcrossed progeny, or the magnitude of inbreeding depression, in invasive plant populations.

While selfing may facilitate naturalization and invasion of non-native plants following a single introduction of one or a few individuals, perhaps a more frequent scenario involves multiple introductions of unrelated individuals into the same non-native location (Dlugosch & Parker 2008; Facon et al. 2008). Admixture can occur when individuals from different native or established non-native sites that may vary in habitat type are introduced to a new location, either simultaneously or asynchronously (Ellstrand & Schierenbeck 2000; Rius & Darling 2014). Outcrossing among individuals from disjunctive populations can increase the amount of additive genetic variation in introduced populations. This could provide nascent populations with the evolutionary potential that may help them cope with novel environmental conditions (Lavergne & Molofsky 2007). Outcrossing can introduce dominant alleles that mask potentially deleterious recessives, which may have become fixed in a small, colonizing population (Lynch et al. 1995). Outcrossing can also result in other heterosic effects, such as generating novel genotypes that produce heterozygous progeny with greater relative fitness compared to both parents (Vergeer et al. 2004; Facon et al. 2005: Verhoeven et al. 2011). However, as with the evolutionary trade-offs that can occur from selfing, short-term negative fitness consequences may occur following outcrossing, particularly if the

parental genotypes are locally adapted to dissimilar environments. Outbreeding depression is the reduction in fitness in progeny of individuals locally adapted to different environmental conditions (Lynch 1991; Edmands 1999). Specifically, outbreeding depression results from the expression of alleles that are detrimental to fitness in the new environment, or that are functionally antagonistic when integrated within a genetic background that it has not evolved in (Frankham et al. 2011). Also, recombination following outcrossing between individuals that are locally adapted to dissimilar environments can break up co-adapted gene complexes and result in outbreeding depression (Fenster et al. 1997; Fenster & Galloway 2000). It has been suggested, however, that populations can recover from outbreeding depression over several generations (Fenster & Galloway 2000; Edmands & Timmerman 2003).

While the mating system of non-native plants certainly plays a role in successful naturalization and invasion, phenotypic plasticity (i.e. a single genotype's ability to respond to different environments through alterations in phenotype; Schlicting 1986; Pigliucci & Hayden 2001) can also influence a colonizing population's response to novel selection pressures (Dudash et al. 2005; Godoy et al. 2011). Whether invasive species express greater plasticity compared to native species or naturalized populations that have not become invasive is a controversial topic and requires more investigation. For example, one recent meta-analysis concluded that invasive species are indeed more plastic compared to native species (Davidson et al. 2011), while another found that the expression of plasticity was similar between natives and non-native congeners (Palacio-López & Gianoli 2011). A hypothesis often used to consider phenotypic plasticity in the context of plant invasions states that populations in invasive regions evolve greater

plasticity compared to populations in the native region or in non-native regions where the species has become naturalized but not invasive (Parker et al. 2003; Richards et al. 2006). This implies that there is genetic variation for plasticity in species, and that plastic genotypes have a fitness advantage in non-native environments. Rapid evolutionary change in other traits has been documented for invasive species (Sakai et al. 2001; Lee 2002; Bossdorf et al. 2005). Rapid evolution in plasticity thus could facilitate invasion in non-native plant populations.

When considering plasticity for fitness traits, a flat reaction norm (i.e. a graph that illustrates a change in phenotype or fitness across environments) may be the best-case scenario for an invasive population ("fitness homeostasis"; Rejmánek 2000; Richards et al. 2006). Richards et al. (2006) deemed this the "Jack-of-all-trades" scenario, which infers that genotypes in the invasive region are more robust across a wide breadth of environments compared to native genotypes (Fig. 7a). This idea of invasive plant species' harboring more robust genotypes and plasticity for fitness has been around for decades, with Baker (1967) referring to it as the "general-purpose genotype" often seen in weeds. Alternatives to the Jack-of-all-trades scenario include the "Master-of-none" scenario (Fig. 7b), which occurs if invasive populations demonstrate greater fitness in favorable conditions compared to natives, and the "Jack-and-Master", which combines the previous two situations and describes a super-invader capable of robust fitness across environments while being opportunistic when the environment is favorable (Fig.7c).

The relationship between selfing, outcrossing, and plasticity has rarely been studied (but see Murren & Dudash 2012). One hypothesis focused on the relationship between inbreeding and plasticity suggests that plants with low levels of heterozygosity

experience developmental instability; therefore, inbred individuals would express greater plasticity compared to outcrossed, more heterozygous individuals (Pederson 1968). There has been little empirical support for the developmental instability hypothesis, but Murren & Dudash (2012) did find some evidence that showed inbred *Mimulus guttatus* individuals demonstrated greater plasticity for stem diameter compared to outcrossed individuals.

In this study, we investigated the independent and joint influences of mating system and plasticity on several floral and vegetative traits associated with fitness on 13 native and non-native populations of the ecological-genetic model species, *Mimulus guttatus* [Phrymaceae], as well as one non-native *Mimulus* population comprised of polyploid individuals. Our goals were to determine whether non-native plants suffered less from inbreeding depression and experienced heterosis when crossed with plants from two different habitat types. We also examined whether non-native plants were able to maintain greater fitness (i.e. express plasticity for fitness traits) when exposed to a stressful, experimental saline watering treatment compared to plants from the native regions. The salinity treatment aimed to mimic conditions found in native populations of *M. guttatus* from the Pacific coast of North America that are constantly exposed to sea spray. We specifically addressed the following questions: 1) Do *M. guttatus* individuals suffer inbreeding depression in traits associated with fitness, and if so, are plants from the non-native regions (i.e. naturalized and invasive) affected for fewer traits than native populations? 2) Do progeny originating from native and non-native individuals experience heterosis (or alternatively, outbreeding depression, which is indicative of local adaptation in the parental populations) following crosses with mates from similar and

dissimilar habitats? 3) Do plants from the non-native regions have a greater ability to express plasticity by maintaining fitness between two watering treatments compared to native populations? Are inbred plants more likely to maintain fitness through plasticity compared to outcrossed plants, thereby supporting the developmental instability hypothesis? 4) Do measurements of traits associated with high selfing rates in *Mimulus* species, namely small flower size and short distances between the anthers and stigma (Ritland & Ritland 1989; Karron et al. 1997), differ between plants from the native, naturalized, and invasive regions?

Methods

Study species

Mimulus guttatus [Phrymaceae] is an herbaceous species that occurs across a native range that spans the west coast of North America, from Mexico to Alaska (e.g., Dudash et al. 1997; Lowry et al. 2008; Wu et al. 2007). *Mimulus guttatus* is an extremely variable species with respect to morphological characteristics, ranging from 0.1 to 1 m in height. Outcrossing rates vary extensively among native populations (Vickery 1974; Ritland 1990; Carr & Fenster 1994). *Mimulus guttatus* is pollinated by hoverflies and bees, but is also self-compatible and able to reproduce asexually via stolons (Murren & Dudash 2012; van Kleunen et al. 2015). Life history is dependent on water availability (Lowry et al. 2008). In sites where water in the local environment dries up in the spring, the plant acts as an annual, while perennial populations are found in areas that remain inundated throughout the growing season. Native perennial populations, the focus of this study, are able to colonize different habitats, including coastal bluffs along the Pacific Ocean, roadside seeps, or riparian habitat along the banks of freshwater streams (Hall & Willis

2006; Lowry et al. 2008). Coastal bluff populations grow in conditions of constant humidity and have an adaptive tolerance for soil salinity, while inland riparian/seep populations are not exposed to a salty environment (Murren et al. 2006; Lowry et al. 2008; Lowry et al. 2009). Because *M. guttatus* harbors genetic variability for tolerance to soil salinity, this environmental factor formed the basis for our between-population outcrosses and exploration into phenotypic plasticity, described below.

In the United Kingdom (UK), *M. guttatus* is considered a harmful invasive that was intentionally introduced as a horticultural species approximately 200 years ago (Truscott et al. 2006; van Kleunen & Fischer 2008). Recently, naturalized populations in New York state and New Brunswick, Canada, received attention (Murren et al. 2009). Little is known of the introduction history of these naturalized populations; however outcrossing rates in the naturalized populations are low compared to those found in native and invasive populations (Berg doctoral dissertation). While native populations can be found in either coastal bluff or riparian/seep habitats, non-native populations in the invasive and naturalized regions are perennials found in the latter habitats (Truscott et al. 2006; Vallejo-Marin *pers. comm.).*

We have also included in our study a population that was initially thought to consist of diploid *M. guttatus* individuals like the other native, naturalized, and invasive populations. This population, NBBR, is one of two populations we sampled in New Brunswick, Canada (NBS is the other). However, we observed differences in morphology between the NBBR individuals and those from other populations in the greenhouse. Also, seed and pollen produced from between-population outcrosses with NBBR individuals were often not viable, to the point where no second-generation seed was produced.

Ancillary tests, including genotyping polymorphic microsatellites designed for *Mimulus* species and chromosome counting, aimed at determining the identity of the NBBR individuals, revealed their polyploid nature and a chromosome number of 48-52 (Berg unpublished data). It has been shown that tetraploid *M. guttatus* individuals are present in its native range ($2n = 56$) at modest frequencies (7/76 populations surveyed; Vickery et al. 1968), and have recently been found in the invasive range in the UK (Simón-Porcar et al. 2017). Therefore, this population was included in the study with the caveat that the taxonomy of its individuals has not specifically been determined.

Field collected seed and hand-pollinations

In 2012 and 2013, seed was collected from eight native populations on the west coast of North America, three naturalized populations on the east coast of North America, and three populations in the invasive range in the United Kingdom (Table 9). The sampled native populations represent a wide swath of the native distribution, including one population in California, three populations in Oregon, one population in Washington, and three populations in Alaska; the three naturalized populations included one from New York and two from New Brunswick, Canada; and the three populations from the invasive range were from the United Kingdom. One of the naturalized populations (NBBR) from New Brunswick, Canada was later determined to consist of polyploid individuals and was treated as a separate region in each analysis (see below). All populations were perennial, and seeds were haphazardly collected from between 20 and 31 maternal families located $\geq$ 1 m apart. Four native populations represented a coastal bluff habitat, while the other

four native populations were from riparian/ seep habitats. The six populations from the naturalized and invasive regions were from riparian/seep habitats.

To produce two generations of seed that were the result of selfing and three different levels of outcrossing, we sowed field-collected seed in January 2014. Seeds from 20-31 maternal families for each of the 14 populations were sown in 2.5" pots filled with Sunshine® LC1 potting mix (SunGro Horticulture®, Agawam, MA), and pots were placed in trays that were bottom watered every two days so that soil was constantly saturated. Trays with pots were placed in a growth chamber set at 4°C in constant darkness for a one week cold stratification period, then brought into the University of Maryland (UMD) greenhouse. During germination in the pollinator-free greenhouse, natural light was used and temperature was set at 4°C at night and 17°C during the day (Vickery 1983). Following germination, two seedlings per maternal family from each of the 14 populations were transplanted into their own pots. After flowering had commenced in February 2014, we conducted within-population outcrosses (hand-pollination between two randomly chosen individuals from the same population) to control for any maternal effects that might confound examination of the morphological characteristics of interest. When fruits ripened in March 2014, seed from these within-population crosses was collected in paper coin envelopes and stored at 4°C. In late August 2014, seed generated from the within-population crosses from at least 15 maternal families/population was sown using the same method described above, with the aim of having five maternal families per population for use in the final fitness assessment. Following transplanting of seedlings and subsequent flowering of plants derived from the within-population outcrossing, hand-pollinations were conducted to

produce the first generation of four cross -types: (a) self pollination; (b) within-population outcrosses (WI); (c) between-population outcrosses, parents from similar habitat (BSIM; pollination from randomly chosen flowering individual from a different population that inhabited a similar habitat, e.g. coastal bluff x coastal bluff); (d) between-population outcrosses, dissimilar habitat (BDIS; pollinations from randomly chosen flowering individual from a different population that inhabited a dissimilar habitat, e.g. coastal bluff x riparian/seep). NBBR polyploid individuals were not used as sires in the crossing program, due to low pollen viability (pollen remained green and moist, opposed to the mature, viable pollen that was characterized by a bright yellow color and a dry, "dusty" quality). The six naturalized and invasive populations represented riparian/seep habitat only. Thus, to conduct between-population outcrosses with a population from dissimilar habitats we used a randomly chosen native population from a coastal bluff habitat. Otherwise, sires for between-population outcrosses were chosen at random and independent of region.

To produce the first generation of each of the four cross-types, four flowers on each of the 15 maternal plants per population were randomly chosen to receive pollen. Each of these four flowers was then randomly designated as one of the four cross-types. For the flower chosen for self-pollination, anthers were separated from the flower and pollen collected on a clean microscope slide (soaked in ethyl alcohol solution and dried between pollinations). Pollen was then applied to the stigma of the same flower, and a successful pollination was determined by watching the lobes of the stigma remain closed for five minutes (Beardsley & Olmstead 2002; Dudash pers. obs.; Mario Vallejo-Marin & Jannice Friedman, pers. comm.). For within-population crosses, the siring individual (and flower

on the sire) was randomly chosen from the same population as the maternal plant, and pollen applied using the same method described. For the flowers representing the two between-population crosses, the sire was randomly chosen from all possible populations that represented the appropriate habitat type; for example, to conduct a between-population, dissimilar habitat cross for a maternal plant from a seep/riparian habitat, a sire was randomly chosen from one of the four coastal bluff populations.

Seed was collected from the first generation of crosses in November 2014. Some of this seed would be used in the final fitness assessment in spring 2015 and was stored in coin envelopes at 4°C, while the rest was sown immediately after collection to produce a second generation of each cross type. First generation selfed seed was grown up and selfed a second time; first generation within-population crossed seed was grown and the individual was crossed with a randomly chosen individual from the same population (not necessarily the same maternal family that acted as sire for the first cross); first generation between-population crossed seed, both similar and dissimilar habitat crosses, was grown and crossed with a randomly chosen sire from the same population that provided the sire for the first generation cross, but not necessarily the same maternal family. Seed collection for the second generation of crosses was completed in February 2015.

Final fitness assessment

In late February 2015, seed that was generated for the first and second generation of crosses was sown and cold stratified in the dark for one week at 4°C, and pots were brought into the greenhouse to germinate under natural light and a constant temperature of 21°C. An exact number of seeds were sown in each pot (40 seeds/pot) so that

germination rates could be assessed in March 2015. Once five or more seedlings germinated from a single maternal family for each cross-type x generation combination, individual seedlings were randomly chosen and transplanted into single pots. The exception was a naturalized population from New Brunswick, Canada (NBBR). The NBBR population produced very few second-generation seeds for any of the four cross-types, and differences in morphology compared to the other *M. guttatus* populations observed in the greenhouse warranted tangential work to examine the taxonomy of NBBR individuals. After further evaluation, including chromosome counting and genotyping (Berg doctoral dissertation), the NBBR population was determined to consist of polyploid individuals ($2n = 48 – 52$). For that reason, NBBR was treated as a fourth region in statistical analyses, and only the first-generation crosses were used due to low viability of second-generation seed for this population. For all populations, five maternal families from each population were randomly chosen to be included in the final fitness assessment (13 populations x 5 maternal families x 4 cross-types x 2 generations x 2 salinity treatments x 5 replicates = 5200, plus 1 population (NBBR) x 5 maternal families x 4 cross-types x 1 generation x 2 salinity treatments x 5 reps = 200) for a total of 5400 individuals.

Once the individuals included in the final fitness assessment were randomly chosen, the 5400 seedlings were transplanted into individual pots containing a 2:1 ratio of vermiculite to perlite potting mix following the advice of the UMD greenhouse staff. This inorganic substrate was chosen in place of the organic potting mix used previously so that plants would be exposed to the control treatment of water and fertilizer, and to the saline treatment of water, fertilizer, and saline solution (50 mM; see below) without the

potential buffering effect of organic material. Throughout the final fitness experiment, plants were grown under natural light. Prior to adding the fertilizer and saline treatments, bottom trays were filled with water only for one week as an acclimatization period before adding fertilizer and salt treatment. Following transplanting, the five replicates representing each individual cross (population x maternal family x cross type x generation x treatment) were assigned randomly to one of five benches in the UMD greenhouse, each bench representing a block in the statistical analyses. Each bench held 34 randomly assigned trays, 17 of which were control treatments and 17 were salinity treatments, with each tray holding approximately 32 pots.

After the acclimatization period of water only, the watering regimes representing experimental treatments were begun (March 27, 2015). We followed the fertilizer protocol from Lowry et al. (2009) and added 6 L of a fertilizer solution (Nutriculture®, Plant Marvel Laboratories, Inc., Chicago Heights, IL) consisting of a nitrogen concentration of 105 ppm to each control tray. For trays requiring the saline treatment, we used the same fertilizer treatment as control trays and added 17.5 mL of 1 M NaCl solution to get a 50 mM saline treatment concentration. This concentration is much less than that found in undiluted ocean water (~500 mM). However, we were interested in simulating groundwater conditions rather than direct contact with concentrated seawater in the form of salt spray. We chose a 50 mM saline concentration based on a pilot study in which several randomly chosen native *M. guttatus* individuals (n = 36) from both riparian/seep and coastal habitats were exposed to a range of saline concentrations, from 0 to 70 mM. Most plant species show the effects of saline stress through reductions in shoot growth and leaf expansion at concentrations above 40mM (Munns & Tester 2008).

Results of the pilot study showed that at concentrations of 50 mM and above, new leaf growth rate was significantly different compared to a control treatment with no saline (Appendix A). Based on these results, we chose a 50 mM saline solution to examine whether plants originating from the invasive region, which are all riparian/seep populations, express greater plasticity compared to plants originating from the native and naturalized regions. We also examined the NBBR population comprised of polyploid individuals for both control and salinity treatments.

Control and saline treatments were recharged every four days by emptying the bottom trays and replacing them with new solution. Plants began flowering on April 2nd, 2015 and we began taking floral measurements that would continue for the duration of the experiment. The experiment concluded when 45% of plants had flowered, which occurred on May 1st, 2015.

Fitness traits

To assess fitness in selfed and outcrossed progeny from the four regions, we pooled plants grown in the control and saline treatments and measured morphological traits. (see Analyses section below for motivation to pool data for analysis of inbreeding depression and heterosis/outbreeding depression).

We measured seven vegetative and floral traits associated with fitness: germination rate, aboveground biomass, plant height, stem diameter, number of stolons, number of reproductive units ("ru's", included flower buds, flowers, and fruits), and probability of flowering. We measured germination rates by sowing 40 seeds per pot and counting seedlings in the 540 pots (each pot held a unique population x maternal family x cross

type x generation combination) one week after observing first germination (first week March 2015). Just before plants were harvested and placed in bags to be dried and weighed for aboveground biomass, each plant's height and stem diameter (measured with calipers at the second node) was measured, and stolons and ru's counted. Aboveground biomass was measured following harvest of all plants, which occurred from May 1st to May 8th, 2015. Stems were cut at soil level and just above any stolons that may have been present. Loose soil was removed and plants were placed in labeled brown paper bags and immediately placed in drying ovens on the UMD campus at 50°C for at least 13 days. Dried plants were weighed in summer 2015.

We also assessed two floral traits associated with mating system in *Mimulus* species, floral size and stigma/anther distance, from the day the first plant flowered until one day before the harvesting of plants began. We monitored all plants closely during this period, and collected these measurements when an individual flowered for the first time. To measure floral size, we calculated a floral shape index that was simply the product of a flower's length and width. Stigma/anther distance was measured using calipers to measure the length in millimeters between the top of the tallest anther and the top of the stigma. The stigma/anther distance and floral size was measured for one or two flowers per plant.

Analyses

All analyses were conducted in R version 3.3.1 (R Foundation for Statistical Computing, 2016). We first tested for an effect of generation to determine whether the measures of each trait differed between the first and second generation. We ran a preliminary analysis

with plants from the control treatment and the saline treatment pooled (i.e. all plants included in the study irrespective of the watering treatment they were grown in), and a model that included the fixed effects of generation, cross type, and their interaction on each fitness variable separately. As random variables, we included block and maternal family nested in population:

$$Y \sim \text{generation*cross type} + (1|\text{block}) + (1|\text{population:maternal family})$$

We found no significant effect of generation on any of the traits (Berg unpublished data). We then ran subsequent analyses on plants grown in the control and saline treatments separately, and used the same model as before. Again, we found no effect of generation on traits in either treatment, so first and second generation plants were pooled for all subsequent analyses.

For all analyses of inbreeding depression, heterosis, floral size, and stigma/anther distance, plants grown in the control and saline treatments were pooled. The decision to pool data from the control and salinity treatments was made to maximize statistical power in detecting inbreeding depression, heterosis/outbreeding depression in the progeny originating from native, naturalized, invasive, and NBBR regions. We did, however, run preliminary analyses to examine data from the control and saline treatments separately. With a few exceptions, patterns of inbreeding depression and heterosis in each watering treatment resembled those found in analysis of pooled data (Supplemental Table 1).

Several of the response variables examined in this study had a high likelihood of being correlated (e.g. aboveground biomass vs. plant height), so we calculated correlation coefficients to quantify interdependence between each pair of response variables (Supplementary Table 2).

Regional level analysis of fitness traits among cross types to test for inbreeding depression and heterosis

To test whether maternal region and cross type affected measurements of seven fitness traits, we used (generalized) linear mixed-effects models and REML analysis of variance. These models were implemented in the '*lmer'* function (for normally distributed response variables) or '*glmer*' function (for response variables characterized by the Poisson or binomial distributions) found in the '*lme4*' package. As fixed terms, we included the maternal region, cross type (selfed, within-population cross, WI, outcross between populations from similar habitat, BSIM, and outcross between populations from dissimilar habitat, BDIS), and their interaction. As random terms, we included block and maternal family nested within population:

$$Y \sim \text{region*cross type} + (1|\text{block}) + (1|\text{population:maternal family})$$

The exception to this was the "germination rate" response variable, which did not include the random "block" variable because pots occupied a relatively small space on one greenhouse bench (each pot contained a seedling representing a unique population x maternal family x cross type x generation combination). We used a binomial error distribution for germination rate and for probability of flowering, and a Poisson error distribution for number of stolons and number of reproductive units (Table 10). Because the '*lme4*' package does not include the degrees of freedom for random variables in the model, we used the '*lmerTest*' package to adjust degrees of freedom in the denominator of each model and then re-calculate the *P*-value before reporting in the text.

For each response variable, we tested the significance of the interaction term, maternal region x cross type, by first removing the interaction and then comparing models using a likelihood-ratio test. In instances where differences between the models with and without the interaction term were not significant, we reported the Chi-square value for main effects from the model that omitted the interaction term (see Table 10 caption). Next, we performed Tukey's HSD contrasts to separately test for differences between maternal regions and cross types within regions for each fitness trait using the '*glht*' function in the '*multcomp*' package to perform *z*-tests. A significant signal for inbreeding depression within a region was detected when measures of the selfing cross type were significantly lower compared to WI crosses; heterosis was detected when either BSIM and/or BDIS crosses were significantly greater than WI crosses; and outbreeding depression was detected when WI crosses were significantly greater than BSIM or BDIS crosses.

To quantify the proportion of variance explained by the fixed and random effects in each model, we calculated the conditional R^2 . This method of reporting the proportion of explained variance in mixed models is described in Nakagawa & Schielzeth (2013), and values were calculated in the '*MuMIn*' package using the function '*r.squaredGLMM*'. Means for each region x cross type combination are illustrated in Fig. 8 and reported in Supplemental Table 3. Variance components from the model are reported in Supplemental Table 4.

Phenotypic plasticity and maintenance of fitness in response to saline treatments among regions and cross types

We assessed phenotypic plasticity for six fitness traits with the specific aim of determining whether *M. guttatus* progeny originating from the non-native regions demonstrated greater relative fitness in response to an optimal watering regime with fertilizer (the control treatment) compared to progeny originating from the native regions. We were also interested in whether progeny originating from the non-native regions would maintain fitness across both watering regimes (control vs. increased salinity treatment) more consistently than progeny from the native region. To address the developmental instability hypothesis, which states that inbred progeny should be more plastic compared to progeny produced by outcrossing, we also examined the effect of cross type on any plastic response to the watering regime treatment.

We first used generalized linear mixed-effects models and REML analysis of variance in the package '*lme4*' to model the fixed effects of maternal region, treatment (control treatment consisting of optimal fertilizer and water regime, and a stressful watering regime that was similar to the control treatment but for the addition of 17.5 mL of saline), and their interaction. The random terms in the model were block and maternal family nested within population (Table 11). As in the GLMM analyses for inbreeding depression and heterosis, we used '*lmerTest*' package to adjust degrees of freedom in the denominator of each model and then re-calculate the *P*-value before reporting in the text. Next, we made pairwise comparisons between regions for each fitness trait and each treatment by performing Tukey's HSD contrasts (Table 12). Specifically, this allowed us to determine whether one region had greater fitness compared to another for each treatment separately, and provides tests of significance between regions and watering treatments that correspond to the reaction norms (Fig. 9). To investigate the

developmental instability hypothesis and determine whether selfed plants expressed greater plasticity for fitness compared to the three other cross types, we ran a model that included watering treatment, cross type, and their interaction as the fixed effects, and block and maternal family nested within population as random effects (Table 13). When the effect of the treatment x cross type interaction was significant, we examined how the cross types behaved differently in response to watering treatments and specifically whether selfed individuals were able to maintain fitness (i.e. no difference between treatments for selfed individuals) while other cross types were not.

Regional level analysis floral size and stigma/anther distance

To test for differences among regions for two floral traits associated with mating system in the *M. guttatus* species complex (Fishman et al. 2002), floral size and stigma/anther distance, we used linear mixed-effects models and REML analysis of variance implemented in the '*lmer*' function found in the '*lme4*' package in R. As the fixed term, we included the region. As random terms, we included block and maternal family nested within population. Cross types and watering regime treatments were pooled for these analyses.

Results

Correlations between fitness traits

There was a significant correlation between each pair of fitness response variables at the $P < 0.001$ level (with the exception of the comparison between ru's and stolons, which were positively correlated $P < 0.002$; Supplementary Table 2). There were particularly strong associations between aboveground biomass and both stem diameter ($r = 0.47$) and

plant height (r = 0.42). All correlations were positive except for that between plant height and stolons, which showed a negative relationship between variables (r = -0.06).

Inbreeding depression and heterosis/outbreeding depression

Germination rate

Progeny originating from the native region were the only plants to suffer significant inbreeding depression with regard to germination, and progeny from the three *M. guttatus* regions (excluding the NBBR population) experienced heterosis, either following BSIM or BDIS outcrosses (Table 10). Contrastingly, polyploid progeny from NBBR population did not experience heterosis. The germination rate was significantly lower in the polyploid NBBR population (12.5%) compared to the native, naturalized, or invasive regions comprised of diploid *M. guttatus* individuals, which had similar germination success (63%, 55%, 62%, respectively; Fig. 8A). This can be attributed to the nearly complete failure of second-generation NBBR seeds to germinate.

Probability of flowering

There was no evidence of inbreeding depression regarding probability of flowering and only the progeny from the native region produced by BSIM outcrossing experienced significant heterosis for this trait (Table 10). Progeny from the naturalized region had the highest percentage of plants that flowered overall (65%), followed by progeny from the invasive region, native region, and NBBR population (51%, 40%, 23%, respectively; Fig. 8B).

Stem diameter

Progeny from the native region were the only individuals to experience inbreeding depression for stem diameter, and progeny from the naturalized region were alone in experiencing heterosis (Table 10). Progeny from the invasive region produced the largest stems overall (5.95 mm), while progeny from the native region produced significantly smaller stems than other regions (4.17 mm; Fig. 8C).

Plant height

Progeny from the native and invasive regions experienced inbreeding depression with respect to plant height; there were no examples of heterosis for either the BSIM or BDIS outcross types with regard to plant height (Table 10). Progeny from the naturalized and invasive regions grew taller compared to native and NBBR progeny (35.18 mm, 33.83 mm, 26.12 mm, 25.56 mm, respectively; Fig. 8D).

Number of stolons

With respect to the number of stolons produced, native progeny experienced inbreeding depression. However, these individuals also demonstrated heterosis following BDIS outcrosses, indicating a lack of local adaptation for this trait (Table 10). While the effect of maternal region was significant overall, we only found one marginally significant pairwise contrast in a post hoc test; the NBBR progeny had more stolons on average (3.51 stolons) compared to native region (3.03 stolons; Tukey's HSD, $P = 0.09$, Table 10 & Fig. 8E).

The number of ru's

We found no evidence of inbreeding depression with respect to the average number of reproductive units per plant ("ru's", includes flower buds, flowers, and fruits), and the only case of significant heterosis was found in invasive progeny produced from BDIS outcrossing (Table 10). Progeny from the naturalized and invasive regions producing significantly more ru's overall (9.63 ru's & 8.27 ru's, respectively) compared to the progeny from the native region and the NBBR population (5.32 ru's & 2.85 ru's, respectively; Fig. 8F).

Aboveground biomass

Progeny from the native and invasive regions experienced inbreeding depression with regard to aboveground biomass. Native progeny also demonstrated significant heterosis following both types of outcross, BSIM and BDIS, while invasive progeny experienced heterosis following BDIS outcrossing only (Table 10). Progeny originating from the invasive and naturalized regions produced significantly more biomass overall (2.72 g & 2.09 g, respectively) than the progeny from the native region and the NBBR population (1.66 g & 1.75 g, respectively; the naturalized vs. NBBR was significant at the $P < 0.1$ level, while all other significant contrasts in the analysis were at the $P < 0.001$ level; Fig. 8G).

Plasticity for fitness in a stressful saline treatment and test of developmental instability hypothesis

Flowering probability

The probability of flowering was significantly greater in the naturalized and invasive plants compared to the native and NBBR plants in both watering treatments, the control with no saline and the 50 mM saline treatment (Table 12 & Fig. 9A). Progeny from the native and invasive regions, as well as the NBBR population, were able to maintain their ability to flower in the increased salinity treatment relative to the control treatment. Interestingly, a greater percentage of naturalized plants flowered in the saline treatment compared to the control treatment. For all four regions, each of the four cross types maintained a similar flowering probability in both treatments (Table 13, no significant effect of treatment for each cross type in the four regions, $P > 0.05$).

Stem diameter

Progeny originating from the invasive region had a significantly larger stem diameter on average among all regions for both treatments (Table 12), but invasive plants grown in the salinity treatment had significantly smaller stem diameters compared to those grown in the control treatment (Table 13; Fig. 9B). In contrast, progeny from the native, naturalized, and NBBR regions had smaller stems than invasive plants but were able to maintain stem diameter size across the two treatments. Although the general effect of saline on invasive progeny was negative regarding stem diameter, when we examined the effect of watering treatment on each cross type separately, we found that selfed plants were able to maintain stem diameters across the two treatments (Table 13; no significant effect of treatment on selfed plants).

Plant height

Progeny originating from the invasive and naturalized regions had greater height in both treatments compared to plants from the native region and NBBR population (Table 12; Fig. 9C). Plants from each of the four regions were significantly shorter when grown in the saline solution compared to plants grown in the control treatment; generally, plant height was also reduced by the salinity treatment in all four cross types, regardless of region (Table 13).

Number of stolons

When the number of stolons was analyzed for each watering regime separately, the only significant difference was that progeny from the NBBR population grown in the control treatment produced more stolons than progeny from the native and invasive regions also grown in the control treatment (Table 12 & Fig. 9D). Progeny from the native and invasive regions, and from the NBBR population, maintained the number of stolons produced across both watering regime treatments; however, progeny from the naturalized region increased stolon production in the saline treatment, and this increase was driven by progeny produced by outcrossing with BSIM plants (Table 13; significant effect of treatment on BSIM cross type in the naturalized region).

Number of ru's

Progeny from the invasive and naturalized regions produced significantly more ru's compared to the native and NBBR plants in both watering regime treatments (Table 12 & Fig. 9E). While naturalized plants were able to maintain the number of ru's produced across both treatments, plants from the invasive region grown in the salinity treatment

actually produced more ru's compared to those grown in the control treatment (Table 13). This increase was seen in all four cross types in the invasive progeny.

Aboveground biomass

Progeny originating from the invasive region had greater aboveground biomass compared to all other regions, and this occurred in both the control and salinity treatments (Table 12 & 14; Fig. 9F). Naturalized progeny also produced more biomass than native progeny in both treatments. While plants from the naturalized region and the NBBR population maintained biomass production across both treatments, plants from the invasive and native regions showed a significant reduction in biomass in the saline treatment compared to the control treatment (Table 13). Interestingly, the reduction in biomass in native and invasive progeny grown in saline was driven by plants produced by outcrossing (WI, BSIM, & BDIS); selfed plants from both regions maintained biomass production across both treatments (Table 13).

Differences in expression of plasticity among cross types (developmental instability hypothesis)

Given our experimental design, there were a total of 24 opportunities to detect a difference in plasticity between selfed and outcrossed progeny between the two watering treatments, within a given region (six fitness traits x four regions; Table 13). We found only three instances of greater plasticity for fitness expressed by selfed compared to outcrossed progeny. Specifically, we found that selfed progeny from the native region maintained fitness between the two watering treatments for aboveground biomass while

the three outcross types (WI, BSIM, and BDIS) showed significantly less biomass in the salinity treatment; selfed progeny from the NBBR population maintained fitness for plant height while outcrossed progeny showed a decrease in plant height in the salinity treatment; and selfed progeny from the invasive region were the only cross type to maintain fitness for stem diameter (Table 13). In contrast, we found 15 instances where the selfed and the three types of outcross progeny within a region behaved in a similar manner in response to the salinity treatment, either by maintaining fitness or by showing a significant response to the treatment (Table 13). Lastly, there were just two instances where one or more of the three outcross types maintained fitness between the two watering treatments while selfed progeny were significantly affected by the salinity treatment (stem diameter, native WI and BDIS outcross progeny maintained fitness while selfed and BSIM progeny were significantly affected by the treatment; plant height, BSIM outcross progeny maintained fitness while the other three cross types were affected).

Differences in stigma/anther distance and floral size among maternal regions

Among the *M. guttatus* native, naturalized, and invasive regions, plants from the invasive region had larger flowers compared to native and naturalized plants, but the stigma/anther distance was similar among the three regions (Fig. 8A & 8B). Progeny produced from the NBBR polyploid *Mimulus* population had significantly greater floral size and stigma/anther distance than progeny from all three *M. guttatus* regions.

Discussion

The ability to self-fertilize without drastically reducing fitness, to experience heterosis following outcrossing with unrelated individuals, and to express adaptive plasticity under maladaptive conditions have all been suggested as mechanisms that may facilitate establishment and invasion by non-native plants (Baker 1967; Barrett et al. 2008; Eckert et al. 2010). Traditionally, studies of invasion that focused on these have only made comparisons between native and invasive populations, or among differentiated invasive populations (Richardson et al. 2000). However, to fully understand the mechanisms that allow a non-native population to progress from an established component of a novel location to one that spreads aggressively beyond the point of initial introduction, studies must compare traits among populations from the species' native, naturalized, and invasive regions. Here, we show that non-native *Mimulus guttatus* populations from the naturalized and invasive regions experience inbreeding depression following self-fertilization ("selfing") for fewer fitness traits compared to native populations. *Mimulus guttatus* individuals from both native and non-native regions also experienced an increase in fitness following outcrossing, suggesting that these individuals can benefit from introductions from outside populations. However, native individuals also experienced heterosis for some traits and the result did not seem to depend as much on whether the sire was from a similar or dissimilar habitat as it did with non-natives. Regarding adaptive plasticity, we found that the expression of phenotypic plasticity for fitness traits may be common for *M. guttatus* when exposed to maladaptive environments. Individuals from both native and non-native regions were able to maintain fitness for at least some traits in the stressful, high salinity watering treatment. However, the non-native

populations had greater fitness in both optimal watering conditions and saline conditions compared to native populations for nearly all of the traits examined. This pattern of greater fitness across environments by non-native individuals compared to natives resembles the "Jack-and-Master" scenario proposed by Richards et al. (2006), and suggests that adaptive plasticity plays a role in the establishment success of introduced *M. guttatus* individuals. Below, we discuss patterns of inbreeding depression, heterosis, and adaptive plasticity in native and non-native populations of *M. guttatus* found in this study.

Inbreeding depression in native and non-native regions

Plants from the non-native regions, specifically the naturalized region in eastern North America (including the NBBR polyploid population from New Brunswick, Canada) and the invasive region in the UK, experienced inbreeding depression for fewer traits following selfing when compared to native plants from western North America (Table 10; Fig. 8). In fact, we found no evidence for inbreeding depression in the two naturalized populations and the NBBR population; however, our inability to detect inbreeding depression in these naturalized populations was confounded by the fact that these populations were highly clonal. Populations from the invasive region expressed inbreeding depression for only two of seven traits, plant height and aboveground biomass, and these two traits were correlated (Supplemental Table 2). In contrast, populations from the native region expressed inbreeding depression for five traits (only flowering probability and the number of reproductive units, or "ru's", were not negatively affected by selfing in native individuals). In this section, we discuss the implications of

inbreeding depression in the context of native population persistence and establishment in locations outside of the native distribution.

With regard to inbreeding depression in native populations in this study, the results presented here are consistent with the outcomes of other research on the same topic. Past studies on *M. guttatus* also found high incidences of inbreeding depression in native populations (Willis 1993; Fu & Ritland 1994; Carr et al. 1997; Dudash et al. 1997). However, most of these studies involved annual populations with no comparisons to inbreeding depression in non-native populations. Our results are novel because we show that the non-native *M. guttatus* individuals in the naturalized and invasive regions (and in the NBBR population) may be able to use selfing as a strategy to, at the very least, become established without experiencing the same detrimental effects on fitness seen in native individuals.

The pattern of inbreeding depression we found in native and non-native individuals is at least partially attributed to two characteristics of *M. guttatus* populations: the amount of genetic diversity held in populations within each region, and the history of introduction of *M. guttatus* into the naturalized and invasive regions. Regarding the former, it is necessary to understand that the manifestation of inbreeding depression requires genetic variation, and selfing exposes deleterious recessive alleles that might otherwise be hidden in heterozygotes to natural selection (Lande & Schemske 1985; Charlesworth & Willis 2009). However, selfing reduces heterozygosity, and therefore genetic variation, by 50% each generation (Lande & Schemske 1985; Carr & Dudash 2003). Populations with a history of selfing, including introduced, mixed-mating plant populations that begin small, may purge deleterious recessives thereby reducing the harmful effects of inbreeding

depression with each successive round of self-fertilization. This could be true for at least one of the naturalized populations, NBS. In a related study, we found that individuals in this population were essentially homozygous for 12 microsatellite and intron-based polymorphic markers (Berg doctoral dissertation, Chap. 2). Plants from the other two naturalized populations, FC and the polyploid NBBR population, had relatively high genetic diversity, on par with populations from the native and invasive regions (observed heterozygosity = 0.23 for FC, 0.49 for NBBR). However, these populations were similar to NBS in that they had very low genotypic diversity; they were highly clonal with very few multilocus genotypes representing the number of individuals sampled. This makes it difficult to detect inbreeding depression because within-population crosses between individuals that are the same genet is effectively the same as self-fertilization. Therefore, without further sampling of maternal lines that represent dissimilar genets, and subsequent within-population outcrosses, we cannot make the determination that self-fertilization definitely results in lower fitness in NBS, FC, and NBBR. However, the low genetic diversity in NBS individuals may indicate that they are "proficient selfers" that may have outcompeted more maladapted genotypes following introduction of the original cohort of individuals. These early colonizers may have also persisted through a difficult introduction marked by inbreeding depression, and subsequently purged the genetic load responsible.

To our knowledge, this is the first study to include the polyploid NBBR *Mimulus* population, so historical records are absent from the literature. Determining the genetic basis of inbreeding depression, whether it is attributed to the partial dominance hypothesis or the overdominance model, can be difficult in general (Dudash & Carr

1998) and perhaps more so when considering polyploids due to the increased interactions between alleles and loci (Husband & Schemske 1997). Under the partial dominance hypothesis, polyploid individuals should have higher frequencies of deleterious mutations, which by itself might suggest greater potential for inbreeding depression. However, while the heterozygosity in diploids decreases by 50% after each generation of selfing, the reduction of heterozygosity should occur more gradually in polyploids (Soltis & Soltis 2000). Because polyploidy provides a buffer against loss of heterozygosity following selfing, the NBBR individuals may have escaped inbreeding depression in this study. Or, as mentioned above, these individuals may have purged deleterious alleles responsible for inbreeding depression following introduction. However, our conclusions here should be accepted with caution as we do not know for certain the taxonomy of the NBBR individuals.

Populations from the invasive region showed some evidence for inbreeding depression (Table 10). Like native populations, these populations have relatively high genetic diversity (average observed heterozygosity among three populations = 0.26; Berg doctoral dissertation, Chap. 2), which may be due to an introduction history that involves repeated introductions as a horticultural species (Truscott et al. 2008; van Kleunen & Fischer 2008; Puzey & Vallejo-Marin 2014). These populations were also shown to have relatively high outcrossing rates (average outcrossing rates in invasive populations = 0.78; Berg doctoral dissertation, Chap. 1). Frequent outcrossing would make it difficult to purge deleterious alleles responsible for inbreeding depression for, in the case of the UK populations studied here, aboveground biomass and plant height. Another recent study also found inbreeding depression for biomass in introduced *M. guttatus* populations from

the UK, as well as from New Zealand (van Kleunen et al. 2015). This suggests that, through outcrossing, invasive populations in the UK maintain some genetic load for biomass and plant height (traits that were correlated in this study, r = 0.42, $P < 0.001$, see Supplemental Table 2) that is expressed following self-fertilization. One interpretation of these results is that populations in the UK were successful in establishing and spreading throughout the region not because they are proficient selfers, but because the multiple introductions increased genetic diversity and the probability of persisting following escape from gardens in the region.

Heterosis and local adaptation in native and non-native regions

We showed that populations from the native region demonstrated heterosis for germination, probability of flowering, number of stolons, and aboveground biomass (Table 10). While heterosis for the first two traits depended on the type of outcross (heterosis for probability of flowering occurred following BSIM crosses, and heterosis for number of stolons occurred following BDIS crosses), heterosis for germination and aboveground biomass occurred after both types of outcrossing. This suggests that native populations may receive a boost in germination success and biomass from immigrants regardless of what type of habitat the incoming genotypes were from. Heterosis has also been demonstrated in native *M.guttatus* populations for survival and number of flowers produced (Lowry et al. 2008). The frequent occurrence of heterosis in native *M. guttatus* populations suggests a surprising lack of local adaptation, and the potential to benefit from immigrants from a range of habitat types. The fact that fitness traits increased significantly after outcrossing with individuals from similar and dissimilar habitat types

suggests that genetic load, perhaps due to drift, can be mitigated by admixture (Lee 2002). The presence of drift load is supported by the fact that we found little evidence for admixture among these native populations in a related study (Berg doctoral dissertation, Chapter 2). Another consideration for conservationists is whether admixture between native populations and non-native *M. guttatus* from invasive populations may boost performance and result in invasive behavior in the native region. We are, however, not aware of any examples of this occurring in wild populations.

The populations from the naturalized and invasive regions also experienced heterosis for several traits each. However, unlike the native populations which experienced heterosis for three traits each for BSIM and BDIS outcross types, naturalized and invasive populations experienced heterosis more frequently following one particular outcrossing type. For naturalized populations, heterosis occurred in three traits (stem diameter, number of stolons, and biomass) following BSIM outcrosses and in only one (germination success) following BDIS outcrosses. The opposite result occurred with outcrossed individuals from the invasive region, where heterosis was detected for three traits (germination success, number of ru's, and biomass) following BDIS outcrosses and for one trait (germination success) following BSIM outcrosses. This pattern reveals that naturalized populations may demonstrate local adaptation, to the extent that admixture with individuals from the same riparian/seep habitat results in heterosis, while mating with individuals from coastal populations results in no benefit or outbreeding depression. This result is not unprecedented, as heterosis following admixture between divergent lineages is not a prerequisite for successful colonization (Chapple et al. 2013; Ordóñez et al. 2013). Further, while we found little evidence of heterosis in naturalized populations

following BDIS outcrosses, we also did not detect outbreeding depression in naturalized individuals following the same type of BDIS outcrosses (no negative z-scores in the BDIS vs. WI outcross types, Table 10). However, it has been suggested that it may take several generations before the manifestation of outbreeding depression (Edmands 2007). Studies of the F_2 and later generations are rare, but one known case showed that outbreeding depression was delayed until the F_3 generation and attributed to an additional generation of recombination (Fenster & Galloway 2000). Our study looked at only two generations, and there were no significant differences in the fitness of naturalized BDIS outcrossed progeny between the two generations (this provided the motivation to pool data from the two generations; see Methods). It is possible that if outcrossing between naturalized individuals and immigrants from coastal populations was sustained beyond two generations, outbreeding depression may occur.

The NBBR population did not experience heterosis at all, whether the outcrosses were BSIM or BDIS. This would support the hypothesis that NBBR individuals do not share the same ploidy level with the diploid *M. guttatus* individuals from the other 13 populations examined in this study. Seed and pollen produced from between-population outcrosses with NBBR individuals were often not viable, to the point where little to no second-generation seed was produced. The fact that admixture with *M. guttatus* individuals does not result in heterosis in the NBBR population may be considered as a positive result from a conservation standpoint, if that means that accidental introduction of immigrants into this naturalized population will not represent a boost in fitness that leads to spread beyond the NBBR population's present boundaries.

Contrastingly, heterosis in individuals from the invasive region occurred mainly following outcrossing with individuals from the dissimilar coastal habitat, demonstrating that the results of admixture among non-native populations of *M. guttatus* are variable. Progeny that resulted from BSIM outcrosses, that is with individuals that originated from riparian/seep habitat, did not experience an increase in fitness compared to within population outcrossing. This may suggest that the relatively large amount of genetic variation in the invasive populations studied here is common to riparian/seep genotypes, so that outcrossing with immigrants from this same habitat type results in a negligible boost in fitness. Outcrossing with individuals from a dissimilar habitat (i.e. coastal bluffs), on the other hand, might lead to heterosis, novel genotypes, and further ability to invade new locations. Only a few studies have assessed the importance of admixture on invasive plant populations (Wolfe et al. 2007; Keller & Taylor 2010; Mullarkey et al. 2013). A study used molecular markers to demonstrate considerable admixture in invasive and native populations of *Silene vulgaris* [Caryophylaceae], but the invasive populations benefitted more from heterosis in terms of fruit production (Keller & Taylor 2010). Another study showed that invasive populations of *Allaria petiolata* [Brassicaceae] experienced heterosis following between-population outcrossing while demonstrating no signs of inbreeding depression in selfed progeny (Mullarkey et al. 2013).

Finally, we must mention that non-genetic causes may underlie the heterosis response in *M. guttatus* individuals from the invasive region, and the lack of response in naturalized individuals, following the BDIS crosses. The BDIS crosses represent admixture between previously divergent evolutionary lineages. For this to occur in a

natural setting, the situation would imply multiple introductions from different source populations. Therefore, in places where admixture occurs, and all else being equal, greater propagule pressure is being exerted compared to locations that experience only single introductions. This correlation between admixture and propagule pressure has been shown in some introduced species, including the invasive ladybird beetle (Kajita et al. 2012). In this sense, propagule pressure can confound the apparent genetic benefits produced by admixture, and the prolific mixing of divergent lineages in some sites where a species has become invasive may instead be a situation where admixture is a passenger to the true driver of invasion success, propagule pressure (Hufbauer et al. 2013; Rius & Darling 2014). *Mimulus guttatus* populations in the naturalized region of eastern North America may simply have lacked that intense propagule pressure that has occurred in the UK due to the species' reputation as a horticultural favorite in Europe. It has been suggested that in studies of invasion success, accounting for propagule pressure should be used as a 'null model' (Colautti et al. 2006), and this approach would further our understanding of why some introduced *M. guttatus* populations have become invasive while others have not. However, these studies are difficult to conduct, in part because of the lack of historical records of introduction for many non-native populations.

Phenotypic plasticity for fitness traits

We examined adaptive plasticity in native and non-native populations of *M. guttatus* utilizing the framework provided by Richards et al. (2006). Specifically, we compared plasticity expressed in progeny originating from naturalized and invasive regions (and from the NBBR population) to that expressed in progeny originating in the native region

to determine whether non-natives fit one of the scenarios described in the framework. We found that *M. guttatus* individuals from each region, native, naturalized, and invasive, were able to express plasticity for several fitness traits. This result supports other studies that suggest that invasive species and/or populations do not harbor greater genetic variation for plasticity than natives (Godoy et al. 2011; Palacio-López & Gianoli 2011). However, individuals from the naturalized and invasive regions demonstrated greater fitness in both favorable (control treatment) and stressful (salinity treatment) conditions for five of six traits (the exception was the number of stolons; Table 12, Figs. 9A-F).

Our results demonstrate that non-native *M. guttatus* populations represent the "Jack-and-Master" scenario regarding plasticity for fitness described by Richards et al. (2006) and suggest that, along with a low susceptibility to inbreeding depression and potential for heterosis following admixture, plasticity for fitness traits acts as a mechanism that facilitates establishment of this species outside of its native distribution. Also, because populations from both non-native regions display the pattern of Jack-and-Master, it suggests that adaptive plasticity is not necessarily a pre-requisite for invasion by non-native *M. guttatus* populations. Naturalized populations have established in eastern North America, but not spread aggressively in the landscape. However, the "Jack-and-Master" characteristic may instead allow for increased niche breadth in non-native individuals, giving them a better chance to at least establish in novel locations. It is also likely that other factors, such as a shorter residence time compared to invasive populations, a limited amount of propagule pressure, lack of genetic diversity, or environmental conditions constrains the spread of naturalized *M. guttatus* populations in eastern North America.

Native populations, while often demonstrating lower fitness than the non-native populations in both watering treatments, were able to express plasticity for fitness as well, maintaining fitness between the two environments for several traits. Whether invasive plant populations are able to express greater plasticity compared to native populations remains a contentious topic in invasion ecology (Sexton et al. 2002; Parker et al. 2003; Davidson et al. 2011; Godoy et al. 2011). For example, a study of the invasive tree *Melaleuca quinquenervia* [Myrtaceae] found some evidence that non-native populations were more plastic than native population in their response to variation in pH (Kaufman & Smouse 2001). Contrastingly, no evidence of the evolution of plasticity (response to shade) was found regarding invasive populations of the shrub species *Clidemia hirta* [Melastomataceae] (DeWalt et al. 2004). Our results also suggest a lack of difference between native and non-native *M. guttatus* populations to express phenotypic plasticity for fitness. Instead, we revealed that while plasticity for fitness is common among regions, naturalized and invasive populations are more robust in a variety of conditions. This may be due to a selective advantage of larger, more prolific plants being able to colonize and spread in new environments, an idea related to the evolution of increased competitive ability hypothesis (EICA; Blossey & Nötzold 1995). A past study supports our findings by showing that *M. guttatus* individuals from invasive populations in the UK and New Zealand produced twice as many flower-bearing branches as native plants (van Kleunen & Fischer 2008). To our knowledge, no study has investigated a trade-off between traits related to defense against herbivory (e.g. trichome production; Holeski 2007) and fitness in non-native populations of *M. guttatus*. Investigating

differences in fitness and defense traits between native and non-native *M. guttatus* populations would be an interesting area of inquiry.

We also found evidence for the polyploid NBBR individuals maintaining fitness between the two watering treatments. However, like the native populations, the fitness of NBBR individuals was lower compared to naturalized and invasive individuals for all traits but number of stolons. Contrary to what we found in this study concerning *M. guttatus*, there is evidence for polyploid cytotypes of a species expressing greater plasticity compared to diploid conspecifics. A recent study found that introduced tetraploid cytotypes of the invasive plant species *Centaurea stoebe* [Asteraceae] expressed greater plasticity in traits associated with rapid growth compared to native diploids, and that the plasticity was adaptive (Hahn et al. 2012). Interestingly, the magnitude of plasticity in non-native tetraploid genotypes was similar to native tetraploids, leaving the authors to conclude that the plasticity that polyploidy affords in *C. stoebe* may increase its invasiveness but does not seem to have evolved in the introduced range.

Inbreeding and plasticity

We found little evidence for inbred plants expressing greater plasticity for fitness than outcrossed plants, contrary to what would be expected under the developmental stability hypothesis. The lack of a relationship between inbreeding and phenotypic plasticity in this study agrees with the results of some past studies that have investigated this topic. A recent study involving a congener of *M. guttatus*, *M. ringens*, did not detect a relationship between inbreeding and plasticity after measuring a number of traits in the greenhouse and in field sites (O'Halloran & Carr 2010). In contrast, a field experiment performed by

Murren and Dudash (2012) found that inbred plants were more plastic with regard to stem diameter when plants originating from two native *M. guttatus* populations were grown across native and non-native sites. Our results suggest that self-fertilization and phenotypic plasticity act independently on the phenotype (Cheptou & Donohue 2011). However, most studies have focused on structural traits, and more research is needed to understand whether an interaction between mating system and plasticity in mixed-mating plants may influence other types of traits, such as physiological processes (Ivey & Carr 2005; Auld & Relyea 2010).

Conclusions

Selfing can provide reproductive assurance to plant species following introduction to regions outside of the native distribution, where pollinators and mates may be scarce (Baker 1967). Our results show that, indeed, *Mimulus guttatus* populations from the naturalized and invasive regions expressed less inbreeding depression compared to native populations. This suggests that selfing may have played a part in the establishment success of non-native populations. However, we cannot say whether selfing also facilitated invasion by the populations in the UK because the relatively benign naturalized populations in eastern North America appear to be proficient selfers (and likely also reproduce asexually; Berg doctoral dissertation, Chap. 2) without spreading throughout the recipient landscape. Other factors besides mating system, including limits on suitable habitat or a lack of the residence time required to overcome maladaptations, may also be inhibiting spread of the naturalized populations.

Heterosis was not a unique phenomenon among populations from the non-native regions; in fact, native populations showed a surprising lack of local adaptation and

benefitted from between population outcrossing regardless of which habitat type their mate originated from. Heterosis in the non-native populations appeared to be more dependent on habitat type of the individual that they mate with, and naturalized populations may have demonstrated local adaptation by benefitting the most from outcrosses with individuals from a similar habitat type (i.e. riparian/seep). In the invasive region, individuals benefitted most when outcrossed with mates from the dissimilar coastal bluff habitat, which exist in the native region only. While most countries regulate the import of alien plant species, there are often loopholes that allow propagules of species that had been established prior to legislation becoming enacted (New Zealand Government 2015). That means current legislation may not be sufficient to prevent admixture between native North American *M. guttatus* genotypes and countries where the species has become established. Our findings suggest that both inbred and outcrossed progeny are capable of establishing populations in non-native regions.

The reaction norm of a non-native plant species can contribute to invasion success by maintaining fitness in stressful environments (flat reaction norm) and/or by exploiting favorable conditions (steeper and higher fitness reaction norm; Baker 1967). A third scenario represents what we found with *M. guttatus* populations from the naturalized and invasive regions, a combination of both robustness across environments and much greater fitness in favorable conditions (the Jack-and-Master scenario; Richards et al. 2006). Our study is one of the few to identify both robustness *and* opportunism following an investigation of plasticity for fitness in an invasive plant species (Milberg et al. 1999; Gerlach & Rice 2005). While we found scant evidence for an interaction between selfing and plasticity in this potentially invasive species, the subject certainly requires more

investigation to determine the extent to which these important mechanisms overlap and facilitate invasion.

Table 4.1 Location and approximate population size of 8 *Mimulus guttatus* populations from the native region in western North America, two from the naturalized region in eastern North America, and three from the invasive region in the UK. A third naturalized population was a polyploid *Mimulus* population (NBBR). Population sizes are estimates based on observation only.

Native region populations	Code	Latitude	Longitude	Approximate # individuals in population
Seward, AK (1)	AKS1	60.07021	-149.28102	> 1000
Seward, AK (2)	AKS2	60.12142	-149.25660	>200
Anchorage, AK	AKA	63.33945	-148.49102	>1000
Shelton, WA	WA	47.23089	-123.08862	>1000
Cloverdale, OR	OR05	45.14347	-123.58188	>100
Haceta Head, OR	OR04	44.08163	-124.07605	>500
Otter Point, OR	OR03	42.27994	-124.25329	>1000
Bodega Bay, CA	BB1	38.31701	-123.07117	>1000
Naturalized region				
Bass River, New Brunswick, Canada (polyploid)	NBBR	46.32904	-65.06621	>500
Fly Creek, NY	FC	42.44391	-74.58212	>1000
Springfield, New Brunswick, Canada	NBS	45.41486	-65.49202	>500
Invasive region				
Brampton, Norfolk, UK	BRA	52.7681	-1.27985	>100
Dunblane, Perthshire, UK	DBL	56.18861	-3.96608	>300
Houghton Lodge, Hampton, UK	HOU	51.09699	-1.5084	>100

Table 4.2 Summary of (generalized) linear mixed models used to test for inbreeding depression, heterosis, and outbreeding depression in seven fitness traits in 13 *Mimulus guttatus* populations and one *Mimulus* population comprised of polyploid individuals (NBBR). The sample size (N) and overall mean for each variable is reported in the first column. The fixed variables in each full model included maternal region, cross type, and their interaction. The random components in each full model included block, and maternal family nested within population. The exception to this model format was that used to analyze germination rate, which omitted the random block variable (see Methods for explanation). The conditional R^2 is the proportion of variance explained by both the fixed and random effects. Specific hypotheses (i.e. contrasts between cross types to examine inbreeding depression, heterosis, or outbreeding depression) were examined using Tukey HSD *post hoc* z-tests. Negative estimates indicate that the first cross type in the contrast had a lower value than the second, and a positive estimate indicated the opposite. Values in bold indicate significance at the specified value and evidence for inbreeding depression, heterosis, or outbreeding depression, depending on the contrast. WI, within-population outcrosses; BSIM, between-population outcrosses with populations from similar habitat (e.g. seep x seep or coastal x coastal); BDIS, between-population outcrosses with populations from dissimilar habitat (e.g. seep x coastal). Means (SE) for each region x cross type are illustrated using bar charts in Fig. 9 and shown in Supplemental Table 1. The variances of the random effects are presented in Supplemental Table 4.

[a]No significant difference between models with and without the interaction term (maternal region x cross type) included; results of main effects are from model with the interaction term removed

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; values in italics are marginally significant ($p \leq 0.1$)

				Selfing v. WI cross *(if WI > self, evidence for inbreeding depression)*		BSIM v. WI *(if BSIM > WI, evidence for heterosis; if WI > BSIM, then outbreeding depression and evidence for local adaptation)*		BDIS v. WI *(if BDIS > WI, evidence for heterosis; if WI > BDIS, then outbreeding depression and evidence for local adaptation)*	
response variable (error distribution)	χ^2 maternal region (df = 3)	χ^2 cross type (df = 3)	χ^2 maternal region x cross type (df = 6)	estimate	z	estimate	z	estimate	z
germination (normal) N = 560 mean = 58%	**22.33*** **	**140.11*** **	**38.62*** **	*native*: - 0.16 *natural*: -0.04 *invasive*: 0.01 *NBBR:* -0.03	**-5.69*** ** -0.46 0.13 -0.49	*native*: 0.07 *natural*: 0.12 *invasive*: 0.29 *NBBR:* -0.09	**2.53*** 1.60 **6.53*** ** -1.38	*native*: 0.12 *natural*: 0.20 *invasive*: 0.18 *NBBR:* -0.06	**4.27*** ** **2.56*** **4.01*** ** -0.82
flowering probability (binomial)[a] N = 5400 mean = 45%	**10.80***	**50.23*** **	13.00	*native*: -0.16 *natural*: -0.19 *invasive*: -0.31 *NBBR:* -0.31	-1.49 -0.92 -1.90 -1.90	*native*: 0.38 *natural*: 0.07 *invasive*: 0.09 *NBBR:* 0.09	**3.61**** 0.33 0.55 0.55	*native*: 0.25 *natural*: 0.15 *invasive:* 0.39 *NBBR:* 0.39	*2.34* 0.70 *2.36* *0.08*
stem diameter (normal)[a] N = 5326 mean = 4.7mm	**221.53*** **	**34.90*** **	14.01	*native*: -0.63 *natural*: 0.03 *invasive*: -0.37 *NBBR:* - 0.61	**-3.08*** 0.10 -1.91 - 1.53	*native*: 0.25 *natural*: 1.00 *invasive*: -0.28 *NBBR:* 0.02	1.21 **3.21**** -1.48 0.06	*native*: 0.34 *natural*: 0.33 *invasive*: -0.07 *NBBR:* 0.05	1.66 1.08 -0.37 0.12
plant height	**143.11*** **	**70.21*** **	10.94	*native*: -5.56 *natural*: -0.52	**-3.92*** ** -0.24	*native*: 3.11 *natural*: 2.34	2.20 1.09	*native*: 3.02 *natural*: 1.73	2.16 0.81

(normal)[a] N = 5305 mean = 29.2cm				*invasive*: -4.74 *NBBR:* -3.06	**-2.66*** -1.58	*invasive*: -0.31 *NBBR:* 0.23	-0.17 0.12	*invasive*: 4.09 *NBBR:* 2.27	*2.30* 1.16
# stolons (poisson)[a] N = 5336 mean = 3.1	**8.75***	**49.27*****	16.81	*native*: -0.34 *natural*: - 0.02 *invasive*: -0.08 *NBBR:* 0.04	**-2.79*** -0.07 -0.51 0.08	*native*: 0.08 *natural*: 0.93 *invasive*: -0.04 *NBBR:* 0.33	0.69 **2.50*** -0.61 0.66	*native*: 0.43 *natural*: 0.49 *invasive*: 0.40 *NBBR:* 0.12	**3.51**** 1.31 2.44 0.25
# ru's (poisson)[a] N = 5275 mean = 6.5	**75.61*****	**15.00****	6.75	*native*: -1.45 *natural*: -0.52 *invasive*: -1.25 *NBBR*: 0.11	-1.55 -0.29 -0.80 0.08	*native*: 0.42 *natural*: 0.76 *invasive*: -0.48 *NBBR:* 0.75	0.45 0.43 -0.31 0.52	*native*: -0.45 *natural*: 3.10 *invasive*: 4.75 *NBBR:* 3.54	-0.49 1.75 **3.04*** 2.44
aboveground biomass (normal)[a] N = 5311 mean = 2.0g	**155.12*****	**235.06 *****	16.83	*native*: - 0.51 *natural*: - 0.04 *invasive*: -0.47 *NBBR:* -0.17	**-4.64 ***** - 0.17 **-3.33**** -0.50	*native*: 0.31 *natural*: 0.67 *invasive*: 0.21 *NBBR:* -0.03	**2.81*** **3.16**** 1.50 -0.09	*native*: 0.48 *natural*: 0.38 *invasive*: 0.72 *NBBR:* 0.19	**4.36***** 1.79 **5.10 ***** 0.55

Table 4.3 Results from model investigating plasticity in native and non-native *M. guttatus* populations. To investigate differences in plasticity in response to increased salinity among the native, naturalized, and invasive *M. guttatus* regions (plus the NBBR polyploid *Mimulus* population), we ran a statistical model with regions, watering regime treatments (control vs. increased salinity), and their interaction as fixed factors for six response variables related to fitness. Block and maternal family nested in population were random factors in the model. A significant interaction indicates that the response to the two watering regime treatments depended on region. For pairwise comparisons between regions in each watering regime treatment separately, see Table 12.

response variable (error distribution)	$\chi 2$ maternal region (df = 3)	$\chi 2$ treatment (df = 2)	$\chi 2$ maternal region x treatment (df = 6)
probability of flowering (binomial)	**76.69*** **	1.02	**10.07***
stem diameter (normal)	**207.44*** **	**12.09*** **	**16.20** **
plant height (normal)	**124.17*** **	**268.32*** **	**51.36*** **
# of stolons (poisson)	**7.62***	**5.05***	**4.12**
# of ru's (poisson)	**74.16*** **	**23.77*** **	**79.55*** **
biomass (normal)	**231.00*** **	**159.19*** **	**198.64*** **

Table 4.4 Pairwise comparisons between regions in each watering regime treatment (control and increased salinity) were made using Tukey's HSD post hoc z-tests that followed the full model described in Table 11. Significant differences are in bold, and means (SD) for each region being compared are reported in the order they appear at the top of the column. These results can be used in conjunction with Figure 3 to identify significant differences between regions in each reaction norm.

*p < 0.05; **p < 0.01; ***p < 0.001; values in italics are marginally significant (p ≤ 0.1)

	invasive vs. native		**invasive vs naturalized**		**invasive vs. NBBR**		**naturalized vs.native**	
Treatment	control	saline	control	saline	control	Saline	control	saline
	z-score mean(SD)							
flowering probability	***3.17****	***769.8*****	***-2.60****	***-840.8*****	***3.21****	***1623.4*****	***5.65*****	***1958.5*****
	0.50(0.50) vs. 0.40(0.49)	0.52(0.50) vs. 0.40(0.49)	0.50(0.50) vs. 0.61(0.49)	0.52(0.50) vs. 0.70(0.46)	0.50(0.50) vs. 0.27(0.45)	0.52(0.50) vs. 0.20(0.40)	0.61(0.49) vs. 0.40(0.49)	0.70(0.46) vs. 0.40(0.49)
stem diameter	***13.75*****	***11.13*****	***5.30*****	***4.26*****	***4.58*****	***3.84*****	***5.59*****	***4.59*****
	6.23(2.40) vs. 4.18(2.43)	5.66(2.38) vs. 4.15(2.34)	6.23(2.40) vs. 5.16(2.53)	5.66(2.38) vs. 4.88(2.44)	6.23(2.40) vs. 4.69(2.37)	5.66(2.38) vs. 4.49(2.35)	5.16(2.53) vs. 4.18(2.43)	4.88(2.44) vs. 4.15(2.34)
plant height	***5.89*****	***10.55*****	*-2.06*	*0.01*	***2.39****	***5.26*****	***7.41*****	***9.02*****
	34.31(13.10) vs. 28.76(12.97)	33.35(13.00) vs. 23.50(12.00)	34.31(13.10) vs. 37.13(13.24)	33.35(13.00) vs. 33.29(13.07)	34.31(13.10) vs. 29.09(12.94)	33.35(13.00) vs. 22.18(12.90)	37.13(13.24) vs. 28.76(12.97)	33.29(13.07) vs. 23.50(12.00)
number of stolons	*-0.95*	*0.31*	*-1.05*	*- 1.19*	***- 3.01****	*- 1.31*	*0.40*	*1.65*
	2.89(1.50) vs. 3.00(1.87)	3.08(1.48) vs. 3.05(1.73)	2.89(1.50) vs. 3.05(1.84)	3.08(1.48) vs. 3.31(3.17)	2.89(1.50) vs. 3.62(1.61)	3.08(1.48) vs. 3.41(1.84)	3.05(1.84) vs. 3.00(1.87)	3.31(3.17) vs. 3.05(1.73)
number of ru's	***4.80*****	***6.71*****	*- 1.86*	*- 0.76*	***2.55****	***4.39*****	***6.46*****	***6.65*****
	7.49(11.45) vs. 5.23(9.20)	9.05(11.81) vs. 5.39(9.27)	7.49(11.45) vs. 9.78(12.20)	9.05(11.81) vs. 9.49(10.16)	7.49(11.45) vs. 3.39(6.82)	9.05(11.81) vs. 2.34(5.04)	9.78(12.20) vs. 5.23(9.20)	9.49(10.16) vs. 5.39(9.27)
aboveground biomass	***12.17*****	***12.44*****	***6.97*****	***3.71****	***5.66*****	***4.16*****	***2.35****	***6.35*****
	2.92(1.34) vs. 1.85(1.11)	2.50(1.20) vs. 1.47(1.04)	2.92(1.34) vs. 2.09(1.27)	2.50(1.20) vs. 2.09(1.20)	2.92(1.34) vs. 1.78(1.08)	2.50(1.20) vs 1.73(1.03)	2.09(1.27) vs, 1.85(1.11)	2.09(1.20) vs. 1.47(1.04)

Table 4.4 continued Pairwise comparisons between NBBR and native populations, and NBBR vs. naturalized populations.

	NBBR vs. native		**naturalized vs. NBBR**	
Treatment	control	saline	control	Saline
	z-score mean(SD)			
flowering probability	*-1.94* 0.27(0.45) vs. 0.40(0.49)	***-1526.1**** 0.20(0.40) vs. 0.40(0.49)	***4.54**** 0.61(0.49) vs. 0.27(0.45)	***2463.7**** 0.70(0.46) vs. 0.20(0.40)
stem diameter	*1.57* 4.69(2.37) vs. 4.18(2.43)	*1.15* 4.49(2.35) vs. 4.15(2.34)	*1.35* 5.16(2.53) vs. 4.69(2.37)	*1.23* 4.88(2.44) vs. 4.49(2.35)
plant height	*0.22* 29.09(12.94) vs. 28.76(12.97)	*-0.60* 22.18(12.90) vs. 23.50(12.00)	***3.50**** 37.13(13.24) vs. 29.09(12.94)	***5.08**** 33.29(13.07) vs. 22.18(12.90)
number of stolons	***2.71**** 3.62(1.61) vs. 3.00(1.87)	*1.53* 3.41(1.84) vs. 3.05(1.73)	*- 2.26* 3.05(1.84) vs. 3.62(1.61)	*- 0.56* 3.31(3.17) vs. 3.41(1.84)
number of ru's	*- 0.47* 3.39(6.82) vs. 5.23(9.20)	*- 1.55* 2.34(5.04) vs. 5.39(9.27)	***3.52**** 9.78(12.20) vs. 3.39(6.82)	***4.68**** 9.49(10.16) vs. 2.34(5.04)
aboveground biomass	*- 0.30* 1.78(1.08) vs. 1.85(1.11)	*1.40* 1.73(1.03) vs. 1.47(1.04)	*1.45* 2.09(1.27) vs. 1.78(1.08)	*1.88* 2.09(1.20) vs 1.73(1.03).

Table 4.5 Summary of (generalized) linear mixed models used to test for plasticity for fitness in four cross types in the three *M. guttatus* regions, plus one *Mimulus* population comprised of polyploid individuals (NBBR). Each region was analyzed independently and the fixed variables in each full model included watering regime treatment (control vs. increased salinity), cross type, and their interaction. The random components in each full model included block and maternal family nested within population. To address the developmental instability hypothesis, which states that selfed progeny express greater plasticity (in this case, for fitness traits) compared to outcrossed progeny, Tukey HSD post hoc z-tests were used to examine the effect of treatment on each cross type. The developmental instability hypothesis was supported when there was no effect of treatment on selfed individuals and a significant effect on outcrossed individuals. These cases are highlighted in gray. Values in bold indicate significance at the specified value. WI, within-population outcrosses; BSIM, between-population outcrosses with populations from similar habitat (e.g. seep x seep or coastal x coastal); BDIS, between-population outcrosses with populations from dissimilar habitat (e.g. seep x coastal). The variances of the random effects are presented in Supplemental Table 5.

response variable (error distribution)				
flowering probability (binomial)				
native region		Chi square	*df*	*P*
	treatment	0.10	1	0.75
	cross	9.94	3	**0.02**
	treatment x cross	5.18	3	0.16
	self cross	0.19	1	0.66
	WI	1.38	1	0.24
	BSIM	2.93	1	0.08
	BDIS	0.70	1	0.40
naturalized region		Chi square	*df*	*P*
	treatment	8.19	1	< **0.01**
	cross	1.53	3	0.67
	treatment x cross	0.33	3	0.95

	self cross	3.17	1	0.07
	WI	1.16	1	0.28
	BSIM	2.83	1	0.09
	BDIS	1.40	1	0.24
invasive region		Chi square	*df*	*P*
	treatment	0.49	1	0.48
	cross	7.98	3	< **0.05**
	treatment x cross	0.93	3	0.82
	self cross	0.13	1	0.72
	WI	0.00	1	1.00
	BSIM	0.01	1	0.94
	BDIS	1.26	1	0.26
NBBR		Chi square	*df*	*P*
	treatment	1.48	1	0.22
	cross	7.92	3	< **0.05**
	treatment x cross	0.99	3	0.80
	self cross	0.28	1	0.59
	WI	1.23	1	0.27
	BSIM	1.01	1	0.31
	BDIS	0.00	1	1.00
stem diameter (normal)				
native region		Chi square	*df*	*P*
	treatment	0.13	1	0.71
	cross	27.33	3	< **0.001**
	treatment x cross	4.31	3	0.23
	self cross	6.34	1	<**0.01**
	WI	0.22	1	0.64
	BSIM	8.72	1	<**0.01**
	BDIS	0.08	1	0.78
naturalized region		Chi square	*df*	*P*
	treatment	2.28	1	0.13
	cross	13.32	3	< **0.01**

	treatment x cross	1.46	3	0.69
	self cross	0.05	1	0.82
	WI	2.42	1	0.12
	BSIM	0.91	1	0.34
	BDIS	1.85	1	0.17
invasive region		Chi square	*df*	*P*
	treatment	39.87	1	**< 0.001**
	cross	5.07	3	0.17
	treatment x cross	2.15	3	0.54
	self cross	1.51	1	0.22
	WI	24.57	1	**< 0.001**
	BSIM	24.20	1	**< 0.001**
	BDIS	33.75	1	**< 0.001**
NBBR		Chi square	*df*	*P*
	treatment	1.01	1	0.31
	cross	3.86	3	0.28
	treatment x cross	1.99	3	0.57
	self cross	0.34	1	0.56
	WI	0.76	1	0.38
	BSIM	0.21	1	0.65
	BDIS	1.67	1	0.20
plant height (normal)				
native region		Chi square	*df*	*P*
	treatment	268.83	1	**< 0.001**
	cross	48.39	3	**< 0.001**
	treatment x cross	1.70	3	0.64
	self cross	84.66	1	**< 0.001**
	WI	72.84	1	**< 0.001**
	BSIM	67.88	1	**< 0.001**
	BDIS	48.40	1	**< 0.001**
naturalized region		Chi square	*df*	*P*
	treatment	20.24	1	**< 0.001**
	cross	2.39	3	0.49

	treatment x cross	0.28	3	0.96
	self cross	14.30	1	< **0.001**
	WI	9.70	1	< **0.002**
	BSIM	2.42	1	0.11
	BDIS	10.52	1	< **0.001**
invasive region		Chi square	*df*	*P*
	treatment	4.02	1	0.06
	cross	25.04	3	< **0.001**
	treatment x cross	0.57	3	0.90
	self cross	0.70	1	0.40
	WI	1.17	1	0.28
	BSIM	0.61	1	0.43
	BDIS	2.22	1	0.14
NBBR		Chi square	*df*	*P*
	treatment	35.13	1	< **0.001**
	cross	8.25	3	< **0.05**
	treatment x cross	9.29	3	< **0.03**
	self cross	3.00	1	0.08
	WI	5.90	1	< **0.05**
	BSIM	6.25	1	< **0.05**
	BDIS	31.32	1	< **0.001**
# of stolons (Poisson)				
native region		Chi square	*df*	*P*
	treatment	0.91	1	0.34
	cross	42.08	3	< **0.05**
	treatment x cross	1.70	3	0.64
	self cross	0.44	1	0.51
	WI	0.11	1	0.74
	BSIM	0.02	1	0.87
	BDIS	1.94	1	0.16
naturalized region		Chi square	*df*	*P*
	treatment	4.12	1	**0.04**

	cross	10.03	3	**0.02**
	treatment x cross	5.70	3	0.13
	self cross	0.96	1	0.33
	WI	0.39	1	0.53
	BSIM	8.14	1	**< 0.01**
	BDIS	0.25	1	0.61
invasive region		Chi square	*df*	*P*
	treatment	3.46	1	0.07
	cross	12.44	3	**< 0.01**
	treatment x cross	1.18	3	0.76
	self cross	2.69	1	0.10
	WI	3.72	1	**<0.05**
	BSIM	0.13	1	0.72
	BDIS	0.53	1	0.47
NBBR		Chi square	*df*	*P*
	treatment	0.61	1	0.43
	cross	0.70	3	0.87
	treatment x cross	1.32	3	0.72
	self cross	0.01	1	0.95
	WI	0.18	1	0.67
	BSIM	2.74	1	0.10
	BDIS	0.04	1	0.84
# of ru's (Poisson)				
native region		Chi square	*df*	*P*
	treatment	2.62	1	0.11
	cross	5.34	3	0.15
	treatment x cross	16.72	3	**< 0.001**
	self cross	0.43	1	0.51
	WI	0.01	1	0.91
	BSIM	0.94	1	0.33
	BDIS	0.47	1	0.49
naturalized region		Chi square	*df*	*P*

	treatment	0.82	1	0.36
	cross	1.45	3	0.69
	treatment x cross	29.10	3	< **0.001**
	self cross	1.92	1	0.17
	WI	0.05	1	0.82
	BSIM	0.33	1	0.56
	BDIS	0.72	1	0.40
invasive region		Chi square	*df*	*P*
	treatment	79.41	1	< **0.001**
	cross	16.95	3	< **0.001**
	treatment x cross	25.70	3	< **0.001**
	self cross	10.30	1	< **0.001**
	WI	74.28	1	< **0.001**
	BSIM	13.67	1	< **0.01**
	BDIS	10.97	1	< **0.001**
NBBR		Chi square	*df*	*P*
	treatment	16.48	1	< **0.001**
	cross	18.97	3	0.22
	treatment x cross	18.97	3	< **0.001**
	self cross	2.80	1	0.09
	WI	29.92	1	< **0.001**
	BSIM	2.34	1	0.12
	BDIS	3.44	1	0.06
aboveground biomass (normal)				
native region		Chi square	*df*	*P*
	treatment	182.12	1	< **0.001**
	cross	93.99	3	< **0.001**
	treatment x cross	38.23	3	< **0.001**
	self cross	2.21	1	0.10
	WI	92.23	1	< **0.001**
	BSIM	59.07	1	< **0.001**
	BDIS	60.79	1	< **0.001**
naturalized region		Chi square	*df*	*P*

	treatment	0.00	1	0.95
	cross	14.94	3	< **0.002**
	treatment x cross	2.02	3	0.56
	self cross	1.58	1	0.21
	WI	0.01	1	0.92
	BSIM	0.05	1	0.82
	BDIS	1.20	1	0.27

invasive region		Chi square	*df*	*P*
	treatment	42.40	1	< **0.001**
	cross	73.25	3	< **0.001**
	treatment x cross	29.60	3	< **0.001**
	self cross	1.37	1	0.24
	WI	0.05	1	0.82
	BSIM	20.48	1	< **0.001**
	BDIS	52.31	1	< **0.001**

NBBR		Chi square	*df*	*P*
	treatment	0.25	1	0.61
	cross	1.11	3	0.77
	treatment x cross	3.60	3	0.30
	self cross	0.02	1	0.88
	WI	0.80	1	0.37
	BSIM	1.40	1	0.24
	BDIS	1.18	1	0.28

Supplemental Table 1. Summary of results for tests of inbreeding depression (IBD) and heterosis for six fitness traits in each of the four regions. Methods and models were the same as those used to compile the results shown in Table 10, however the data shown here were divided into the control and saline treatments rather than pooled. As with the pooled data, data from the control and saline treatment showed that IBD occurred only in the native and invasive regions. There are three instances where significant IBD occurred only in the control treatment and not the saline treatment (for stem diameter, both native and invasive plants suffered IBD in control treatment only; for stolons native plants suffered IBD in control only). Patterns of heterosis shown here (when progeny from between population crosses have greater trait means compared to within population crosses) were also similar to the pooled data, with a few exceptions. For example, here heterosis for plant height is detected in invasive plants grown in the control treatment; however, in the pooled data the effect is only marginally significant ($P < 0.1$). Another exception between the pooled data set results and those shown here is that no heterosis for number of stolons was detected when data was separated into the two treatments, but was found in native and naturalized plants when data was pooled.

	Selfed vs. within population cross (WI) Inbreeding depression?		BSIM vs. WI *heterosis or outbreeding depression when populations from similar habitats crossed?*		BDIS vs. WI *heterosis or outbreeding depression when populations from dissimilar habitats crossed?*	
Response	control estimate	saline estimate	control estimate	saline estimate	control estimate	saline estimate
aboveground biomass	Native: **0.71*** **	**0.29***	**0.29***	**0.33***	**0.47*** **	**0.48*** **
	Naturalized: 0.12	0.06	**0.69***	**0.67***	0.43	0.33
	Invasive: **0.42***	**0.53** **	**0.52** **	0.11	**1.15*** **	0.29
	NBBR: 0.31	0.04	0.06	0.11	0.04	0.43
plant height	Native: **5.42** **	**5.53*** **	2.94	3.33	2.60	3.63
	Naturalized: 0.14	0.76	0.20	1.8	1.64	1.8
	Invasive: 3.7	4.2	0.91	0.13	**5.69***	4.15
	NBBR: 3.4	2.7	0.85	0.19	**6.7***	1.62
stem diameter	Native: **0.79** **	0.46	0.29	0.22	0.27	0.44
	Naturalized: 0.05	0.11	**1.23***	**0.79** **	0.34	0.34
	Invasive: **0.55***	0.22	0.33	0.25	0.13	0.03
	NBBR: 0.89	0.34	0.05	0.09	0.17	0.02
stolons	Native: **0.40***	0.28	0.05	0.13	0.31	0.56
	Naturalized: 0.17	0.06	0.56	1.27	0.58	0.37
	Invasive: 0.07	0.11	0.03	0.24	0.50	0.30
	NBBR: 0.07	0.15	0.60	0.08	0.00	0.28
ru's	Native: 1.36	1.57	0.09	0.74	0.69	0.22
	Naturalized: 1.70	0.59	0.95	0.49	3.57	2.60
	Invasive: 0.38	2.19	0.52	1.70	**5.47** **	4.20
	NBBR: 0.72	0.98	0.04	1.47	2.96	**4.20***
probability of flowering	Native: 0.20	0.12	0.19	**0.59*** **	0.24	0.22
	Naturalized: 0.29	0.10	0.01	0.14	0.15	0.16
	Invasive: 0.37	0.27	0.09	0.09	0.26	0.54
	NBBR: 0.72	0.37	0.15	0.09	0.20	0.54

Supplemental Table 2. Correlations between five response variables, data from the four regions pooled.

	r	t	Df
biomass v. plant height	0.42	32.22***	4885
biomass v. stem diam.	0.47	37.88***	4932
biomass v. ru's	0.32	23.57***	4948
biomass v. stolons	0.22	15.77***	4940
plant height v. stem diam.	0.34	25.74***	5254
plant height v. ru's	0.30	22.48***	5257
plant height v. stolons	-0.06	-4.71***	5254
stem diameter v. ru's	0.19	14.50***	5322
stem diameter v. stolons	0.08	5.81***	5320
ru's v. stolons	0.04	2.99**	5334

Supplemental Table 3. Fitness trait means (SE) for four cross types in the native, naturalized, and invasive regions of *M. guttatus* (and one polyploid *Mimulus* species in the naturalized region, NBBR). Values are for each region x cross type combination, and data from the two watering regime treatments were pooled. The means shown here are illustrated using bar charts in Fig. 8.

	native				naturalized				Invasive			
	self	WI	BSIM	BDIS	self	WI	BSIM	BDIS	self	WI	BSIM	BDIS
germination rate	0.45	0.61	0.69	0.74	0.44	0.48	0.60	0.67	0.50	0.49	0.79	0.67
	(0.02)	(0.02)	(0.02)	(0.02)	(0.06)	(0.05)	(0.05)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)
flowering probability	0.33	0.37	0.45	0.42	0.60	0.65	0.66	0.68	0.42	0.49	0.51	0.59
	(0.01)	(0.01)	(0.01)	(0.01)	(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)
stem diameter (mm)	3.55	4.15	4.41	4.52	4.70	4.66	5.67	5.00	5.74	6.13	5.84	6.06
	(0.05)	(0.13)	(0.04)	(0.05)	(0.08)	(0.08)	(0.34)	(0.08)	(0.14)	(0.08)	(0.07)	(0.05)
plant height (cm)	20.46	25.81	28.93	29.02	34.044	34.76	35.47	36.38	29.45	33.61	33.80	38.38
	(0.48)	(0.46)	(0.44)	(0.40)	(0.73)	(0.71)	(0.88)	(0.70)	(0.68)	(0.65)	(0.66)	(0.61)
# of stolons	2.63	2.97	3.06	3.41	2.81	2.81	3.76	3.31	2.85	2.93	2.81	3.32
	(0.06)	(0.06)	(0.05)	(0.06)	(0.12)	(0.13)	(0.29)	(0.12)	(0.08)	(0.08)	(0.08)	(0.08)
# of ru's	4.23	5.69	6.07	5.25	8.17	8.87	9.50	11.86	6.23	7.51	6.91	12.34
	(0.29)	(0.37)	(0.34)	(0.30)	(0.66)	(0.71)	(0.85)	(0.93)	(0.50)	(0.62)	(0.56)	(0.88)
aboveground biomass (g)	1.08	1.58	1.90	2.06	1.79	1.83	2.51	2.20	2.12	2.60	2.81	3.32
	(0.03)	(0.03)	(0.03)	(0.03)	(0.08)	(0.07)	(0.09)	(0.08)	(0.06)	(0.07)	(0.07)	(0.07)

Supplemental Table 4. Variance components of the random effects in the (generalized) linear mixed model analysis testing for inbreeding depression, and heterosis for traits in *Mimulus guttatus*. Maternal family was nested within population. The results for the fixed effects are shown in Table 10. Note that there are no residual variances for binomial or Poisson variables.

	germination (binomial)	probability of flower (binomial)	stem diameter (normal)	plant height (normal)	# of stolons (Poisson)	# of ru's (Poisson)	biomass (normal)
Block	-	0.00	0.01	0.85	0.06	0.38	0.02
Family(Population)	0.009	0.02	0.91	54.48	0.37	27.48	0.34
Residual	-	-	0.40	115.40	-	-	0.87

Supplemental Table 5. Variance components of the random effects in the (generalized) linear mixed model analysis testing for plasticity for fitness among cross types in each region (Table 5). Maternal family was nested within population. Note that there are no residual variances for binomial or Poisson variables.

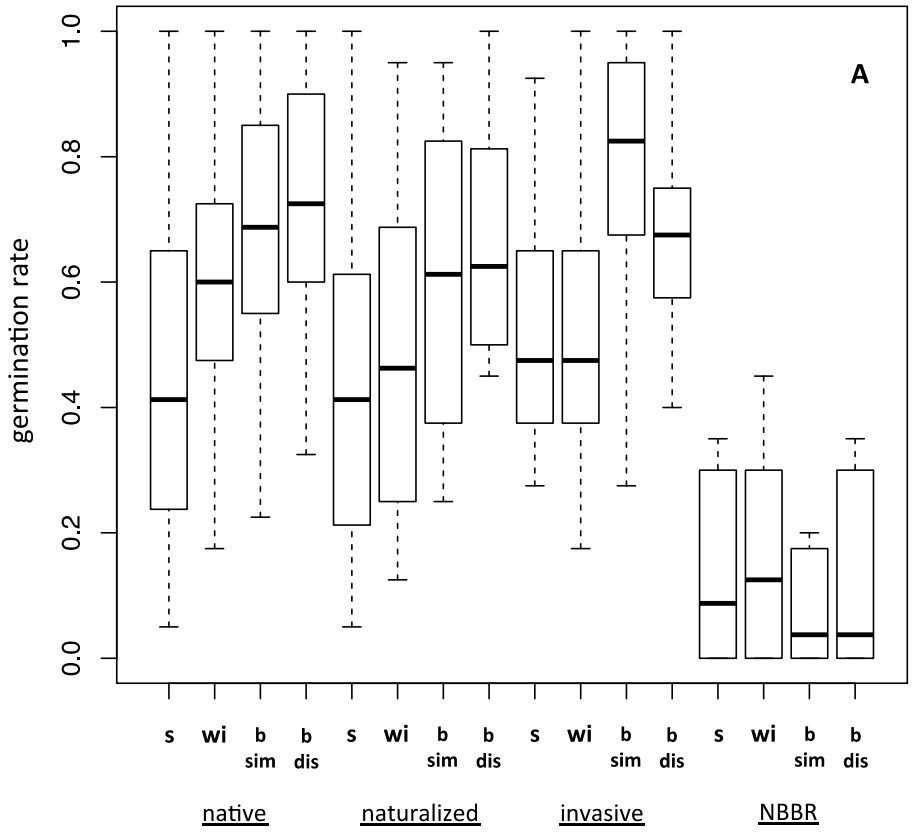
	probability of flower (binomial)	stem diameter (normal)	plant height (normal)	# of stolons (Poisson)	# of ru's (Poisson)	biomass (normal)
Block	0.00	0.01	0.89	0.06	0.38	0.02
Family(Population)	0.05	1.50	72.50	0.39	30.71	0.36
Residual	-	3.99	110.37	-	-	0.83

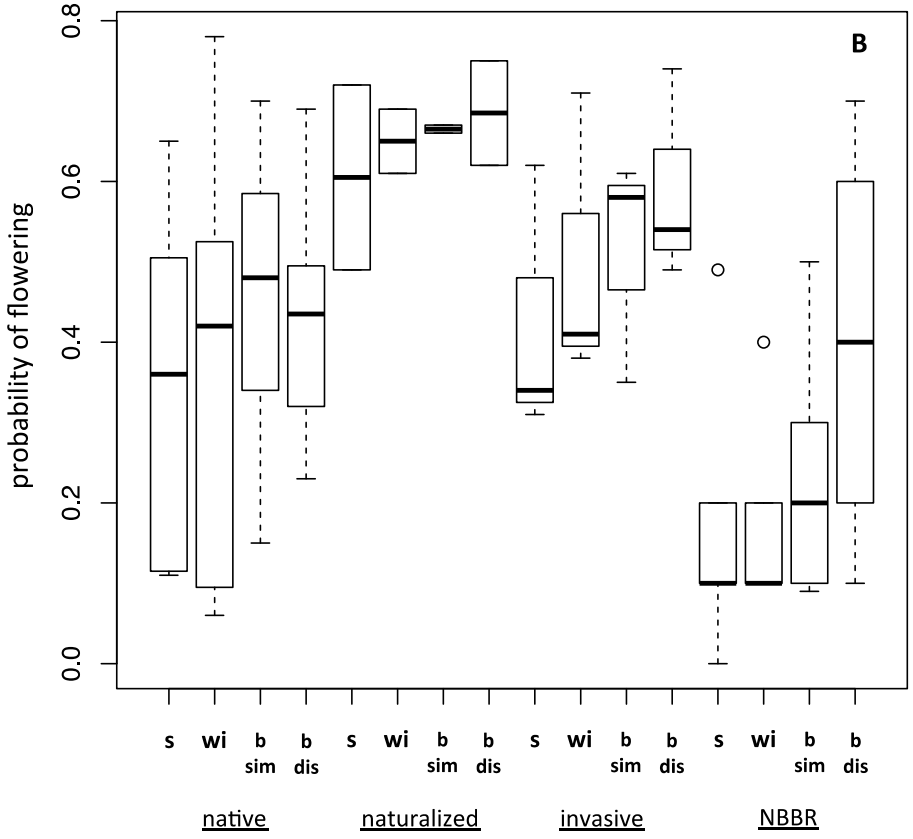
Supplemental Table 6. Tukey's HSD contrasts between cross types after pooling data from the four regions (native, naturalized, invasive, and NBBR) and two watering regime treatments (control vs. increased salinity). The full model included cross type as the fixed effect, with block (except for 'germination' variable, see Methods for explanation) and maternal family nested within population included as random effects. WI, within-population outcrosses; BSIM, outcrosses between populations from similar habitats; BDIS, outcrosses between populations from dissimilar habitats. Error distribution for the full model depended on the response variables (see Table 10 for error distributions). Values in bold type indicate a significant difference between cross types in the contrast.

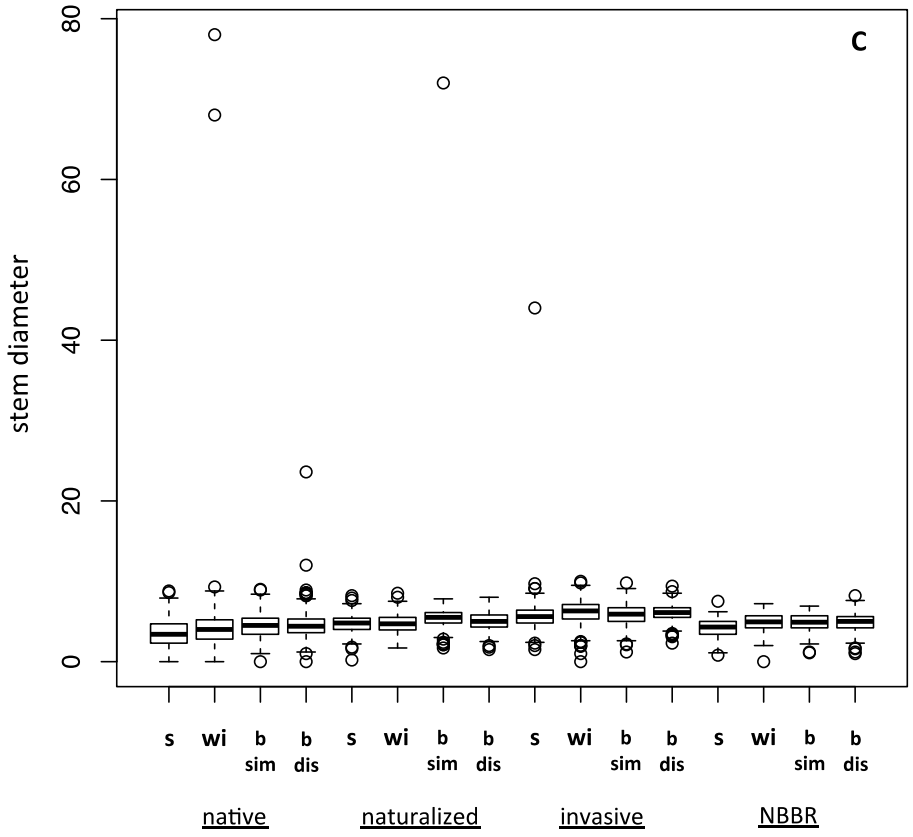
	self vs. WI		WI vs. BSIM		WI vs BDIS		BSIM vs. BDIS	
	estimate	*P*	estimate	*P*	estimate	*P*	estimate	*P*
germination rate	**-1.08**	**<0.001**	**-0.86**	**<0.01**	**-1.18**	**<0.001**	-0.32	0.71
flowering probabiity	-0.23	0.54	-0.34	0.20	-0.36	0.16	-0.01	0.99
stem diameter	**-0.47**	**0.02**	-0.23	0.49	-0.23	0.47	0.00	1.00
plant height	**-4.14**	**<0.001**	-2.31	0.13	**-3.38**	**<0.01**	-1.07	0.75
# of stolons	**-0.08**	**0.05**	**-0.05**	0.28	**-0.13**	**<0.001**	**-0.07**	**0.05**
# of ru's	-0.18	0.61	**-0.37**	**<0.05**	-0.23	0.41	0.13	0.77
aboveground biomass	**-0.41**	**<0.001**	**-0.33**	**<0.01**	**-0.51**	**<0.001**	-0.18	0.24

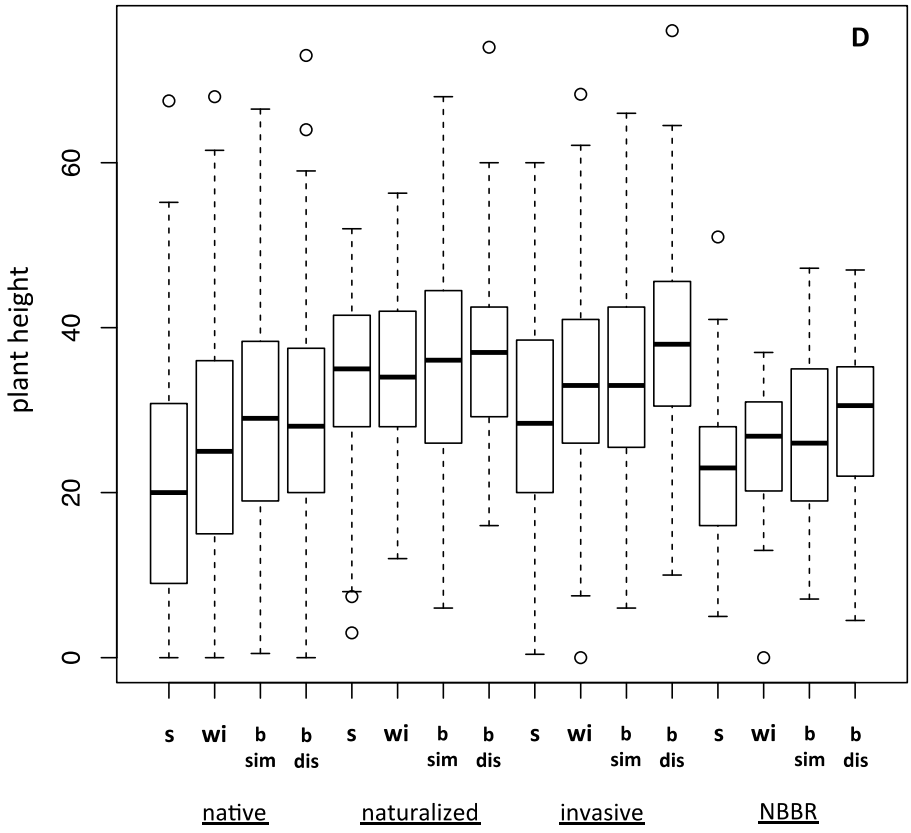
Figure 4.1 Theoretical framework for plasticity in fitness traits. Invasive populations represented by black circles, native by open circles. a) fitness of invasives is more robust across an environmental gradient compared to natives; b) invasives are more opportunistic in favorable habitats compared to natives; c) invasives are both robust across environments and more opportunistic in favorable environment. Figure reproduced from Richards et al. (2006).

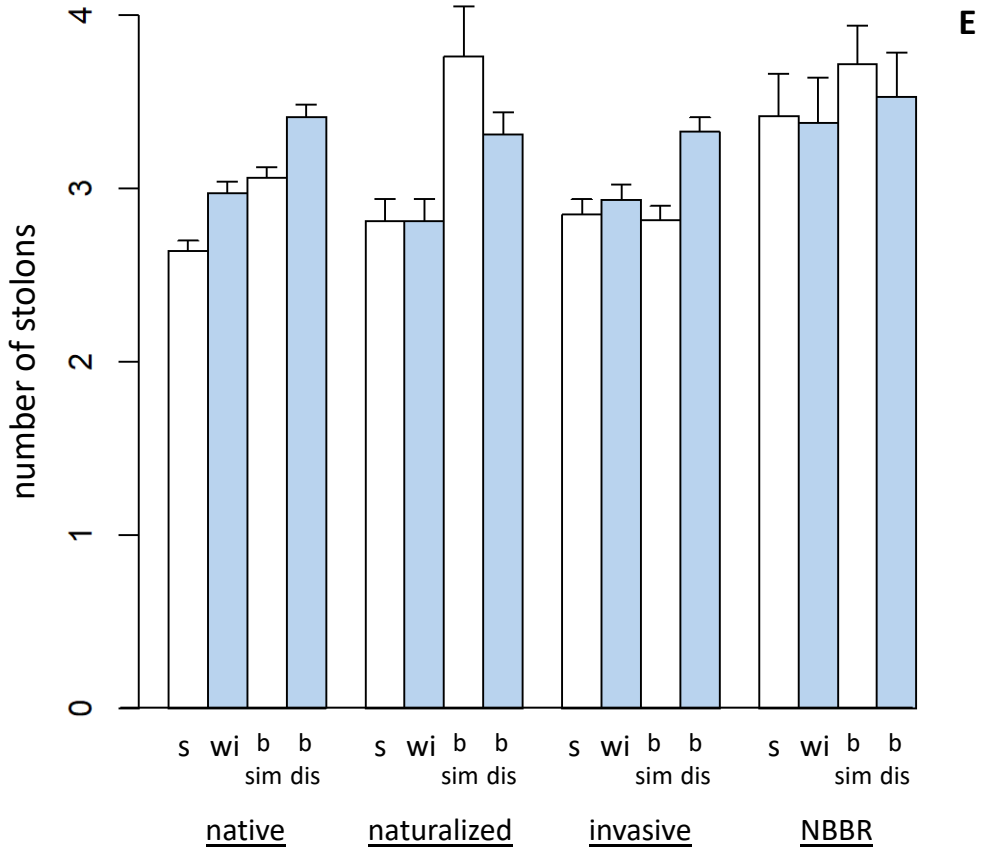
Figure 4.2 Measures of seven fitness traits for native, naturalized, and invasive *M. guttatus* regions, plus one region comprised of polyploid *Mimulus* individuals (NBBR). The four categories within each region represent the four cross types analyzed in the study: s, self-fertilized; wi, within-population crosses; bsim, between-population outcrosses, mates from similar habitat; bdis, between-population outcrosses, mates from dissimilar habitat. When wi plants in a region had significantly greater values for a trait compared to selfed plants, this was evidence for inbreeding depression. When bsim and/or bdis plants had greater values compared to wi plants, this was evidence for heterosis, and the opposite indicated outbreeding depression occurred (for statistical differences between cross types within regions, refer to Table 10). Boxplots show medians and quartiles for each crosstype within region; bar graphs show means and standard errors. For these analyses of inbreeding depression, heterosis, and outbreeding depression, data from the two watering regime treatments (control vs. saline) were pooled.

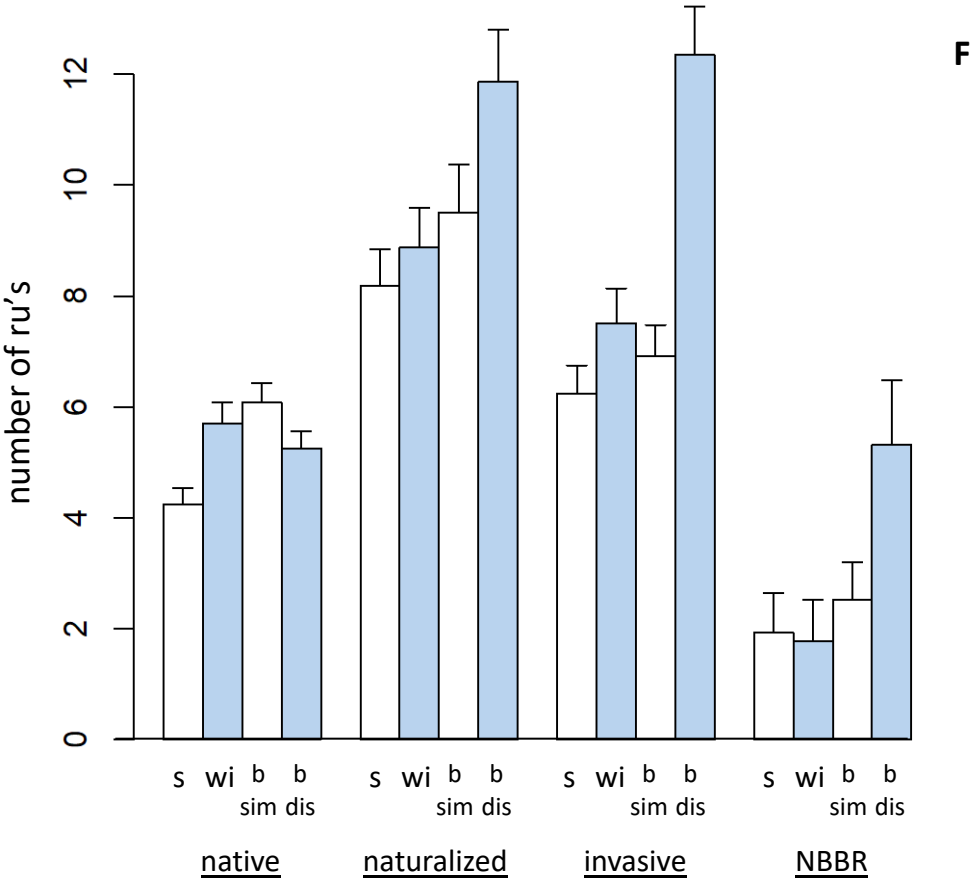



B
probability of flowering
0.0
0.2
0.4
0.6
0.8
s
wi
b sim
b dis
native
naturalized
invasive
NBBR


C
stem diameter
0
20
40
60
80
s
wi
b sim
b dis
native
naturalized
invasive
NBBR


D
plant height
0
20
40
60
s
wi
b sim
b dis
native
naturalized
invasive
NBBR


E
number of stolons
0
1
2
3
4
s
wi
b sim
b dis
native
naturalized
invasive
NBBR


F
number of ru's
0
2
4
6
8
10
12
s
wi
b sim
b dis
native
naturalized
invasive
NBBR

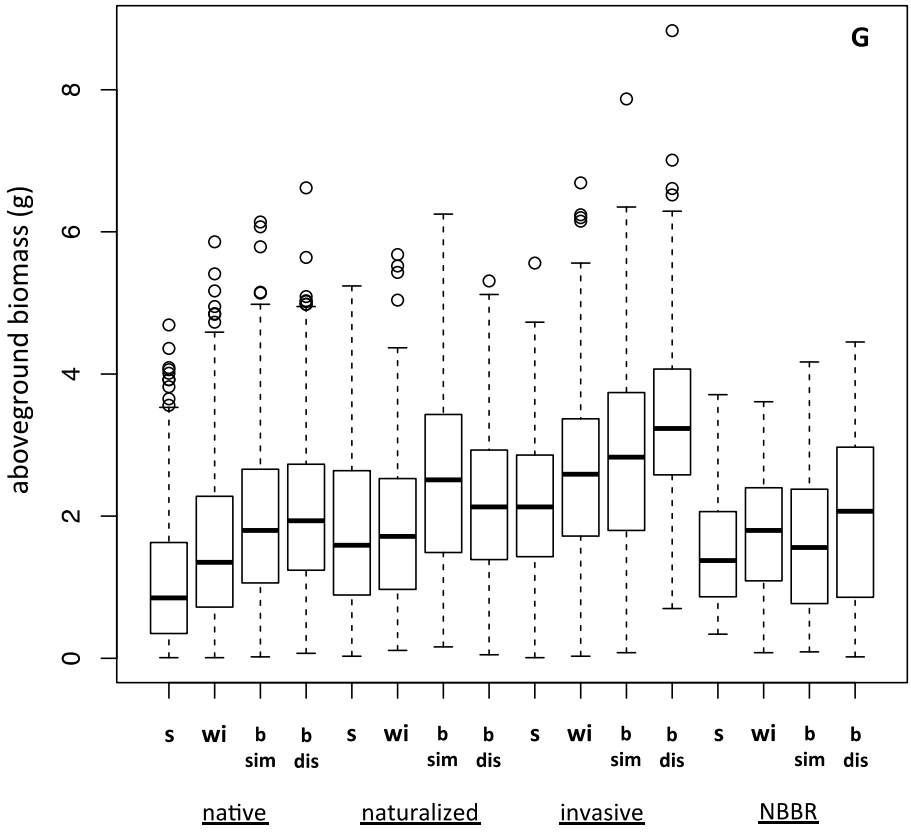
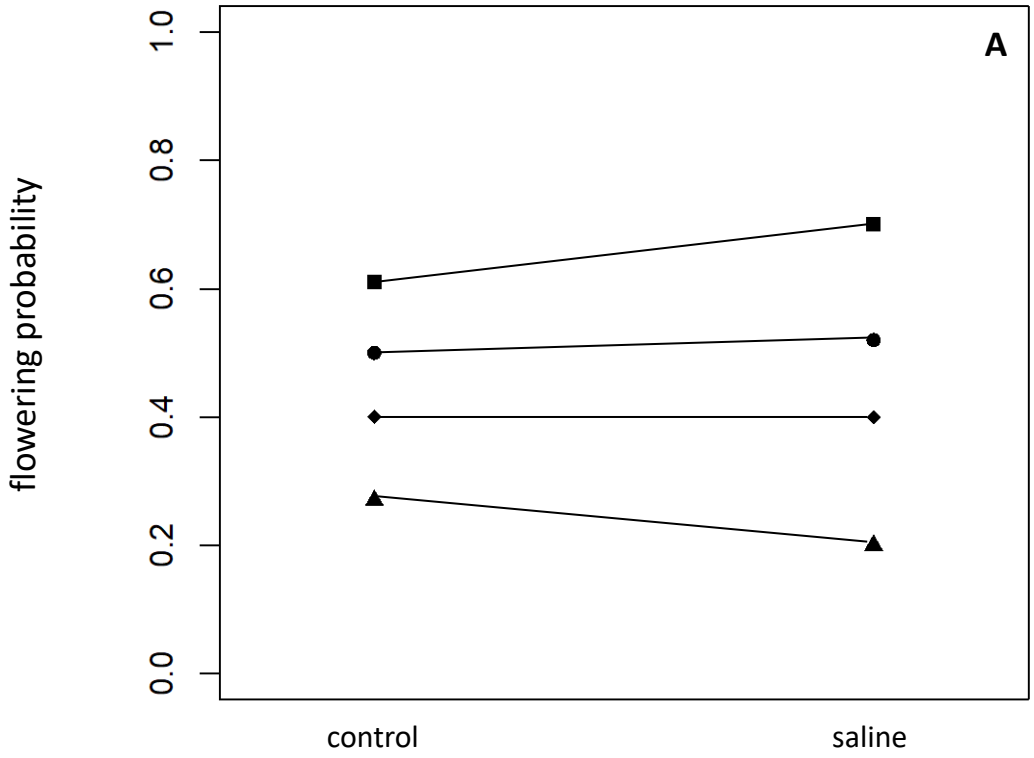
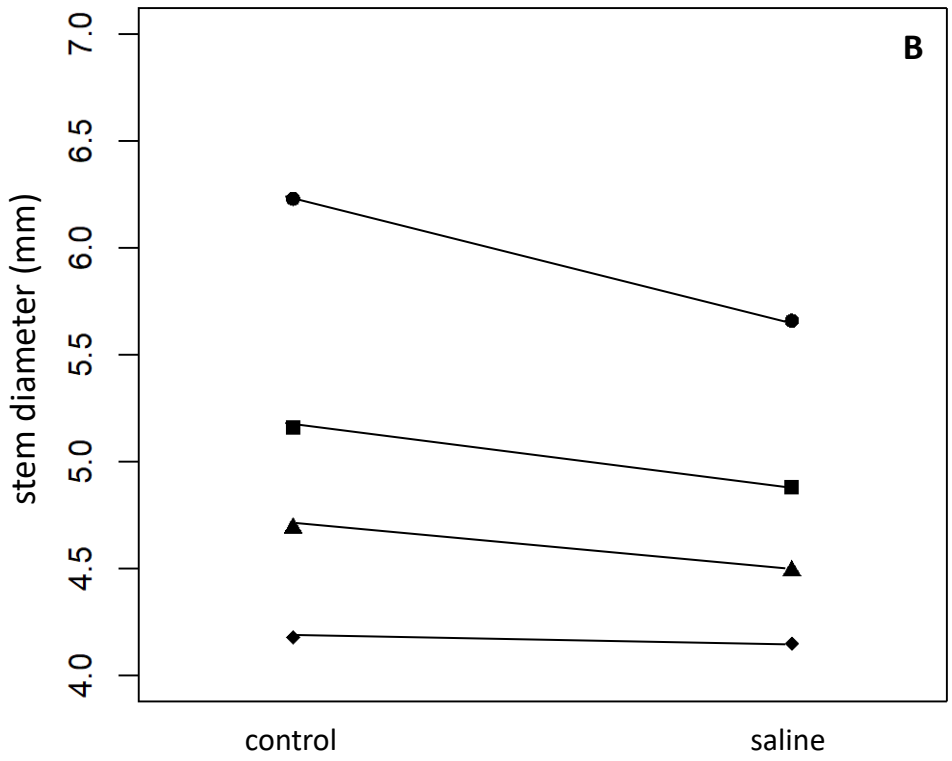
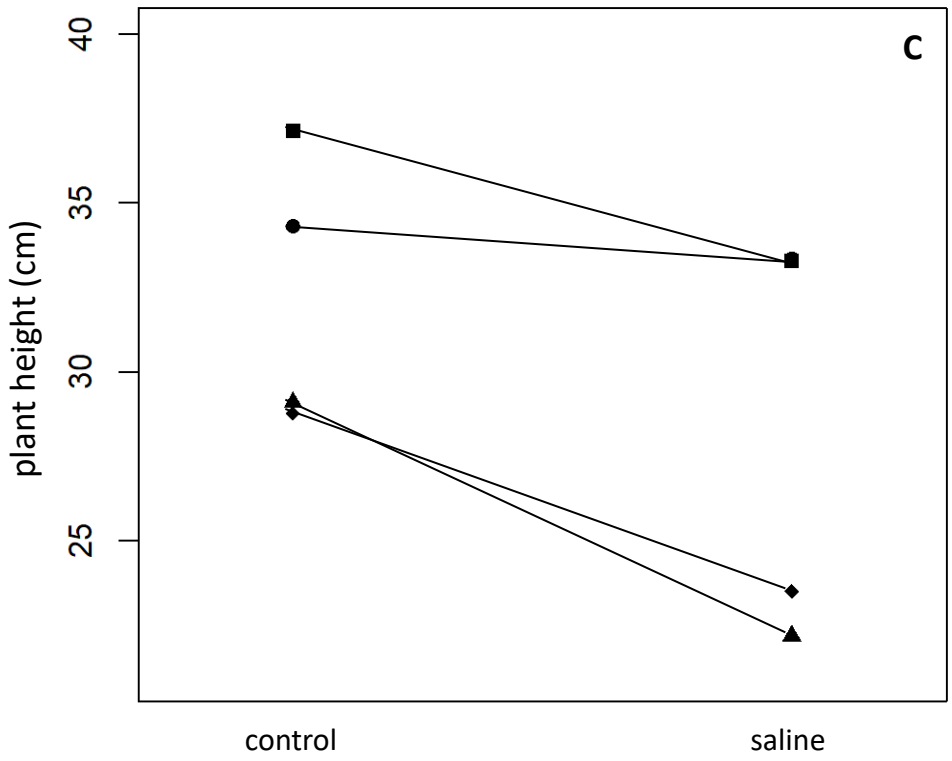
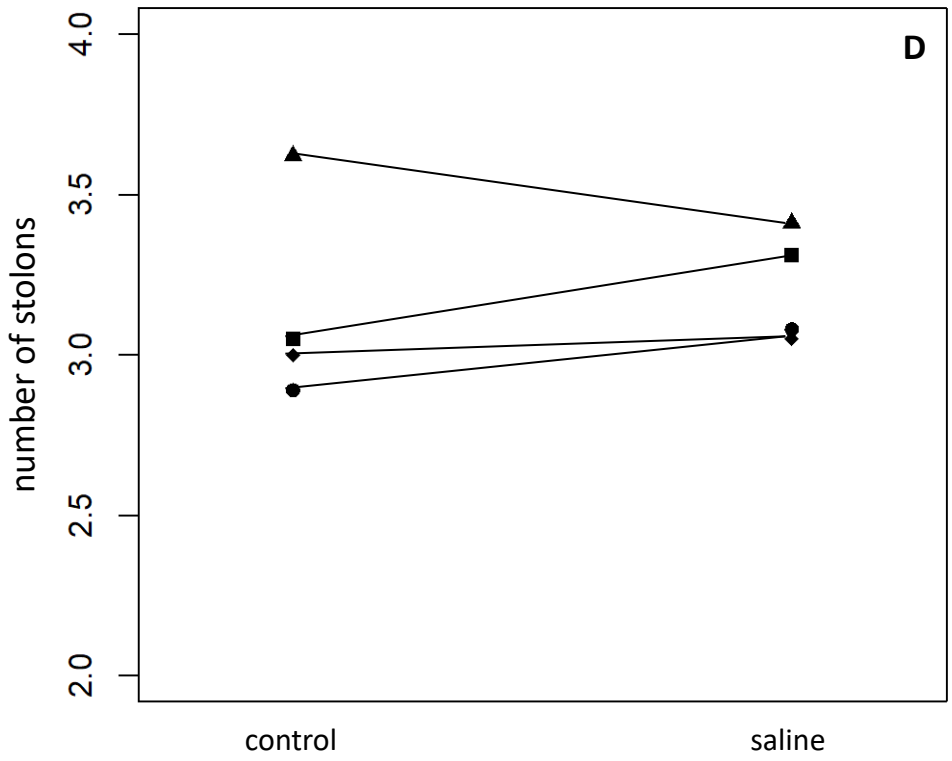

G
aboveground biomass (g)
0
2
4
6
8
s
wi
b sim
b dis
native
naturalized
invasive
NBBR

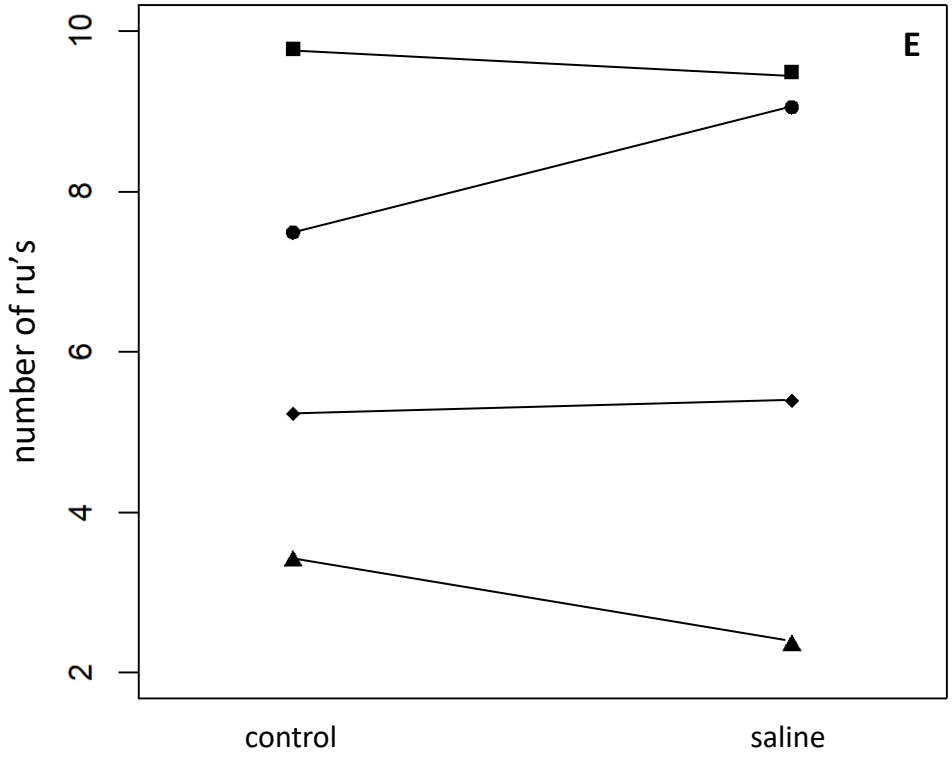
Figure 4.3 Reaction norms illustrating plasticity for six fitness traits among the four regions in two watering regimes. Diamonds represent the native region, squares the naturalized region, circles the invasive region, and triangles the NBBR population. For significant differences between regions in each treatment, see Table 12; for significant effect of treatment on each cross type within regions, see Table 13.




B
stem diameter (mm)
4.0
4.5
5.0
5.5
6.0
6.5
7.0
control
saline


C
plant height (cm)
40
35
30
25
control
saline


D
number of stolons
4.0
3.5
3.0
2.5
2.0
control
saline


E
number of ru's
2
4
6
8
10
control
saline

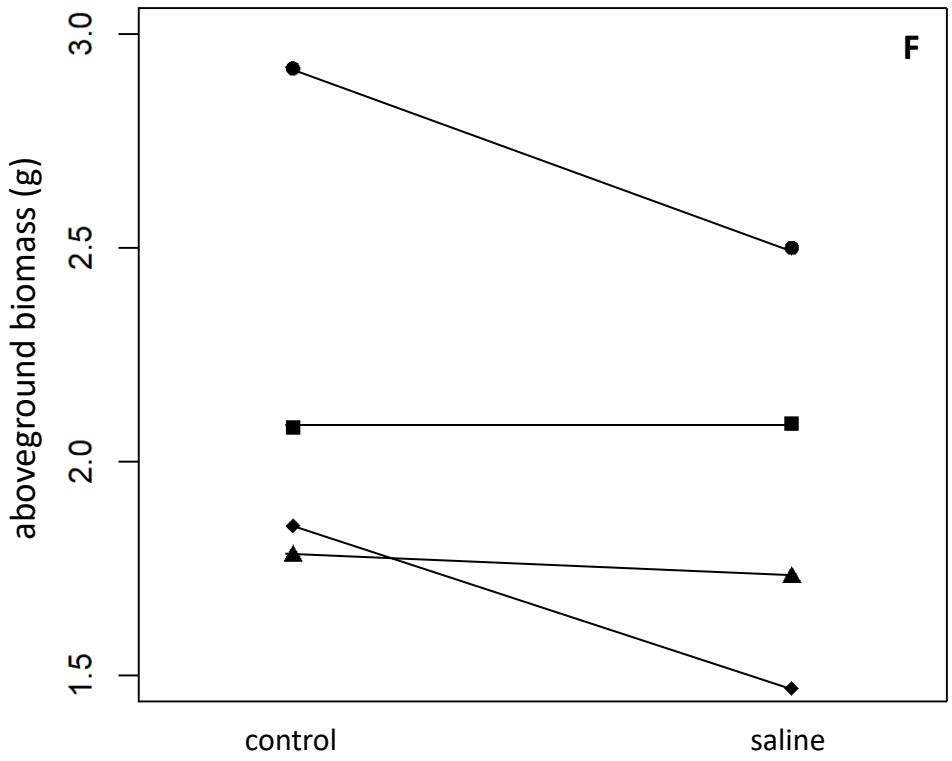
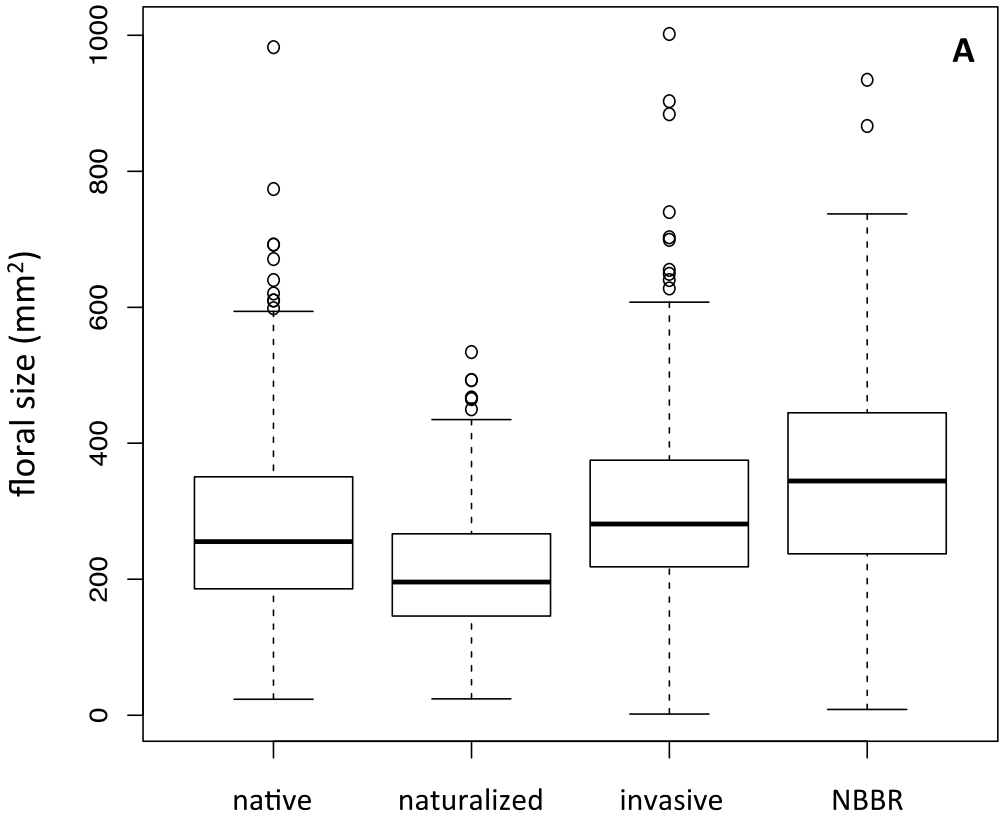
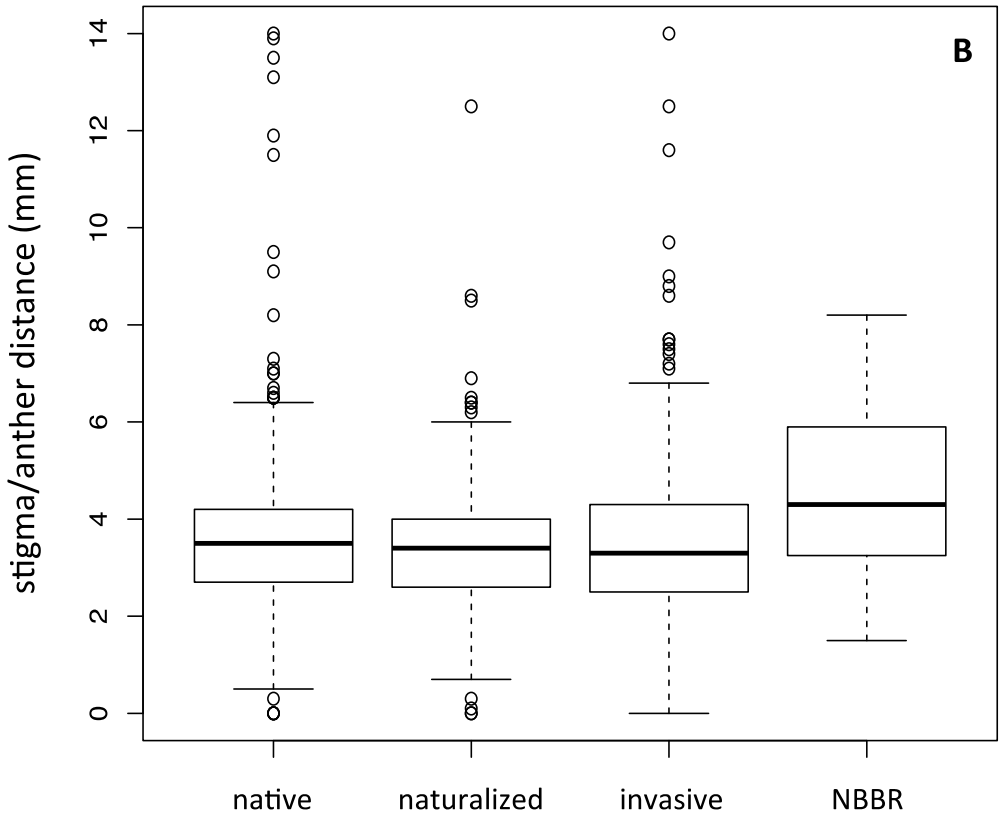

F
aboveground biomass (g)
3.0
2.5
2.0
1.5
control
saline

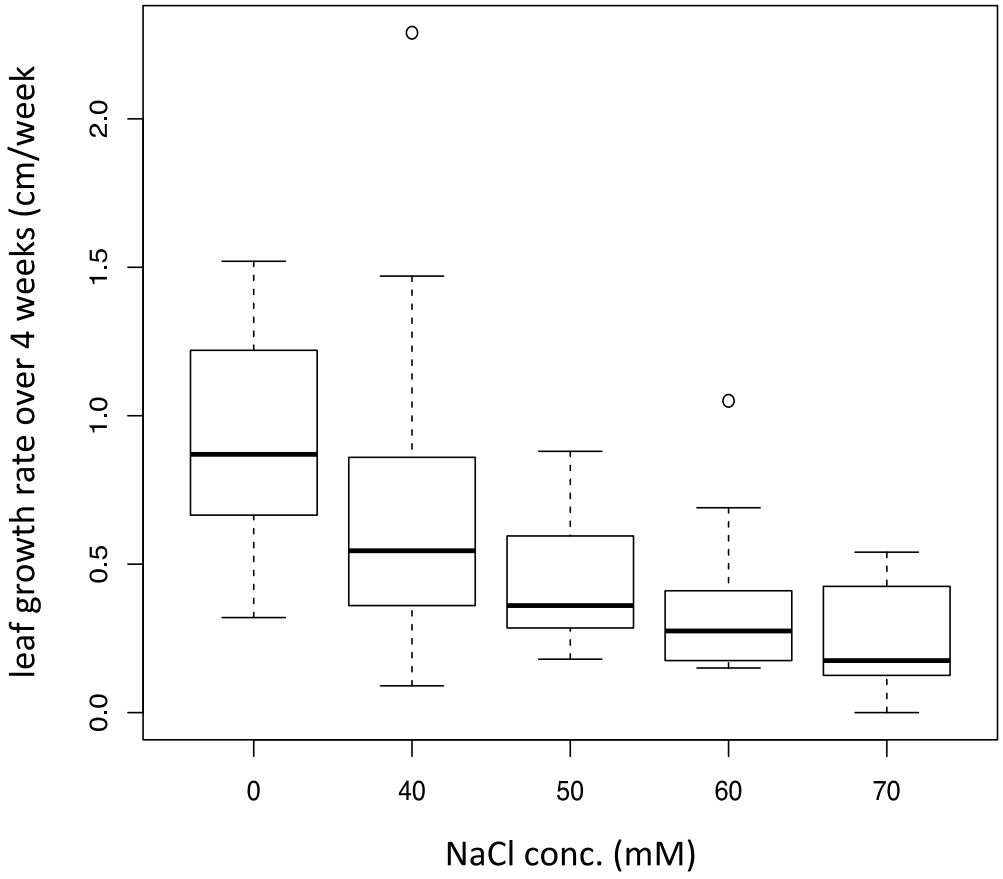
Figure 4.4 Graphs showing A) Floral size, which was the product of floral length and floral width, and B) the distance between the stigma and the closest anther. Boxes show medians and quartiles.




B
stigma/anther distance (mm)
0
2
4
6
8
10
12
14
native
naturalized
invasive
NBBR

Appendices

Appendix A. Result of pilot study conducted to determine concentration of saline solution used in fitness experiment. Sample size for each treatment is 36 randomly chosen individuals.



Appendix B. Authorship and intended target journal for contribution for each chapter.

Berg JA, Dudash MR, Zimmer EA (*in prep*) Chapter 2: Progeny array analysis to estimate outcrossing rates, inbreeding coefficients, and inbreeding depression among native, naturalized, and invasive populations of Mimulus guttatus [Phrymaceae]. *Intended for* Evolution

- Berg collected and analyzed all data. Berg wrote the manuscript with comments from Dudash and Zimmer. All authors contributed to the study design.

Berg JA, Dudash MR, Zimmer EA (*in prep*) Chapter 3: Genetic diversity and population structure among native, naturalized, and invasive populations of common yellow monkeyflower, *Mimulus guttatus* [Phrymaceae]. *Intended for* Molecular Ecology

- Berg collected and analyzed all data. Berg wrote the manuscript with comments from Dudash and Zimmer. All authors contributed to the study design.

Berg JA, Zimmer EA, Dudash MR (*in prep*) Chapter 4: Selfing, heterosis, and plasticity for fitness may facilitate establishment success in non-native populations of common monkeyflower, *Mimulus guttatus* [Phrymaceae]. *Intended for* Molecular Ecology

- Berg collected and analyzed all data. Berg wrote the manuscript with comments from Dudash and Zimmer. All authors contributed to the study design.

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