Genotypic diversity, reproductive strategies, and natural selection in non-native populations of *Mimulus guttatus*

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Statement of originality

I hereby confirm that this PhD thesis is an original piece of work conducted independently by the undersigned and all work contained herein has not been submitted for any other degree.
All research material has been duly acknowledged and cited.

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General abstract

The success of non-native species exposed to environmental conditions may depend on how the species adapt to new conditions. For this reason, non-native species offer the opportunity to understand evolutionary mechanisms such as natural selection that can promote adaptive evolution in new conditions, and also to investigate whether intraspecific admixture may serve as a stimulus for invasion by increasing fitness or a cost to fitness due to outbreeding depression. In addition, high performance of introduced species may be accomplished by a combination of multiple reproductive strategies (e.g., vegetative and sexual reproduction) that can contribute to dispersal and colonization ability. In this research, the herbaceous *Mimulus guttatus* native to North America and naturalized in United Kingdom (UK) is used to investigate: (1) the level of genotypic (clonal) diversity and genetic variation in non-native populations; (2) the effect of resource availability on the relative investment of sexual and clonal reproduction; (3) the level of phenotypic variation among non-native populations; and (4) patterns of natural selection in its introduced range, and evidence of outbreeding depression in admixed experimental populations.

The genotyping study using single nucleotide polymorphisms reveals that non-native populations show a wide variation of genotypic diversity and that the largest percentage of genetic variation is within populations either in native or introduced ranges. A common garden glasshouse experiment with non-native populations indicates that limited space intensifies the trade-off between sexual and clonal lateral spread, and suggests that populations under limited space conditions (e.g., high-density population) may have to invest less in sexual reproduction than in clonal lateral expansion. A survey of natural *M. guttatus* populations in UK indicates that production of flowers is favoured in places with low precipitation and high temperatures where production of stolons is limited in *M. guttatus*. The field experiment with F2 individuals from three crosses between introduced and native populations shows that admixed individuals from introduced populations have higher population growth rate due to increased survival, clonality, and seed production than admixed individuals from introduced and native populations, consistent with outbreeding depression. Selection through sexual fitness favours

large floral displays, large vegetative traits, clonal spread, and early flowering in the non-native range. The results presented in this thesis indicate that clonal and sexual reproduction are integrated strategies that contribute to population growth rate, and the alternative investment in both traits in different environments may contribute to the colonization of the species in different habitats. Natural selection has an important role in the naturalization of a highly diverse species such as *M. guttatus*, and intraspecific admixture is not always beneficial in the introduced range as it may result in outbreeding depression, which further suggests the potential of non-native populations to evolve by adaptation.

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Chapter 1: General introduction

1 Non-native species in the study of contemporary adaptation and evolution

Human trade has been governing the introduction of species beyond their native ranges resulting in the overlap of species distributions that naturally would not happen because of geographical barriers (Hulme 2009; Capinha et al. 2015). Once introduced, the species can become naturalized, being able to self-sustain without human intervention (Richardson et al. 2000). Naturalised species can become invasive resulting in negative impacts for the native species and ecosystem with extended consequences for the economy and human well-being. Although the magnitude of the impact on native biodiversity may depend on the non-native species life-form and ecosystem type (Hejda et al. 2009; Pyšek et al. 2012), research on plant invasions has shown that, in general, non-native species significantly reduce fitness, growth, abundance and diversity of native plants, and alter animal fitness and abundance (Vilà et al. 2011). In line with the concerns about the negative impacts, studies of biological invasions have been trying to identify traits that are associated with invasiveness of plant species with the aim to predict future invasions (van Kleunen et al. 2010) mainly directed to management programs (Drenovsky et al. 2012). However, non-native species are exposed to novel environmental conditions that may lead to evolutionary changes, which in turn may contribute to the success of introductions. Therefore, non-native species offer a great opportunity to study ecological and evolutionary processes in contemporary time.

A growing number of studies have shown rapid evolution in invasive species. A demonstration of rapid evolution is provided by Guo et al. (2014) with the invasive lineage of the wetland grass *Phragmites australis*. Comparing the North American invasive haplotype M lineage and its European ancestor under identical environmental conditions, Guo and co-authors showed that the invasive lineage exhibited different biomass allocation patterns and photosynthetic traits compared to its European ancestor group, indicating post-introduction evolution in these traits. In another example Vandepitte et al. (2014) found evidence of genetic

evolution in the herbaceous plant *Sisymbrium austriacum* by demonstrating allele frequency shifts between native and introduced populations, and in herbarium specimens over time. Many of the genetic shifts observed are not at random across the genome (as expected from genetic drift), but instead underlie flowering time, which suggests evolution by natural selection (Vandepitte et al. 2014). Evolution in introduced populations can be an adaptive response to natural selection and many studies have shown that invasive species are locally adapted as frequently as native species (reviewed in Oduor et al. 2016). Selection of traits that increase performance of invasive species can exacerbate the negative effects on native communities, particularly for highly invasive species. For example, the invasive herb *Lythrum salicaria* has adapted to latitudinal clines in North America as a result of divergent natural selection for flowering time and plant size within less than 100 years (Colautti and Barrett 2013). Therefore, understanding the process of adaptation such as natural selection can help predict how invasive species succeed and spread. More generally, studies with introduced species can give insight into how species will respond to future climate change, given that the new environmental conditions in climate and biotic interactions experienced by introduced species could be similar to the magnitude of change predicted for species following climate change (Moran and Alexander 2014).

1.1 Consequences of introduction on population genetic variation

In many cases, the introduction of a species involves only a small number of individuals resulting in a population bottleneck, a severe reduction in population size (Novak and Mack 2005). Random changes in allele frequencies from one generation, genetic drift, is exacerbated in small populations (Fisher 1930; Wright 1931; Nei et al. 1975). When a population is small, rare alleles have a higher chance to be lost from one generation to the next by genetic drift, which results in decrease of allelic diversity (Allendorf and Luikart 2007a) . An example of bottleneck effects on introduced populations is the study of the paper wasp *Polistes chinensis antennalis* native to Japan and South Korea. According to microsatellite analysis, introduced populations of this wasp species in New Zealand have reduced allelic diversity that likely happened because of bottlenecks following independent introductions (Tsuchida et al. 2014). Another consequence of genetic drift is the

loss of heterozygosity, although the effect of bottlenecks on expected heterozygosity is smaller as compared to the effect on allelic diversity (Nei et al. 1975). In small populations, however, the mating between relatives (i.e., inbreeding) can increase the proportion of homozygotes relative to heterozygotes, which can increase the probability of recessive deleterious alleles being expressed in inbred individuals, that in turn may reduce fitness by inbreeding depression (Allendorf and Luikart 2007b). As number of alleles and heterozygosity are measures of genetic diversity present within populations, reduced levels of both metrics means that bottlenecks reduce genetic diversity (Fisher 1930; Wright 1931; Nei et al. 1975). Therefore, given that natural selection and evolution needs genetic diversity, reduced genetic variation in populations that have passed through strong bottlenecks may affect the ability of an introduced species to succeed and become invasive in a new environment.

Many studies have compared the genetic diversity between native and introduced populations with the null hypothesis of lower genetic diversity in introduced populations relative to native populations. Some studies have shown loss of genetic diversity in the introduced range (Dlugosch and Parker 2008; Zhao et al. 2013; Hagenblad et al. 2015). However, other studies have shown that introduced species have similar or higher levels of genetic diversity compared to native populations (Genton et al. 2005; Erfmeier and Bruelheide 2011). Even the species that experience loss of genetic diversity are able to thrive and become invasive (e.g., Hagenblad et al. 2015). Therefore, in many introduced species genetic variation is not limiting and bottlenecks do not seem to cause a great impact on the success of introduction (Uller and Leimu 2011).

Many studies suggest that successful introductions are facilitated by the role of multiple introductions acting as a source of genetic variability (Dlugosch and Parker 2008; Ray and Quader 2014; Oduor et al. 2015). Multiple introductions from different source populations can bring new genotypes and opportunity of recombination, which may reduce genetic bottlenecks and, in turn, increase genetic variance (Rius and Darling 2014). Admixture among genotypes from different sources may increase standing genetic variation and provide the genetic material for adaptive evolution of introduced species. Comparative studies have used as evidence of multiple introductions, higher or equivalent genetic diversity of introduced populations relative to native populations and sympatric occurrence in

the introduced range of genotypes from geographically different native populations (Genton et al. 2005; Ray and Quader 2014; Oduor et al. 2015).

Neutral genetic markers such as microsatellites have been used in many studies to assess the amount of genetic differentiation of introduced species (Dlugosch and Parker 2008). However, microsatellites can present homoplasy, null alleles and variable mutation patterns among loci, which may influence common measures used to estimate genetic diversity and population structure, and conclusions about the degree of genetic differentiation in natural populations (Putman and Carbone 2014). New genetic markers such as single nucleotide polymorphisms (SNPs) are abundantly distributed throughout the genome with potentially higher resolution compared to other markers such as microsatellites (Morin et al. 2004). These features of SNPs can improve our ability to characterize and compare genetic variation as well as to develop phylogeographic analysis between native and introduced populations that may help indicate potential a region or regions of native source populations (Cristescu 2015).

1.2 Intraspecific admixture in non-native species

Intraspecific admixture promoted by multiple introduction events has been suggested to increase fitness of non-native species and, as a consequence, favour invasion (Rius and Darling 2014). Admixture can have different outcomes for offspring that depend on the resulted genetic combinations and can be magnified with increasing geographic and genetic distance between parental populations (Edmands 2009; Dlugosch et al. 2015). The positive outcome of admixture involves benefits for plant fitness that are manifested in short and long time scales (Rius and Darling 2014).

The long-term advantages of admixture are the production of novel genotypes and the increase of genetic variation that is necessary for natural selection and adaptive evolution (Hamilton and Miller 2016). For introduced species, increase of genetic variability by admixture could be particularly important since small colonizing populations can show reduced genetic diversity due to founder effects and genetic drift (Dlugosch and Parker 2008; Bock et al. 2015). At short-time scales, offspring of admixture populations can have higher fitness than parental populations as a result of heterosis (e.g., Keller and Taylor 2010; Keller et

al. 2014; van Kleunen et al. 2015; Hahn and Rieseberg 2017). The manifestation of heterosis can be explained by different genetic causes: (i) heterosis as a result of overdominance happens when heterozygous genotypes show higher fitness over homozygous genotypes for a given locus; (ii) the dominance results in heterosis when dominant alleles from one parent are favoured over deleterious recessive alleles from another parent, which can reduce inbreeding depression; (iii) and, lastly, heterosis can be manifested by an epistatic interaction of genes that favour fitness (Lynch 1991). Heterosis may be important at the initial stage of introduction as has been shown to be lost in subsequent generations because of recombination (e.g., Keller et al. 2000; Edmands 2009). Thus, admixture is a central subject in biological invasions that could increase fitness of non-native species and contribute significantly to the initial establishment of introduced populations as well as improve the capacity of range expansion (Rius and Darling 2014).

Although admixture can improve fitness, it can also have a negative impact as a consequence of outbreeding depression. Outbreeding depression can happen when admixture results in genetic costs for the offspring such as the breakdown of co-adapted genes or genetic incompatibilities that decrease fitness independently of the environment (Price and Waser 1979; Schaal and Leverich 2005). In addition, outbreeding depression can be environment-dependent in the case of admixture resulting in offspring that are less locally adapted (Verhoeven et al. 2011). Outbreeding depression happens after recombination of different genotypes from subsequent generations. For example, comparisons of fitness among parents and hybrids from different generations of intraspecific crosses (F1 and F2) in the copepod *Tigriopus californicus* showed that F1 hybrids had an increase in fitness, whereas F2 hybrids had a decrease in fitness relative to their parents (Edmands 2009). Fitness means of F1 and F2 hybrids of *T. californicus* can be explained by dominance and break down of co-adapted genes, respectively, that were both magnified by increasing genetic distance between their parents (Edmands 2009). The positive and negative effects of admixture on the fitness of non-native populations are important to understand what promotes invasions and evolution during non-native species range expansion.

Mixing of divergent gene pools in the context of biological invasions can happen under different scenarios: populations can outcross when introduced simultaneously; native populations can be introduced in different periods and make

contact after population expansion; native populations can cross to a non-native population already established, because of recurrent introductions; and admixture can happen among populations within the native and introduced range. The genetic and fitness outcomes of admixture under these different scenarios may be different and species-specific. For locally adapted native populations, it may be advantageous to limit admixture as mixing of genotypes could break adapted gene combinations and, consequently, decrease fitness. Conversely, recently introduced individuals that are not yet locally adapted in the invasive range may increase fitness by admixture, and it is suggested that intraspecific admixture is associated with fitness more in the invasive range than in the native range (Verhoeven et al. 2011). However, non-native populations can become locally adapted (Oduor et al. 2016) and admixture can disrupt local adaptation resulting in environment-dependent outbreeding depression. In *Silene vulgaris*, individuals from the invasive range presented higher fitness than native individuals and fitness was correlated with multilocus heterozygosity which was correlated with the level of admixture among invasive genotypes but not among native genotypes (Keller et al. 2014). In contrast, in an experimental study with the common ragweed, hybrid vigour was observed in the first generation offspring (F1) from parents within the native range, but no significant heterosis was observed in the F1 offspring derived from crosses between invasive genotypes. The only observed heterosis was for the F1 offspring derived from the most geographically distant populations (Hahn and Rieseberg 2017). To date, few studies have performed crosses between native and introduced populations to access fitness consequences of admixture. Van Kleunen et al. (2015), conducted a greenhouse experiment with *Mimulus guttatus* individuals and demonstrated that F1 offspring derived from crosses between native and introduced genotypes showed heterosis in terms of sexual and clonal reproduction, and biomass production compared to F1 genotypes from within and between population crosses, suggesting that ongoing introductions may increase performance of already established individuals. Although there is support that admixture increases fitness of non-native species, many studies focus on the F1 generation (e.g., Wolfe et al. 2007; van Kleunen et al. 2015; Hahn and Rieseberg 2017), which cannot account for the possibility of outbreeding depression manifested in later generations; or growing individuals under greenhouse conditions that limit measures of fitness (e.g., Bailey and McCauley 2006). Therefore, because of the short term effect of

heterosis more studies using later generations (e.g., F2) from crosses within and between ranges in combination with field estimation of fitness are needed to access the importance of intraspecific admixture for the success of non-native populations.

1.3 Adaptation following biological introductions

The introduction of species beyond their native range through human transport whether intentional or accidental usually exposes non-native species to environmental conditions that differ from the conditions experienced in the native range at some aspect (e.g., climatic conditions and different pollinators) (Holt et al. 2005). The extent to which a species is able to match the phenotype with the environment will determine the extinction or the success of the introduction. If the introduced range resembles the environments experienced by the source populations in the native range, then individuals may be pre-adapted to the new environment, possessing traits or trait combinations that may help during establishment (Guo et al. 2014). The escape of specialist natural enemies in the new environment, the increase of competitive ability as a result of less investment in costly defensive traits (e.g., Zou et al. 2008) and phenotypic plasticity (Hahn et al. 2012) can also contribute for the success of introduction. However, considering the different environmental conditions in the introduced range, natural selection can act upon individuals' phenotypes and, given sufficient genetic variation for selected traits, lead to adaptive evolution in the introduced range that may contribute to survival and range expansion.

Previous comparative studies have suggested that evolution in introduced species occurs frequently, based on significant differences in traits among populations from native and introduced ranges in common garden experiments (reviewed in Felker-Quinn et al. 2013; Turner et al. 2014). Many species have showed greater size (Blumenthal and Hufbauer 2007) and fecundity (Parker et al. 2013) in introduced populations compared to native populations. Non-adaptive evolutionary forces like founder effects and genetic drift can be involved in evolution of introduced populations. For instance, variation of some traits of *Silene latifolia* and *S. vulgaris* in introduced populations along an environmental gradient is better explained by shared history of neutral loci (for example, number of flowers

in European *S. vulgaris* and number of fruits in *S. latifolia*), but other traits such as number of leaves in *S. vulgaris* showed phenotypic differentiation along a gradient of climate as a result of adaptation (Keller et al. 2009). However, as the effects of bottlenecks seem to be of minor importance for many introduced species (Uller and Leimu 2011), natural selection could be the major force promoting phenotypic differentiation between native and non-native populations.

Studies in the introduced range have used evidence of natural selection acting on non-native populations in similar latitudinal and altitudinal clines of phenotypic variation in the native and introduced range as observed in many introduced species like *Hypericum perforatum* (Maron et al. 2004) and *Lithrum salicaria* (Montague et al. 2008). Adaptive evolution can also be detected by selective sweeps in the genome (Puzey and Vallejo-Marín 2014) and by an excess of quantitative trait variation in comparison with genetic neutral variation based on the analysis of Qst-Fst comparisons (e.g., Chun et al. 2011; Shirk and Hamrick 2014). However, phenotypic selection analysis to assess the relationship between a specific trait and fitness is a direct way to measure natural selection and determine which traits are targets of selection (Colautti and Lau 2015). Estimates of selection such as selection differentials, which measures the direct selection of a trait and correlated traits; and selection gradients, which measures direct selection of a trait after controlling for correlated traits, can be used to assess the strength and direction of natural selection (Lande and Arnold 1983). A recent meta-analysis by Colautti and Lau (2015) including invasive animal and plant species revealed that selection differentials were stronger in introduced species than in native species, but selection gradients were similar between ranges, which can be explained by changes in genetic covariances and weaker genetic constrains in invasive species. Nonetheless, Colautti and Lau's analysis is based on a small number of studies of phenotypic selection in natural populations available in the introduce range (five plants and four animal studies) compared to the same type of studies in the native range (149 studies in the native range). Hence, more studies of phenotypic selection are needed in the introduced range, given the importance of natural selection to adaptive evolution between native and introduced ranges.

1.4 The balance between sexual and clonal reproduction for plant ecological success

Many plants are able to combine sexual reproduction by production of seeds and asexual reproduction by clonal propagation that participate in the dispersal and maintenance of populations (Richards 1997). The allocation to both modes of reproduction often has different and equally important genetic and ecological consequences for plant populations. From a genetic perspective, clonal reproduction can be either disadvantageous because production of clones can decrease genetic diversity by favouring crosses among clones (Charpentier 2001) or be advantageous by preserving highly successful genotypes (Ren and Zhang 2007). In contrast to clonal reproduction, sexual reproduction promotes recombination and genetic diversity, thus the balance between both types of reproduction can modify population genetic structure (Chen et al. 2007; Pollux et al. 2007). From an ecological perspective, sexual reproduction may be important for colonization of new environments (Wilk et al. 2009) and clonal reproduction may help to overcome the difficulty of seedling establishment in unfavourable conditions such as in riparian habitats (Cronk and Fennessy 2001). The fitness gains promoted by clonal reproduction allow the dominance of clonal species in diverse habitats such as places in high latitudes and aquatic environments (Cronk and Fennessy 2001; Ye et al. 2014).

Sexual and clonal reproduction can occur simultaneously, which in many cases can result in a trade-off (e.g., Ronsheim and Bever 2000; Prati and Schmid 2000; Thompson and Eckert 2004). The availability of resources in the environment can be determinant for the expression of trade-offs. Both types of reproduction can compete for the same finite pool of resources resulting in a phenotypic trade-off, whereas when resources are plenty individuals can distribute enough resources for many traits which can hide the expression of a trade-off (van Noordwijk and de Jong 1986). A phenotypic trade-off can be an expression of a genetic trade-off that happens when a locus affects more than one trait with alleles affecting traits in opposite directions and with different fitness consequences (Roff 2002). However, even genetically based trade-offs can change the direction or magnitude under different environmental conditions that alter physiological processes, which in turn

affect trait production (Stearns 1989b). As a consequence of trade-offs and phenotypic plasticity of traits, different environments may favour one type of reproduction over the other. For instance, van Kleunen et al. (2001) detected an increase of sexual reproduction and a decrease in vegetative reproduction with increasing density in populations of the clonal herb *Ranunculus reptans.* In a study with three populations of *Dicentra canadensis,* Lin et al. (2016) demonstrated that sexual reproduction occurred with more frequency in the population located in continuous forest, while clonality was more common in populations from fragmented forests. Clonal investment in fragmented forests could be a strategy to compensate for the scarcity of mates for sexual reproduction in small fragment areas (Lin et al. 2016). Moreover, different investment in sexual and clonal reproduction can contribute to local adaptation as a result of divergent natural selection (e.g., Lowry et al. 2008). Thus, because clonal and sexual reproduction trade-off, a plant will invest in either more clonal or more sexual reproductive strategy to maximize survival and reproduction given that an environmental condition and different reproductive strategies can have consequences for population genetics, fitness and adaptation.

Clonal reproduction is often a trait observed in many invasive plant species (Silvertown 2008). The variety of environmental conditions in which widespread invasive species occur suggest that allocation to clonal and sexual reproduction varies. Indeed, for the invasive weed *Spartina alterniflora*, the relative investment in sexual and asexual reproduction changes according to inundation death (Xiao et al. 2011). Clonal reproduction decreases with inundation depth, whereas sexual reproduction increases (Xiao et al. 2011). The allocation of sexual and clonal reproduction can contribute to the dynamics of dispersal and colonization. For instance, Piquot et al. (1998) showed that sexual reproduction is favoured in newly established populations and clonal reproduction in old populations. Piquot et al. (1998) suggested that sexual reproduction is selected among populations and contribute to colonization of new sites, whereas clonality is selected within populations and contributes to population growth. The importance of clonal and sexual reproduction for dispersal, colonization and local dominance was indicated for a few invasive species (Dong et al. 2006; Kettenring et al. 2016), therefore it is important to know what conditions influence the expression of trade-offs between

sexual and clonal reproduction to understand the processes involved in the range expansion of invasive species.

1.5 Study species: *Mimulus guttatus*

1.5.1 Native *M. guttatus*

Mimulus guttatus DC. (yellow monkeyflower, Phrymaceae) is an herbaceous species within the *M. guttatus* complex (*M. guttatus* and congeneric species) that is native from western North America and distributed from Mexico to Alaska (Grant 1924; Wu et al. 2008). *M. guttatus* is a hermaphroditic and outcrossing species that is capable of self-fertilization. Ritland and Ganders (1987) observed that selfing rates vary from 28% to 66% (mean of 48%) across five populations of native *M. guttatus* from British Columbia, Canada. Most native populations occur as diploid, although Vickery et al. (1968) found that six out of 28 *M. guttatus* native populations were tetraploids. Flowers have bilateral symmetry with two pairs of anthers with different lengths that can stand above or below the stigma. Stems grow upright, can reach approximately one meter height, bear opposite leaves at each node, and produce lateral branches and flowers from meristems in the axis of the leaves (Figure 1). Some lateral branches root in the soil forming stolons that serve as vegetative reproduction. Stolons when detached from the mother plant form ramets that produce flowers in the following season.

Figure 1. An individual of *Mimulus guttatus* in the field.

M. guttatus has been the subject of ecological, genetic and evolutionary studies since 1940s, which can be attributed to the presence of key characteristics that make it a model species: Interfertile populations that are variable in terms of phenotype, life-history and life-cycle; incomplete reproductive isolation; high fertility and extensive genetic and genomic information (Wu et al. 2008). In the native range, the species occupies a large geographical area along latitudinal and altitudinal gradients (Kooyers et al. 2015) that includes extreme environments such as serpentine areas (Gardner and Macnair 2000) and cooper mine tailings (MacNair and Christie 1983). *M. guttatus* forms two ecotypes with annual and perennial populations. Annual populations occupy inland areas characterized by hot and dry summers. *Mimulus* plants in these areas die when soil dries out in midsummer. In contrast, populations of perennial plants occur close to rivers and small streams where soil stays wet year-round (Lowry et al. 2008). Genetic diferentiation in phenotype between annual and perennial populations in comonn gardens was observed by van Kleunen (2007) that demonstraded consitent genetic differences among populations and families within populations in life-history and phenotypic traits. On average, however, annual plants produce larger flowers with reduced

anther-stigma separation, more sexual reproduction and start to produce flowers early than perennial plants, which let van Kleunen suggets that contrasting diferences between populations are adaptive (van Kleunen 2007). Indeed, reciprocal transplant studies using annual and perennial populations pairs have shown that native genotypes have higher fitness than non-native genotypes, which demonstrates that plants from different ecotypes are locally adapted (Lowry et al. 2008; Hall and Willis 2006; Hall et al. 2006; 2010). For example, Hall and Willis (2006) found divergent selection and local adaptation in flowering time in annual and perennial populations of *M. guttatus*. In a reciprocal transplant experiment with a pair of annual and perennial plants in each native habitat, Hall and Willis showed that a seasonally dry inland environment at Iron Mountain, Cascades, which is inhabited by annuals favours early flowering, while late flowering is favoured in continually wet sites at Oregon`s Pacific coast, which is inhabited by perennials. Selection of early flowering in seasonally dry environments may be an adaptation to produce seeds before summer when annual individuals die due to soil drought. Recently, Friedman et al. (2015) using high throughput genome sequencing on crosses between annual and perennial parents found that flowering time and number of stolons are correlated and are affected by the same quantitative trait loci (QTLs). Shared genetic basis for flowering time and stolons showed by Friedman et al. (2015) and also involving other floral and vegetative traits (Hall et al. 2006; Mojica et al. 2012) could reflect alternative phenotypes as a result of divergent selection in different environments that contributed to the formation of annual and perennial ecotypes.

1.5.2 Non-native *M. guttatus*

Mimulus guttatus was introduced and became naturalized in eastern North America, European countries (e.g., Poland, Belgium, Germany, and United Kingdom-UK) and New Zealand (Roberts 1964; Murren et al. 2009; Tokarska-Guzik and Dajdok 2010; Vallejo-Marin and Lye 2013). According to herbarium records the first introduction of the species in United Kingdom was in 1812 (Roberts 1964; Parker et al. 1975) and, currently, the species occurs solely as a perennial herb that can be found widespread around UK by rivers, small streams and in waterlogged areas.

Introduced populations have mixed mating system (outcrossing and self-fertilizing), but to date selfing rate is unknown. Most *M. guttatus* introduced populations occur as diploid and to date there is only a recent study that confirms the presence of one mixed diploid–tetraploid population of *M. guttatus* in UK at the northern end of the range in the Shetland Isles (Simón-Porcar et al. 2017). Phenotypic comparisons between diploid and tetraploid individuals in the introduced range revealed that tetraploids have later flowering time and larger flowers, leaves and stems than diploids (Simón-Porcar et al. 2017). Studies have shown that the occurrence of *M. guttatus* in UK is mainly linked to areas with disturbed sediment, bare sediment availability, high soil moisture and open canopy, while abundance increases under low competition (Truscott et al. 2008a). Multisite comparisons in the field and experiments demonstrated that *M. guttatus* decreases species richness; thus, having a negative effect on the community, although the impact to the native plant community can be considered minor since most of the species affected by *M. guttatus* are widespread native species or other non-native species (Truscott et al. 2008b). Characteristics such as high propagule pressure (*M. guttatus* produces thousands of seeds per fruit, see chapter 5) and long release period of seeds (beginning of August to the end September) are likely to favour *M. guttatus* spread in UK, especially in populations close to rivers with high-flow events, which can be further enhanced by the dispersal of stolons with high colonization and regeneration capacity (Truscott et al. 2006).

The establishment of *M. guttatus* outside the natural range provides the opportunity to investigate how genetic composition and phenotype change in the new environment, and the process involved. Natural selection has already been shown to operate on flower size in non-native populations of *M. guttatus* from eastern North America in a phenotypic selection analysis comparing non-native and native populations in the field (Murren et al. 2009). In northern Europe, a study developed by van Kleunen and Fischer (2008) suggested that variation of *M. guttatus* in non-native regions is caused by adaptive evolutionary process based on a greenhouse experiment that showed seven non-native populations (three from New Zealand and four from Scotland) producing more floral stems as compared to 17 native populations. Puzey and Vallejo-Marín (2014) developed a whole-genome resequencing study of 10 native populations and 14 non-native populations to analyse genetic diversity and detect genomic signals of selection (all populations

but one were represented by a single resequenced genome). This study detected selection sweeps in UK not shared with native population samples, which is consistent with positive selection that could have happened in the ancestral native population or after introduction. In addition, this study showed a genome-wide reduction of genetic diversity in the introduced range and evidence that UK populations form a single and separate group from North American populations suggesting that non-native populations have a common ancestry and originated from a single introduction or few introductions of populations from the same area. Vallejo-Marin and Lye (2013) developed a genetic study using 12 microsatellites with *M. guttatus* from UK and showed that most of the genetic variation is within populations rather than between populations. These previous studies mentioned give important insights of genetic and phenotypic changes, and adaptation of *M. guttatus* outside the native range. Our study contributes to characterize phenotypic and genetic variation with a bigger sample size of *M. guttatus* within its introduced range, and also determines the role of admixture and natural selection for the success of introduction, indicating which traits have been target of selection in UK.

1.6 Research questions

The main goal of the thesis was to understand how non-native plant species adapt to new environmental conditions. As a study system, I used the herbaceous species *M. guttatus*, a native to North America that became naturalized in UK to answer the following questions in four chapters:

Chapter 2: What is the level of genetic and genotypic (clonal) variation of introduced populations in the UK?

Hypotheses: (1) Genetic variation genetic structure. A previous genetic study using 12 microsatellites detected little evidence of population genetic structure in UK populations of *M. guttatus* (Vallejo-Marin and Lye 2013). However, microsatellites may have low resolution to detect population structure compared to other markers such as SNPs (Morin et al. 2004). Thus, if low population structure is a result of low marker resolution, I predict that using SNPs will help detect such structure. In contrast, if low population structure is a consequence of a combination

of a recent introduction of *M. guttatus* in UK and extensive gene flow, I predict that SNPs will also fail to reveal clear patterns of population differentiation.

(2) Genotypic (clonal) variation. Introduced populations of *M. guttatus* reproduce both sexually and asexually (van Kleunen 2007). However, the relative importance of these two modes of reproduction is unknown. I predict that populations should combine these modes of reproduction as each of them may help population establishment at different stages. For example, seed propagules can establish after long distance dispersal and form new populations. Clonal reproduction can become more important following population establishment when intra- and inter-specific competition make seedling establishment more difficult. Hence, I expect that populations display a range of relative levels of clonal and sexual reproduction. In order to estimate the contribution of sexual and asexual reproduction, I estimated genotypic (clonal) diversity. I expect low genotypic diversity (low proportion of genets relative to the number of sampled ramets) if populations have intense clonal reproduction. If introduced populations invest more in sexual reproduction, I expect high genotypic diversity (high proportion of genets relative to sampled ramets).

In order to answer the question posed above, I genotyped 383 individuals from 10 populations from the native range in North America and 14 populations in the UK using 62 SNP markers to investigate the genotypic diversity of non-native populations and the population genetics of introduced and native populations.

Chapter 3: What is the importance of environmental factors such as nutrients and space for the expression of trade-off between clonal and sexual reproduction in introduced populations?

Hypothesis: Sexual and clonal reproduction can compete for the same pool of resources resulting in a trade-off between these traits (e.g., Thompson and Eckert 2004). A previous study has shown that in *M. guttatus* sexual reproduction occurs at the expense of clonal reproduction (van Kleunen 2007). However, trade-offs among traits are expected to be detected under low resource conditions and may change in sign and magnitude when individuals are exposed to high resource conditions. Space and nutrients are environmental factors that could influence the

investment in both traits, because bare sediment is linked to occurrence and number of patches of *M. guttatus* (Truscott et al. 2008a) and nutrients are necessary for plant development. Hence, if limited conditions of space or nutrients influence the expression of trade-offs in *M. guttatus*, I predict that under good conditions of space or nutrients the relationship between sexual and clonal reproduction will be positive and under restricted conditions sexual and clonal reproduction will trade-off.

In order to answer the question above, I developed a common garden glasshouse experiment with 712 individuals from 13 populations of *M. guttatus* from the UK. Plants were exposed to treatments with different combinations of nutrients and space availability. I measured sexual (number of flowers) and clonal reproduction (clonal lateral spread) to analyse changes in the relative investment in both traits.

Chapter 4: (i) What is the variation in sexual and clonal reproduction, as well as vegetative and flower morphology among populations of *M. guttatus* in its introduced range in the UK? (ii) What is the effect of plant density on reproductive allocation and phenotypic variation?

Hypothesis: (1) Phenotypic variation in non-native populations. In the native range, temperature and precipitation regulates soil water availability, which is the selective agent for annual and perennial populations (Oneal et al. 2014). Annual populations that are characterized by high sexual reproduction can be found in places in which soil moisture is reduced due to high temperatures and low precipitation during summer, whereas perennial populations that are more clonal than annuals can be found in places with cool temperatures that stays permanently wet during the whole year (Lowry et al. 2008). I hypothesize that if temperature and precipitation variables associated with soil moisture are the drivers of phenotypic variation in non-native *M. guttatus*, populations in places that limit clonal reproduction with high temperature and low precipitation will invest more in sexual reproduction, and populations in places where sexual reproduction is limited such as in cold places populations would invest more in clonal reproduction, reduced herkogamy and big flowers as a way to secure reproduction.

(2) Effect of population density on morphological and reproductive traits. Population density has been shown to affect plant growth and reproductive

allocation in other clonal species (van Kleunen et al. 2001). If density affects morphological and phenotypic traits, competition for resources in populations occurring at high density will affect vegetative growth and reproductive allocation. Individuals will invest less in vegetative growth, and allocate more resources either to clonal reproduction, as a strategy to increase competition ability and survival, or sexual reproduction as a strategy to increase dispersal to environments without competition.

In order to answer the questions above, I surveyed 32 natural populations (507 individuals in total) from different localities around the UK. In each population, I measured floral and vegetative traits, and estimated density by percentage of coverage of *M. guttatus* individuals and other species within $1m^2$ quadrats.

Chapter 5: (i) Does source of origin affect the fitness of admixed individuals from crosses between native and introduced populations? (ii) What is the pattern of selection acting on floral and vegetative traits in the introduced range?

Hypothesis: I hypothesize that populations of *M. guttatus* in the UK are locally adapted, therefore I expect that admixture between native and introduced populations will break down adaptations resulting in individuals with lower fitness than individuals derived from admixture among introduced populations. In addition, patterns of selection in the introduced range will favour traits associated with perenniality in *M. guttatus* such as increase clonality, later flowering time and larger size.

In order to answer these proposed questions, I generated three arrays of F2 segregant progeny of *M. guttatus* derived from crosses between an introduced population and a native population (one annual and one perennial). I planted 1188 F2 individuals in a field common garden experiment and measured population growth rates (λ) as an estimate of fitness. I also measured floral, vegetative and life-history traits to detect patterns of selection in *M. guttatus* UK.

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Chapter 2: Genetic variation and clonal diversity in introduced populations of *Mimulus guttatus* assessed by genotyping at 62 single nucleotide polymorphism loci

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Abstract

Single nucleotide polymorphisms (SNPs) are increasingly being used to study non-native populations. SNPs are relatively information poor on a per locus basis, but allow genotyping more loci than others markers (e.g., microsatellites) and have the advantage of consistent allele calls between studies. We investigated the utility of a newly developed set of SNP markers, suitable for high throughput genotyping to characterise genotypic variation and population structure in non-native populations of the facultative clonal herb *Mimulus guttatus* in the United Kingdom (UK). We analysed 62 SNP markers and using a high throughput platform genotyped 383 individuals from 10 populations from the native range in North America and 14 populations in the UK. We found wide variation in genotypic diversity within UK populations, indicating reproductive strategies that vary from mostly clonal to mostly sexual. All but one UK population were, on average, more closely related to each other than to North American populations, and the exceptional UK population showed strong affinity to native Alaskan plants. A small number of SNPs can detect patterns of clonality and broad-scale relationships between native and introduced populations. However, elucidating population structure at a finer scale will require genotyping individuals at greater depth.

2 Introduction

Traditionally, genetic markers such as microsatellites and amplified fragment length polymorphisms (AFLPs) have been used to study patterns of genetic variation within and between introduced populations of numerous taxa (Dlugosch and Parker 2008; Rollins et al. 2013). Although microsatellites are highly polymorphic, the presence of homoplasy, null alleles, relatively small numbers of loci used per study (ca. 10–20), and little consistency of allele calls between studies, can limit their utility to infer population genetic structure and diversity (Putman and Carbone 2014). The increased accessibility to other markers, such as single nucleotide polymorphisms (SNPs), is widening the genetic toolkit available to investigate the genetic properties of introduced populations.

SNPs are markers with low information content per locus (SNPs are usually biallelic), but abundantly distributed throughout the genome, which yields a broad sampling of different genomic regions (Morin et al. 2004; Helyar et al. 2011). SNPs can be more informative than microsatellites in analyses of population structure, especially when there is high population admixture (Haasl and Payseur 2010), potentially providing increased resolution to detect even low levels of population genetic differences (Brumfield et al. 2003; Morin et al. 2004). The development of new and more economic technologies for SNP genotyping (Burrell et al. 2015; Funk et al. 2016) has resulted in SNPs being increasingly applied to population genetic studies of both model (e.g., Catchen et al. 2013) and non-model organisms (e.g., Martin et al. 2016), partly because SNPs can be more easily genotyped in high throughput platforms compared to other markers such as microsatellites. The use of SNPs to investigate the population structure of introduced populations is on the rise (Cristescu 2015), but more studies are needed to determine whether SNP markers can be successfully used to elucidate changes in genetic variation and population structure at the short time scales that characterise biological invasions.

The yellow monkey flower *Mimulus guttatus* DC. (Phrymaceae) provides an ideal study system to investigate the potential of SNP markers to characterise genetic variation in introduced populations. Previous studies have shown that SNP variation is relatively high in this species, even within populations (Kelly et al. 2013; Flagel et al. 2014). In addition, the availability of a reference genome sequence for *M. guttatus* (Hellsten et al. 2013) allows designing genotyping assays

that require a priori knowledge of the DNA sequence surrounding a particular SNP. *M. guttatus* is a, mostly, diploid taxon (2n = 28), which has served as a model system in ecological and evolutionary studies in its native range for more than 50 years, and has recently become a model for studying biological invasions (Truscott et al. 2006; Murren et al. 2009; Vallejo-Marin and Lye 2013; Puzey and Vallejo-Marín 2014; van Kleunen et al. 2015). *M. guttatus* is native to Western North America, ranging from Mexico to Alaska. In the last 200 years, this taxon has been introduced to Eastern North America, continental Europe, Britain and Ireland, and New Zealand (Murren et al. 2009; Tokarska-Guzik and Dajdok 2010; Vallejo-Marin and Lye 2013). The species represents an example of a successful introduced plant with potential for rapid adaptation (Bodbyl Roels and Kelly 2011; Puzey and Vallejo-Marín 2014), and one which can alter native species richness composition (Truscott et al. 2008).

M. guttatus is widespread in the United Kingdom (UK) where it occurs as a perennial herb (Preston et al. 2002). Its populations are found in wet habitats, including bogs and river banks (Vallejo-Marin and Lye 2013) with plants capable of reproduction via sexual (seed) and asexual (vegetative propagation) means (Truscott et al. 2006). Individual plants may produce several thousand small seeds, which can be transported by abiotic (e.g., wind and water) and biotic vectors (e.g., birds and deer; Vickery et al. 1986; Truscott et al. 2006). Asexual reproduction occurs via lateral stems that root at the nodes and clonal fragments can be transported down watercourses, particularly during high-flow events, and have high regeneration and colonisation capacity (Truscott et al. 2006). The relative contribution of sexual and asexual reproduction to the composition of introduced populations has not been established yet.

To date, few attempts have been made to characterise the genetic diversity of *M. guttatus* in its introduced range. For instance, van Kleunen and Fischer (2008) used five allozyme markers to study genetic variation in seven native and seven introduced populations of *M. guttatus* in the UK and New Zealand. These authors did not find significant differences between native and introduced ranges in terms of allozyme variation. Vallejo-Marin and Lye (2013) studied 12 UK populations of *M. guttatus* using 12 microsatellite markers, and showed that ca. 50% of the genetic variation was distributed within and 50% between introduced populations. In a subsequent study, Puzey and Vallejo-Marín (2014) used genome resequencing of

10 UK and 12 populations from North America to analyse genetic diversity and selection of *M. guttatus* in the UK. All populations, except one, were represented by a single resequenced genome. This study showed a genome-wide reduction of genetic diversity in the introduced range, and identified candidate genome regions under selection in the introduced range.

Here we used a relatively small number of SNP markers designed for high throughput genotyping, to investigate genotypic (clonal) diversity and the population genetics of introduced populations of *M. guttatus*. Specifically, we analysed 383 individuals from 10 native and 14 introduced populations from the UK, using a panel of 62 biallelic SNPs. Our study addressed two main questions: (1) what is the level of genetic and genotypic (clonal) variation of introduced populations in the UK? (2) Can a small number of SNP markers be used to elucidate the genetic relationships between native and introduced populations? Our study complements previous work based on fewer markers (van Kleunen and Fischer 2008; Vallejo-Marin and Lye 2013) or fewer individuals per population (Puzey and Vallejo-Marín 2014).

2.1 Materials and methods

2.1.1 Development of SNP markers

Our initial goal was to generate a panel of SNP markers that are variable within introduced UK populations, and which could be analysed using the GoldenGate genotyping assay with VeraCode technology in the Illumina BeadXpress platform (Illumina, Sand Diego, California). Briefly, this genotyping method uses locus- and allele specific oligonucleotides to hybridise genomic DNA attached to paramagnetic particles. A subsequent PCR step attaches fluorescent labels in an allele-specific manner, and the PCR product is then hybridised onto VeraCode beads. The optical signature of the VeraCode beads can then be individually scanned and analysed in a BeadXpress Reader (Illumina 2010). This technology is a high throughput genotyping platform that can be applied over hundreds of individuals.

The GoldenGate assay requires the *a priori* identification of SNP loci and the surrounding sequence in order to develop the necessary oligonucleotides for genotyping. To identify SNPs that are polymorphic within the UK populations, we used pyrosequencing (454 GS-FLX Titanium; Roche Applied Science, Indianapolis, Indiana) of a pooled sample of 10 individuals from 10 populations distributed across the UK (Supplementary Table 1). Field-collected leaf samples from each individual were collected in plastic bags with self-indicating silica gel and sent to Ecogenic GmbH (Balgach, Switzerland), for DNA extraction, preparation of a reduced representation library, and sequencing. We obtained 49,910 reads comprising 26,032,247 bases for an approximate coverage of the sequenced *M. guttatus* genome of 0.06x (26/430 Mb). All quality filtering was applied after mapping.

Sequence data were aligned to the *M. guttatus* version 2.0 reference genome (an individual from Iron Mountain, Oregon; see www.mimulusevolution.org) using Bowtie 2 (Langmead and Salzberg 2012). Only one mapping position per read was kept, and PCR duplicates were identified and removed. We excluded positions with more than 50x coverage. After filtering, the average coverage per genotyped position was 8.87x. We searched for biallelic sites with more than one allele within the UK samples, obtaining a list of 1813 SNP candidates. We excluded SNPs located near (within 125 bp on either side of the SNP) mononucleotide repeats longer than 3 bp, and/or near microsatellites. From this subset, we selected 178 loci sampled to be as evenly distributed as possible across the 14 major linkage groups (normally *M. guttatus* has 14 chromosomes, n = 14). The selected number of loci per linkage group was chosen proportionally to the size of the linkage group. We also included six additional loci near the quantitative trait loci (QTL) for vernalisation and life history. Of these SNP loci, four were inside an inversion region known to distinguish annuals and perennials *M. guttatus* populations (Lowry and Willis 2010) and two others were close to QTLs underlying critical photoperiod and vernalisation in *M. guttatus* (Friedman and Willis 2013) (Supplementary Table 2). The subset of 184 SNP loci was then analysed for designable primers for the GoldenGate assay using Illumina software, and unsuitable loci were discarded. A designability score ranging from 0 to 1 that evaluates the quality of each SNP in the genotyping assay was given by Illumina. To select the final set of 144 loci for the SNP genotyping panel, we chose a subset with designability scores of 1 (highest),

and an overall quality score of >0.90, and randomly selected from loci meeting these criteria to reach 144, including the 6 loci near known QTLs for vernalisation and life history.

2.1.2 Population sampling

Between 2010 and 2013, we collected fresh leaves from 10 to 20 individuals in 14 perennial populations of *M. guttatus* in the UK. We collected leaves from individuals at least 1 m apart and placed them in plastic bags with silica gel. Although we did not analyse the ploidal level of each individual included in this study, a recent study indicate that all the populations analysed here, except one, were composed exclusively of diploids. The exception is the Shetland population near Quarff (QUA), which has been found also to contain autotetraploids (Simón-Porcar et al. 2017). We do not know whether autotetraploids were included among the QUA samples examined. Nevertheless, because cytotypes are partially, spatially segregated within the QUA population and the sampling for this study was done within diploid patches, the inclusion of tetraploids seems unlikely. For the native range, we selected 10 populations distributed from California to Alaska, so as to encompass as much of the range as possible. Eight of these populations have been recorded to be capable of a perennial life history (Table 1), while two others have been recorded as annuals (Table 1). For these 10 native populations, we collected leaf tissue from individuals grown at the plant growth facilities at the University of Stirling. These native individuals were obtained by germinating seeds from the *Mimulus* collection, Willis Lab, Duke University. This collection contains both field-collected seeds, and seeds obtained through a single round of self-pollination (not inbred lines) of field-collected or greenhouse-grown plants. Three native populations were composed exclusively of field-collected seeds, while seven populations consisted of a mix of field- and greenhouse-collected seeds (Table 1). In all cases, we only sampled one individual per maternal family. In total, we sampled 383 individuals from 14 populations in the UK and 10 populations from North America (Figure 1).

Table 1. Characteristics of the 10 introduced and 14 native populations analysed in this study. Life history: Perennial (P), annual (A). Source: FCL = Field-collected leaf; FCS = Field-collected seed; GCS = Greenhouse collected seed. Sample size: number of individuals genotyped, N = number of individuals successfully genotyped and analysed. # of loci: Loci amplified per individual, averaged over all individuals.

Population Code	Life history	Location	Latitude	Longitude	Source	Sample size	N	# of loci
United Kingdom								
HAM	P	Hamnavoe, Isle of Yell	60.503	-1.099	FCL	20	19	60.2
QUA	P	Quarff, Shetland	60.104	-1.226	FCL	20	20	57.3
BKN	P	Balnakeil, Sutherland	58.575	-4.767	FCL	20	20	61.5
ELP	P	Elphin, Sutherland	58.06	-5.027	FCL	20	19	57
PAC	P	River Livet, Speyside	57.354	-3.336	FCL	10	10	59.3
DBL	P	Dunblane, Perthshire	56.187	-3.965	FCL	20	20	57.7
VIC	P	Victoria Bridge, Northern Ireland	54.763	-7.453	FCL	21	21	59
COB	P	Colebrooke River, Northern Ireland	54.339	-7.359	FCL	19	19	58.2
CER	P	Cerrigydrudion, Wales	53.005	-3.549	FCL	20	18	58.3
SGI	P	Houghton, St. Gilles	52.887	0.869	FCL	20	18	56.5
BRA	P	Brampton, Norfolk	52.768	1.297	FCL	20	18	59.5
HOU	P	Houghton Lodge, Hampshire	51.096	-1.508	FCL	20	20	56.9
CRO	P	Crowan, Camborne, Cornwall	50.162	-5.293	FCL	20	20	57.6
FAL	P	Falmouth, Cornwall	50.135	-5.095	FCL	19	19	55.3
North America								
ALA	P	Port Frederick, Chichagof Island, Alaska	58.06	-135.68	FCS/GCS	9	9	57.6
WLB	A/P	Graham Island, Haida Gwaii, British Columbia	53.355	-131.933	FCS	15	15	55.1
CPB	A	Moresby Island, Haida Gwaii, British Columbia	53.171	-131.784	FCS/GCS	9	9	56.8

HOC	P	Hood Canal, Mason, Washington	47.385	-123.147	FCS/GCS	13	13	56.1
HEC	A/P	Heceta Beach, Lane, Oregon	44.135	-124.122	FCS/GCS	15	15	55.8
ANR	A/P	Angelo Reserve, Mendocino, California	39.736	-123.631	FCS/GCS	8	8	58.1
LMC	A	Lower Mendocino County, California	38.863	-123.083	FCS	15	15	56
WTB	P	Wright's Beach, Sonoma, California	38.405	-123.096	FCS	10	10	56.4
DFAL	P	Fales Hot Springs, Mono, California	38.355	-119.41	FCS/GCS	14	14	55.5
DAV	A/P	Davenport Beach, Santa Cruz, California	37.024	-122.217	FCS/GCS	6	6	58.1

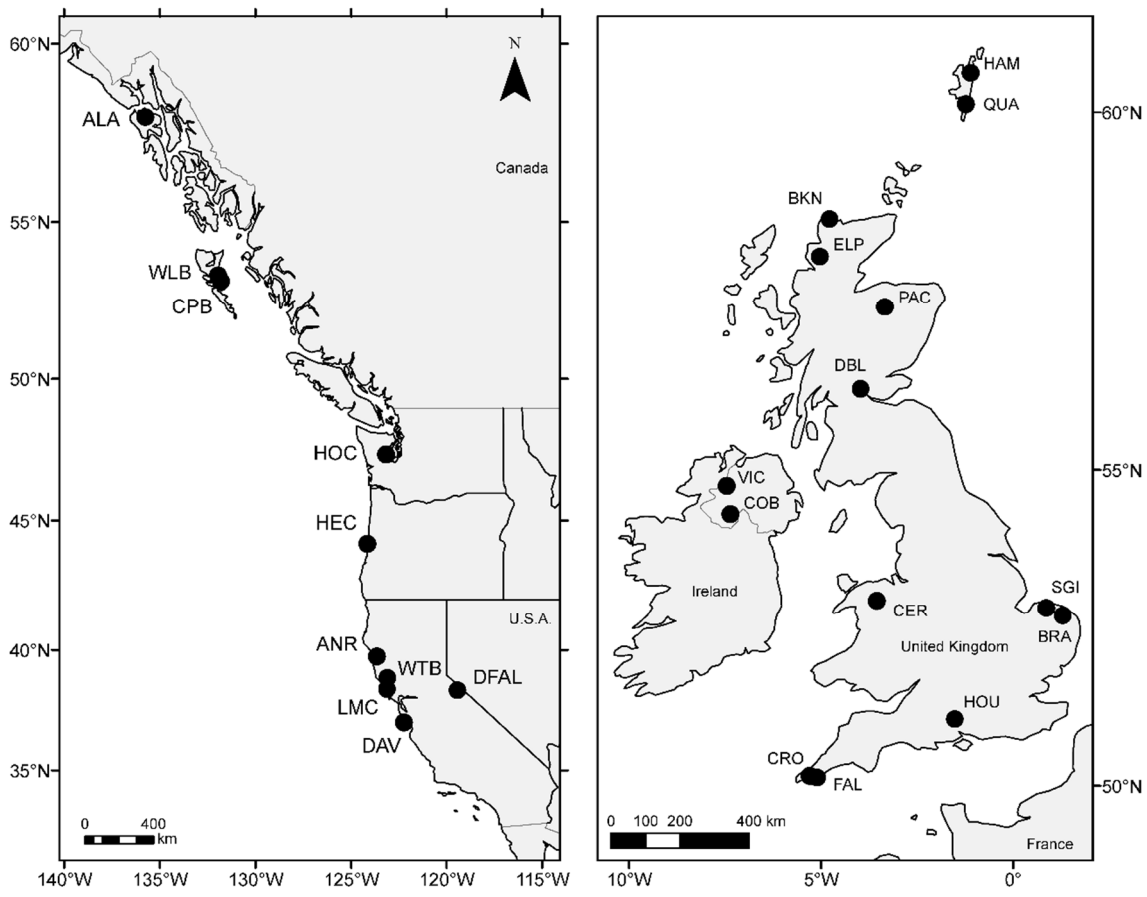


Figure 1. Populations of *Mimulus guttatus* sampled for this study in North America (left) and the UK (right). Population codes as in Table 1.

2.1.3 SNP genotyping

To obtain DNA for SNP genotyping we used dry leaf tissue from the 383 individuals. DNA was extracted using DNeasy Plant Kits (QIAGEN; Manchester, UK), and RNase A, and eluted in 50–200 µl of Tris-EDTA buffer. The concentration of double-stranded DNA measured in a fluorometer (Qubit 2.0, High Sensitivity assay, ThermoFisher Scientific) ranged from 1 to 11 ng µl−1 (total yield 45–2200 ng). To increase DNA concentration, we used a Speedvac and reduced the final volume to ca. 20 µl.

The DNA samples were genotyped in 384-plex at the University of Sheffield for 144 SNPs using our custom GoldenGate/VeraCode assay on the BeadXpress platform. Genotypes were scored in *Genome Studio v. 2011.1* (Illumina) with a Genotype Call Score (GC) threshold of 0.25, as recommended by Illumina for GoldenGate products. GC is a metric that indicates the relative

confidence of the genotype call. Poorly performing samples, that is, those with low genotype call rates, and low 10% GC scores were excluded. The size of genotype cluster boundaries (corresponding to each of the three possible genotypes at each locus) was adjusted manually as needed. Loci in which genotypes could not be clearly assigned to separate genotypic clusters were omitted (see Results for final sample sizes). The Genome Studio genotype report was edited with a custom programme in R version 3.0.3 (R Core Team 2014) to generate population genetic files for downstream analyses.

2.1.4 Analyses of genetic diversity

One of the constraints of this study is that the individuals genotyped were obtained using two different sampling strategies: Samples from UK populations were collected from adult plants directly in the field, while samples from North American populations were derived from both field-collected seeds, and from seeds obtained in the greenhouse after self-pollinating field-collected plants (Table 1). Thus, while UK samples represent genotypic and genetic variation of naturalised populations, the artificial round of selfing of some North American samples will have caused a deficit of heterozygotes, and potentially decreased allelic diversity due to the reduction in effective population size caused by inbreeding. Therefore, our analysis of genotypic diversity is restricted to UK populations. However, for illustration we also provide estimates of allelic diversity for North American populations, but keep in mind that these likely represent a lower bound of the diversity of native populations.

2.1.5 Identification of unique multilocus genotypes (MLGs) and genotypic (clonal) diversity in introduced UK populations

In clonal organisms, such as in *M. guttatus*, asexual reproduction can result in one genetic individual (genet) being represented by multiple physiologically independent units (ramets) (Harper 1977). As a first step in characterising the genetic diversity of introduced populations, we identified unique multilocus genotypes (MLGs). A shared MLG among multiple individuals within a population can be used to infer clonal membership to the same genet. To identify MLGs, we

used the statistical package *poppr v. 2.2.1* (Kamvar et al. 2014; Kamvar et al. 2015) in R. The minimum genetic distance to distinguish different MLGs was calculated using the cutoff_predictor function and a relative dissimilarity distance matrix (threshold = 0.5) (Kamvar et al. 2014). Requiring a minimum genetic distance before distinguishing different MLGs allows the same MLG to differ slightly due to, for example, genotyping error. Using the identified MLGs, we estimated the following components of genotypic (clonal) diversity in each population: Shannon's diversity index (*H*), Simpson's index (λ), genotypic evenness (E), and the ratio of MLGs per individual (genets to ramets; G:N).

2.1.6 Genetic diversity of introduced populations in the UK

For the field collected samples of *M. guttatus* in the UK, we estimated average allelic richness per population using a rarefaction approach to correct for differences in sample size using the R package *diveRsity* (Keenan et al. 2013). We also estimated observed heterozygosity (*Ho*), expected heterozygosity (*He*), and the inbreeding coefficient (*Fis*). Confidence intervals for *Fis* were obtained using 999 bootstrap replicates. We calculated these estimates for two data sets: one containing all individuals, including multiple instances of the same MLG, and a second data set including only unique MLGs (the "clone-corrected" data set). The population from Balnakeil, North West Scotland (BKN) was excluded from the second analysis as it consisted of only one MLG (see Results).

For illustration purposes, we also calculated allelic richness, *Ho*, and *He* for a data set including all individuals from the native North American populations. Inbreeding coefficients were not calculated for these populations. As expected, each of these seed-derived individuals had unique MLGs within populations (data not shown). Comparisons of allelic richness and heterozygosity between regions (native vs. introduced) should be treated with caution, keeping in mind that some individuals from the native range are the product of artificial self-fertilisation.

2.1.7 Population genetic structure and relationships between native and introduced populations

To determine the genetic relationships among native and introduced populations, as well as the distribution of genetic variation within regions, we used analyses that do not require assumptions about specific population genetic models, such as Hardy–Weinberg equilibrium, or reproductive modes, such as no asexual reproduction. First, we calculated pairwise genetic distances between populations using Prevosti's distance, with populations nested within region (native vs. introduced), as implemented in *poppr*. We then used the population distance matrix to calculate a neighbour joining (NJ) tree, and assessed the support for each node, using 1000 bootstrap replicates by using the package *ape* (Paradis et al. 2004). Second, we carried out a principal component analysis (PCA) of individual genotypes using the *glpca* function from the *adegenet* package (Jombart and Ahmed 2011). The PCA loadings of the first two principal components were then averaged across individuals within populations to create a population-level PCA graph. To partition the genetic variability among individuals, populations, and regions, we carried out a nested Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) using *Genalex* ver. 6.502 (Peakall and Smouse 2012), with statistical significance based on 999 permutations. AMOVA was also computed separately for UK and North America data sets to estimate the genetic variability within each region. All analyses were conducted both on the full and the clone-corrected data sets.

2.2 Results

From 144 genotyped SNPs, 79 could not be reliably genotyped (i.e., they could not be assigned to separate genotype clusters during analysis) and were excluded. Three monomorphic loci were also removed (SNPs: 7_14497816; 13_6989143, and 7_17992333), yielding a final number of 62 successfully genotyped polymorphic SNP loci, of which 2 were near the selected QTLs and 3 within the chromosomal inversion in linkage group 8 (Supplementary Table 2). Analyses of the data excluding the loci in the inversion region and near QTLs did not qualitatively change our population genetic results (data not shown). The lack of a strong signal from SNPs near QTLs is perhaps not very surprising. Linkage disequilibrium in *M. guttatus* decays rapidly (Brandvain et al. 2014), and our SNP loci may be far enough

from the focus of selection that they effectively behave as the other sampled SNPs. Even loci within the inverted region may have had enough time to recombine (in individuals homozygous for the inversion) as the inversion is old and covers thousands of base pairs (Twyford and Friedman 2015). Therefore, the results presented below were obtained using all 62 SNP loci. From the 383 individuals analysed, 8 did not amplify at any loci and were excluded. The final data set consisted of 62 SNPs and 375 individuals (Table 1). The average number of loci amplified per individual ranged between 55.1 (WLB, Queen Charlotte Islands) and 61.5 (BKN, North West Scotland) (Table 1).

We identified 270 unique MLGs among the 375 individuals analysed in both native and introduced ranges. In the UK, the overall ratio of MLG per individual genotyped was 62% (G:N; 163:261), while in North America this ratio was 95% (109:114; two MLGs occurred in both North American and UK populations). Table 2 shows genotypic (MLG) diversity calculated separately for each population in the UK range only. Population BKN from Northern Scotland had the lowest genotypic diversity, with a single MLG identified among 20 sampled individuals (G:N = 0.05). Other populations with low genotypic diversity were ELP and BRA, which had G:N ratios of 32% and 56%, respectively. In contrast, populations DBL, HOU, PAC, QUA, and VIC had G:N ratios of 90% or higher, as well as relatively high values at other diversity indices (Table 2). To the extent that unique MLGs represent individual genets, our results indicate that UK populations vary widely in the relative contribution of sexual (seed) and asexual (clonal) reproduction, ranging from highly clonal (e.g., BKN) to highly sexual (e.g., DBL).

Table 2. Genotypic diversity in introduced populations of *M. guttatus* in the UK. Unique multilocus genotypes (MLG) were identified using a minimum genetic dissimilarity threshold of 0.5. Assuming that individuals belonging to the same MLG in a given population belong to the same genet, the ratio of G:N (number of MLGs [G, genets] divided by the number of individuals [N, ramets] analysed) estimates the degree of clonality in the population. A value of one indicates purely sexual reproduction, while a value of near zero indicates purely clonal reproduction.

Population	Shannon (H)	Simpson (λ)	Evenness	G/N
BKN	0	0	--	0.05
BRA	1.89	0.77	0.58	0.56

CER	2.63	0.92	0.89	0.83
COB	2.77	0.93	0.90	0.89
CRO	2.69	0.93	0.90	0.80
DBL	3.00	0.95	1.00	1.00
ELP	1.12	0.51	0.51	0.32
FAL	2.58	0.91	0.82	0.79
HAM	2.63	0.92	0.89	0.79
HOU	2.93	0.95	0.97	0.95
PAC	2.16	0.88	0.95	0.90
QUA	2.93	0.95	0.97	0.95
SGI	2.63	0.92	0.89	0.83
VIC	2.91	0.94	0.96	0.90

The 14 UK populations analysed here had an average allelic richness of 1.74 ± 0.04 (mean ± S.E.) when all individuals were included, and 1.75 ± 0.04 when only unique MLGs were analysed (Table 3). Mean observed heterozygosity ranged between 0.16 (for population HOU) to 0.60 (for BKN), with an average across populations of *Ho* = 0.31 ± 0.03 (*Ho* = 0.26 ± 0.02, when calculated for unique MLGs only). Average gene diversity (*He*) across UK populations was 0.32 ± 0.02 (range 0.14–0.39; Table 3). The mean inbreeding coefficient (*Fis*) calculated using all UK individuals was 0.09 ± 0.01 (*Fis* = 0.13 ± 0.02, when only unique MLGs are included). Individual populations showed significant deviations from Hardy–Weinberg in *Fis* values, from heterozygote excess (BKN, BRA, and ELP) to heterozygote deficit (CRO, DBL, HOU, and QUA; Table 3). Negative *Fis* values (heterozygote excess) are unlikely to be simply a consequence of the relatively small number of individuals sampled per population, but instead they may reflect excess heterozygosity associated with reproduction via clonality. Indeed, negative *Fis* values disappeared in all but one population (ELP) when analysing unique MLGs only, which suggests that excess heterozygosity in some populations is inflated by clonal reproduction. For native North American populations, allelic richness and average gene diversity across populations were very similar to UK populations (allelic richness = 1.76 ± 0.01; He = 0.31 ± 0.01), while, as expected, observed heterozygosity was lower (*Ho* = 0.13 ± 0.01, range 0.08–0.18).

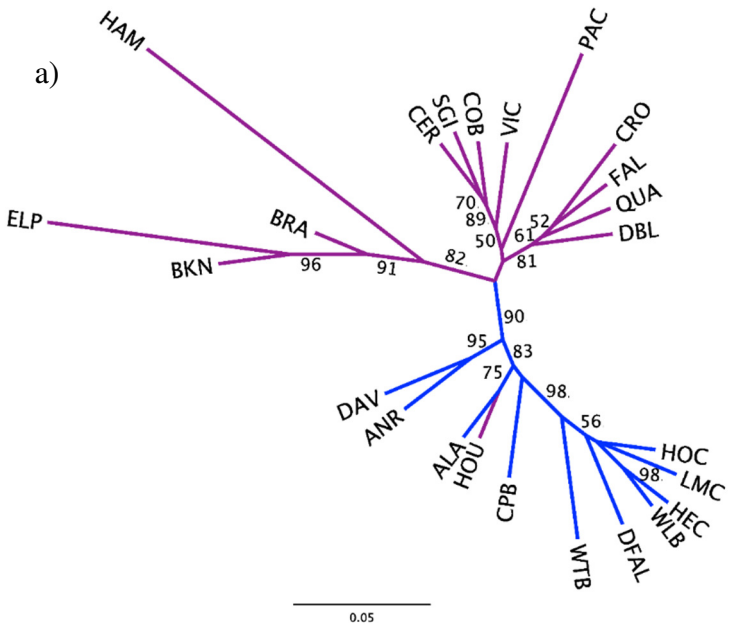
Table 3. Genetic diversity estimates of *M. guttatus* populations in the non-native range in the United Kingdom. Estimates were calculated for the full data set, as well as using "clone-corrected" data, which includes only unique multilocus genotypes

(MLGs); estimates obtained using only unique MLGs are shown in parenthesis. For illustration, diversity estimates are also shown for North American samples, although these likely represent a lower bound estimate of the diversity of native populations due to the additional generation of selfing used to generate these samples. Allelic richness was calculated using a rarefaction method to account for different sample sizes between populations. An asterisk for the *Fis* coefficient indicates that the 95% confidence interval calculated using 999 bootstrap replicates does not overlap zero. Notice that some indices are not calculated for population BKN as it consists of a single MLG.

Population	**Allelic richness**		H_o		H_e		F_{is}	
United Kingdom								
BKN	1.58	--	0.6	--	0.3	--	-1.00*	--
BRA	1.78	(1.8)	0.47	(0.35)	0.35	(0.35)	-0.24*	(0.02)
CER	1.82	(1.81)	0.37	(0.31)	0.36	(0.35)	0.00	(0.11)
COB	1.81	(1.80)	0.32	(0.28)	0.36	(0.35)	0.10	(0.19)
CRO	1.82	(1.83)	0.23	(0.23)	0.34	(0.36)	0.32*	(0.33*)
DBL	1.85	(1.84)	0.23	(0.23)	0.36	(0.36)	0.31*	(0.31*)
ELP	1.29	(1.38)	0.24	(0.21)	0.14	(0.15)	-0.44*	(-0.30*)
FAL	1.83	(1.80)	0.31	(0.27)	0.35	(0.33)	0.08	(0.17*)
HAM	1.60	(1.62)	0.26	(0.26)	0.24	(0.25)	0.10	(0.11)
HOU	1.82	(1.81)	0.16	(0.16)	0.36	(0.35)	0.53*	(0.54*)
PAC	1.62	(1.62)	0.20	(0.2)	0.24	(0.25)	0.15	(0.16)
QUA	1.84	(1.83)	0.24	(0.24)	0.35	(0.35)	0.29*	(0.30*)
SGI	1.81	(1.80)	0.33	(0.29)	0.35	(0.34)	0.11	(0.20)
VIC	1.86	(1.86)	0.35	(0.33)	0.39	(0.38)	0.09	(0.15)
Mean ± SE	1.74 ± 0.04	1.75 ± 0.04	0.31 ± 0.03	0.26 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.09 ± 0.01	0.13 ± 0.02
Population	**Allelic richness**		H_o		H_e			
North America								
ALA	1.80		0.15		0.34			
ANR	1.80		0.18		0.32			
CPB	1.84		0.14		0.34			
DAV	1.77		0.17		0.32			
DFAL	1.70		0.08		0.29			

HEC	1.71	0.11	0.29
HOC	1.77	0.12	0.31
LMC	1.71	0.10	0.30
WLB	1.74	0.12	0.32
WTB	1.76	0.12	0.31
Mean ± SE	1.76 ± 0.01	0.13 ± 0.01	0.31 ± 0.01

The NJ trees obtained using a matrix of pairwise genetic distances are shown in Figure 2. Both trees, obtained with either the full data set (Figure 2 a) or using only unique MLGs (Figure 2 b), placed native and all but one of the introduced populations in separate clades. The exception was the introduced population HOU (Hampshire), which was nested within the clade containing native populations.



b)

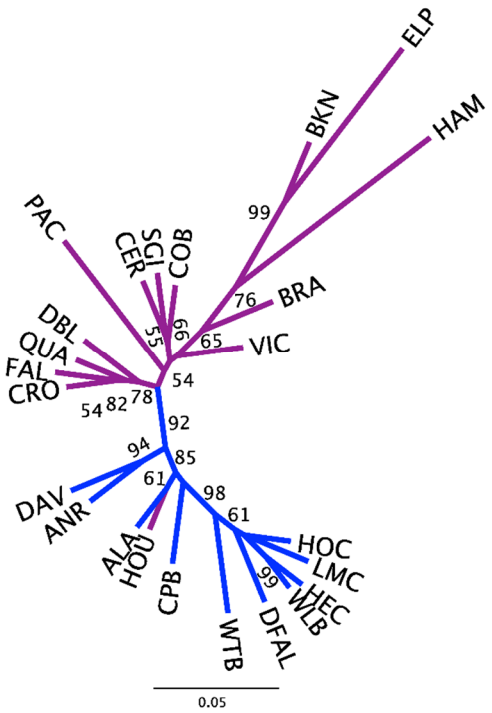


Figure 2. Neighbour Joining (NJ) trees depicting the relationships among 10 native North American populations and 14 introduced populations in the United Kingdom. The cladogram was built using pairwise genetic distances (Provesti's distance) between populations. a) Tree estimated using all 375 individuals from 24 populations. b) Tree estimated using only 270 unique multilocus genotypes (MLGs).

The results of the PCA conducted using unique MLGs also showed a clear separation between most native and introduced populations along the first two principal components (Figure 3). Again, the exception was HOU, which was placed closer to native populations (ALA, Chichagof Island, Alaska). Interestingly, introduced populations showed a wider spread over the two first principal components, while native populations were only partially differentiated in the first, but not in the second principal component (Figure 3). Together, these results indicate that the 62 SNP loci analysed here have limited resolution to detect population differentiation within the native North American range, but are sufficient to distinguish between most native (North American) and introduced (UK) populations.

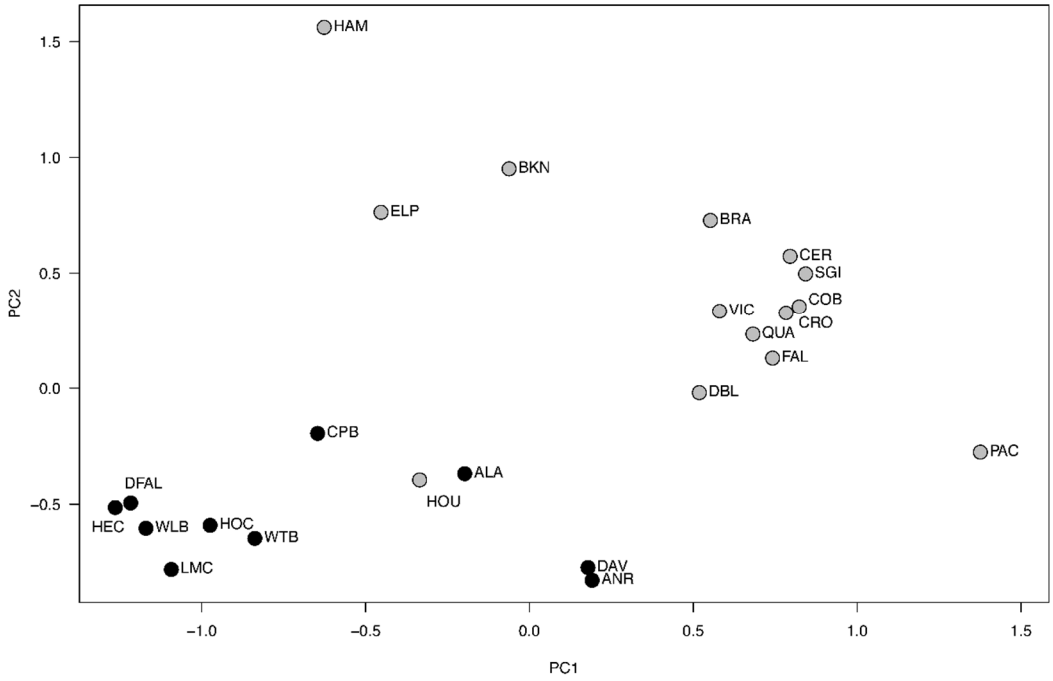


Figure 3. Relationship among *M. guttatus* populations in both native (black symbols) and introduced ranges (grey symbols) as inferred from principal component analysis (PCA) of the clone-corrected data set. Data points represent population averages of the first two principal components (PC1, PC2).

AMOVA on the full data set showed that 11% of the genetic variation occurred between native and introduced regions, and 10% of the genetic variation occurred among populations within regions (Table 4). Most genetic variation in our data set occurred within individuals (47%), followed by among (32%) individuals. The AMOVA on unique MLGs showed similar results, although in this case, variation among individuals explained a slightly larger proportion of variance than variation within individuals (45% vs. 39%, Table 4). When the AMOVA was conducted separately for each region, we found qualitatively similar patterns, with the UK showing 63% (52% for the analysis with unique MLGs only) of the variation within individuals, 22% (39%) among individuals, and 15% (9%) among populations. For North American populations, the variance partitioning was 71% within individuals, 27% among individuals, and only 2% among populations (Table 4).

Table 4. Nested analysis of molecular variance (AMOVA) of *M. guttatus* individuals from the UK and North America. Values in parentheses indicate estimates based on the analysis with only unique multilocus genotypes ("clone-corrected" data set). All North American individuals belonged to unique multilocus genotypes. All variance estimates are statistically significant (P< 0.01; P-value based on 999 permutations).

Source of variation	Degrees of freedom	Variance Estimated	% of variance	P-value
United Kingdom and North America				
Among Regions	1	1.681	11%	0.001
Among Populations	22	1.638	10%	0.001
Among Individuals	351	4.993	32%	0.001
Within Individuals	375	7.443	47%	0.001
United Kingdom only				0.001
Among Populations	13	2.192	15%	0.001
Among Individuals	247	3.156	22%	0.001
Within Individuals	261	9.155	63%	0.001
North America only				
Among Populations	9	0.239	2%	0.002
Among Individuals	104	9.282	27%	0.001
Within Individuals	114	3.522	71%	0.001

2.3 Discussion

Our study on *M. guttatus* showed that a relatively small subset of 62 SNP markers analysed in 375 individuals can be used to genetically distinguish most native and introduced populations, but is insufficient to detect finer patterns of genetic structure within regions. At a broader scale our genetic analysis support the hypothesis of Puzey and Vallejo-Marín (2014) that most UK populations share a similar origin and may have been introduced from a geographically limited subset of native populations. Most UK populations (except HOU) form a separate

clade/group in the NJ and PCA analyses (Figures 2 and 3) and thus seem to be have been derived from the similar material. Previous historic records and genomic work suggest that the origin of UK plants is from somewhere in the North Pacific (Northern Canada or Alaska; Puzey and Vallejo-Marín 2014). Compatible with this speculation, the HOU population shows a strong affinity with ALA, the Alaskan population from Chichagof Island, which is also relatively close to the other UK populations in the multivariate analysis (Figure 3). Thus, although we cannot show that all UK populations come from a single locality, we suggest that their origin is broadly the same geographic area, and therefore they represent a subset of the overall range of distribution of *M. guttatus.*

We found that in the introduced range, most genetic diversity at the studied markers occurred within populations (85–91%), and only a small fraction could be attributed to variation among populations (9–15%) (Table 4). Similarly, population structure detected with this SNP panel within the native range was weak, with only 2% of the variation occurring between populations. The lower resolution of SNP markers in North America compared with the UK could be partly explained by ascertainment bias as the design panel included mostly UK populations (e.g., McTavish & Hillis 2015). Ascertainment bias could be avoided in future studies by including a broader sample of individuals in the design panel, or using genotyping techniques that do not develop markers a priori for specific subsets of individuals (e.g., genotype by sequencing; Narum et al. 2013).

The lack of detectable population structure within native or introduced regions may reflect, in part, the number and type of markers used. Previous studies that used thousands of SNP markers (Brandvain et al. 2014; Puzey and Vallejo-Marín 2014; Twyford and Friedman 2015) have detected some geographic structure in the native range. Similarly, studies that used more variable length polymorphic markers (microsatellites and intron-based markers) have indicated relatively high levels of population structure in North America as measured with both AMOVA (39% of the variation occurs between populations) and Fst (average pairwise Fst values of 0.32 and 0.55 for annual and perennial populations, respectively; Lowry et al. 2008). In the introduced range, the ability to detect population differentiation also seems to depend on marker type. A study of seven *M. guttatus* introduced populations from the UK and New Zealand, using five allozymes markers, found small and non-significant differentiation within the introduced range (Scotland: Fst

= 0.05; New Zealand: Fst = 0.12) (van Kleunen and Fischer 2008). In contrast, a study that included 12 UK populations of *M. guttatus* genotyped at 12 microsatellite and intron-based markers detected relatively high population differentiation (AMOVA: 47% variation between populations; ΦST = 0.468; (Vallejo-Marin and Lye 2013). Therefore, we expect that detecting finer population structure within geographic regions by using SNP markers will require larger numbers of loci than the 62 analysed here, particularly given the high dispersal potential of *M. guttatus* by both seeds (Levine 2001) and vegetative propagules with high colonisation rates (Truscott et al. 2006), as well as the short time since the introduction of *M. guttatus* to the UK (ca. 200 years).

The pattern of genetic variation in the native and invasive range can influence invasion dynamics (Dlugosch et al. 2015). Both previous work and our results in *M. guttatus* indicate that individual populations contain a significant amount of the total genetic variation (e.g., 52% of the variation in North America is contained within populations; Lowry et al. 2008). Given the large amount of variation within populations in the native range, the introduction of *M. guttatus* into the UK could have brought a significant fraction of the standing genetic variation, even from a single introduction event, resulting in relatively diverse introduced populations. In fact, the resequencing study by Puzey and Vallejo-Marín (2014) showed that introduced populations still harbour approximately 50% of the variation observed in the native range. Genetic diversity within introduced populations can enable rapid evolutionary responses to new environments from standing variation (Barrett and Schluter 2008).

We found that introduced UK populations of *M. guttatus* range from entirely clonal to entirely sexual; however, on average, most populations rely to some extent on combining sexual and asexual reproduction. In one extreme, all individuals in the highly heterozygous population BKN (Table 3) belonged to the same MLG, a pattern that is consistent with the hypothesis that all sampled individuals belong to a single clonally propagated genet. At the other extreme, all DBL individuals were assigned to distinct MLGs suggesting that all reproduction was sexual, at least at the spatial scale examined here (individuals separated by >1 m) (Table 2). Nevertheless, most populations had G:N ratios consistent with partial clonality (Table 2), as is common in plants capable of vegetative propagation (Vallejo-Marín et al. 2010). In the UK, *M. guttatus* often occurs in riparian habitats, and previous

work has shown that stem fragments can be dispersed during high-flow events (Truscott et al. 2006). The determinants of the contribution of sexual vs. clonal propagation to the growth of individual populations are unknown, but it is possible that seed and vegetative propagules play different roles during the establishment and spread of local populations. For example, the small seeds of *M. guttatus* could allow long-distance dispersal events, while clonal propagation could facilitate local spread and ecological dominance (Pysek 1997; Truscott et al. 2006). The ability to combine clonal and sexual reproduction may facilitate invasions (Liu et al. 2006). For example, in *Phragmites australis* (Cav.) Trin. ex Steud., sexual reproduction facilitates dispersal and colonisation, while clonality allows the expansion of local populations (Kettenring et al. 2016). The ability to combine both modes of reproduction may be one of the reasons why *P. australis* is one of the most successful invasive plants in North America. Therefore, the extent of clonality in individual populations does not only affect the genotypic diversity in the introduced range, but could also reflect the ecological dynamics of populations at different stages of the colonisation process.

2.4 Conclusions

Although SNPs can be highly informative for the detection of population genetic structure in invasive taxa (e.g., Puzey and Vallejo-Marín 2014), the number of loci genotyped can limit the ability to uncover patterns of genetic structure within geographic regions. The relatively high levels of genetic diversity in the introduced range of *M. guttatus* may be explained, in part, by the large fraction of total variation contained in single populations in the native range. It would be important to establish if introduced populations of *M. guttatus* in other non-native regions (e.g., Faroe Islands, Continental Europe, and New Zealand) are similarly genetically diverse. Our genotyping approach using a small SNP panel allowed us to genotype a much larger number of individuals (375) than would be possible in most ecological studies using other SNP genotyping approaches such as whole-genome sequencing. However, new tools such as RAD-seq and genotyping-by-sequencing (GBS) are promising for studies of population diversity and structure as they have the advantage of being simple and highly reproducible methods of reduced

representation library that can be used across a large number of individuals to generate thousands of SNPs (Narum et al. 2013). Moreover, the discovery of SNP by GBS is developed in the population of interest and not *a priori* in a subset of individuals, which minimizes ascertainment bias (Heslot et al. 2013). These techniques will be useful to investigate the fine-scale population structure of other invasive species, particularly as they can also be applied to non-model taxa that lack previously available genomic data.

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2.7 Supplemental data

Supplementary Table 1. Samples pooled and sequenced to generate the SNP genotyping panel.

Individual	Population	Latitude	Longitude
10-AYR-10	Ayr, River Ayr	55.4612	-4.625
10-CER-10	Cerrigydrudion, Denbigshire, Wales	53.0059	-3.549
10-DBL-20	Dunblane, Perthshire	56.1886	-3.966
10-HOU-17	Houghton Lodge, Hampshire	51.0969	-1.508
10-QUA-47	Quarff, Shetland	60.1045	-1.227
10-TOM-23	Tomintoul, Moray	57.2549	-3.368
12-CRO-5	Crowan, Cornwall	50.1629	5.2933
12-PAC-39	Packhorse Bridge, River Livet, Speyside	57.3545	3.3363
12-TRE-17	Tremar Coombe, Cornwall	50.4980	4.4647
12-VIC-18	Victoria Bridge, Northern Ireland	54.7633	7.4538

Supplementary Table 2. List of SNPs tested in this study. The Table includes those loci used in the final analysis of 62 SNPs ("Loci included in the analysis"), as well as those loci that were tested but could not be successfully genotyped ("Loci not used in the analysis"; see Methods). Scaffold and position of each loci in the *M. guttatus* reference genome v2.0 are provided (scaffold number = linkage group number). N = number of individuals successfully genotyped at each locus. SNPs inside the inversion region or near QTLs are indicated with suffixes.

Locus	Scaffold	Position	N
Loci included in the analysis			
1_1560357	1	1560357	359
1_3311498	1	3311498	367
1_9521371	1	9521371	318
2_1299261	2	1299261	363

2_2689280	2	2689280	362
2_3327931	2	3327931	364
2_6267916	2	6267916	352
2_10677833	2	10677833	358
2_17189629	2	17189629	372
2_19077691	2	19077691	334
3_4782783	3	4782783	374
3_8891171	3	8891171	367
3_15949046	3	15949046	285
3_18860864	3	18860864	375
4_1221493	4	1221493	359
4_1970497	4	1970497	371
4_4668320	4	4668320	363
4_14812597	4	14812597	366
4_19450247	4	19450247	279
5_1767055	5	1767055	365
5_2108122	5	2108122	354
5_15240426	5	15240426	372
5_16621878_QTL	5	16621878	336
5_18032640	5	18032640	346
5_20590913	5	20590913	350
6_163496	6	163496	365
6_1628753	6	1628753	365
6_3809606	6	3809606	360
6_4457113	6	4457113	340
7_5990979	7	5990979	374
8_674757	8	674757	371
8_2779201 INVERSION	8	2779201	361
8_3526849 INVERSION	8	3526849	240
8_6739828 INVERSION	8	6739828	363
8_11385282	8	11385282	356
8_16703743	8	16703743	375
8_17461279	8	17461279	360
9_13721353	9	13721353	374
9_14823219	9	14823219	366
9_15122290	9	15122290	348
10_7976577	10	7976577	371
10_19213396	10	19213396	264
11_579718	11	579718	362
11_1417846_QTL	11	1417846	364
11_5132610	11	5132610	315
11_10333703	11	10333703	303
12_1406603	12	1406603	363
12_5697229	12	5697229	344

12_22881122	12	22881122	245
12_26269967	12	26269967	361
13_786671	13	786671	366
13_14542575	13	14542575	351
13_15848108	13	15848108	366
13_18323557	13	18323557	364
13_20589955	13	20589955	339
13_21200723	13	21200723	363
14_2171704	14	2171704	367
14_7336038	14	7336038	369
14_8822514	14	8822514	313
14_20766252	14	20766252	359
14_25015701	14	25015701	320
14_26097287	14	26097287	303

Loci excluded from the analysis		
1_41344	1	41344
1_658292	1	658292
1_4703652	1	4703652
1_5735224	1	5735224
1_12909793	1	12909793
2_154107	2	154107
2_939118	2	939118
2_15998982	2	15998982
2_18023641	2	18023641
3_10245727	3	10245727
3_13160852	3	13160852
3_14618623	3	14618623
3_18549322	3	18549322
4_3174976	4	3174976
4_3887071	4	3887071
4_6057132	4	6057132
4_9264627	4	9264627
4_17979731	4	17979731
4_21077127	4	21077127
5_183367	5	183367
5_707171	5	707171
5_3785823	5	3785823
5_4688655	5	4688655
5_6485944	5	6485944
5_12655865	5	12655865
5_24164971	5	24164971
6_2549065	6	2549065
6_14461710	6	14461710

6_16788223_QTL	6	16788223
6_19414945	6	19414945
7_800691	7	800691
7_1551683	7	1551683
7_2424000	7	2424000
7_4823700	7	4823700
7_8575707	7	8575707
7_10977961	7	10977961
7_14497816	7	14497816
7_17992333	7	17992333
8_2004979_INVERSION_QTL	8	2004979
8_22909413_QTL	8	22909413
8_23757567_QTL	8	23757567
9_129202	9	129202
9_1384489	9	1384489
9_4494057	9	4494057
9_10727971	9	10727971
9_12139418	9	12139418
10_1081594	10	1081594
10_1993776	10	1993776
10_3152449	10	3152449
10_5403190	10	5403190
10_10635065	10	10635065
10_15459185	10	15459185
10_16820219	10	16820219
10_18519721	10	18519721
11_202365	11	202365
11_1092448	11	1092448
11_2356281	11	2356281
11_2930652	11	2930652
11_11082005	11	11082005
11_16211033	11	16211033
11_23069758	11	23069758
11_23979308	11	23979308
11_25510238	11	25510238
12_720512	12	720512
12_7744848	12	7744848
12_12376336	12	12376336
12_15279359	12	15279359
12_20591289	12	20591289
12_24330723	12	24330723
12_25416038	12	25416038
13_43006	13	43006
13_6120219	13	6120219

13_6989143	13	6989143
13_16838327	13	16838327
13_19269970	13	19269970
13_20144740	13	20144740
14_436691	14	436691
14_3460169	14	3460169
14_5263278	14	5263278
14_10438762	14	10438762
14_14205794	14	14205794
14_16121601	14	16121601

Chapter 3:Trade-off between sexual and clonal reproduction changes in magnitude under different resource availability in naturalized *Mimulus guttatus*

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Author`s comments: POP participated in the design of the study, collected the data, developed the statistical analyses and drafted the manuscript; MVM conceived the study as well as helped design the study and contributed to the final draft of the manuscript.

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Abstract

Clonal growth is a common feature in non-native plant species and it is suggested to facilitate invasions. In general, sexual and clonal reproduction compete for the same resources which results in a trade-off. However, plants can be very plastic to the environment and change the relative allocation between sexual and clonal traits in a way that could alter the magnitude and direction of trade-offs depending on the amount of resources available. Here, we studied individuals from 13 introduced populations of the herbaceous *Mimulus guttatus* from the United Kingdom grown in a common garden under different resource combinations (space availability and nutrients). We tested the effect of different growing conditions on the expression of trade-offs between clonal (stolon size) and sexual reproduction (number of flowers). We found that the trade-off between clonal and sexual reproduction changes according to resource availability. Although, on average, individuals in the smaller space treatment produced smaller stems, fewer flowers, and shorter stolons compared to plants with more space, the trade-off between sexual and clonal reproduction was stronger in the treatment with less space availability. We found no effect of nutrients on the expression of the trade-off or phenotypic traits among treatments. Shifts in the magnitude of the trade-off between sexual and clonal reproduction, may result in different combinations of reproductive strategies favoured across environments. Our results suggest that plants in space-limited environments (e.g., occurring at high density) should be more strongly constrained to invest in both types of reproduction, resulting in individuals investing less in sexual investment than clonal propagation. In contrast, plants in less competitive environments may still be able to simultaneously invest in both sex and clonal propagation. We therefore predict that mixed reproductive strategies should characterise low-density populations while mostly clonal investment should occur in denser, or older populations.

3 Introduction

Clonality is a common feature in many angiosperms (Kliměs et al. 1997). Clonal plants can reproduce sexually and clonally, and both types of reproduction can provide several ecological advantages for the plant (Zhang et al. 2014). For instance, sexual reproduction allows long distance dispersal of seeds (Levine 2001). Clonality can increase abundance (Herben et al. 2014), since clonal organs can extend horizontally to forage and distribute resources among connected ramets (van Kleunen and Fischer 2001), and once disconnected from the plant can also contribute to dispersal of propagules especially in riverine communities (Truscott et al. 2006). Because of the many ecological advantages of clonal growth, clonal species dominate over non-clonal species in extreme habitats characterized by cold, wet or dry conditions (Pysek 1997; Ye et al. 2014; Klimesova et al. 2012).

Sexual and clonal reproduction are also involved in the process of colonisation and population establishment of non-native populations in new environments. It is known that many widespread invasive weeds are clonal (Silvertown 2008) and also combine with sexual reproduction (e.g., *Spartina alterniflora,* Xiao et al. (2011) and *Phragmites australis,* Kettenring et al. 2016). For example, *Solidago canadensis*, an invasive perennial plant with extensive vegetative reproduction uses the strategy of long distance dispersal of seeds for colonisation of new habitats and expands the population with vegetative reproduction (Dong et al. 2006). Having both types of reproduction may facilitate non-native species naturalization and invasion of new environments.

Investment in sexual and clonal reproduction can result in a trade-off, which can interfere with maximal investment in both traits simultaneously (e.g., Ronsheim and Bever 2000; Prati and Schmid 2000; Thompson and Eckert 2004). Trade-offs can have multiple causes and can be detected in different ways. Trade-offs can be an expression of a genetic trade-off as a result of one gene controlling multiple traits in which multiple expressions of this gene have opposite effects on fitness (i.e., antagonist pleiotropy) (Roff 2002), it can be a phenotypic trade-off resulting from limited availability of meristems to produce multiple traits at the same time (Geber 1990) or a resource-based phenotypic trade-off in which an increment of resources allocated to one trait necessitates a decrease of resources to another trait. There are different methods to detect different levels of trade-offs. According to Reznick

(1985) phenotypic trade-offs are detected by measuring traits to detect negative correlation among them or by experimental manipulations of one trait and observation of the effect on other traits. To demonstrate that trade-offs are under genetic control, studies estimate the negative genetic correlations among traits using covariance among individuals within or among families, among clones or inbred lines or by selection experiments to estimate the correlated change in one trait in response to selection on another (Reznick 1985). In addition, a direct way to detect genetic trade-offs is by developing a genetic study to identify pleiotropic quantitative trait loci affecting a phenotypic trade-off (e.g., Hall et al. 2010; Friedman et al. 2015). Trade-offs expressed in the phenotype may reflect a negative genetic correlation, but a negative phenotypic correlation is not necessarily genetically based as it can result from resource or meristem limitation. It is important to distinguish between phenotypic negative correlations and genetic negative correlations since the former will indicate the direction of natural selection, but the response to selection that results in evolution is determined by the presence of genetic correlations (Stearns 1989b).

The enviroment should be considered when measuring trade-offs, because phenotypic plasticity (i.e., ability of a single genotype to produce different phenotypes according to the environemnt without genetic change) can alter allocation to correlated traits under different environments. For correlated traits that contribute to fitness, plastic changes in sign and magnitude across environments may result in changes in intensity and direction of natural selection (Stearns 1989a). Moreover, our ability to detect trade-offs also depends on the enviroment, as in some cases trade-offs can be expressed only under limited resources or stressful conditions (Reznick 1985; Hansen et al. 2013). When resources are abundant, individuals may supply the demands for production of traits that compete for the same pool of resources and, in turn, negative correlations can become positive. Even the expression of genetic trade-offs can change in different environmental conditions, because the expression of the genetic correlation depends on the genotype's physiological processes that can change plastically according to the environment (Stearns 1989b). For example, in a study with *Impatiens capensis* a genetically based trade-off in meristem allocation between flowers and branches became apparent only in high-resource environments with intense sunlight and no intraspecific competition (Donohue et al. 2000). Thus, trade-offs between sexual

and clonal reproduction are expected to be detected under specific limiting conditions.

In natural populations, availability of nutrients in the soil and density of individuals, which results in competition for space and belowground resources (i.e., nutrients and water), have been shown to influence reproductive trait allocation (Yang and Kim 2016). However, studies investigating the effect of environmental quality on trade-offs have showed mixed results. For instance, in *Sagittaria pygmaea* the trade-off between sexual and clonal reproduction are stronger under moderate nutrients (Liu et al. 2009), but for *Butomus umbellatus* a trade-off in biomass between number of flowers and inflorescence bulbil was only detected under high nutrients (Thompson and Eckert 2004). A previous study with 245 clonal species demonstrated that extensive clonal lateral spread was correlated with decreased sexual reproduction (estimated by the production of seed number and seed mass) (Herben et al. 2015), however this study did not answer the question whether the negative correlation between sexual reproduction and clonal lateral spread is still present under different resource availabilities. We need more information about the effect of resources on trade-offs as it can contribute to our understanding of how environmental heterogeneity modulates sexual and clonal reproduction variation within and among populations to maximize individual performance in the environment.

In the present study, we estimate the effect of different resource levels on the detection of trade-offs between sexual and clonal reproduction in introduced populations. As a model system, we used *Mimulus guttatus* DC. (Phrymaceae), a herbaceous species with sexual reproduction and clonal reproduction by stolons. This species is native to western North America and became naturalized in eastern North America, New Zealand and many countries in Europe, including United Kingdom (UK) (Tokarska-Guzik and Dajdok 2010; Vallejo-Marin and Lye 2013). Native *M. guttatus* populations form two ecotypes of annual and perennial populations (Lowry et al. 2008), while introduced populations in the UK have only a perennial life-cycle. In a previous study by van Kleunen (2007), annual and perennial *M. guttatus* populations were planted in common gardens with different watering treatments (temporary wet and permanently wet) and have showed genetic variation for both sexual and vegetative reproduction within and among populations of *M. guttatus.* In the van Kleunen study, on average, plants from populations with

a perennial life-cycle produced more and longer stolons and started to flower later than annual populations, while number of flowers was higher in plants from annual populations than plants from perennial populations. Moreover, for both annual and perennial plants the number of stolons and the length of the longest stolon were significantly higher in the permanently wet soil than in the temporarily wet soil, which is suggestive of plasticity in investment in clonality under different water availability conditions (van Kleunen 2007). Other studies with reciprocal transplant of populations from both *M. guttatus* ecotypes have shown that genetic-based differences in flowering time and reproduction traits between annual and perennial populations contribute to local adaptation (Lowry et al. 2008; Hall et al. 2010). To date, there is no information about whether trade-offs between sexual and clonal traits in *M. guttatus* is a result of genes in antagonistic pleiotropy. However, a recent study with *M. guttatus,* mapped quantitative trait loci (QTL) using high-throughput resequencing of a cross between a annual and a perennial population, and found extensive pleiotropy for QTLs related to flowering time and number of stolons (Friedman et al. 2015). Given that in *M. guttatus* there is a strong correlation between flowering time and investment in flowers (Ivey and Carr 2012) and stolons, the study by Friedman et al. (2015) suggests that sexual and clonal reproduction investment can also arise from shared QTLs. In a study with introduced *M. guttatus*, van Kleunen and Fischer (2008) developed a glasshouse experiment to characterize the phenotypic divergence among three and four introduced populations of *M. guttatus* from Scotland and New Zealand, respectively, and 17 native populations. Regression analysis with those 24 populations as a function of latitude, revealed that stolon length increases with latitude, while number of flowers decreases and that there were plastic responses of stolon length and other traits such as plant height in response to water soil conditions. Therefore, these studies in the native range with annual and perennials and the study of van Kleunen and Fischer (2008) with three populations from the UK suggest that trade-offs between sexual and clonal reproduction in *M. guttatus* can be mediated by environmental conditions in both it's native and introduced range.

In order to determine the influence of resources on the detection of a reproductive trade-off in *M. guttatus*, we grew plants in common garden with a combination of treatments with different levels of nutrients and space availability. Studies have shown that plant reproductive allocation responds to nutrient

availability (e.g., Liu et al. 2009; Zhang et al. 2014), while bare space influences the occurrence and number of patches of *M. guttatus* in the UK (Truscott et al. 2008b). Specifically, we addressed the following question: What is the relative importance of nutrients and space for the expression of a clonal versus sexual reproduction trade-off in introduced populations?

3.1 Material and Methods

3.1.1 Experimental design

We used *M. guttatus* seeds collected in different populations around the United Kingdom (Figure 1; Supplementary Table 1). In May 2014, we developed a common garden experiment at the Stirling University glasshouse using seeds from 13 populations of *M. guttatus* collected in 2009 and 2010. We did not analyse the ploidy level of each individual in this study, but the previous study of Simón-Porcar et al. (2017) indicates that three populations used in our study (HAM, HOU and CER populations) are composed exclusively of diploids. Moreover, the frequency of polyploid populations of *M. guttatus* is considerably smaller than diploid populations, as indicated by a recent survey in UK (3% or 1/29 sampled populations were polyploid, Simón-Porcar et al. 2017); thus, it is likely that all populations in this study are diploids. We used seven families of each population except for the population 10-ABB and 10-MUK that had six families each. The experiment had four different treatments in a combination of two factors: A space-availability factor corresponding to pots with two different sizes: "small" pots (0.12L in volume; hereafter "size-small" treatment), and "large" (0.37L in volume; hereafter "size-large" treatment); and a nutrient-availability factor, which consisted of whether plants received additional fertilizer or not. Thus, we had plants in the small space availability with and without nutrients and plants in the large space availability with and without nutrients and a total of 712 plants (n = 712).

We planted multiple seeds per maternal family in pots with commercial soil composed of 5% of sand, 15% (0 - 5mm) and 80% (0 - 10mm) of peat; N = 192g/m^3, P_2O_5 = 224g/cm^3 and K_2O = 384 g/cm^3 (Sinclair - All-purpose growing medium). When the majority of the seedlings germinated (after one week), we thinned the

pots to one individual per maternal family. Small pots with less soil volume absorb water at a different rate than larger pots, thus in other to minimize the different soil drying rates among pots with different sizes, large pots and small pots were put in separate trays. Pots were distributed in 48 trays placed on three benches. Trays were randomized once per week and bottom watered every two days. Half of the trays received 50 ml of 1g/1L solution of soluble fertilizer (Scotts Blossom Booster Fertlizer (10% N; 13.1% P; 16.6%K; 1.2% Mg; 1.6% S; 0.12% Fe; 0.06% Mn; 0.02% B; 0.010% Cu; 0.01 Mo; 0.015% Zn) and the other half received no fertilizer. The fertilizer was applied directly in the trays every other week until the end of flowering season in August. The windows of the glasshouse were kept open during the experiment, which allowed the entrance of pollinators. The plants were supplied with artificial light for 16 hours per day.

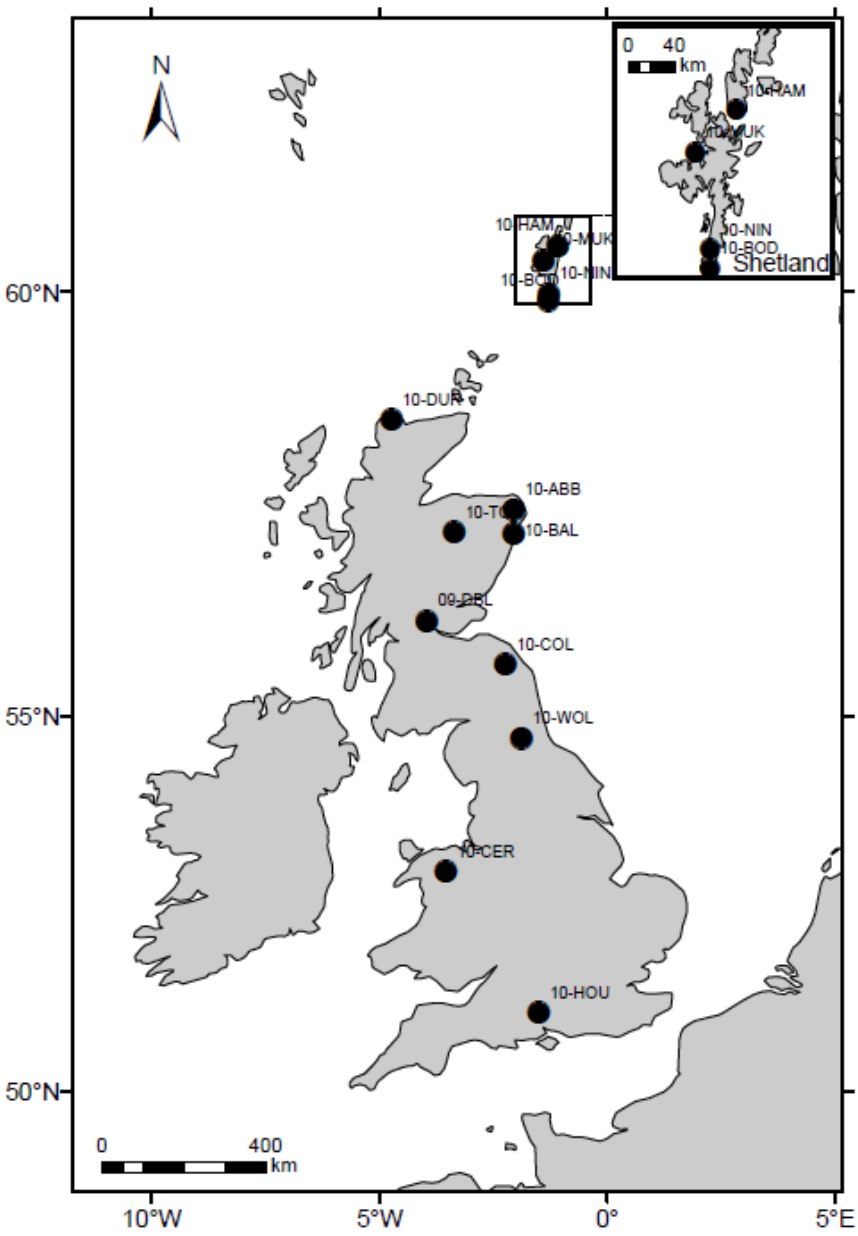

Figure 1. Map showing the localities of the 13 populations of *Mimulus guttatus* used in glasshouse experiment.

3.1.2 Trait Measurements

To estimate the trade-off between clonal and sexual reproduction, we measured five traits at the beginning of August 2014 when plants stopped producing new flowers: Total number of flowers (sum of flowers and fruits); number of stolons (non-flowering lateral branches with roots counted from the base until the third node); plant height, measured as the height of stem from the base of the plant until the top (cm) and stolon size, measured as the length of the longest stolon (cm).

3.1.3 Statistical analysis

Our main goal was to test for the detection of a phenotypic trade-off between clonal and sexual reproduction among treatments. In our study, we used number of flowers as an estimate of sexual reproduction and as an estimate of overall clonal reproduction, we calculated the total stolon length by multiplying the longest stolon length by the number of stolons. We used these traits because a previous glasshouse common garden experiment by van Kleunen and Fischer (2008) with four populations of *M. guttatus* from UK showed that total stolon length (the length of the longest stolon multiplied by number of stolons) varies with latitude of origin in the opposite direction of the relationship between flower number and latitude, which might indicate that these traits change plastically in response to environment across the introduced range.

We used a mixed effects model to assess the influence of the fixed factors: number of flowers, plant height, space and nutrient, together with interactions among space, nutrients and numeric predictors, on the response variable total stolon size. We investigated how plants allocate resources to sexual versus clonal reproduction using the ratio of total stolon length to number of flowers as a response variable and treatments as fixed factors. Mixed-effects models allow for simultaneous analysis of trade-offs among treatments, and permit the use of families nested within populations as a random effect, which allows the intercept to vary among populations and among families within populations and, in turn, provide inferences about the populations as a whole. Moreover, random effects account for the non-independence of individuals from the same family.

We included plant height as a covariate in the models, because it has been shown that this trait can influence the production of flowers and stolons (e.g., Pluess and Stöcklin 2005). We log transformed total stolon length to avoid heteroscedasticity of the residuals in the model. A significant negative regression coefficient between total stolon length and number of flowers indicates a trade-off between clonal and sexual reproduction; and a significant interaction between number of flowers and treatments, means that the trade-off changes among treatments. We also tested the influence of the interaction between space and

nutrients on the relationship between plant height and number of flowers. For this model, the response variable was plant height and the fixed effects were number of flowers, space, and nutrients, with interactions.

Parameter estimates were obtained with all numeric predictors standardized to mean zero and variance one to allow for the comparisons of their relative contribution to the response variable (Schielzeth 2010). We assessed statistical significance of predictors by calculating *P*-values based on Kenward-Roger's approximations of degrees of freedom. All analysis were performed in *R* version 3.4.0 (R Core Team 2017) using the package *lmer*, *lmerTest* for the mixed models.

3.2 Results

On average, in the size-large treatment with and without nutrients, plants were taller, had longer stolons and produced more flowers, but produced similar number of stolons than plants in the size-small treatment with and without nutrients (Figure 2 and 3).

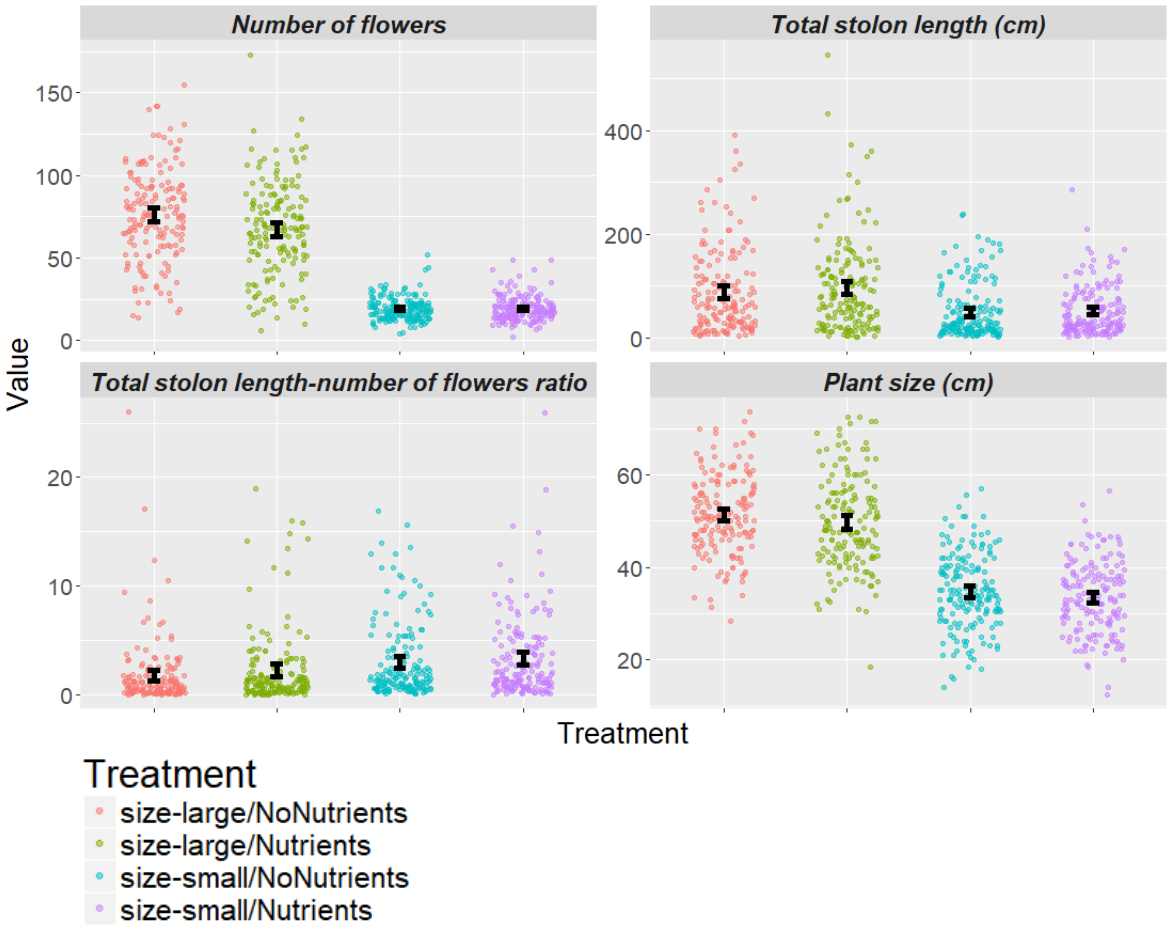

Figure 2. Phenotypic characteristics of individuals from 13 introduced populations of *Mimulus guttatus* grown in a glasshouse experiment with a combination of space (small and large) and nutrient (with and without nutrients) treatments. The mean and 95% confidence interval (95% CI; calculated using a bootstrap approach) are shown in black. Variation along the x-axis (jitter) is provided for each treatment to facilitate the visualisation of the data points.

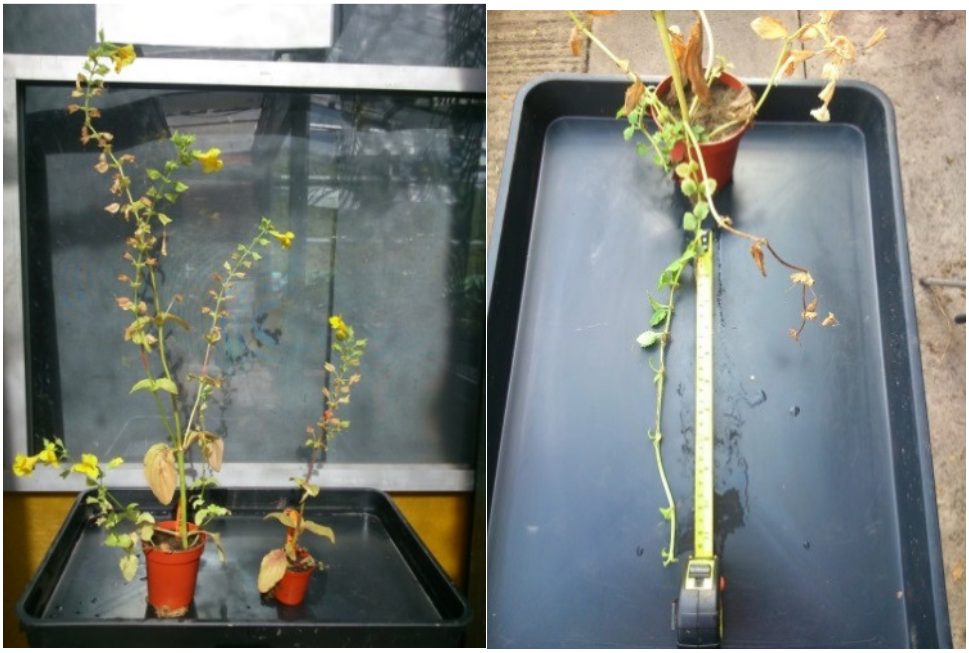

Figure 3. Different individuals of *Mimulus guttatus* grown in different space treatments (left panel), and an example of an individual with a long stolon (right panel).

We found a significant effect of space (F value = 52.5, $P < 0.00$), plant height (F value = 5.14, $P = 0.02$), number of flowers (F value = 26.97, $P < 0.00$), and the interaction of space x number of flowers (F value = 6.22, $P = 0.01$) on total stolon length (Table 1A).

Table 1. Statistically significant standardized estimates from the mixed model analysis testing the effect of space treatment and covariates on the response variables total stolon length (log transformed) (A) and plant height (B) and (C) ratio of total stolon length to number of flowers. For all models, the fixed effect nutrients

and interactions were not significant with $P < 0.05$ and were excluded from the model. *P* values were calculated using Kenward-Roger`s approximation of denominators degrees of freedom using the package *lmerTest*. *P* values represent the significance of the difference between the mean of each source and the intercept, which is the mean of the size-large treatment. *** $P < 0.00$, * $P < 0.05$.

A) Response variable: Log (Total stolon length)	**Source**	**Estimate**	**Standard Error**	***P* value**
	Intercept	4.25	0.13	< 0.00 ***
	Size-small	-1.57	0.21	< 0.00 ***
	Number of flowers	-0.39	0.06	< 0.00 ***
	Plant height	0.13	0.05	0.02 *
	Size-small x Number of flowers	-0.68	0.27	0.01 *
B) Response variable: Plant height	**Source**	**Estimate**	**Standard Error**	***P* value**
	Intercept	48.31	1.31	<0.00***
	Size-small	-24.97	1.63	<0.00***
	Number of flowers	0.03	0.01	0.03*
	Size-small x Number of flowers	0.52	0.06	<0.00
Response variable: Ratio of total stolon length to number of flowers	**Source**	**Estimate**	**Standard Error**	***P* value**
	Intercept	0.89	0.21	<0.00 ***
	Size-small	0.90	0.25	<0.00 ***
	Plant height	-0.00	0.00	0.67
	Size-small x Plant height	-0.02	0.00	<0.00 ***

The effect of number of flowers on the response variable total stolon length was significant and negative (Table 1A), which indicates a phenotypic trade-off between clonal and sexual reproduction (Figure 4). Patterns of correlation among families can be used to proximate genetic correlations (e.g., Brodie III and Brodie 1999). In our study, the analysis of family means showed qualitatively the same effect of flowers and space on total stolon length using all individual values (data not shown), which could demonstrate an underlying genetic basis of the phenotypic

trade-off (i.e., genetic trade-off), however the analysis using family means was limited by low statistical power due to small number of families (89 in total). Therefore, the results presented were obtained using all individual values.

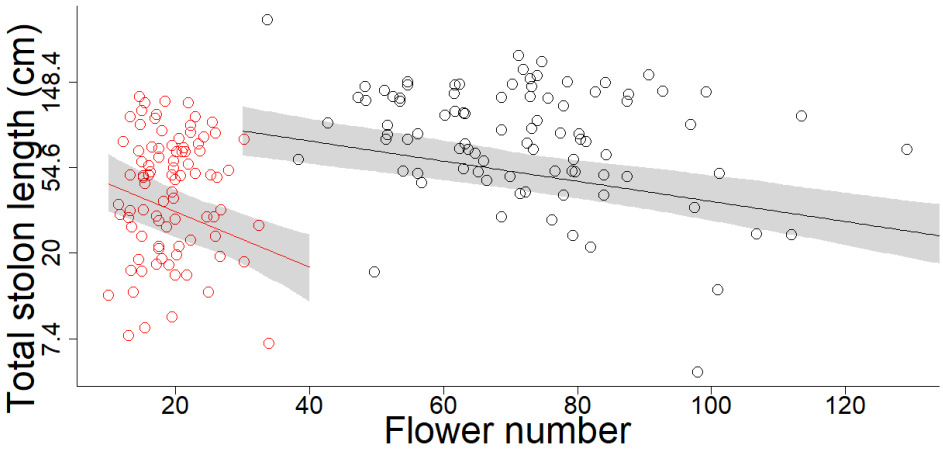

Figure 4. The effect of number of flowers on total stolon length in *Mimulus guttatus*. Lines are partial coefficients after controlling for others variables in the mixed effect model (Table 1A). Red points represent family means and the red line represents the partial regression line from the size-small treatment. Black points represent family means and the black line represents the partial regression line from the size-large treatment. Grey area represents 95% bootstrapped confident intervals. The Y-axis was log back transformed for illustration.

As indicated by the significant interaction space x number of flowers, the slope of the effect of number of flowers on total stolon length changed between the size-large and size-small treatments. In the size-small treatment the relationship between the two reproductive variables was more negative in comparison with the size-large treatment (Table 1A). In other words, the trade-off between clonal and sexual reproduction was stronger under limited space and was alleviated under conditions with high space availability. The three-way interactions space x nutrients x number of flowers (F value = 1.41, $P = 0.23$), and space x nutrient x plant height (F value = 0.10, $P = 0.74$), and the two-way interaction nutrient x number of flowers (F value = 1.19, $P = 0.27$) were not significant and were excluded from the model. Plant height positively influenced total stolon length independently of treatments

(Table 1A, Figure 5) and the effect of space on total stolon length was still significant after accounting for plant height (Table 1A).

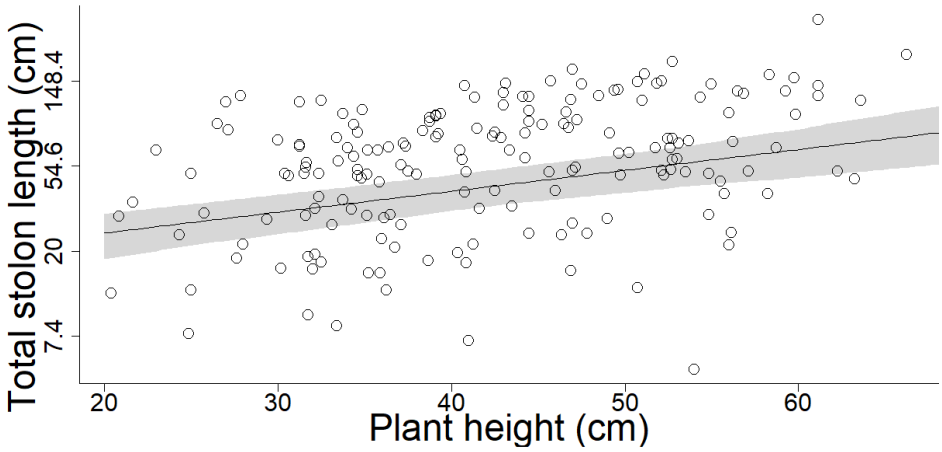

Figure 5. Effect of plant height on total stolon length in *Mimulus guttatus*. Line is the significant partial coefficient after controlling for others variables in the mixed effect model (Table 1A) and points represent family means. Grey area represents 95% bootstrapped confident intervals.

We found a significant effect of space (F value = 231.77, $P < 0.00$), number of flowers (F value = 89.52, $P = 0.03$) and the interaction term space x number of flowers (F value 74.22, $P < 0.00$) on plant height. In other words, plants in the size-large treatment had taller stems than plants in the size-small, independently of nutrients (F value = 1.99, $P = 0.15$). The three-way interactions number of flowers x space x nutrients (F value = 0.28, $P = 0.59$), and the two-way interactions number of flowers x nutrients (F value 0.35, $P = 0.55$) and space x nutrients (F value = 0.11, $P = 0.73$) were not significant and excluded from the model. The variables plant height and number of flowers had a significant positive relationship that changed in magnitude between the size-small and size-large treatments (Table 1B, Figure 6), which indicates that as plants grow, more flowers are produced, particularly under restricted conditions in the size-small treatment. Moreover, we found a significant effect of space on the ratio of total stolon length to number of flowers (F value = 12.50, $P < 0.00$). In the size-small treatment, the ratio of total stolon length

to number of flowers was higher than in the size-large treatment (Table 1C), which indicates that investment in flowers was smaller than total stolon length in the size-small treatment.

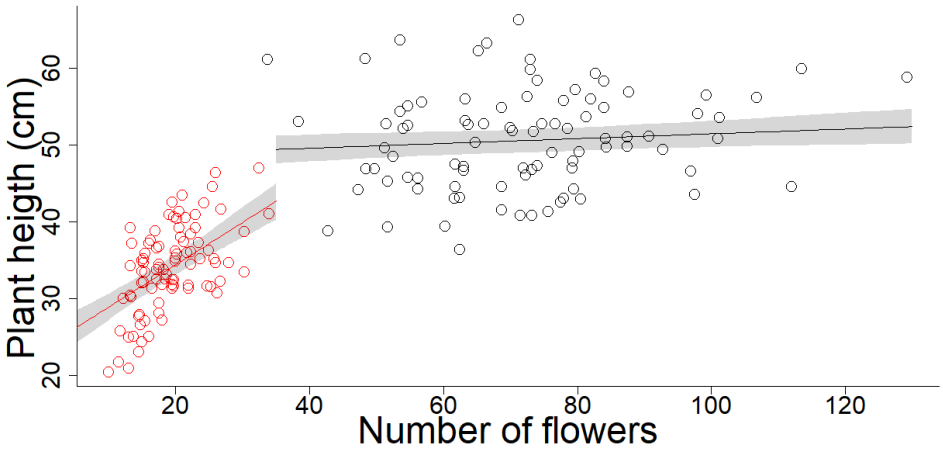

Figure 6. Relationship between plant height and number of flowers in Mimulus guttatus. Lines are significant coefficients after controlling for others predictors in the mixed effect model (Table 1B). Red points represent family means and the red line represents the partial regression line from the size-small treatment. Black points represent family means and the black line represents the partial regression line from the size-large treatment. Grey area represents 95% bootstrapped confident intervals.

3.3 Discussion

Our study showed a trade-off between clonal expansion and flower investment. The trade-off, demonstrated by a negative correlation between total stolon length and number of flowers, varied in magnitude under different space treatments (size-large and size-small), supporting the prediction that a trade-off would be stronger under limited resources. Moreover, we found that for introduced *M. guttatus* in the UK, the relative effect of space is more important than availability of nutrients for the expression of the trade-off between sexual and clonal reproduction.

The trade-off between total stolon length and flower number was stronger in the treatment that limited space. In natural conditions, space could be a primary limiting resource for plants under high density, which can increase competition for

space as well as soil nutrients and water intake among individuals and, in turn, alter the allocation of resources between sexual and clonal reproduction (e.g., Prati and Schmid 2000). Changes in the magnitude and direction of trade-offs across environments may be explained by phenotypic plasticity (Stearns et al. 1991). We hypothesized space limitation changing the magnitude of a trade-off between total stolon length and number of flowers could be a phenotypic plastic response to the limited intake of resources. Future studies are needed to test for plasticity of *M. guttatus* reproductive traits in different environments. In clonal plants the extent of plasticity could be determined by experimentally growing clonal replicates in different environments (e.g., Ronsheim and Bever 2000; Mal and Lovett-Doust 2005).

The demonstration that the observed phenotypic trade-off has a genetic basis (i.e., genetic trade-off) was not possible, because the small number of families limited the analysis of trait correlation among families. However, Friedman et al. (2015) analysed individuals from a cross between an annual and perennial *M. guttatus* population and showed that stolon number and flowering time are positively correlated and controlled by at least four genomic regions with pleiotropic effects. In native *M. guttatus*, flowering time is positively correlated with number of flowers (Ivey and Carr 2012), therefore it is possible that number of flowers and clonality traits such as stolon number and length could be affected by the same genes in opposite directions resulting in a trade-off (antagonistic pleiotropy). Future studies could use quantitative trait locus mapping (QTL analysis) to detect loci affecting the expression of number of flowers and clonality on a segregating population derived from a cross between a highly clonal (with many long stolons) and a highly sexual (with many flowers) *M. guttatus* individual from the introduced range.

The large phenotypic variation among individuals observed in our treatments could reflect maternal carryover effects. Although maternal effects have been shown to decrease with time and significantly affect early life history traits such as germination and seedling size instead of later traits such as number of flowers and stolons (Roach and Wulff 1987), Galloway (1995) showed that phenotypic variation in flowering time and number of flowers among native *M. guttatus* genotypes can be caused by variation in seedling size due to maternal effects. However, the seeds of introduced *M. guttatus* are minuscule (less than 0.02

mg; Truscott et al. 2006) and do not vary appreciably in size and germination between the source populations used here (personal observation). Hence, although we do not have data on seedling size, we suggest that there were no differences in maternal seed provisioning in our study and the variation observed in introduced *M. guttatus* likely reflects environmental and genetic effects as observed by van Kleunen (2007) with native *M. guttatus* populations.

Different environmental factors can affect the expression of trade-offs. For some species, trade-offs are only detected at low nutrient availability (Biere 1995). In our experiment, nutrients were not limiting for the detection of the trade-off, although we cannot exclude the possibility that higher levels of nutrients could affect reproductive traits. For instance, in *Sagittaria pygmaea*, the trade-off between sexual and clonal reproduction was apparent in moderate nutrient level, but not in low nutrient, because individuals invested in photosynthetic traits that need fewer resources, and could produce additional resources to invest in both reproductive traits; and in high nutrient treatments, there were plenty of resources for allocation resulting in no trade-off among reproductive traits (Liu et al. 2009). It is possible that the concentration of nutrients applied to the trays was not sufficient to reveal an effect on the phenotype or that the nutrients present in the commercial soil diminished the differences between treatments even after nutrient addition. We suggest that future studies should investigate the effect of nutrient addition on trade-offs by developing an experiment with stronger differences in availability of nutrients among treatments and growing all plants in a neutral soil (i.e., soil without nutrients).

Our results showed that the trade-off between total stolon length and number of flowers changed in magnitude in response to space availability, and the ratio of total stolon length to number of flowers increased in the size-small treatment. Change in the ratio of traits can result in populations with different reproductive strategies under different conditions of space such as in populations with different densities. For example, in *M. primuloides* density and climatic conditions affect the investment in clonal reproduction (Douglas 1981). Populations of *M. primuloides* showed high investment in clonal reproduction at middle altitude in California, likely because high densities at low altitude and harsh climatic conditions at high altitude decreased plant size and resulted in less clonal reproduction. Conversely, sexual investment in populations from high altitude seems to be an adaptation to

greater potential dispersal and seedling establishment (Douglas 1981). Our experiment with limited availability of space suggests that non-native *M. guttatus* plants growing in benign environments (e.g., low-density populations) may invest in both clonal growth and flowers, whereas plants under limited conditions (e.g., high-density populations) will invest less in sexual reproduction than clonal growth. Our findings indicate that the increased ratio of stolon length to flower investment is an indirect effect of plant height in the size-small treatment (Table 1C). The results suggest that small space availability decreases plant height which concomitantly reduces allocation to production of flowers because plants produce more flowers with increasing plant height. Our result complements a previous study with *M. guttatus* from the UK that revealed mixed levels of genotypic (clonal) diversity in most populations, but highly sexual or highly clonal signals in other populations (Pantoja et al. 2017). Populations of *M. guttatus* in the UK occur in variable places such as riverine and waterlogged habitats with different levels of disturbance (e.g., flooding, Truscott et al. 2006) that could create open spaces and result in populations with different sizes and densities, and a previous study showed that sediment availability is a limiting factor for occurrence and number of patches of *M. guttatus* in the UK (Truscott et al. 2008). Therefore, individuals could be exposed to environmental heterogeneity that could affect population establishment and also the alternative investment in sexual and clonal reproduction, which could facilitate the colonisation of different habitats.

3.4 Conclusions

Our results show a trade-off between stolon length and number of flowers, and that the expression of this trade-off is environment dependent. Limited resources, driven by low availability of space rather than nutrients, intensify the negative relationship between clonal spread and flower production suggesting that in populations occurring at different densities, individuals will have to invest in alternative reproductive traits to better fit into the environment resulting in populations with different levels of clonality and sexual reproduction. We predict that under good conditions of space, such as in populations with low density and competition, individuals will invest in both traits with the same intensity resulting in mixed reproductive strategies. Conversely, limited space in populations occurring at high

density individuals will result in populations being more clonal than sexual. Future studies are needed to test the relative allocation between sexual and clonal reproduction in the field where populations have different densities.

3.5 Acknowledgments

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3.7 Supplemental material

Supplementary Table 1. Geographic location of *M. guttatus* populations from United Kingdom used in the experiment.

Population code	Location	Latitude (N)	Longitude (W)
10-HOU	Houghton Lodge, Hampshire	51.096	1.508
10-CER	Denbighshire**,** Wales	53.005	3.549
10-WOL	Walsingham, Durham	54.726	1.887
10-COL	Coldstream, Scottish Borders	55.654	2.240
09-DBL	Dunblane, Perthshire	56.187	3.965
10-BAL	Balmedie, Aberdeenshire	57.237	2.063
10-TOM	Tomintoul, Moray	57.254	3.367
10-ABB	Deer Abbey, Aberdeenshire	57.523	2.057
10-DUR	Durness, Sutherland	58.568	4.747
10-BOD	Boddam, Shetland	59.904	1.302
10-NIN	St**.** Ninians, Shetland	59.977	1.300
10-HAM	Hamnavoe, Isle of Yell, Shetland	60.503	1.099
10-MUK	Muckle Roe, Shetland	60.348	1.413

Chapter 4: Allocation to sexual and clonal reproduction in populations of a non-native plant across different climatic conditions and population densities

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Author's comments: POP designed the study, collected the data, carried out the statistical analyses and drafted the manuscript; VIS carried out the flow cytometry analysis, as well as helped design the study and collect data; MVM helped conceive and design the study.

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Abstract

Many perennial plants combine the capacity to reproduce via both sexual and vegetative (clonal) means. The costs and benefits of different reproductive strategies vary in different environments and, therefore, the relative allocation to sexual and asexual reproduction is expected to change across climatic gradients. In this study, we analysed variation in sexual and clonal reproduction, as well as vegetative traits and flower morphology, in 32 populations of *Mimulus guttatus* (Phrymaceae) in its introduced range in the United Kingdom. We also studied the effect of plant density on reproductive allocation and phenotypic variation. As hypothesised, we found that the relative investment in sexual and clonal reproduction is associated with climatic variation. We found that the number of stolons increased in populations exposed to overall cooler climates, which receive higher precipitation in the summer, and experience lower seasonality. In contrast, populations produce more flowers in warmer climates, with drier summers, and with stronger seasonality. We found no effect of plant density on reproductive allocation or phenotypic characteristics. Our results suggest that climatic conditions mediate the investment in sexual and asexual reproduction even in recently (<200 years) established populations of non-native species. Investment in reproduction via seeds may be favoured in climatic conditions where seasonality in temperature and precipitation may limit clonal growth in drought-intolerant species such as *M. guttatus*. In contrast, cooler, wetter, and more stable climates may favour clonal growth over sexual reproduction as suggested by previous surveys in the native range of *M. guttatus*, and by broad surveys across taxonomic groups.

4 Introduction

The success of introduced populations, measured either as persistence or abundance, depends on how well they adapt to the conditions in the invaded range (Colautti and Lau 2015). During range expansion of an introduced plant species, the availability of pollinators and environmental conditions such as temperature and precipitation may change. In wide invaded ranges, different conditions may promote phenotypic differentiation among introduced populations (Liu et al. 2016). The extent of differentiation among populations is very variable across species and populations (Weber and Schmid 1998; Mozdzer et al. 2016), and could be the outcome of adaption to different selective pressures and/or phenotypic plasticity that directly affects the species establishment and spread at larger scales (Liao et al. 2016).

Climatic variation is one of the most important factors determining patterns of population differentiation in plants (Beerling 1993; Mclean et al. 2014; Wang et al. 2015), particularly affecting the reproduction success (Bykova et al. 2012). Cold environments in high latitudes and altitudes can restrict outcrossing rates due to scarcity of pollinators (Arroyo et al. 1985). Under such conditions, self-compatible plants, as a strategy of reproductive assurance, might modify floral traits to increase self-fertilization, for instance reducing anther stigma separation (reduced herkogamy; de Vos et al. 2012) or producing bigger flowers to attract pollinators (Olsson and Ågren 2002). In addition, clonal reproduction varies under gradients of climatic conditions. For example, a study with herbaceous species in China revealed that the proportion of clonal plants relative to non-clonal plants decreased with increasing temperature and precipitation (Ye et al. 2014). As a consequence, the relative investment in sexual and clonal reproduction can vary according to the environment (Young et al. 2002), thus it is expected that plants may invest in different reproductive strategies under different climatic conditions. One of the most important factors determining the success of invasive populations is their effective reproduction, therefore advantageous changes in reproductive strategies under climatic conditions should facilitate invasion success (Barrett et al. 2008).

Differences in reproductive strategies among populations can also result from different selective pressures in populations with different successional stages

and densities. Theoretical and simulation studies have suggested that genotypes with high fecundity and dispersal capacity will be selected in newly founded populations, because of the high ability of these genotypes to occupy empty spaces (Olivieri et al. 1995; Ronce and Olivieri 1997). As the population ages, density increases and competition intensifies, which gradually selects for genotypes that are better competitors and invest more in vegetative growth and survivorship rather than fecundity (Olivieri et al. 1995; Ronce and Olivieri 1997). Piquot et al. (1998) extended this for sexual and clonal reproduction and found that initial populations of *Sparganium erectum* invested more in sexual reproduction, while old populations more on clonal reproduction. In another study, populations of *Ranunculus reptans* allocated more resources to seed production in dense populations than in low density populations, which could be the result of phenotypic plasticity of seeds that increase dispersal to new sites with less competition (van Kleunen et al. 2001). During colonization, invasive species will have varying levels of population density and competition that can lead to rapid evolution of traits associated with dispersal and result in differentiation among populations of an invasive species.

Here we use *Mimulus guttatus* DC. (Yellow monkeyflower, Phrymaceae) to study patterns of phenotypic variation in reproductive traits among non-native populations. *M. guttatus* is native to western North America, from Mexico to Alaska and introduced to Europe and some other parts of the world approximately 200 years ago (Roberts 1964). Although in the native range *M. guttatus* has annual and perennial life-cycles, in the United Kingdom (UK) the species has become widespread as a perennial herb (Vallejo-Marin and Lye 2013). *M. guttatus* is hermaphrodite and partially self-fertilising (Ritland 1989), and also reproduces clonally by vegetative propagation of stolons.

Previous studies in the native range indicated that soil water availability is the main selective pressure for many traits of *M. guttatus* (e.g., sexual and clonal investment, flower size and phenology, Hall and Willis 2006; Lowry et al. 2008; Oneal et al. 2014). In the introduced range, a study by van Kleunen and Fischer (2008) characterized phenotypic variation in non-native *M. guttatus*; in a glasshouse experiment, van Kleunen and Fischer compared phenotypic divergence among three and four introduced populations of *M. guttatus* from Scotland and New Zealand, respectively, and 17 native populations. Analysing these 24 populations together, they showed that corolla width, plant size and herkogamy do not have a

relationship with latitude, whereas stolon length increases with latitude and number of flowers decreases, which suggest that *M. guttatus* has adapted to climatic conditions associated with latitude in the native and invasive range, and that this variation is likely a result of adaptive evolution. Van Kleunen and Fischer's study showed an association between reproductive traits and latitude, but did not look at whether climatic variables are associated with reproductive allocation. Our study complements the van Kleunen and Fischer (2008) study by analysing the effect of climatic variables on phenotypic differentiation among introduced populations of *M. guttatus*.

Here we characterized investment in sexual and clonal reproduction, plant size, floral traits associated with self-fertilization and vegetation density from 32 populations of *M. guttatus* around UK and asked the following questions: What is the variation in sexual and clonal reproduction, as well as vegetative growth and flower morphology among populations of *M. guttatus* in its introduced range in the United Kingdom? And what is the effect of plant density and climatic variables on reproductive allocation and phenotypic variation? I hypothesize that populations in places with high temperature and low precipitation, i.e. conditions related to low soil moisture, will limit clonal reproduction and plants will invest more in sexual reproduction. In contrast, populations in cold places will invest more in clonal reproduction and will show limited sexual investment, reduced herkogamy and small number of big flowers as a way of reproductive assurance. If competition for resources in populations occurring at high density affects vegetative growth and reproductive allocation, individuals will invest less in vegetative traits, and allocate more resources either to clonal reproduction, as a strategy to increase competition ability and survival, or sexual reproduction as a strategy to increase dispersal to environments without competition.

4.1 Material and Methods

4.1.1 Population sampling

We identified *M. guttatus* populations in the field using morphological characteristics (Stace 2010), and later confirmed that the sampled populations were

diploid by assessing relative genome size in a subset of individuals using flow cytometry on fresh leaf tissue as described in Simón-Porcar et al. (2017). We surveyed 32 populations (507 individuals in total) from different localities around UK during a field survey in the summer of 2014. In order to sample the extremes of the climatic conditions found in the UK, we focused our sampling efforts in the northern and southern ends of the distribution of *M. guttatus* in the UK. Specifically, we sampled populations in Scotland (Stirlingshire, Highlands, Moray and Shetland), southern England (Cornwall, Devonshire, and Hampshire) and southeast England (West Sussex). The northernmost population was located in Hamnavoe, Shetland Islands and the southernmost population in Crowan, England (Supplementary Table 1). Throughout our sampling area, we found populations in a variety of habitats close to streams and rivers, and in waterlogged areas, roadside ditches and abandoned fields with small cobbles (Figure 1).

Figure 1. Examples of habitats of *Mimulus guttatus* in the United Kingdom sampled for this study. Clockwise from top-left panel: Balnakeil, Scotland (BKN); Uplowman, England (UPL); Maryburg, Scotland (MAR); Dalmore, Scotland (DAL).

4.1.2 Sampling design and data collection

Most populations consisted of patches with varying densities of individuals and isolated individuals between them. For this reason, we measured density of each population with a stratified sampling protocol. First, we mapped patches of individuals with XY coordinates in each population. Next, we performed a line-intercept sampling by placing linear transects of 2-362 meters of length to span the total area of each population. Finally, we placed 1-12 $1m^2$ quadrats in each population using stratified sampling; we assigned a proportional number of quadrats to each category "*Mimulus* patches" and "areas with isolated individuals of *Mimulus*", and placing quadrats randomly within each category.

In each quadrat, we recorded the percentage of coverage of *M. guttatus,* other species, and bare space. We summed the percentage of coverage of *M. guttatus* and other species within $1m^2$ to estimate population density. We sampled three *M. guttatus* ramets at random from each quadrat to measure phenotypic traits. We used these three ramets to measure plant size, as the height of the stem (cm); stem diameter, measured at the base of the stem (mm); number of stolons (lateral branches with roots); number of floral stems (branches with flowers); length of the biggest leaf (mm); number of fruits and number of flowers (sum of total number of buds, flowers and fruits). In addition, we collected one flower from each ramet to measure the following floral traits: corolla width (mm), corolla height (mm) and herkogamy (mm), the spatial separation of stigma and the longest anthers.

4.1.3 Environmental variables

In other to determine the effect of climatic variables on phenotypic traits, first we obtained 19 climatic variables for every population from the data base of WorldClim (Hijmans et al. 2005) using the spatial resolution of 10 minutes (~18 Km^2). These variables include annual averages, extreme records (minimum and maximum) and quarterly summaries of temperature and precipitation, for an explanation of each variable see Supplementary Table 3. Because climatic variables covary, we reduced the dimensionality of the 19 standardized climatic variables by

applying a principal component analysis using *FactorMinoR* package (Le et al. 2008) in R version 3.4.0 (R Core Team 2017) and extracted the value of the first two principal components (PC1 and PC2) for each population.

4.1.4 Morphological and reproductive allocation analyses

We identified variables that measured the same trait or were correlated by applying a Pearson correlation test, and only kept one representing plant size, floral traits and reproduction (see Table 4 in results). Thus, for the remaining analysis, we used number of flowers, number of stolons, plant height, corolla width and herkogamy as response variables. We also calculated the proportion of stolons (number of stolons divided by number of flowers and stolons) and included this as another response variable to assess the investment of clonality relative to total sexual and clonal reproduction. Next, we conducted separate mixed effect models for each trait with the environmental variables, PC1 and PC2, and density as fixed effects. Quadrat was nested within population and included as a random effect. For the analyses of number of flowers, number of stolons and proportion of stolons, we also included plant height as a co-variate (fixed effect), because plant size often affects flower and stolon production (e.g., Schmidt et al 1995; Pluess and Stöcklin 2005). We used this model structure for our six response variables with model error structure varying according to the response variable; for number of flowers and number of stolons: generalized linear model with Poisson error structure; for proportion of stolons: generalized linear model with binomial error structure; for plant height, corolla width and herkogamy, linear mixed model. Plant height was square root transformed to normalize the residuals. All the predictors were standardized to one standard deviation and mean zero. The use of standardized predictors is useful for interpretation and comparison of the relative importance of estimate effects in cases where the predictors have different scales such as density and plant height used in our analysis (Schielzeth 2010).

In the linear mixed models, we assessed statistical significance of coefficients by calculating *P*-values with Kenward-Roger approximation of denominators degrees of freedom using the package *lmerTest* (Kuznetsova et al. 2016). A simulation study showed that Kenward-Roger method results in smaller

bias of Type I error when calculating denominators degrees of freedom relative to others such as Satterthwaite method and Wald test in linear mixed models (Spilke et al. 2005). Finally, to identify whether variation was associated with population, we dropped population from each model and assessed statistical significance of the effect of population using Chi-squared tests. We conducted all the analysis in R version 3.4.0 (R Core Team 2017).

4.2 Results

4.2.1 Phenotypic variation and density

We observed substantial variation in many traits measured in the field that is summarized in Table 1. The highest coefficient of variation (CV %) values were for number of flowers (150.81), number of floral stems (116.24), number of fruits (170.79), herkogamy (100) and number of stolons (83.82). The lowest value was for corolla width (20.9) (Table 1). The effect of population on trait variation was significant for the traits analysed: number of flowers ($\chi^2 = 3791$, $P < 0.001$), number of stolons ($\chi^2 = 221.65$, $P < 0.001$), plant size ($\chi^2 = 416.08$, $P < 0.001$), corolla width ($\chi^2 = 126.41$, $P < 0.001$) and herkogamy ($\chi^2 = 4.19$, $P = 0.04$). Average density over quadrats within each population varied from 37.5% in KIL to 100% in DAR, FUN, and SIN populations.

Table 1. Summary of traits measured in the field. Number of individuals (n), mean, standard deviation (SD), minimum, maximum and coefficient of variation (CV %).

Trait	n	Mean	SD	Minimum	Maximum	CV%
Number of Flowers	460	20.05	30.23	1	433	150.81
Number of Floral stems	454	1.8	2.1	0	27	116.24
Number of Stolons	455	4.47	3.75	0	26	83.82
Number of fruits	460	14.38	24.57	0	374	170.79
Plant size (cm)	447	61.38	32.33	7	200	52.67
Stem thickness (mm)	480	6.42	4.17	0.4	29	65.01

Leaf length (mm)	442	45.21	25.28	10.1	170.5	55.34
Corolla width (mm)	271	27.85	5.82	12.6	46	20.9
Corolla height (mm)	270	22.14	5.35	9.5	37	24.19
Herkogamy (mm)	293	3.17	3.17	-13	33.1	100

4.2.2 Relationship between traits, climatic variables and population density

The first two principal components cumulatively explained 73.16% of variation in climatic variables (PC1 = 51% and PC2 = 22%). Most of the variables (16 out of 19) had moderately high loading values on PC1 (> 0.14), except for minimum temperature of coldest month, mean temperature of driest quarter and mean temperature of coldest quarter (Supplementary Table 2). In PC1, most of the temperature variables had negative loadings, except for minimum temperature of coldest month, while all precipitation variables had positive loadings (Supplementary Table 2). The plot of variables factor map shows the climatic variables projected into the area spanned by the two PCs and indicate the correlation between the variables and the PC (Figure 2). The factor map shows that PC1 correlated positively with many precipitation variables including annual precipitation (bio 12), precipitation of the wettest month (bio 13) and precipitation of wettest quarter (bio 16). The variables that most negatively correlated with PC1 were annual temperature range (bio 7) and temperature seasonality (bio 4). PC2 summarizes variation in a combination of temperature and precipitation variables, such as mean temperatures of quarterly periods, mean annual temperature, quarterly precipitation, precipitation seasonality and isothermality. The plot of variables factor map shows that the many temperature variables were highly positively correlated with PC2 such as mean temperature of driest quarter (bio 9), mean temperature of the coldest quarter (bio 11) and mean annual temperature (bio 1). Among the precipitation variables the most positively correlated with PC2 was precipitation seasonality (bio15). The most negatively correlated with PC2 was precipitation of the warmest quarter (bio 18) (Figure 2).

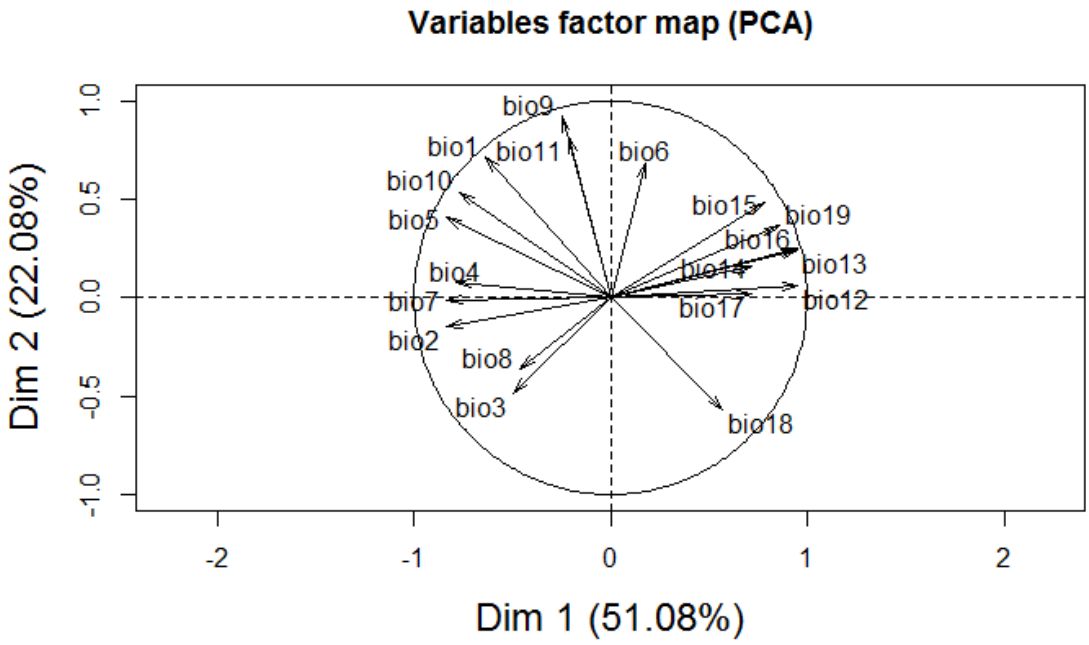

Figure 2. Plot showing the correlation between climatic variables and the two principal components (PC) from the principal component analysis (PCA). The first PC is labelled as Dim 1 and accounts for 51.08% of the total variation. Dim 2 is the second PC with an additional 22% of the variation. The perpendicular projection of the arrows to the dimension represents the correlation (loadings) of the variable with each PC. Longer arrows account for a larger amount of the total variance. Arrows are labelled according to the supplementary Table 3. Bio one to 11 represents temperature variables and bio 12 to 19 precipitation variables.

A plot of the first and second principal components of climatic variables illustrates the distribution of populations of *M. guttatus* studied here and shows that PC2 separates populations from high and low latitudes in two groups (Figure 3).

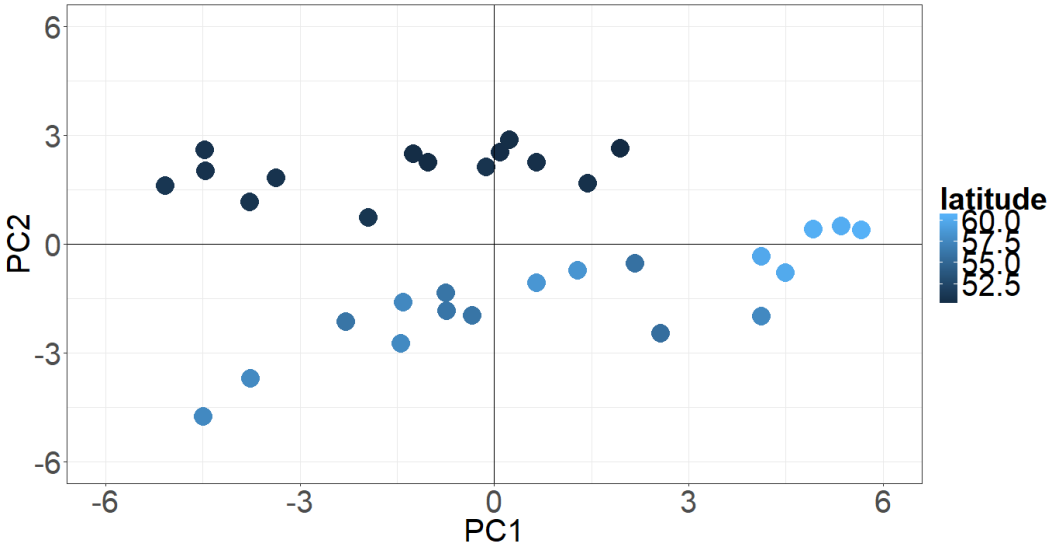

Figure 3. Plot of the two principal components in the analysis of 19 climatic variables obtained for 32 populations of *Mimulus guttatus* in United Kingdom. Points are coloured according to latitude.

We found a positive effect of PC2 on number of flowers (Table 2A, Figure 4A). In contrast, PC2 was negatively associated with number of stolons (Figure 4B) and proportion of clones (Figure 4C). The association of plant height with both number of flowers and stolons was positive, which indicates that as plant grows the production of flowers and stolons increases (Table 2A). Plant height, corolla size and herkogamy were not associated with PC1, PC2 or density (Table 2B, Figure 5). Pearson correlation coefficients revealed that PC2 was negatively correlated with latitude (r = -0.78, Table 3).

Table 2. A) Generalized linear mixed model results for the fixed effects of PC1, PC2, plant height and density on number of flowers, number of stolons and proportion of stolons (number of stolons divided by number of stolons and flowers). B) Linear mixed models results for the fixed effect of PC1, PC2 and density on plant height, corolla width and herkogamy. For linear mixed models *P* values were calculated using Kenward-Roger`s approximation of denominators degrees of freedom using the package *lmerTest.* Est. = Standardized estimates. Bold numbers means statistical significant of standardized estimates with $P < 0.05$.

A)	**Explanatory variables**											
	PC1			**PC2**			**Density**			**Plant height (cm)**		
	Est.	F-value	*P*	Est.	F-value	*P*	Est.	F-value	*P*	Est.	F-value	*P*
Number of flowers	0	0.01	0.82	**0.14**	8.48	<0.01	-0.01	0	0.76	**0.21**	122.38	<0.01
Number of stolons	0.03	1.63	0.21	**-0.08**	4.3	0.03	-0.05	2.08	0.23	**0.16**	13.1	<0.01
Proportion of clones	0.02	0.46	0.57	**-0.21**	17.01	<0.01	-0.01	0.84	0.83	**-0.26**	21.82	<0.01
B)	**Explanatory variables**											
	PC1			**PC2**			**Density**					
	Est.	F-value	*P*	Est.	F-value	*P*	Est.	F-value	*P*			
Plant height (cm)	0.11	2.59	0.74	0.33	3.79	0.06	0.16	2.54	0.1			

Corolla width (mm)	0.24	0.75	0.39	0.55	2	0.16	-0.37	1.49	0.22
Herkogamy (mm)	-0.05	0.31	0.57	-0.05	0.2	0.64	0.02	0.02	0.88

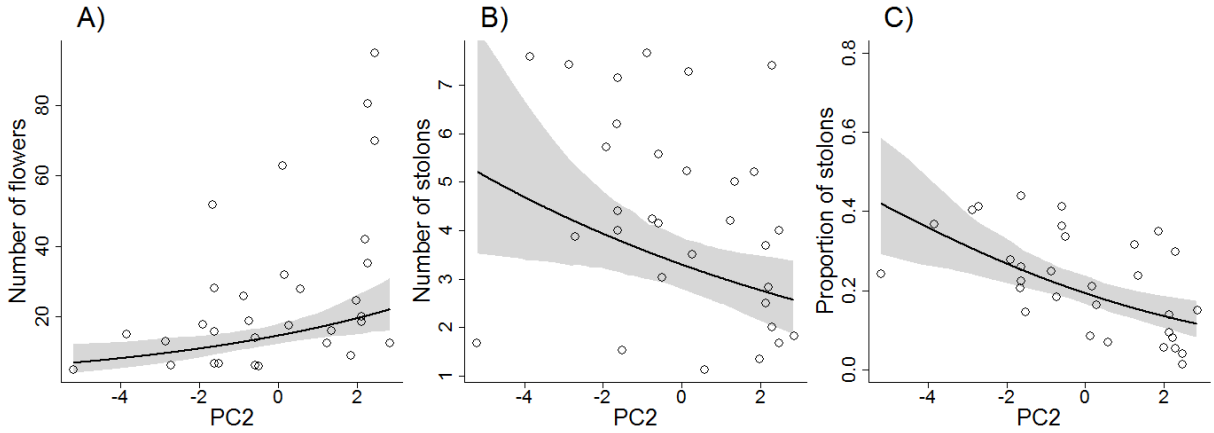

Figure 4. A) Relationship between number of flowers and PC2, B) Relationship between number of stolons and PC2, C) Relationship between proportion of stolons (number of stolons divided by total number of stolons and flowers) of *Mimulus guttatus* and PC2. Lines represent partial effect from the generalized linear mixed effect model (Table 2A). Points are average of individuals within populations. Grey area represents bootstrapped 95% confident intervals.

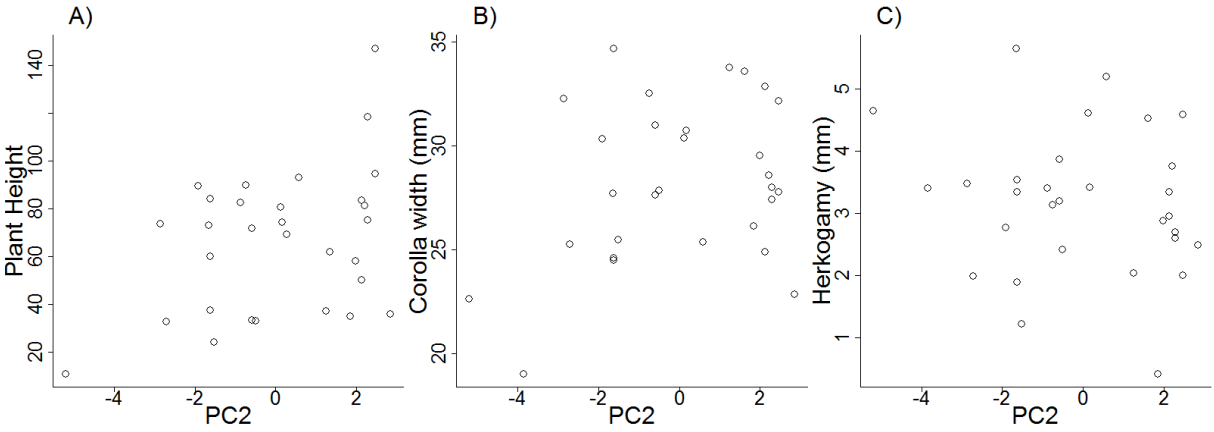

Figure 5. Non- significant relationships between PC2 and plant height (A), corolla width (B) and herkogamy (C) of *Mimulus guttatus*. Points represent average of individuals within populations.

Table 3. Pearson correlation coefficients of latitude, PC1, PC2, floral and morphological traits measured in individuals of *Mimulus guttatus* in the field.

	Plant height (cm)	Stem thickness (mm)	Leaf length (mm)	Number of flowers	Number of fruits	Number of flowering stems	Corolla width (mm)	Corolla height (m)	Herkogamy (mm)	Latitude	PC1	PC2
Plant height (cm)		0.78	0.16	0.43	0.37	0.37	0.44	0.28	0.1	0.06	0.11	0.14
Stem thickness (mm)			0.06	0.72	0.46	0.39	0.4	0.35	0.12	0	-0.28	0.35
Leaf length (mm)				0.41	0.32	0.39	0.38	0.27	0.11	-0.19	-0.14	0.17
Number of flowers					0.98	0.96	0.13	0	0	-0.11	-0.05	0.15
Number of fruits						0.93	0.08	-0.03	0.04	-0.06	0	0.12
Flowering stems number							0.1	0.03	0	-0.13	-0.09	0.12

Corolla width (mm)								0.5	0.16	0.21	0.09	0.1
Corolla height (mm)									0.09	0.1	-0.16	-0.2
Herkogamy (mm)										0.06	0.05	-0.05
Latitude											0.43	-0.78
PC1												0.2
PC2												

4.3 Discussion

In our field survey of *Mimulus guttatus* populations in the introduced range, we found significant variation in plant size, investment in sexual and clonal reproduction, flower size and herkogamy among populations. A combination of temperature and precipitation variables associated with PC2 influence the absolute and relative investment in flowers and stolons. We showed that as temperature decreases, there is more investment in clones relative to the number of flowers. Moreover, places with low precipitation during warm months of the year and high precipitation seasonality favour sexual reproduction. Further work, e.g., with common garden experiments, is needed to distinguish whether this variation in investment is due to phenotypic plasticity or genetic variation. We found that sexual and clonal reproductive traits, likewise plant height, flower size and herkogamy, are not density-dependent. Our findings suggest that, in *M. guttatus*, different combinations of temperature and precipitation favour differential investment in sexual or asexual reproductive modes.

4.3.1 Effect of climatic variables on trait variation

Consistent with a growing number of studies that have found patterns of phenotypic variation along latitudinal and climatic gradients in other plant species (e.g., Weber and Schmid 1998; Kollmann and Bañuelos 2004; Mozdzer et al. 2016), we found that a combination of temperature and precipitation conditions change sexual and clonal reproductive allocation among populations of *M. guttatus* in the UK. In general, clonal reproduction is favoured in climates with high precipitation, with little seasonality and low temperatures. Interestingly, sexual investment showed the opposite pattern with populations in warm climates with dry summers producing many flowers relative to stolons. Different investment in clones and flowers in populations from different climates found in our study is consistent with the type of habitats that select for high investment of sexual reproduction in annuals as opposed to high investment of clonal reproduction in perennials across native populations of *M. guttatus*. Perennial native populations are locally adapted to

coastal areas with cool temperatures that maintain soil moisture year-round and to inland areas close to streams and rivers that retain permanently wet soil. In contrast, annual plants are adapted to inland habitats that have hot summers with low precipitation in which soil drought kills annual individuals in the beginning of the summer (Lowry et al. 2008). Surveys around the UK have shown that the percentage of soil moisture is higher in the northern part of the country than in the southern part (Henrys et al. 2014), thus we suggest that soil moisture could mediate the effect of latitude on allocation to flowers and stolons. For a drought intolerant species such as *M. guttatus*, high investment in stolons under high precipitation and in flowers under low precipitation conditions, seems to be an adaptation to soil water availability that in native *M. guttatus* favours different reproduction strategies.

Site-level variables not measured in our study could influence phenotypic variation among populations. For instance, soil texture can influence infiltration and retention of water available to the roots (Kramer and Boyer 1995), the frequency and duration of river flow events determines soil water saturation after high water levels that may influence survival of deposited stolons (Truscott et al. 2006). In addition, soil nutrients can influence the allocation to different reproductive modes, as in habitats with favourable soil nutrient content many perennial plants tend to promote clonal growth over sexual reproduction, possibly because seeds are costlier than clones and high nutrients may reduce the need to produce seeds that could serve as a mechanism of escape from sites with scarcity of nutrients, through seed dispersal (reviewed in Yang and Kim 2016). Moreover, the different investment in flowers among *M. guttatus* populations in UK could be the result of shorter days in the north and longer days in the south influencing flowering time and number of flowers. For example, in *Prunella vulgaris*, it was suggested that higher seed set in the south than in the north is a consequence of a longer summer season in the south selecting for individuals that grow for longer periods before flowering and thereby invest more resources in producing many flowers, while shorter days in the north select for early flowering individuals that produce few flowers (Winn and Gross 1993). Further studies measuring the relationship between flowering time and sexual reproduction in non-native *M. guttatus* are needed to investigate if day length influence sexual reproduction. Finally, the observed phenotypic patterns may be influenced by sampling populations at different phenological phases during the

summer. For instance, a population may have a small number of flowers because it was sampled at the start of the flowering season and not during peak flowering. However, in our study, populations from the south of the UK with many flowers (sum of number of buds, flowers and fruits) were sampled first (7-22 of July), before northern populations with fewer flowers (24 of July to 25 of August), suggesting that we started the phenotypic measurements in the peak of flowering of *M. guttatus* in UK and our results are not biased by sampling date.

Our results also agree with the general observation that clonal growth is favoured in cold climates (Ye et al. 2016). Investment in clonal reproduction has advantages under conditions that limit sexual reproduction, as clonal organs can photosynthesize and store reserves, being less energetically costly than sexual reproduction (Watson 1984). Therefore, if low temperatures limit sexual reproduction, a shift to clonal reproduction in places with low temperatures could be a safe strategy to secure reproduction under unfavourable conditions.

The results of our study support previous phenotypic clines of relative investment in sexual and asexual reproduction observed in a few introduced *M. guttatus* populations (van Kleunen and Fischer 2008). Van Kleunen and Fischer grew plants from 17 native and seven invasive populations from Scotland and New Zealand in a common garden and found a positive correlation between latitude and vegetative reproduction, and a negative correlation with sexual reproduction investment. Although they did not test the effect of climatic variables, our result is in line with their study since PC2 has a negative relationship with clonality (Table 2A) and a negative correlation with latitude (r = - 0.78, Table 3). Greenhouse common garden studies using family comparisons in *M. guttatus* have shown that sexual and clonal reproduction have a genetic basis and that the investment in flowers occurs at the expense of stolons, consistent with a trade-off among these traits (van Kleunen 2007; Baker and Diggle 2011). In our study, we show that the investment in clonal and sexual traits in *M. guttatus* changes among perennial populations in the introduced range according to climatic conditions. If trade-offs are the result of negative genetic correlations, selection for increase sexual investment in one environment will also select for decreased clonal investment. Future work with common garden experiments combined with measurements of natural selection on sexual and clonal reproduction will help explain if divergent

selection is involved in the observed population differentiation among *M. guttatus* populations.

Environmental conditions can also affect floral morphology. For instance, cold environments may select for reproductive assurance through self-fertilization in plants, due to the low abundance of pollinators (Arroyo et al. 1985). In *M. guttatus,* autonomous selfing is associated with a decrease in anther-stigma separation and small flowers (Ritland and Ritland 1989; Carr and Fenster 1994, Fenster and Ritland 1994; Robertson et al. 1994; Van Kleunen and Ritland 2004), and also in other self-compatible plants (e.g., Vallejo-Marín and Barrett 2009). In our study, however, we did not find a relationship between environmental variables and reduced herkogamy, or modification of flower size. Similarly, herkogamy and flower size have shown to be environmentally independent in other species (Kay and Picklum 2013). Our result agrees with van Kleunen and Fischer (2008), who showed no variation in herkogamy and flower size within three introduced *M. guttatus* populations from UK and four from New Zealand. However, our results contradict Bodbyl Roels and Kelly (2011) who showed, in the absence of pollinators, rapid evolution of smaller anther-stigma distance and higher autonomous seed set in annual *M. guttatus* in only five generations. A possible explanation for our findings is that *M. guttatus* is not pollen limited, due to sufficient pollinator visits throughout the UK, and does not depend on high self-pollination to secure reproduction. Consequently, genetic variation for herkogamy may not be under selection or floral traits are less environmentally plastic than, for example, number of flowers. A recent common garden study with 24 species of herbaceous angiosperms in Central Europe showed that invasive species are able to attract as many pollinators as non-native and native species, suggesting that pollen limitation may not restrict the spread of invasive species, particularly when they become naturalized (Razanajatovo and van Kleunen 2016). Many British native bumblebee species, for instance, effectively pollinate *M. guttatus* in the UK (Robertson et al. 1999). Even if density of pollinators is lower in north than in the south of the UK, *M. guttatus* has the advantage of clonal reproduction as a way of reproductive assurance (Dole 1992). Additionally, van Kleunen (2007) found that *M. guttatus* populations with annual life-cycles had smaller flowers and anther–stigma separation than plants from populations with perennial life-cycles, which suggests that autonomous seed set is more important in annual than in perennial plants. Our

results indicate that floral morphology, which facilitates self-fertilization as a reproductive assurance mechanism under heterogeneous environments, may not always be important in invasive species, particularly for perennial species, such as *M. guttatus,* that harbour clonal growth as a complement to sexual reproduction.

4.3.2 Effect of population density on trait variation

Contrary to our expectation, we did not detect an effect of population density on sexual and clonal reproduction along with other traits such as plant size and flower size. The reproductive behaviour of clonal plants in populations with different densities across studies is inconsistent. For example, individuals of *Ranunculus reptans* invested more in sexual reproduction (number of flowers) than clonality (number of rosettes) in high-density populations (van Kleunen et al. 2001), but for *Fragaria virginiana* allocation to seed production remained constant in plants grown at different densities (Holler and Abrahamson 1977). Interspecific competition has been shown to increase the lateral spread of stolons in some species (Price et al. 1996; van Kleunen and Fischer 2001). Thus, it is possible that density could affect lateral spread of stolons, instead of number of stolons in *M. guttatus*. In our study, the measure of stolon lateral spread in the field was not feasible, because the extensive clonal propagation of many plants (with ramets spreading more than a meter from the original rosette) prevented us from accurately measuring the length of stolons. The development of greenhouse experiments with and without interspecific competition would allow a more complete measure of clonality that includes number of stolons and stolon spread (e.g., stolon length x number of stolons).

In some cases reproductive allocation could be an indirect response of plant size to density, because in many species plant size determines the start of reproduction and number of flowers produced (Liu et al. 2008). In a competition experiment with the clonal plant *Geum reptans*, for instance, many plants reproduce only by stolons, probably because under competition most of the individuals are small and do not reach a minimum size to produce flowers (Pluess and Stöcklin 2005). Plant size was not affected by density, but had an almost significant effect of PC2. It is possible that plant size, measured as stem length, in our study, was too

simple to quantify plant growth and instead a more complex measure of plant size that includes upright and lateral size (e.g., stem length x rosette diameter) could enable us to detect the effect of density and PC2 on plant size. However, we found that, even after accounting for plant size, PC2 was significantly related to production of stolons and flowers. This result suggests that number of stolons and flowers produced by individuals of *M. guttatus* are not limited by competition and, instead, among population variation of these traits respond to factors associated with climatic variables such as temperature and precipitation.

4.4 Conclusion

We showed that the relative investment in clonal and sexual reproduction in non-native *M. guttatus* in the UK is environment-dependent and is influenced by a combination of temperature and precipitation variables that differ along a latitudinal gradient. Flower production in non-native *M. guttatus* is favoured in places where high temperatures and low precipitation in the south prevent clonal reproduction, probably because of intolerance to soil drought as observed in native populations. Conversely, clonal reproduction seems to be an alternative strategy to sexual reproduction in places with cooler, wetter and stable seasonality climates towards the north. This result suggests that for a highly diverse species, such as *M. guttatus*, phenotypic differences in reproductive allocation can be mediated by contrasting climatic conditions across its introduce range just 200 years after species introduction.

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4.6 Supplemental material

Supplementary Table 1. Population code, geographic coordinates and location name. Locations are ordered from south to north. Density (%) is the sum of *Mimulus guttatus* and other species coverage averaged over quadrats within each population.

Population code	Latitude (N)	Longitude (W)	Location name	Density %
14-CRO	50.1522	-5.2849	Crowan - England	72.7
14-EAS	50.2165	-3.7131	East Prawle - England	82.0

14-DAR	50.3298	-3.5738	Dartmouth - England	100
14-MOO	50.4515	-4.4861	Moors water - England	70.0
14-TCO	50.4981	-4.4657	Higher Tremar Combe - England	70.0
14-SOU	50.6016	-3.7677	Southwett - England	54.0
14-CHA	50.6741	-3.8553	Chagford - England	75.0
14-BOG	50.7972	-0.6982	Bognor Regis Plant Centre - England	76.0
14-HUN	50.8107	-0.7888	Hunston - England	52.5
14-FUN	50.8614	-0.8561	Funtington - England	100
14-SIN	50.9113	-0.7533	Singleton - England	100
14-UPL	50.9351	-3.4117	Uplowman - England	66.0
14-TOU	51.0744	-3.1238	Kingston St. Mary - England	62.0
14-HOU	51.0970	-1.5084	Houghton Lodge Garden – Portsmouth, England	80.0
14-LYN	55.6508	-3.6151	Lyne water - Scotland	61.9
14-GLA	55.8724	-4.2811	River Kelvin, Glasgow - Scotland	55.0
14-RIV	56.1216	-3.9327	Riverside, Stirling - Scotland	32.8
14-STI	56.1300	-3.9640	Stirling - Scotland	70.5
14-TIL	56.1472	-3.7448	Tilicoultry - Scotland	76.2
14-BRI	56.1557	-3.9512	Bridge of Allan - Scotland	62.0
14-MAR	57.5723	-4.4274	Maryburg - Scotland	64.5
14-GAR	57.6150	-4.6734	Garve - Scotland	68.0
14-KIL	57.6300	-3.5757	Kinloss - Scotland	37.5
14-DAL	57.6830	-4.2652	Dalmore - Scotland	73.3
14-POR	57.6939	-2.9259	Portessi - Scotland	80.6
14-BLA	58.4831	-5.1132	Blairmore - Scotland	93.2
14-BKN	58.5750	-4.7683	Balnakeil - Scotland	87.4
14-BOD	59.9041	-1.3027	Boddam, Shetland - Scotland	79.7
14-NIN	59.9777	-1.3003	St. Ninians Road, Shetland - Scotland	74.0
14-WEI	60.2548	-1.2917	Weisdale, Shetland - Scotland	90.0
14-MUK	60.3480	-1.4137	Muckle Roe, Shetland - Scotland	79.6
14-HAM	60.5034	-1.0993	Hamnavoe, Shetland - Scotland	55.8

Supplementary Table 2. Loadings of the 19 environmental variables from WorldClim database on the two first principal component (PC) analysis. Variables are ordered from the largest to smallest loadings values in PC2.

Variables	PC1	PC2
Mean Temperature of Driest Quarter	-0.08	0.45
Mean Temperature of Coldest Quarter	-0.06	0.39
Annual mean temperature	-0.2	0.35
Minimum Temperature of Coldest Month	0.05	0.33
Precipitation of Warmest Quarter	0.18	-0.27
Mean Temperature of Warmest Quarter	-0.24	0.26
Isothermality	-0.15	-0.23
Precipitation Seasonality	0.25	0.23
Maximum Temperature of Warmest Month	-0.26	0.19
Mean Temperature of Wettest Quarter	-0.14	-0.17
Precipitation of Coldest Quarter	0.27	0.17
Precipitation of Wettest Month	0.3	0.12
Precipitation of Wettest Quarter	0.3	0.11
Mean Diurnal Range	-0.26	-0.07
Precipitation of Driest Month	0.23	0.07
Temperature Seasonality	-0.25	0.03
Annual Precipitation	0.3	0.03
Temperature Annual Range	-0.26	0
Precipitation of Driest Quarter	0.23	0

Supplementary Table 3. Definition of the 19 climatic variables from WorldClim according to http://www.worldclim.org/.

Variable	Definition
Bio 1 Annual Mean Temperature	The annual mean temperature
Bio 2 Annual Mean Diurnal Range	The mean of the monthly temperature ranges (monthly maximum minus monthly minimum).
Bio 3 Isothermality	Isothermality quantifies how large the day-to-night temperatures oscillate relative to the summer-to-winter (annual) oscillations
Bio 4 Temperature Seasonality	The amount of temperature variation over a given year
Bio 5 Max Temperature of Warmest Month	The maximum monthly temperature occurrence over a given year
Bio 6 Min Temperature of Coldest Month	The minimum monthly temperature occurrence over a given year
Bio 7 Annual Temperature Range	A measure of temperature variation over a given period.
Bio 8 Mean Temperature of Wettest Quarter	This quarterly index approximates mean temperatures that prevail during the wettest season
Bio 9 Mean Temperature of Driest Quarter	This quarterly index approximates mean temperatures that prevail during the driest quarter
Bio 10 Mean Temperature of Warmest Quarter	This quarterly index approximates mean temperatures that prevail during the warmest quarter

Bio 11 Mean Temperature of Coldest Quarter	This quarterly index approximates mean temperatures that prevail during the coldest quarter
Bio 12 Annual Precipitation	This is the sum of all total monthly precipitation values.
Bio 13 Precipitation of Wettest Month	This index identifies the total precipitation that prevails during the wettest month.
Bio 14 Precipitation of Driest Month	This index identifies the total precipitation that prevails during the driest month
Bio 15 Precipitation Seasonality	This is a measure of the variation in monthly precipitation totals over the course of the year
Bio 16 Precipitation of Wettest Quarter	This quarterly index approximates total precipitation that prevails during the wettest quarter
Bio 17 Precipitation of Driest Quarter	This quarterly index approximates total precipitation that prevails during the driest quarter
Bio 18 Precipitation of Warmest Quarter	This quarterly index approximates total precipitation that prevails during the warmest quarter
Bio 19 Precipitation of Coldest Quarter	This quarterly index approximates total precipitation that prevails during the coldest quarter

Chapter 5: Natural selection and outbreeding depression suggest adaptive differentiation of non-native populations of *Mimulus guttatus*

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*This chapter was submitted to Evolution

Abstract

Studies documenting natural selection in non-native populations represent a key step towards understanding the role of adaptive evolution during rapid evolutionary change. In addition, analysing the fitness of admixed individuals can be used as a tool to uncover the benefits (e.g., heterosis) and costs (outbreeding depression) of admixture between genetically differentiated populations. Here we use experimental F2 crosses between native and introduced populations of *Mimulus guttatus* to estimate the pattern of natural selection in its introduced range, and to seek evidence of outbreeding depression in admixed experimental populations. The F2s combined the maternal genome of an introduced individual with the paternal genome of either native or introduced populations. We found that the introduced × introduced cross had the fastest population growth rate due to increased winter survival, clonality, and seed production. Our analysis also revealed that selection through sexual fitness favoured large floral displays, large vegetative traits and flower size, clonal spread, and early flowering. Our results indicate a source-of-origin effect, which is consistent with outbreeding depression exposed by mating between introduced and native populations, and demonstrates the potential for adaptive evolution in introduced populations. Together with recent genomic evidence of selection in introduced *M. guttatus*, our findings point to an important role of natural selection in the establishment and spread of non-native populations.

5 Introduction

Biological introductions, the introduction of populations beyond their native range, present individuals with novel environmental challenges. Although only some introduced species become invasive and result in severe costs for local biodiversity, ecosystem services, and the economy (Pimentel et al. 2005; Pysek et al. 2012), all successful introductions require that populations become established and spread under new conditions. Both adaptive and non-adaptive processes contribute to the success of biological introductions. Successful establishment and spread of non-native species can occur without adaptive evolution in the novel range, for example through the escape from native herbivores, parasites and competitors (Keane and Crawley 2002; Mitchell and Power 2003), through phenotypic plasticity (Riis et al. 2010), or through pre-existing adaptations that evolved in the native range in response to similar selective pressures (Henery et al. 2010; Elst et al. 2016). However, non-native populations often experience novel environments that may result in natural selection (Maron et al. 2004; Colautti and Barrett 2013) and, potentially, adaptive evolution in the introduced range (Lee 2002; Colautti and Lau 2015).

One process that may facilitate biological invasions is admixture between genetically differentiated populations (Lavergne and Molofsky 2007; Dlugosch et al. 2015; Hahn and Rieseberg 2017), but whether admixture benefits or hinders introduced populations is a matter of debate, and could be system-specific (Rius and Darling 2014; Hamilton and Miller 2016). For example, admixture can facilitate biological invasions through an increase in adaptive genetic diversity (Dlugosch et al. 2015) and evolutionary potential of introduced populations (Hamilton and Miller 2016). In the short term, admixture can deliver immediate benefits through heterosis (Keller and Taylor 2010; van Kleunen et al. 2015; Hahn and Rieseberg 2017), particularly when populations are inbred and genetically uniform. However, admixture between genetically differentiated populations can be costly, as admixed populations can suffer from outbreeding depression (Price and Waser 1979; Schaal and Leverich 2005; Rius and Darling 2014). The cost of admixture can be environment-independent, for example when gene flow breaks down co-adapted gene complexes or when it brings together genetic

incompatibilities between previously isolated populations (Etterson et al. 2007). In addition, admixture can produce phenotypes that are poorly suited to the local ecological conditions (Verhoeven et al. 2011; Rius and Darling 2014), resulting in environment-dependent outbreeding depression. The cost of admixture on the fitness of introduced populations can be amplified by evolution in the introduced range. For example, genetic drift, which may be particularly strong in the early stages of invasion when populations are small (Lee 2002), will increase genetic differentiation—and potentially the cost of admixture—either among introduced populations or between native and introduced populations (Dlugosch et al. 2015). Moreover, adaptive evolution in the introduced range would result in local adaptation (Verhoeven et al. 2011; Oduor et al. 2016), which may be disrupted by admixture, amplifying environment-dependent outbreeding depression. Studies of the effect of admixture on introduced populations can help in understanding how some populations may become invasive (Dlugosch et al. 2015) and provide insights into evolutionary processes faced by organisms colonising new environments, particularly in an era when human-mediated transport is breaking down reproductive barriers between previously isolated populations (Vallejo-Marin and Hiscock 2016).

Previous work has demonstrated that natural selection can be an important force in introduced plant populations, and cause adaptive evolution, sometimes rapidly (Colautti and Lau 2015). Natural selection in introduced populations can be inferred in a number of ways, including through the detection of genetic clines (Maron et al. 2004; Montague et al. 2008; Colautti and Barrett 2013), comparing fitness of native and introduced populations in common gardens (reviewed in Colautti et al. 2009), Q_{ST}-F_{ST} comparisons (e.g., Chun et al. 2011), and through genome scans (Bock et al. 2015). One direct way to detect selection in introduced populations is by conducting a phenotypic selection analysis in the introduced range (Colautti and Lau 2015). Estimates of selection, including selection gradients, measure selection on individual traits (Lande and Arnold 1983) and provide direct evidence of the pattern of natural selection. Measuring natural selection in natural or naturalised populations can be challenging because under directional and stabilising selection, phenotypic variation in the population is generally reduced through time. As a consequence, the statistical power to detect the relationship between fitness and quantitative traits is decreased (Schluter 1988). Artificial

crosses between genetically distinct populations are a powerful tool that can be used to increase the frequency of rare genotypes in experimental studies of natural selection (Conner 2003). This approach can also be used to increase phenotypic variation in studies of selection in introduced populations, including generating the ancestral variation potentially eliminated by selection. To date, relatively few studies have measured natural selection in introduced populations with or without the help of artificial crosses (reviewed in Colautti and Lau 2015), and more studies are needed to characterise natural selection in the novel range.

Here we estimate patterns of natural selection on introduced populations with different admixed origins. As a study system we used *Mimulus guttatus* DC. (Phrymaceae), a flowering plant native to western North America that has been introduced to, among other places, eastern North America, Europe, including the British Isles, and New Zealand (Murren et al. 2009; Tokarska-Guzik and Dajdok 2010; Vallejo-Marin and Lye 2013). The history of invasion of *M. guttatus* is best documented in the United Kingdom (UK), where there are historic records of the first arrival of this species as well as of the potential sources of origin of extant populations (Puzey and Vallejo-Marín 2014; Pantoja et al. 2017). Briefly, *M. guttatus* was introduced into the UK in 1812, and became naturalised by the 1830s (Puzey and Vallejo-Marín 2014). Although the exact source of origin is unknown, previous work suggest that *M. guttatus* was introduced from populations in the north Pacific, and historical records suggest that it may have originated in Alaska (Puzey and Vallejo-Marín 2014; Pantoja et al. 2017). Previous work on *M. guttatus* suggest that genetically-based phenotypic differentiation in the introduced range (UK and New Zealand) is likely due to adaptive rather than non-adaptive processes (van Kleunen and Fischer 2008). Genomic scans of native and introduced populations of *M. guttatus* using re-sequencing approaches indicate that natural selection has played a role in shaping introduced populations in the UK (Puzey and Vallejo-Marín 2014). Moreover, a recent experimental study investigated the phenotypic effects of admixture in introduced populations of *M. guttatus* (van Kleunen et al. 2015), and suggested that admixture could improve sexual and asexual reproduction. F1 crosses between native (both annual and perennial ecotypes) and introduced populations from the UK and New Zealand displayed heterosis in the probability of flowering, flower number, clone size and biomass in a greenhouse study (van Kleunen et al. 2015). However, heterosis was only

observed for crosses between native and introduced ranges, but not when comparing within- vs. between-population crosses in the introduced range. Heterosis in admixed populations is potentially important because gene flow from the native range could magnify the invasive potential of *M. guttatus*, and indicate that the benefits of heterozygosity outweigh potential costs associated with outbreeding depression (van Kleunen et al. 2015). However, heterosis can sometime be a transient phenomenon that breaks down in the F2 as the parental genomes recombine (but see Willi et al. 2007). Therefore, further work is needed to confirm whether the heterosis observed in greenhouse conditions in the F1 generation, extends to field conditions and to subsequent generations.

To investigate the effect of admixture on plant fitness under field conditions, and to establish the pattern of selection on individual traits, we used experimental pollinations to generate F2 crosses between native and introduced populations of *M. guttatus*. Specifically, we generated three arrays of F2 segregant progeny of *M. guttatus*, which combined the maternal genome of an introduced individual with the paternal genome of either: (1) a native Alaskan perennial, (2) a native Californian annual, or (3) another introduced British perennial. The objective of analysing F2 crosses was two-fold: First, we wanted to increase the amount of phenotypic variation among experimental plants to facilitate the detection of natural selection (Conner 2003). Second, we wanted to create admixed individuals to investigate whether population-of-origin shapes the fitness consequences of admixture, particularly whether crosses between native and introduced populations have lower fitness compared to crosses between introduced populations, as predicted by the outbreeding depression hypothesis. It is important to stress, that the goal of using F2s in this experiment was not to recreate natural variants or mimic current opportunities for admixture in the introduced range, but to reshuffle the genetic variation of an introduced population among three different backgrounds (two native and another introduced). To this end, the two native populations were chosen to represent two contrasting native backgrounds that bracket the variation in life history observed in *M. guttatus* (annual vs. perennial), while the two introduced populations reflect some of the variation observed in the non-native range. Our study addressed three specific questions: (i) Does source-of-origin affect the fitness of admixed individuals resulting from crosses between native and introduced populations? (ii) Does introduced × introduced admixture result in

higher fitness than native × introduced admixture as predicted by the outbreeding depression hypothesis? (iii) What is the pattern of selection acting on floral and vegetative traits in the introduced range? Our overarching goal is to understand the role of natural selection in the evolution of non-native populations.

5.1 Materials and Methods

5.1.1 Plant material

Introduced populations of *M. guttatus* were collected as seeds from two localities in Scotland: Dunblane, Perthshire (DBL; 56.19° N, 3.96° W), and Coldstream, Scottish Borders (COL; 55.65° N, 2.24° W). Both Scottish populations have a perennial life history, and propagate profusely via clonal reproduction. Clonal growth occurs mostly by rooting of lateral stems (stolons). Native populations were obtained from seed collections and herbarium specimens. To represent an annual life history, we selected a population from Lower Mendocino County, California (LMC; 38.86° N, 123.08° W). Individuals from this annual population come from a seasonally wet habitat that dries over the summer, and do not reproduce clonally. Seeds from LMC were provided by the Willis Lab, Duke University. Previous analysis using genome resequencing has shown that the LMC population belongs to a genetically distinct southern native clade, not closely related to the introduced populations of *M. guttatus* currently found in the British Isles (Puzey and Vallejo-Marín 2014). To represent a perennial life history, we selected a native population collected in the Alaskan peninsula at the confluence of the Alagnak and Nonvianuk rivers (ALASKA; 59.02° N, 155.85° W). The seeds were sampled from a herbarium specimen collected by the US National Park Service for the University of Alaska Museum Herbarium (ALA; ALAAC accession V142998). Although this specific population has not been previously analysed using genetic markers, our work suggests that Alaskan populations are genetically close to British material (Pantoja et al. 2017; Vallejo-Marin and Puzey, *unpublished*). Individuals from this ALASKA population grown in the University of Stirling's controlled environment facilities produce many long stolons.

5.1.2 Creation of experimental crosses and F2s

We manually crossed introduced and native populations to generate three arrays of F2 segregant progeny. These arrays combined the maternal genome of an introduced individual (DBL) with the paternal genome of either: (1) a native Alaskan perennial (ALASKA), (2) a native Californian annual (LMC), or (3) an introduced British perennial (COL). Our experimental design of using single individuals from each population to generate the F2s mean that we have necessarily included just a subsample of the variation in each population. However, by using the F2s we were able to study hundreds of individuals with genomes reshuffled by recombination. In choosing this design we have also assumed that differences between populations are larger than average individual differences within populations, and assumption likely to be met based on our previous work with these populations. We grew seeds from each of the four parental populations at the controlled environment facilities at the University of Stirling. Seeds were germinated and grown in 9cm pots (0.37L) filled with Modular Seed compost (Sinclair, Lincoln, UK), placed in flooded plastic trays. The growth chamber (Snijder, Microclima) was kept at 24°C/16hr light and 16°C/8hr dark cycles with 70% relative humidity and 80% illumination. A single individual from population DBL formed by two rounds of self-fertilisation and single-seed descent from a wild-collected seed (09-DBL-10-2) was used as the maternal parent to create F1 hybrids in December 2014. As the paternal parent of the F1 generation we used either an individual grown from a field-collected seed (V142998-5 or 10-COL-24-1, for ALASKA and COL crosses, respectively), or an individual obtained after self-fertilisation of a field collected plant (G-LMC-25; LMC cross).A single individual from each of these three hybrid lines was then self-fertilised in February 2015 to generate three F2 segregant populations. Hereafter, each of these three crosses are referred to as ALASKA (DBL × ALASKA), LMC (DBL × LMC), and COL (= DBL × COL).

5.1.3 Field experiment

We set up a field plot at the experimental gardens of the University of Stirling in May 2015. The experimental plot measured approximately 15 x 17m, and was previously used as an experimental wildflower meadow. Before the experiment, all vegetation was removed, and the soil was ploughed twice with an agricultural rotavator. The plot was divided into three spatial blocks each of approximately 15.4 x 4.7m. In order to mimic more closely the waterlogged environments where *M. guttatus* can be found in the British Isles, we installed a permanent watering system in each block, which consisted of a 16mm x 100m dripline that delivered water at 30cm intervals at a rate of 1.6 litres per hour at each drip point (Netafim, Lancashire, UK). Each block was watered in cycles of 48 hours of supplemental water alternated with 24 hours of no supplemental water.

Seeds from each F2 cross for the field experiment were first germinated on 27 May in 9cm pots in a growth chamber in 18h/6h light/dark cycles at 24°C/16°C and 70% relative humidity. Planting *Mimulus* seeds directly in the field is unfeasible due to their very small size, and requirement for surface germination, which make them prone to be washed away by wind or rain. Germination rate was assessed for each F2 cross in a separate experiment. After two weeks (two true leaf stage), seedlings were individually transplanted into plug trays filled with Modular Seed compost and placed in a research glasshouse with natural light and average day temperature of 18.8°C, for acclimation before transplanting them to the field. On June 17th, we transplanted seedlings with four to six true leaves from the glasshouse to their final location in the field plot. Each block consisted of 396 individuals from different crosses planted at random in 11 rows, and separated 0.40 m from each other. The field experiment consisted of 396 plants of each cross for a grand total of 1,188 *Mimulus* individuals.

We monitored individual plants for survival, growth, and both sexual and clonal reproduction until the end of the growth season (end of September 2015), and measured vegetative and reproductive traits at the onset of flowering for each individual (see *Selection* section). We recorded summer survivorship and total flower and fruit production at the end of the growth season (29 September to 3 October 2015), and winter survivorship at the beginning of the following spring (25 March 2016). To estimate seed production, we randomly selected 45 individuals

(15 per cross) and collected the seeds of 4-21 (average = 11.7) mature, non-dehisced, fruits produced at different times over the summer (August- September) for each individual. We then pooled the seeds from all fruits, weighed them, and divided by the number of fruits used, to obtain an estimate of seed production (in grams) per fruit. To convert this estimate to seed number, we counted and weighted 470-920 seeds per individual (average = 604 seeds), and obtained the number of seeds per gram. The number of seeds per fruit was then calculated as seed production per fruit (g) * number of seeds per gram. The average across the 15 individuals from each cross was used as an estimate of seed number per fruit. To estimate germination rates, we sowed 400 seeds from each F2 cross in 20 9cm-pots, placed in flooded trays in a polytunnel near the experimental plot, and counted the number of seedlings after eight days.

5.1.4 Estimate of total number of clones

Clone number was initially estimated at the time of flowering, and weekly for the following four weeks after the flowering season started. Initially, clone number remained relatively constant, but towards the final stage of the experiment, we observed that some plants (particularly COL individuals) also produced clones later in the season. Unfortunately, clone number was not systematically recorded at the end of the season. Because an estimate of clone number per cross type is needed for demographic analysis, we therefore conducted a subsequent experiment to estimate the average total number of clones produced per individual by the end of the growing season in each of the F2 crosses. At the beginning of July 2016, we germinated and transplanted 24 new plants from each of the F2 crosses. Each individual was placed in a large rectangular container (37 x 24 x 6cm) filled with All Purpose compost (Sinclair, Lincoln, UK). The containers were kept flooded and placed in the glasshouse. Every week for the next three months we counted the number of clones produced. A clone was defined as a lateral stem (stolon) that rooted at the nodes. This type of clonal propagule could be produced either from the lower nodes in the main stem, or from secondary nodes in lateral branches that had produced flowers. At the end of the experiment, we counted the total number

of clones per plant and obtained an average clone production per cross to use in the demographic analysis.

5.1.5 Demographic analysis

In order to investigate the relative performance of each F2 cross type under field conditions, we built stage-structured matrix population models (Caswell 2001) using the life cycle graph proposed by Peterson et al. (2016) to describe a perennial, clonal population of *Mimulus guttatus* with an annual time step. This life cycle graph describes a population sampled at the beginning of the growth season, after germination, but before vegetative growth has occurred, which in our experiment represented early June. At this point, individuals exist in one of three stages (Peterson et al. 2016): seeds, seedlings, and rosettes (Figure 1). In our model, seeds that have not germinated by the spring census can remain dormant in the seed bank, and survive to next census with probability *D*. Seedlings represent individuals that successfully germinated, from seeds produced the previous year or surviving in the seedbank, and established. Rosettes are individuals that persist from the previous year either through survival or through clonal propagation from another rosette. Seedlings and rosettes can contribute to both sexual and clonal reproduction within a growing season. Both sexual (seed) and clonal reproduction (stolons) occur after the census, which in our experiment was approximately from July to September.

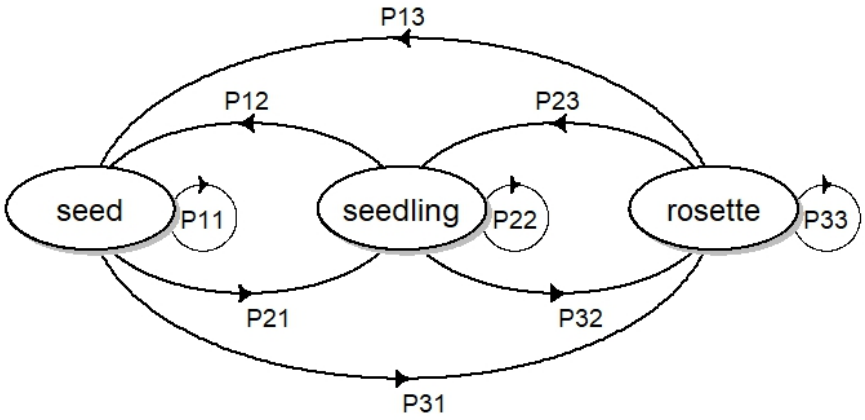

Figure 1. Life cycle graph showing the three life stages of *Mimulus guttatus* based on a yearly census at the start of the growing season in early June. Transitions: P11 = seed to seed; P12 = seedling to seed; P13 = rosette to seed; P21 = seed to seedling;

P22 = seedling to seedling; P23 = rosette to seedling; P31 = seed to rosette; P32 = seedling to rosette; P33 = rosette to rosette.

Transition parameters were estimated from the field and glasshouse experiments. For each F2 cross type, we calculated the following seven vital rates by averaging individual values: germination proportion (*G*); proportion of individuals that flowered (*Gr*); survival to the end of the summer (*Sn*); mean number of clones produced by the end of summer (*C*); total number of fruits produced (*F*); mean number of seeds per fruit (*Sd*); and probability of surviving the winter, estimated as the proportion of individuals alive at the end of summer that were alive the following spring (*Sw*). Vital rates *G*, *C* and *Sd* were estimated from a subset of the individuals of each cross as described in the previous sections, whereas *Gr*, *Sn*, *Sw*, and *F* were estimated using all available individuals in the field experiment. Two additional vital rates were obtained from the literature as they were not available for our study: The yearly survivorship of seeds in the seedbank (*D*) was obtained from Elderd and Doak (2006), which conducted a seed viability experiment of *M. guttatus* from different populations in the Sierra Nevada, California. We used the estimate of Peterson et al. (Peterson et al. 2016) of the relative recruitment success of seeds relative to clonal propagules (*A*), which they calculated in a natural population of clonal, perennial *M. guttatus* in Stanislaus National Forest, California. *D* and *A* were treated as constant for all F2 crosses (*D* = 0.534, *A* = 0.00067).

The projection matrix ***M*** we used is:

	Seed $_t$	**Seedling** $_t$	**Rosette** $_t$
Seed $_{t+1}$	$D\,(1-G)$	$Gr\ F\ Sd\ (1-G)\ A$	$Gr\ F\ Sd\ (1-G)\ A$
Seedling $_{t+1}$	$D\ G$	$Gr\ F\ Sd\ G\ A$	$Gr\ F\ Sd\ G\ A$
Rosette $_{t+1}$	0	$(C+1)\ Sn\ Sw$	$(C+1)\ Sn\ Sw$

5.1.6 Comparison of population growth rates

In order to compare the performance of populations of different admixed origins, we used the projection matrix for each F2 cross to calculate the population growth rate (λ), which we interpreted as the mean fitness of a population (Lande 1982). We obtained a relative measure of fitness for each F2 cross, by dividing each λ by the population growth rate of the DBL × ALASKA cross (λ^{ALASKA}). We obtained 95% confidence intervals through a non-parametric bootstrap with 10,000 replicates. For each replicate, we resampled with replacement individuals within each cross, while maintaining the original sample size within each cross, and recalculated vital rates and population growth rates (λ).

To test for differences in absolute λ among F2 crosses, we calculated the pairwise differences between crosses ($\theta^{A-B} = \lambda^{A} - \lambda^{B}$) (Caswell 2001). We then used non-parametric randomisations to assess the statistical significance of each pairwise difference in population growth rates. We produced 10,000 datasets with cross type randomised across all individuals, but maintaining the original sample size (number of individuals) in each F2 cross. For each randomised data set, we computed λ for each F2 cross, and compared the observed pairwise difference between crosses (θ^{A-B}) with the distribution of differences derived from the randomised datasets ($^{*}\theta = {}^{*}\lambda^{A} - {}^{*}\lambda^{B}$). We used a two tailed test of the null hypothesis (H_0) that there is not difference among pair of crosses: $P\ [\theta \geq {}^{*}\theta \mid H_0]$ (Caswell 2001).

To further compare F2 crosses and determine the importance of different components of fitness (vital rates) on population growth rate, we took two approaches. First, we carried out a life table response experiment (LTRE), which measures the effect of treatment (F2 crosses) on λ relative to a reference matrix, and quantifies how variation in the transition probabilities, P_{ij}, contributes to variation in population growth rates among treatments (Caswell 2001; Angert 2006). As a reference matrix, we used the mean of the three F2 matrices (Caswell 2001). We obtained 95% bootstrap confidence intervals for the mean values of λ, elasticities and LTRE contributions using 10,000 bootstrap replicates. Second, we conducted a perturbation analysis that allowed us to establish how small changes in the vital rates influence λ (Caswell 2001). For this, we chose to focus on measures of elasticity of vital rates, as they measure the proportional, rather than absolute, response of λ to changes in individual vital rates, and allow comparisons among vital rates with different scales (Caswell 2001; Morris 2002). All demographic

analyses were performed using the package *popbio* (Stubben and Milligan 2007) in *R* version 3.3.3 (R Core Team 2017).

Finally, to examine how large variation in the vital rates *D* and *A* (obtained from native populations of *M. guttatus*) influenced λ, we conducted a simulation analysis. We generated values of *D* ranging from -50% to +50% of the estimated value (0.537), obtained a new projection matrix, and calculated λ for each parameter combination. For *A*, we used values one order of magnitude above or below the observed value (0.00067). These simulations allowed us to establish the potential consequences of under- or overestimating *D* and *A*, on population growth rates (λ).

5.1.7 Selection on floral and vegetative traits through sexual fitness

In this component of the work, we were interested in estimating the pattern of selection acting on floral traits through individuals' contributions to sexual reproductive fitness to complement the previous demographic approach. While the demographic approach allowed us to characterise groups of individuals and integrate all components of fitness in a clonal, perennial plant (e.g., winter survival, probability of flowering, seed set, clonal propagation), our separate analysis of sexual fitness enabled us to relate individual variation in phenotypic traits and sexual reproduction, and estimate the pattern of selection. Because our main focus was on traits expressed only in individuals that flowered (flower size, flower number, time to flowering), we only included flowering plants in this analysis (94% of experimental plants; 1121/1188). Thus, the analysis of floral selection represents only a temporal snapshot of selection through one of the main components of lifetime fitness.

We used Lande and Arnold's (1983) approach to estimate phenotypic selection using regression models. As an estimate of sexual fitness, we used total fruit production. For this analysis, we considered the following phenotypic traits, which were measured at the onset of flowering: (1) Number of days from date of transplant to the field to the first flower opened; (2) plant height (cm); (3) flowering node, counted from the base of the plant to the first reproductive node; (4) corolla width, (5) corolla height, and (6) corolla tube length (mm) averaged over two flowers; (7) leaf width measured at the midpoint (mm), (8) length of the leaf blade, excluding the petiole, (mm); (9) stem diameter measured above the first node from

the ground (mm); and (10) number of stolons. In addition, at the end of the reproductive season (end of September), we measured (11) lateral (clonal) spread, the maximum distance between the two longest horizontal stems (clones; cm); (12) total number of flowers produced; (13) total number of fruits produced. We also estimated (14) average daily floral display (number of flowers open) through weekly surveys from the onset of flowering to the end of the August. Prior to the selection analysis, we carried out a Pearson's correlation analysis to identify strongly correlated variables that could introduce multicollinearity. We identified variables that were strongly correlated ($r \geq 0.70$) and which measured similar traits (e.g., corolla width and corolla height), and only kept one for the remainder of the analysis (see Supplementary Table 1). The variables kept for the selection analyses were: days to flower, corolla width, tube length, daily floral display, number of stolons, plant height, leaf width, and lateral (clonal) spread.

We fitted linear regression models using relative fitness and both linear and quadratic terms (Lande and Arnold 1983) using the function *glm* in *R* version 3.3.3 (R Core Team 2017). Phenotypic traits were standardised to a mean of zero and standard deviation of one (Stinchcombe and Rausher 2001). We fitted separate models for each cross type, which facilitated the interpretation of the selection gradients and took into account the large phenotypic differences observed among the three cross types. Relative fitness was obtained for each cross type separately by log-transforming fruit number (*log(fruit number +1)*) (Vallejo-Marin and Rausher 2007) to improve the normality of the residuals, and then dividing by the mean log-transformed fruit production of the corresponding cross type. We first fitted full models including block as a fixed factor, and all linear and quadratic terms (for stabilising/disruptive selection, only). Then we employed likelihood ratio tests to eliminate quadratic terms, followed by single-term deletion of non-significant linear terms, following the marginality principle (Fox 2016). This approach to model selection produced three separate regression models, one for each cross type, keeping only terms that contributed significantly to explain variation in relative fitness. Quadratic selection gradients indicating stabilising or disruptive selection were obtained from models including both linear and quadratic terms. The quadratic coefficients were doubled to estimate quadratic selection gradients (Stinchcombe et al. 2008). Linear selection gradients, indicating directional selection, were obtained from models including only linear terms (Stinchcombe and Rausher 2001).

Statistical significance of the regression coefficients was assessed using single term deletions and likelihood ratio tests.

5.2 Results

5.2.1 Characterisation of F2 crosses

As expected, the F2 crosses differed in several phenotypic and life history traits when grown under identical field conditions (Tables 1, 2; Figure 2). The DBL × LMC cross (introduced × native annual) flowered most rapidly (24.92 ± 0.34 days from being transplanted into the field; mean ± SE), and produced many (5116 ± 1.63), but relatively small, flowers (Table 1). Vegetatively, this cross had smaller leaves, thinner stems, and much less clonal spread compared to the other F2 crosses. Cross ALASKA (introduced × native perennial) took relatively long to flower (29.07 ± 0.41 days), and produced fewer flowers (19.21 ± 1.17) of intermediate size (Table 1). ALASKA produced leaves of similar size to DBL × LMC, but it was strongly clonal, and had the largest clonal spread of all crosses (67.87 ± 0.98 cm). Finally, cross COL (introduced × introduced) took the longest to flower (34.62 ± 0.41 days), but produced the most flowers (67.45 ± 2.74). Vegetatively, COL had the largest leaves and thickest stems, and had fairly large clonal spread (43.76 ± 0.82 cm).

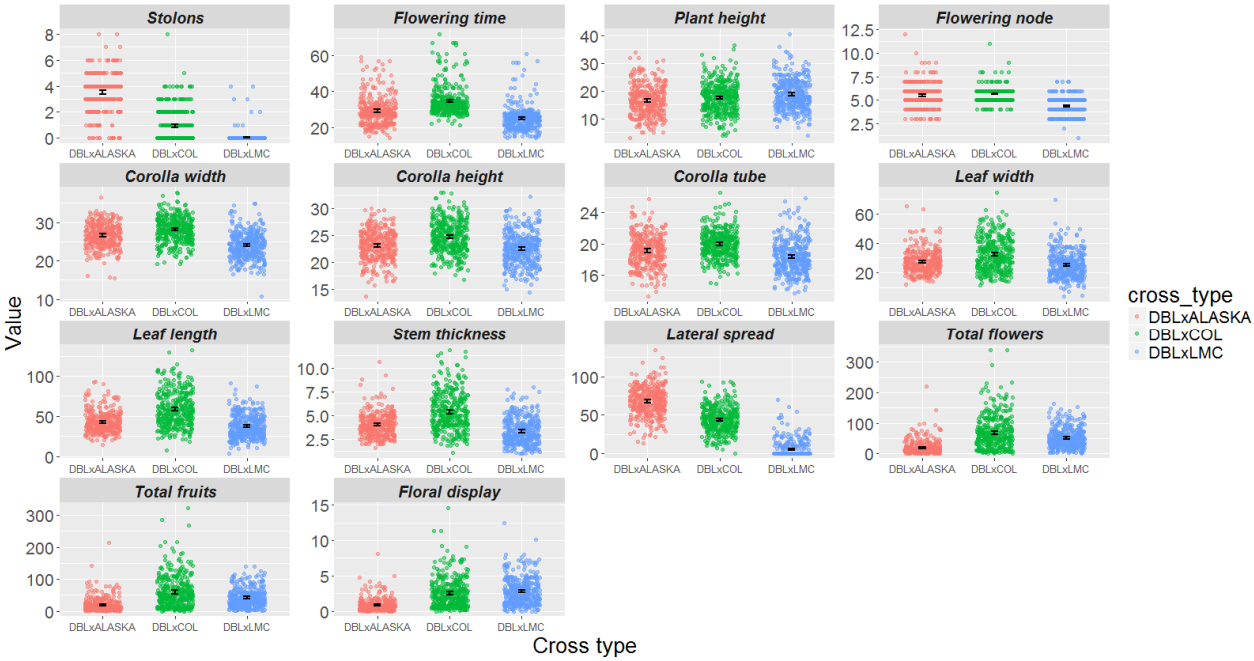

Figure 2. Phenotypic characteristics of the F2 offspring of three crosses between native and introduced populations of *Mimulus guttatus* grown in a field plot in central Scotland. All crosses had an individual from an introduced population (DBL) as the maternal parent in the F1. The paternal parent of the each of the three crosses was a native, perennial (ALASKA), a native, annual (LMC), or another introduced perennial (COL). Units for each trait are provided in Table 1. The mean and 95% confidence interval (calculated using a bootstrap approach) are shown in black. A small amount of variation along the x-axis (jitter) is provided for each cross to facilitate the visualisation of the data points.

Most vital rates were clearly different among F2 crosses, except the probability of surviving to produce at least one flower, which was very high across all crosses (92-99%), (Table 2). In general, LMC was characterised by investment into early sexual reproduction (early and abundant flowering and fruiting), low year-to-year survivorship, and sparse clonality, while ALASKA invested less in sexual reproduction (few fruits), had high summer and winter survivorship, and produce many, and large clones (Table 2). COL delayed investing in reproduction but achieved high fruit number, high clonality, and high year-to-year survivorship. Thus, the three F2 crosses encompassed a range of life strategies from highly sexual to highly clonal.

Table 1. Floral and vegetative characteristics of individuals from the F2 generation resulting from three different crosses between introduced and native population of *Mimulus guttatus*. All crosses had an individual from an introduced population (DBL) as the maternal parent in the F1. The paternal parent of the each of the three crosses was a native, perennial (ALASKA), a native, annual (LMC), or another introduced perennial (COL). The F2 was generated from a single F1 individual in each cross. Mean ± SE (number of individuals). Values calculated using only individuals that flowered. The statistics including all individuals are provided in the Supplementary Material (Supplemental Table 2).

Cross type	Days to flower	Plant height (cm)	Flower node	Corolla Width (mm)	Corolla height (mm)	Corolla tube (mm)	Leaf Width (mm)	Leaf length (mm)	Stem thickness (mm)	Stolons at first flower	Lateral (clonal) spread (cm)	Flower number	Fruit number	Daily floral display
ALASKA	29.074 ± 0.413 (349)	16.577 ± 0.298 (346)	5.512 ± 0.066 (346)	26.595 ± 0.164 (345)	23.112 ± 0.151 (344)	19.083 ± 0.109 (344)	27.16 ± 0.43 (327)	42.752 ± 0.74 (327)	4.008 ± 0.068 (337)	3.537 ± 0.073 (356)	67.872 ± 0.988 (356)	19.211 ± 1.172 (356)	18.843 ± 1.147 (356)	0.842 ± 0.047 (356)
COL	34.62 ± 0.411 (379)	17.502 ± 0.272 (378)	5.675 ± 0.039 (378)	28.115 ± 0.166 (379)	24.791 ± 0.151 (379)	19.939 ± 0.088 (379)	32.189 ± 0.569 (366)	58.616 ± 1.118 (366)	5.348 ± 0.102 (376)	0.907 ± 0.063 (387)	43.76 ± 0.827 (387)	67.452 ± 2.748 (387)	59.63 ± 2.443 (387)	2.497 ± 0.105 (387)
LMC	24.92 ± 0.341 (374)	18.773 ± 0.281 (374)	4.352 ± 0.047 (372)	23.971 ± 0.178 (373)	22.555 ± 0.154 (373)	18.311 ± 0.108 (373)	25.182 ± 0.46 (361)	37.216 ± 0.755 (361)	3.305 ± 0.072 (369)	0.056 ± 0.02 (378)	5.012 ± 0.538 (378)	51.164 ± 1.63 (378)	42.238 ± 1.443 (378)	2.813 ± 0.097 (378)

Table 2. Cross-specific vital rates for *Mimulus guttatus* estimated in an experimental F2 field population in the introduced range in Scotland. *G* = germination rate, *Gr* = proportion of individuals that flowered, *Sw* = mean number of individuals that survived winter, *Sn* = mean number of individuals that survived summer, *C* = mean number of clones, *Sd* = mean number of seeds per fruit, *F* = mean number of fruits, *D* = seed bank survival, *A* = recruitment rate. The values for *D*, and *A* were obtained from previous demographic analyses of *Mimulus* in the native range, as they could not be estimated in our study. The values used here (*D* = 0.534 (Elderd and Doak 2006) and *A* = 0.00067 (Peterson et al. 2016)) were set the same across all F2 crosses. Mean ± SE (sample size). Origin of source populations as follows: DBL = Dunblane, Scotland; ALASKA = Alaskan Peninsula, U.S.A.; COL = Coldstream, Scotland; LMC = California, U.S.A

Cross type	***Gr***	***F***	***Sd***	***G***	***Sn***	***Sw***	***C***
ALASKA	0.92 ± 0.014 (389)	16.982 ± 1.072 (395)	675.995 ± 40.148 (15)	0.925 ± 0.016 (20)	0.98 ± 0.007 (396)	0.943 ± 0.012 (388)	9.417 ± 0.868 (24)
COL	0.987 ± 0.006 (392)	58.275 ± 2.429 (396)	705.799 ± 55.536 (15)	0.93 ± 0.018 (20)	0.987 ± 0.006 (396)	0.985 ± 0.006 (391)	8.609 ± 0.735 (23)
LMC	0.997 ± 0.003 (396)	41.256 ± 1.447 (387)	266.976 ± 38.01 (15)	0.665 ± 0.029 (20)	0.737 ± 0.022 (396)	0.671 ± 0.028 (292)	2.25 ± 0.643 (24)

5.2.2 Performance of F2 crosses in the field

The demographic analysis compared the performance of the three F2 crosses in the field, and identified transitions associated with population growth rates (Supplementary Figure 2). Population growth rates (λ) varied significantly among the three F2 crosses. The lowest population growth rate was for LMC (λ = 6.64; 95% confidence interval = 5.14 – 8.26; $\lambda_{relative}$ = 0.41), while ALASKA had an intermediate value among the three crosses (λ = 16.19; 95% CI = 14.29 – 18.21; $\lambda_{relative}$ = 1.00). COL had the highest population growth rate (λ = 34.67; 95% CI= 29.78 – 39.23; $\lambda_{relative}$ = 2.14). The analysis of pairwise differences in population growth rates confirmed that the introduced × introduced cross performed better in the field than the other two cross types (Figure 3).

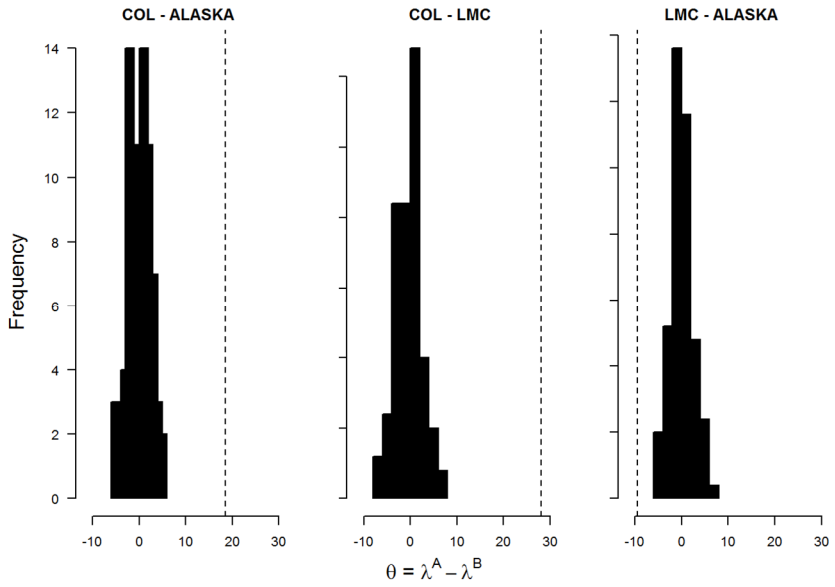

Figure 3. Pairwise comparison of population growth rates (fitness; λ) between three types of crosses of native and introduced *Mimulus guttatus.* For each pairwise comparison, the difference between population growth rates was calculated as θ = λ^A - λ^B, where A and B represent the two cross types being compared. The observed θ for each comparison is shown with dashed, vertical lines. Each histogram represents the distribution of θ under the null hypothesis of no difference between cross types A and B, which was estimated using 10,000 randomisations of the full data set. Population acronyms (COL, ALASKA, and LMC) indicate the paternal parent of each cross; all crosses shared the same maternal parent, DBL. Origin of

source populations as follows: DBL = Dunblane, Scotland; ALASKA = Alaskan Peninsula, U.S.A.; COL = Coldstream, Scotland; LMC = California, U.S.A.

The stable stage structure also varied among F2 crosses (Supplementary Figure 3). At equilibrium, most ALASKA individuals (57%) would occur as adult rosettes at the census stage, whereas for COL the population would consist mostly of newly emerged seedlings (69%), and in both a very small fraction of the population (3 –5%) would persist as seeds in the seed bank. For LMC, the population a larger fraction (28%) would occur as seeds in the seedbank, and the majority (55%) as seedlings.

Elasticity analysis shows that the effect of small changes in individual transition rates (P_{ij}) to population growth varied among F2 crosses (Table 3). For both LMC and COL, high elasticities (e_{ij}) are associated with transitions involving the contribution of sexual reproduction to new seedlings ($e_{22} + e_{23} = 0.722$ and 0.729, respectively). As a consequence, the elasticities for transition rates involving survival and clonality are lower ($e_{32} + e_{33} = 0.237$ and 0.270, for LMC and COL, respectively). In contrast, in ALASKA, the elasticities of transition rates involving survival and clonal reproduction have the largest combined value ($e_{32} + e_{33} = 0.594$), and transitions involving sexual reproduction are lower ($e_{22} + e_{23} = 0.404$). In all F2 crosses, the elasticities of transition rates from (e_{11} and e_{21}) and to the seed bank (e_{12}, and e_{13}) are small to negligible (Table 3). The analysis of the elasticity of lower-level vital rates shows that the main differences between cross types is the relative elasticity of survival and clonality (*Sn, Sw*, and *C*) compared to components related to sexual reproduction (e.g., *Gr, F,* and *Sd*) (Figure 4). In ALASKA, summer and winter survivorship (*Sn* and *Sw*) and clonality (*C*) have the highest elasticities, while in both LMC and COL, the elasticities of these vital rates are lower than those of sexually-related components.

Table 3. Elasticities (e_{ij}) for stage transitions estimated in the F2 generation of three crosses in native and introduced *Mimulus guttatus*. Elasticities represent the proportional sensitivity of population growth rate to small changes in each transition rate. Higher values indicate larger effects of changing a particular transition rate on population fitness (λ). Notice that the transition rate *P31* (seed to

rosette) is set to zero based on the life cycle model used here and in Peterson et al. (2016). Names for each cross type are given in Table 1.

Cross type				
DBL × LMC		**seed$_t$**	**seedling$_t$**	**rosette$_t$**
	seed$_{t+1}$	0.0006	0.0152	0.0048
	seedling$_{t+1}$	0.0200	0.5472	0.1750
	rosette$_{t+1}$	0	0.1798	0.0575
DBL × ALASKA		**seed$_t$**	**seedling$_t$**	**rosette$_t$**
	seed$_{t+1}$	0.0000	0.0004	0.0006
	seedling$_{t+1}$	0.0010	0.1638	0.2402
	rosette$_{t+1}$	0	0.2408	0.3532
DBL × COL		**seed$_t$**	**seedling$_t$**	**rosette$_t$**
	seed$_{t+1}$	0.0000	0.0006	0.0002
	seedling$_{t+1}$	0.0008	0.5327	0.1965
	rosette$_{t+1}$	0	0.1967	0.0725

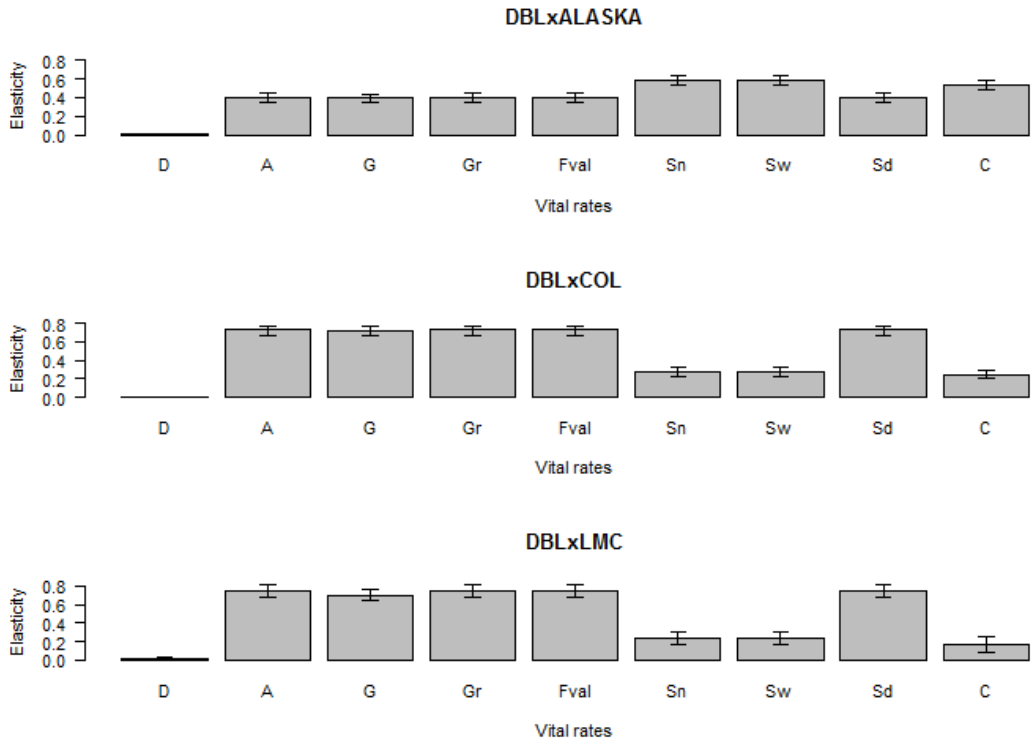

Figure 4. Elasticities (e_{ij}) for individual vital rates estimated in the F2 generation of three crosses of native and introduced *Mimulus guttatus*. Whiskers show the 95% CI for each elasticity estimated using 10,000 bootstrap replicates. *G* = germination

rate, *Gr* = proportion of individuals that flowered, *Sw* = mean number of individuals that survived winter, *Sn* = mean number of individuals that survived summer, *C* = mean number of clones, *Sd* = mean number of seeds per fruit, *F* = mean number of fruits, *D* = seed bank survival, *A* = recruitment rate. Names for each cross type are given in Figure 3.

The LTRE analysis shows that the introduced × introduced cross (COL) outperforms the two other F2 cross types (Figure 5). The decomposition of LTRE into individual transition rates (P_{ij}) indicates that the greater contribution for variation in λ among the cross types, can be attributed to seedling to seedling transitions (P_{22}); in other words, to the contribution of newly merged seedlings via sexual reproduction (Supplementary Figure 4). COL had large, positive contributions for both P_{22} and for transitions from established adults to seedling production (P_{23}). These two transition rates are a function of vital rates related with sexual reproduction and germination. In addition, the variation in population growth rates among crosses was also explained by differences in the transition from seedling to rosette (P_{32}) and from rosette to rosette (P_{33}). In both cases, COL had positive contributions of both transition rates to λ, while LMC had negative contributions. ALASKA had the highest contribution of P_{33} to variation in λ, which probably reflects its higher investment in clonal growth (Tables 1, 2). Calculation of λ using a range of values for seed bank survival ($D \pm 50\%$) and recruitment rate (one order of magnitude on either side of *A*) did not alter the rank order of λ among crosses (results not shown).

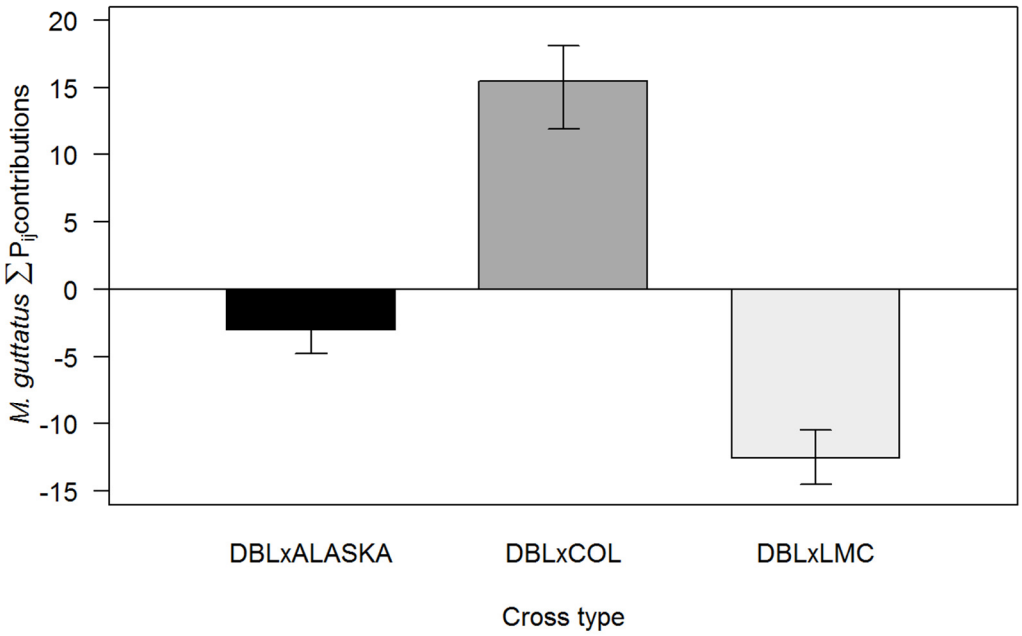

Figure 5. Life table response experiments analysis (LTRE), showing the effect of cross identity on variation in population growth rate (λ, fitness) in three crosses between native and introduced *Mimulus guttatus*. Values on the y-axis indicate the sum of the contribution of all transition rates to population growth rate, relative to the average of the matrices of the three cross types. Whiskers show the 95% CI estimated using 10,000 bootstrap replicates. Names for each cross type are given in Figure 3.

5.2.3 Pattern of selection through sexual fitness

We found positive selection on daily floral display and plant height across all cross types (Table 4). The significant quadratic selection gradients on floral display indicate that selection for larger floral displays decelerates as floral display increases. Selection on plant height also had a quadratic component for two of the three crosses (COL and LMC), indicating decelerating gains in fitness with increased height. In the ALASKA cross, we found positive directional selection on corolla width and leaf width, and negative directional selection for flowering time. In this cross type, we also found selection gradients favouring individuals that start producing flowers at intermediate nodes (Table 4). In COL, selection through fruit

production favoured earlier flowering, and increased lateral spread. In LMC, selection also favoured increased investment in lateral spread, although in both cross types selection on lateral spread was decelerating. In LMC, selection on flower size favoured larger corollas (Table 4). Overall, our results suggest that natural selection favours individuals with large vegetative and reproductive size, and early flowering, but that the pattern of selection on individual traits varies with the phenotypic architecture characterising each cross type.

Table 4. Standardised linear (β) and quadratic (γ) selection gradients estimated in the F2 generation of three crosses of *Mimulus guttatus* in a field population in central Scotland (Stirling). The subscript indicated the paternal parent of each cross: DBL = Dunblane, Scotland; ALASKA = Alaskan Peninsula, U.S.A.; COL = Coldstream, Scotland; LMC = California, U.S.A. All crosses had the same maternal parent (DBL). For each cross type, traits that were not statistically significant (assessed with a likelihood ratio test of nested models) were dropped from the model, except if the quadratic coefficient was significant. Statistical significance of individual coefficients was assessed via single-term deletions and likelihood ratio tests. Linear selection gradients were calculated in a model with linear terms only (Stinchcombe and Rausher 2001). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Trait	β_{ALASKA}	γ_{ALASKA}	β_{COL}	γ_{COL}	β_{LMC}	γ_{LMC}
Flowering time	-0.046***	–	-0.037***	–	–	–
Flowering node	-0.056***	-0.026**	–	–	–	–
Daily floral display	0.307***	-0.226***	0.118***	-0.058***	0.138***	-0.064***
Corolla width	0.034**	–	–	–	0.031**	–
Leaf width	0.041**	–	–	–	–	–
Plant height	0.079***	–	0.059***	-0.030***	0.005	-0.032**

Clonal spread	–	–	0.053***	-0.108***	0.055*	-0.196**

5.3 Discussion

Evidence for adaptive evolution during biological invasions continues to accumulate, but experimental studies of the pattern of selection in the non-native range are still scarce (Colautti and Lau 2015). Quantitative field studies documenting natural selection in non-native populations constitute an important step towards understanding the role of adaptive evolution during rapid evolutionary change. In addition, studies of the fitness of admixed individuals can be used as a tool to reveal outbreeding depression, which is expected when introduced populations are locally adapted (Rius and Darling 2014). Our study shows that the source of origin of admixed populations of *M. guttatus* strongly influences their fitness under field conditions in the non-native range. We found that admixture within the introduced range results in the highest fitness estimated using demographic models that integrate multiple components of fitness, including survivorship, and clonal and sexual reproduction. In comparison, admixture between native and introduced populations conferred lower fitness, particularly when admixture occurs between introduced perennial and native annual ecotypes. Further work is needed to determine the degree to which outbreeding depression is caused by intrinsic genetic interactions (i.e., is environment independent), or by environment-dependent factors mediated by local adaptation. However, quantitative analysis of natural selection revealed several phenotypic traits, including flowering time, flowering node, daily floral display, plant size, and clonal spread that are under selection in the introduced range. Natural selection in field populations of *M. guttatus*, combined with previous evidence of adaptive differentiation (van Kleunen and Fischer 2008), and selective sweeps in introduced populations (Puzey and Vallejo-Marín 2014), indicate a role of adaptive evolution in shaping populations of *M. guttatus* in the British Isles. Our findings suggest that admixture in introduced species is not necessarily beneficial, particularly when introduced populations have evolved to adapt to the new environment and when admixture occurs between potentially maladapted populations.

5.3.1 Phenotypic differentiation among F2 crosses

The phenotypic differences among F2 crosses indicate a genetic basis for several fitness-related traits. Because the F2 crosses all shared the same maternal parent, average differences between them reflect the paternal contribution to phenotypic variation. The F2 cross with a native annual father (LMC) maintained some of the characteristics associated with its annual ancestry including shorter times to flower, smaller vegetative size, little clonality, and abundant flowering. In contrast, the F2 cross with a perennial native father (ALASKA), invested more heavily in clonal and vegetative growth, and less in sexual reproduction. The introduced × introduced cross (COL) invested in both clonal propagation and flower production, which resulted in vegetatively large clones and abundant seed production. The difference in phenotype among F2 crosses, including introduced × introduced crosses, show that at least some of the phenotypic variation of introduced populations is genetically-based, result that is supported by common garden experiments (Dudash et al. 2005; van Kleunen and Fischer 2008; Murren et al. 2009). The presence of genetic variation in the introduced range is important as a prerequisite for evolutionary change. Future studies estimating the level of genetic variation in introduced populations of *M. guttatus*, including estimation of genetic variance-covariance matrices (currently only available for native populations, e.g., Robertson et al. 1994; Scoville et al. 2009), are required to make more accurate predictions of the evolutionary response to selection.

5.3.2 Relative fitness of F2 crosses with different admixture origins

The effect of intraspecific admixture in introduced populations can potentially vary from beneficial to deleterious (Rius and Darling 2014). In the short term, heterosis in the F1 may confer a benefit. In fact, previous work has shown that in some introduced species F1 individuals outperform the parents (Hahn and Rieseberg 2017). High fitness of F1 generations is generally due to overdominance (heterozygosity advantage) or masking of deleterious recessive alleles (Lynch 1991). We did not measure fitness of the F1 generation (which has been done in *M. guttatus* by a previous study; van Kleunen et al. 2015), but instead focused on

second-generation hybrids. Our results show that in F2 individuals under field conditions, admixture between native and introduced populations results in lower fitness than admixture within the introduced range. Although, we concentrate on the F2 generation, it is possible that we could have found heterosis in the F1 generation from native and introduced crosses in our study. Van Kleunen et al. (2015) showed that F1 crosses between *M. guttatus* individuals from different regions can produce offspring of higher fitness than crosses between closely related populations. However, our results imply that if heterosis occurs in crosses between native and introduced populations as it has been previously suggested, it is transitional and does not carry to the F2. Hence, the potential benefits of admixture detected in the F1 generation under controlled conditions may not directly translate to fitness differences in the field or in subsequent generations (Edmands 1999; Keller and Taylor 2010).

But perhaps more importantly, the reduced fitness of native x introduced F2 crosses is consistent with outbreeding depression expressed in the introduced range of *M. guttatus*. The mechanistic causes of outbreeding depression observed in native x introduced crosses remains unknown, and both environment-dependent, and environment-independent factors could be at play (Verhoeven et al. 2011; Rius and Darling 2014; Hahn and Rieseberg 2017). Increased genetic and phenotypic distance between the maternal parent (DBL) and the native populations could explain the observed outbreeding depression (Edmands 1999; Dlugosch et al. 2015). Both ALASKA and LMC populations are more genetically and phenotypically different from introduced population DBL than the other introduced population COL (Puzey and Vallejo-Marín 2014; Pantoja et al. 2017; R. Cumming and M. Vallejo-Marin, unpublished). Increased evolutionary distance (genetic and phenotypic differentiation) can be magnified by adaptation to different environments and increase outbreeding depression (Frankham et al. 2011). Indirect evidence in introduced populations of *M. guttatus* is consistent with some role of adaptive evolution and selection in mediating the observed outbreeding depression. For example, common garden experiments in introduced *M. guttatus* from New Zealand and the UK suggest that phenotypic differentiation in floral production and clone size is structured along latitudinal clines, consistent with adaptive, rather than non-adaptive differentiation (van Kleunen and Fischer 2008). At the genomic level, there is also evidence that selection has acted in introduced UK populations. Using

genome resequencing of 10 native and 10 introduced populations, Puzey and Vallejo-Marín (2014) detected selective sweeps in 5 of the 14 chromosomes of *M. guttatus*. These selective sweeps were absent in the native populations studied, lending support to the hypothesis that selection occurred after the introduction of *M. guttatus* into Europe. Future work comparing the consequences of admixture over a larger range of genetic and phenotypic distances (Edmands 1999), will help disentangling the contribution of environment-independent and environment-dependent factors (including local adaptation) to outbreeding depression in invasive species.

Moreover, to have a more complete understanding of how the background genetic variation within populations varies and affects offspring fitness, it would be useful to include a F2 offspring derived from within-population cross (e.g., DBL x DBL). Although if outbreeding depression is magnified by increasing evolutionary distance among populations (Edmands 1999; Dlugosch et al. 2015), the within-population cross (e.g., DBL x DBL) fitness would be comparable with the between-population cross and higher than the between range crosses found in our study.

Outbreeding depression found in the F2 population from native x introduced crosses can potentially disappear in subsequent generations of *M. guttatus*. Diminishing outbreeding depression could happen because, another outcome of hybridization and recombination is the creation of new genetic variants that may allow for the selection of fit genotypes after many rounds of recombination which would disrupt negative genetic interactions that cause outbreeding depression (e.g., Hwang et al. 2011). For instance, the offspring of the annual legume *Chamaecrista fasciculate* showed strong outbreeding depression in the F3 generation (Fenster and Galloway 2000) but after six generations recovered fitness to the level of F1 performance and was superior to the parents (Erickson and Fenster 2006). Even if outbreeding depression is temporary because natural selection removes it after successive generations, outbreeding depression found in our study is an important step towards understanding the adaptation of *M. guttatus* in the UK. Especially since populations adapted to different environments often show outbreeding depression when crossed, particularly in the F2 or later generations.

Although our experimental design can confidently distinguish the fitness differences of the three F2 crosses analysed here (Figure 3), further studies are needed to make generalisations about why particular native populations produce,

on average, fitter admixed individuals than others. Here we observed that the ALASKA cross had higher fitness than the LMC cross. It is tempting to speculate that the lower fitness of the LMC cross, which had the lowest population growth rate of the three crosses, reflects maladaptation of the annual LMC parental phenotype when grown in the ecological environment found in the British Isles. Annual populations of *M. guttatus* are typically found in seasonally dry inland areas of the native range (Lowry et al. 2008). Drought during the summer favours short life spans and investment in sexual reproduction instead of clonal growth (Lowry et al. 2008; Wu et al. 2010; Kooyers et al. 2015). In contrast, the wet cool summers and mild winters of the British Isles may favour perennial life cycles and investment in clonal growth (Lowry et al. 2008; van Kleunen and Fischer 2008). Indeed, our analysis of selection showed positive, but decelerating selection, on clonal spread in two of the three crosses studied, including the LMC cross. Moreover, the LTRE analysis indicates that transition rates that involve clonality and survival contribute positively to the difference in fitness between cross types (P_{32} and P_{33}; Supplemental Figure 4). The ALASKA cross had higher fitness than the annual cross, which in part is explained by the higher reproductive contribution of surviving adult rosettes through both sexual (P_{23}) and clonal reproduction (P_{33}) compared to the annual cross. In a study of native *Mimulus,* Peterson et al. (2016) also found that vital rates for rosette reproduction (including both sexual and clonal components) contributed to local adaptation of perennial vs. annual forms. Although tentative, our results may help explaining why perennials, but not annuals, have become established in the UK. These results also raise the possibility that niche matching between native sources and the introduced habitats may make some lineages more likely than others to become established and spread following introduction (Holt et al. 2005). Species with variation in life history and broad ecotypic differentiation in the native range, such as *M. guttatus* (Grossenbacher et al. 2014; Peterson et al. 2016), could be a fruitful system to test hypotheses about the role of pre-adaptation and maladaptation during biological invasions.

5.3.3 Combining sexual and clonal reproduction

Many invasive plants combine the capacity for clonal and sexual reproduction (Barrett 2011), and both modes of reproduction could provide an advantage to

introduced species. We found that in the introduced range of *M. guttatus*, a combination of elevated clonal and sexual reproduction conferred the highest fitness. For instance, the higher fitness of the COL cross was associated with the capacity to reproduce vigorously through clonality combined with high fruit production. An increased reproductive effort in both sexual and clonal reproduction characterises the range expansion of other introduced species (Brown and Eckert 2005). We have recently conducted a genetic characterisation of introduced populations of *M. guttatus* in the UK, and showed a large variation in clonal diversity, from populations formed purely by sexually-produced individuals, to mono-clonal populations (Pantoja et al. 2017). The positive combined contribution of clonality and sexual reproduction to population growth and fitness in experimental and naturalised populations of *M. guttatus*, indicate that both modes of reproduction may contribute to the success of introduced populations. It remains to be established the extent to which the variation in clonality in naturalised populations is associated with ecological or temporal dynamics that shift the relative contribution of sexual and clonal reproduction to population structure. Nevertheless, high propagule pressure via seeds, which can be further enhanced by water-borne dispersal of stolons (Truscott et al. 2006), likely favours the spread of riparian invasive plants such as *M. guttatus*, especially in populations subject to high-flow events.

5.3.4 Selection in the introduced range

An increasing number of studies has characterised the pattern of selection in both natural and experimental populations in the introduced range (reviewed in Colautti and Lau 2015). The overall picture emerging from these studies is that natural selection in the introduced range can be as strong or stronger than in native habitats (Colautti and Lau 2015). Our analysis of selection provides the first attempt in quantifying and characterising natural selection in introduced populations of *M. guttatus* outside of North America. Consistent with the general observation of the ubiquity of natural selection (Endler 1986; Kingsolver et al. 2012; Caruso et al. 2017), we find that several floral and vegetative traits in *M. guttatus* are under selection in the invasive range. In particular, selection in the introduced range

favours larger plants that reproduce early, with larger and more numerous flowers, and increased investment in clonal reproduction (Table 4). Importantly, the pattern of selection on some traits (i.e., flowering node, daily floral display, plant height, and clonal spread), is non-linear, indicating diminishing fitness returns with higher trait values. Our analysis of selection supports the hypothesis that selection should favour larger size in the introduced range (Blossey and Notzold 1995). Consistent with our findings, Murren et al. (2009) detected positive selection on flower size and plant height in non-native populations of *M. guttatus* in eastern North America. Studies of native populations of *M. guttatus* have often found positive or stabilising selection on flower size (e.g., corolla size; Hall and Willis 2006; Fishman and Willis 2008), indicating that flower size has continued to be under selection after the dispersal of *M. guttatus* beyond its native habitats. Native populations of *M. guttatus* harbour considerable levels of genetic variation (Puzey et al. 2017), and both vegetative and reproductive traits often display significant heritabilities (e.g., Fenster and Ritland 1994; Robertson et al. 1994; Murren et al. 2009). Genomic analysis of introduced populations suggest that, although diversity is reduced, there is still considerable variation within the introduced range (Puzey and Vallejo-Marín 2014). Therefore, introduced populations may be capable of rapid adaptive evolution. A dramatic demonstration that selection can drive rapid adaptive evolution is provided by the experimental study of Bodbyl Roels and Kelly (2011). These authors showed that an experimental population, initially derived from a single natural population, grown in two different pollination environments (with or without bumblebee pollinators) rapidly evolved distinct genetically-based phenotypes. In the absence of pollinators, *M. guttatus* evolved smaller anther-stigma distances and higher autonomous seed set, in just five generations. Our results suggest that adaptive evolution caused by natural selection in the introduced range in a genetically variable taxon, such as *M. guttatus,* may be a key mechanism in facilitating the naturalisation and spread of non-native species when faced with novel ecological challenges.

Our results suggest that many traits, including flowering time, flower display and clonal lateral spread, are under selection in the introduced range, which poses the question; which selective pressures may be acting on introduced *M. guttatus*? In the native range, water availability determines the life span and imposes strong selection on flowering time, reproductive allocation and floral traits

of *M. guttatus* that contributes to adaptation to different habitats (Hall and Willis 2006; Lowry et al. 2008). Introduced *M. guttatus* populations are drought intolerant (personal observation) and, similar to perennial native populations, inhabit wet places by streams, rivers, ponds and waterlogged grounds suggesting that soil saturation may be an important agent of selection in the introduced range. Soil water availability may be influenced by the level of precipitation, which varies geographically, and results in phenotypic differentiation among populations. In introduced *Arabidopsis thaliana*, for instance, differences in winter precipitation along a longitudinal cline in North America is an important agent of selection for flowering time (Samis et al. 2012). Plants also demonstrate flowering time response to photoperiod, duration of winter (vernalisation) and pollinators (Michaels and Amasino 2000; Sandring and Ågren 2009; Friedman and Willis 2013). Moreover, variation of temperature has been shown to influence the investment in clonal and sexual reproduction. It has been shown that in cold places in northern latitudes, investment in clonality usually increases and sexual reproduction is less common (Johnson et al. 2010; Dorken and Eckert 2011). Besides large scale environmental factors, microhabitat variables such as competition and soil quality act as selective pressures on plants and cause among-population variation within a small scale (Brachi et al. 2011). Future studies could focus on investigating the pattern of phenotypic differentiation among *M. guttatus* populations, and on distinguishing among these and others agents of selection in the introduced range.

5.4 Conclusion

To conclude, our results from admixed individuals of *M. guttatus* demonstrate that, in the introduced range, sexual and clonal reproduction are the fitness components that most contribute to fitness measured as population growth rate, suggesting that combination of multiple reproductive traits facilitate introductions by increasing performance of non-native species. Our data demonstrate that source-of-origin also influences the population growth rate in admixed individuals in the non-native range. Suggesting that admixture is not always beneficial and can result in outbreeding depression in the introduced range when parental crosses present genetic and phenotypic differences as a result of adaptation to different environments, or one of the populations is maladapted to non-native conditions.

Evolution has been demonstrated in non-native and invasive species and often happens in short time scales after introduction (e.g., Turner et al. 2014). Our results demonstrate that morphological and life-history traits are under selection in the non-native range supporting the prediction that evolution by natural selection may be involved in naturalization and expansion of non-native species in the introduced range.

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5.6 Supplemental Material

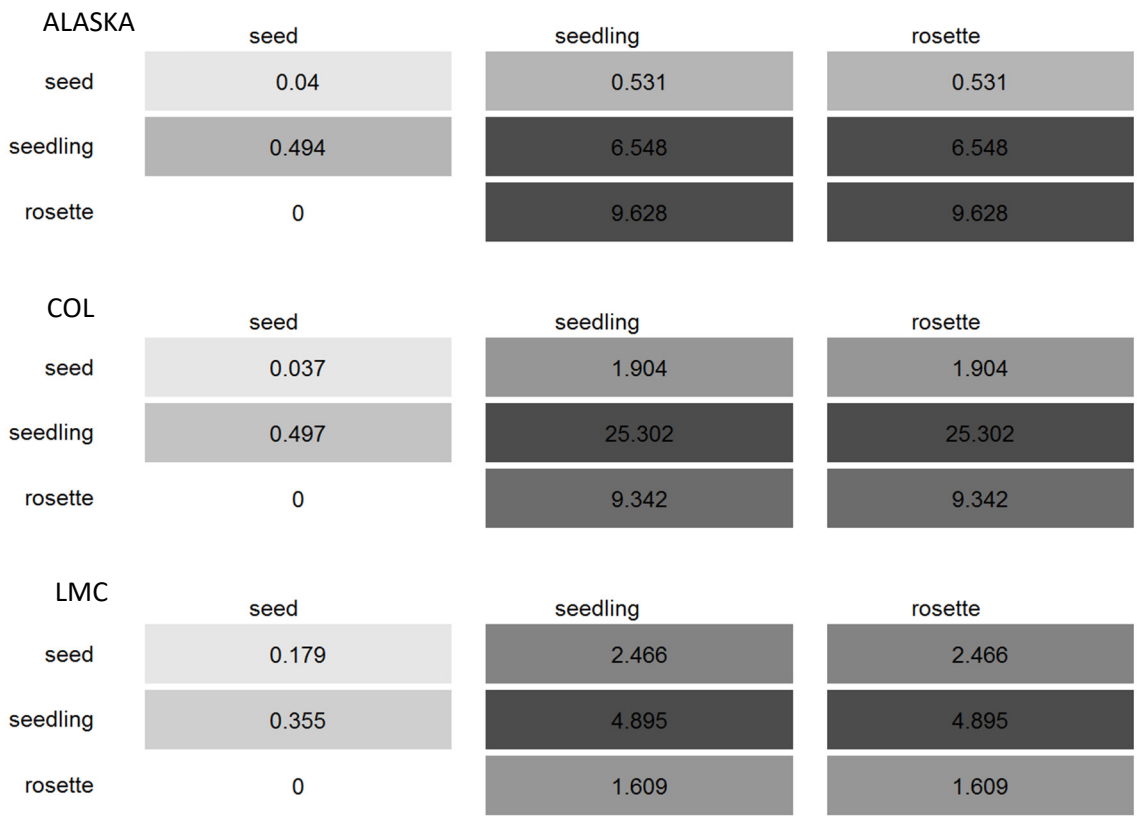
Supplementary Table 1. Pearson's correlation coefficients of 13 traits measured in 1,121 F2 individuals derived from three crosses between native and introduced populations of *Mimulus guttatus*.

	Days to flower	**Plant height**	**Flower node**	**Corolla width**	**Corolla height**	**Corolla tube**	**Leaf width**	**leaf length**	**Stem thickness**	**Clonal spread**	**Flower number**	**Fruit number**	**Floral display**
Stolons	0.08	-0.03	0.29	0.23	0.08	0.14	0.17	0.15	0.18	0.69	-0.16	-0.12	-0.24
Days to flower		-0.06	0.57	0.20	0.17	0.06	0.10	0.11	0.13	0.24	-0.10	-0.08	-0.22
Plant height			0.21	0.38	0.36	0.41	0.56	0.53	0.50	0.00	0.40	0.4	0.45
Flowering node				0.41	0.26	0.25	0.22	0.28	0.31	0.44	-0.01	0.03	-0.13
Corolla width					0.77	0.61	0.47	0.54	0.54	0.39	0.23	0.27	0.11
Corolla height						0.59	0.39	0.43	0.45	0.21	0.24	0.26	0.17
Corolla tube							0.45	0.51	0.50	0.25	0.28	0.3	0.22
Leaf width								0.87	0.78	0.26	0.55	0.56	0.52
leaf length									0.85	0.29	0.61	0.63	0.51

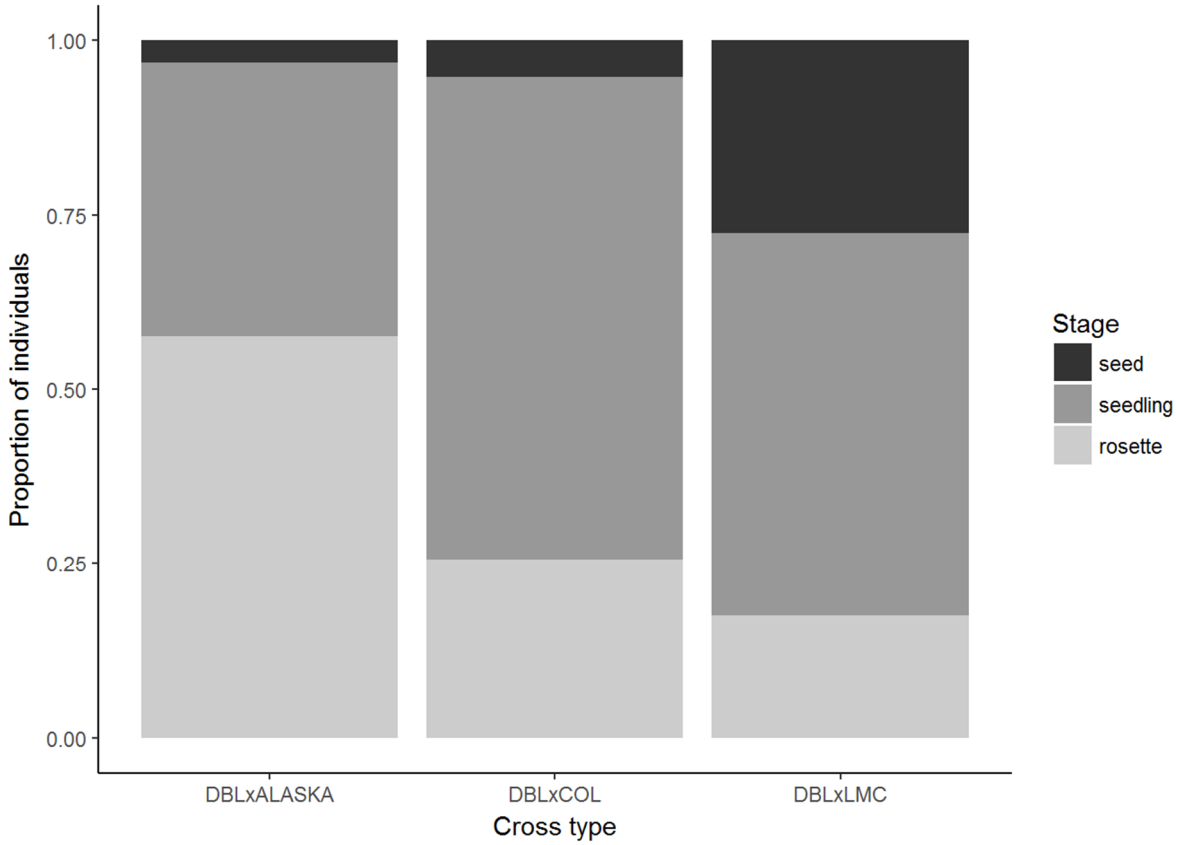
Stem thickness										0.33	0.53	0.54	0.44
Clonal spread											-0.09	-0.04	-0.25
Flower number												0.97	0.82
Fruit number													0.80

Supplementary Table 2. Floral and vegetative characteristics individuals from the F2 generation resulting from three different crosses between introduced and native population of *Mimulus guttatus*. Values for all individuals, including those that did not flower. All crosses had an individual from an introduced population (DBL) as the maternal parent in the F1. The paternal parent of the each of the three crosses were a native, perennial (ALASKA), a native, annual (LMC), or another introduced perennial (COL). The F2 was generated from a single F1 individual in each cross. Mean ± SE (number of individuals).

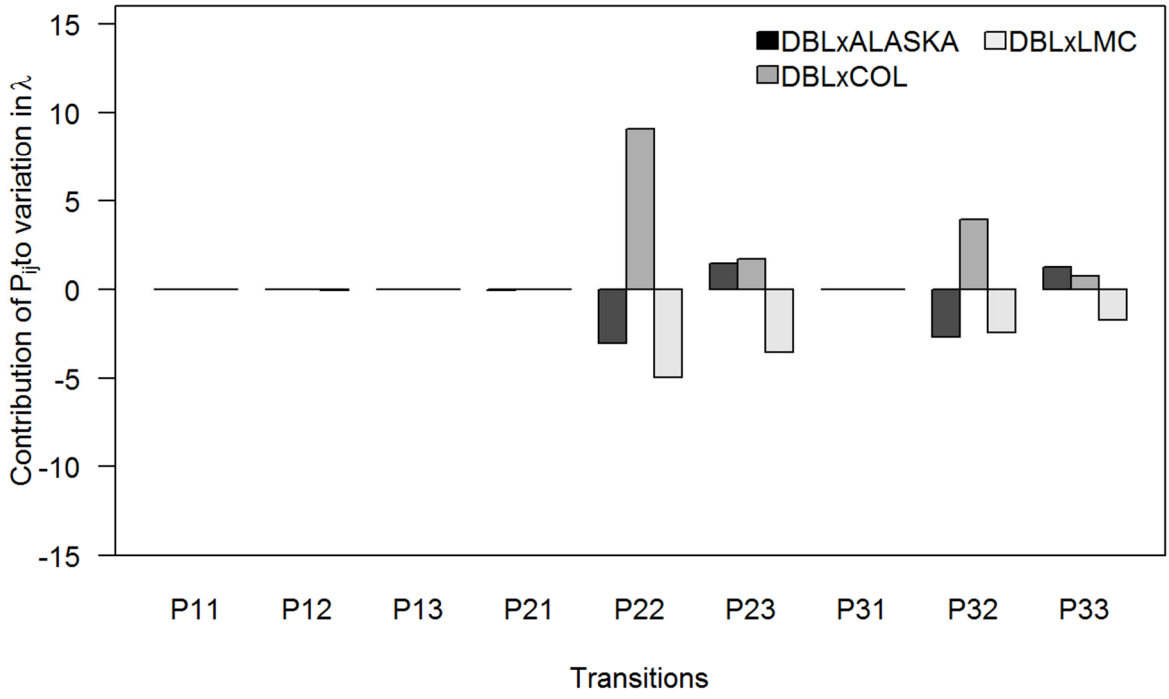
Cross type	Days to flower	Plant height (cm)	Flower node	Corolla Width (mm)	Corolla Height (mm)	Corolla tube (mm)	Leaf width (mm)	Leaf length (mm)	Stem Thickness (mm)	Stolons at first flower	Lateral (clonal) spread (cm)	Flower number	Fruit number	Daily floral display
ALASKA	29.046 ± 0.412 (351)	16.562 ± 0.297 (348)	5.51 ± 0.065 (347)	26.595 ± 0.164 (345)	23.112 ± 0.151 (344)	19.083 ± 0.109 (344)	27.14 ± 0.42 (328)	42.73 ± 0.738 (328)	4.003 ± 0.068 (338)	3.467 ± 0.072 (396)	66.446 ± 0.977 (389)	17.314 ± 1.095 (395)	16.982 ± 1.072 (395)	0.766 ± 0.044 (395)
COL	34.62 ± 0.411 (379)	17.502 ± 0.272 (378)	5.675 ± 0.039 (378)	28.115 ± 0.166 (379)	24.791 ± 0.151 (379)	19.939 ± 0.088 (379)	32.189 ± 0.569 (366)	58.616 ± 1.118 (366)	5.348 ± 0.102 (376)	0.894 ± 0.062 (396)	43.691 ± 0.823 (392)	65.919 ± 2.733 (396)	58.275 ± 2.429 (396)	2.454 ± 0.104 (394)
LMC	24.847 ± 0.334 (386)	18.675 ± 0.277 (386)	4.337 ± 0.046 (383)	23.921 ± 0.174 (385)	22.494 ±0.152 (385)	18.277 ± 0.106 (385)	25.075 ± 0.452 (372)	37.113 ± 0.747 (372)	3.293 ± 0.071 (381)	0.053 ± 0.02 (396)	4.802 ± 0.516 (396)	50.365 ± 1.637 (384)	41.578 ± 1.446 (384)	2.748 ± 0.095 (396)



Supplementary Figure 1. Transition matrices for the F2 progeny of three crosses between native and introduced *Mimulus guttatus*. Cross names as follows: ALASKA = introduced × native perennial; COL = introduced × introduced; LMC = introduced × native annual.



Supplementary Figure 2. Stable stage structure inferred from the demographic analysis of the F2 offspring of three crosses between native and introduced *Mimulus guttatus*. Cross names as follows: ALASKA = introduced × native perennial; COL = introduced × introduced; LMC = introduced × native annual.



Supplementary Figure 3. Life table response experiments analysis (LTRE), showing the effect of cross identity on variation in each transition rate (P_{ij}) in three crosses between native and introduced *Mimulus guttatus*. Cross names as follows: ALASKA = introduced × native perennial; COL = introduced × introduced; LMC = introduced × native annual. Transitions: P11 = seed to seed; P12 = seedling to seed; P13 = rosette to seed; P21 = seed to seedling; P22 = seedling to seedling; P23 = rosette to seedling; P31 = seed to rosette; P32 = seedling to rosette; P33 = rosette to rosette.

Chapter 6: General discussion

The results of this thesis indicate that temperature and precipitation variables alter the production of stolons over flower production, while space increases the trade-off between stolon size and number of flowers. Source-of-origin influences fitness of admixed F2 individuals, measured by population growth rate. The F2 population from among introduced crosses showed high performance, particularly because of clonal and sexual reproduction vital rates, which further highlights the importance of mixed reproductive traits for introduced species. Conversely, the F2 from native and introduced crosses showed low performance consistent with outbreeding depression. Finally, this study indicates the presence of high genetic diversity within populations, which could have served as genetic material for natural selection. Moreover, the data shows natural selection for phenotypic and life-history traits that, together with a previous study about selective sweeps in *M. guttatus* (Puzey and Vallejo-Marín 2014), demonstrates the importance of natural selection for the success of naturalization.

6 Population genetic diversity and genetic structure in non-native populations

Studies of genetic variation in introduced and native ranges have shown evidence of multiple episodes of introduction (Ray and Quader 2014; Shirk and Hamrick 2014; Oduor et al. 2015). Multiple introductions from several different source populations in the native range are used to explain high levels of genetic diversity found in some introduced species (Dlugosch et al. 2015). Our results indicate that most populations from UK are more closely related to each other than to native populations (Chapter 2). Non-native populations form a separate group, except for one population that is closely related to a native population from Alaska. Similarly, a previous study, using 10 resequenced individuals from the UK and 14 from North America, showed that individuals from the UK form a single clade from native individuals and are close to a perennial costal native population from British Columbia, Canada (Puzey and Vallejo-Marín 2014). The genetic similarity within the non-native range indicated by our population genetic study and Puzey and Vallejo- Marín (2014) suggests that the UK populations have originated from the

same area in the Northern part of North America most likely from one or a few introductions from genetically related populations and, therefore represent a small portion of the overall genetic diversity of native *M. guttatus*.

In addition to the number of introductions, the level of genetic structure within the native range can also influence the genetic variation within the introduced range. For instance, if in the native range the level of among population genetic variation is higher than within population genetic variation, then a few introductions might introduce only a small portion of the native genetic variation and multiple introductions can be important to offset the effects of founder effects (Oduor et al. 2015). An important result of the present study is that both native and introduced ranges have a high percentage of genetic variation within populations, which suggests that a substantial amount of standing genetic variation could have been introduced with only a few introduced populations from the same geographical area (Table 4; Chapter 2). Genetically diverse introduced individuals may explain the relatively high levels of genetic diversity in *M. guttatus* from the UK (Table 3; Chapter 2). For invasive species, genetic diversity can facilitate colonization in a short term by increasing the ability of a species to survive, grow and reproduce as experimentally demonstrated with *Arabidopsis thaliana* (Crawford and Whitney 2010). In the long term, the level of standing genetic variation can facilitate rapid evolution by providing the genetic variation necessary for natural selection during the process of adaptation to a new environment (Barrett and Schluter 2008). In addition, sufficient standing genetic variation can promote evolutionary changes in traits important for fitness, such as growth and reproduction, that may enhance the competitive ability of invasive species (e.g., Zou et al. 2008).

6.1 The role of natural selection and admixture for performance in the introduced range

During biological introductions, non-native populations are exposed to biotic and abiotic conditions to which they are not adapted, and although some level of pre-adaptation can confer an advantage at initial establishment, the persistence and range expansion of the species in the new environment may be associated with adaptation by natural selection (Lee 2002; Holt et al. 2005). In Chapter 5, we showed that morphological and life-history traits of *M. guttatus* are under selection

in UK, which could be supported by the high genetic diversity of non-native populations indicated in chapter 2 and by Puzey and Vallejo-Marín (2014). The results showed that selection by sexual fitness in the introduced range favours larger plants that reproduce early, with larger and more numerous flowers, and high investment in clonal reproduction (Table 4; Chapter 5). The patterns of selection differed among crosses, which could be the result of genetic differences among paternal parents shaped by different selection pressures between native and introduced ranges. For example, comparative selection gradient analysis between native and non-native *M. guttatus* populations from eastern North America showed positive selection for flower size only in non-native populations, which is likely a result of differences in environmental conditions resulting in different selection patterns (Murren et al. 2009). On average, the offspring from different crosses showed phenotypic differences in common garden (Figure 2; Chapter 5) indicating that the traits analysed have a genetic influence, but future studies using multiple populations from the UK are needed to determine the level of quantitative genetic variation. A further study could obtain information about heritability of traits and test the probability of a response to selection in multiple populations from across the UK. Given that previous studies demonstrated that floral and vegetative traits have heritable genetic variation in native populations of *M. guttatus* (e.g., Fenster and Ritland 1994; Robertson et al. 1994), traits under selection suggest that adaptive evolution should be possible in non-native *M. guttatus.*

Figure 1. Left-hand side: An individual from the COL cross (introduced x introduced parents). Right-hand side: An individual from the ALASKA cross (introduced x native perennial parents).

A main finding of the field experiment was that the COL cross (introduced x introduced parents) showed higher population growth rate (λ) than LMC (introduced x annual native parents) and ALASKA crosses (introduced x perennial native parents). Better performance of COL is explained by high investment in clonality and, particularly, seed reproduction as demonstrated by elasticity and decompositions of LTRE analysis (Table 3; Supplemental figure 3; Chapter 5). Similarly, a previous demographic study with the same transition rates used in chapter 5 showed that the vital rates that most contribute to population growth of perennial native *M. guttatus* are rosette production and fertility (Peterson et al. 2016). In another study with invasive species of the family Commelinaceae, clonality was more important than sexual reproduction for population growth rate (Burns 2008). The importance of different types of reproduction for population growth rate alternate among species and may be associated with different benefits offered by each reproductive mode. Clonality can contribute to abundance (Herben et al. 2014) and local dominance, whereas seed output contributes to long-distance dispersal of seeds, especially, downstream in riverine plant communities (Levine 2001). Indeed, fecundity is associated with plant invasiveness in many species (Mason et al. 2008; Jelbert et al. 2015). Therefore, sexual and clonal reproduction represent different strategies that can contribute to population dynamics and fitness of invasive species. For *M. guttatus*, seeds and stolons can contribute to dispersal and spread in the UK, especially in populations close to rivers with high-flow events (Truscott et al. 2006).

Low population growth rates of LMC and ALASKA crosses are consistent with outbreeding depression in non-native *M. guttatus.* Outbreeding depression can be driven by genetic incompatibilities that are independent of the environment (Lynch 1991) or via disruption of local adaptation (e.g., Houde et al. 2011). We cannot indicate in this study the cause of outbreeding depression, but it is possible that genetic distance between native and non-native populations result in outbreeding depression. Introduced *M. guttatus* populations are more distantly

related to native populations than with each other (Puzey and Vallejo-Marín 2014; Chapter 2). Moreover, outbreeding depression can be an additional indication that *M. guttatus* is a consequence of local adaptation, because admixture among populations adapted to different environment can break down genetic interactions that confer higher fitness in a given environment.

6.2 Investment in sexual and clonal reproduction in the introduced range

Studies characterising traits in introduced and invasive species have shown that clonal growth is a common feature and suggested that it can facilitate invasion of some species (Cadotte et al. 2006; Silvertown 2008, but see Gassó et al. 2009). Throughout this thesis, I have shown that clonal reproduction in combination with sexual reproduction have important consequences for population fitness and phenotypic divergence among populations, and probably influence population genetic and genotypic diversity. In this study, genotypic (clonal) diversity analysis, using SNP genotyping, revealed that populations of *M. guttatus* in the UK varied from highly sexual to highly clonal, although most populations showed intermediate levels of clonal and sexual reproduction (Chapter 2; Table 2). The low inbreeding coefficient (*Fis*) values in some highly clonal populations suggest that clonality may be responsible for the excess of heterozygotes (Chapter 2). Simulations studies demonstrated that high rates of clonal reproduction will decrease levels of genotypic diversity, but increase heterozygosity (Balloux et al. 2003). An excess of heterozygotes due to clonality was also suggested using empirical data for another plant species (Stoeckel et al. 2006). Invasive plants display different levels of genotypic diversity (e.g., Zhang et al. 2010; Li and Dong 2009), and environmental factors that influence the relative investment in reproductive traits have been shown to determine the level of clonal diversity within populations. For instance, disturbance events affect clonal diversity of populations in the herbaceous *Ranunculus ficaria* (Reisch and Scheitler 2009). In some populations of the aquatic plant *Sparganium emersum*, high water velocity prevents individuals from emerging from the water, which reduces sexual reproduction and results in low genotypic diversity. In populations with slow water velocity genotypic diversity increases, because individuals are able to emerge from the water

and reproduce sexually (Pollux et al. 2007). The different levels of genotypic diversity found in 13 populations studied in Chapter 2 gave the first indication that populations in the UK are exposed to environmental heterogeneity modifying the investment in sexual and clonal reproduction, which affects the amount of clonality within and among populations.

Chapters 3 and 4 showed how the environment influences the investment in sexual and clonal reproduction in British *M. guttatus* populations. In the native range, *M. guttatus* occurs in mesic habitats, e.g. close to springs and streams, and seasonal variation in soil water conditions determines the relative investment in sexual and clonal reproduction, the life span of annual and perennial populations, and mortality of plants when soil gets too dry in summer (Lowry et al. 2008). The field survey of non-native natural populations showed that climatic variables associated with soil moisture, such as mean temperature of the driest and coldest period and precipitation of the warmest period affect the relative investment in number of stolons and number of flowers in opposite directions (Chapter 4). In places with cold temperatures and high precipitation, plants invest more in stolons than flowers, whereas in warmer and drier places plants reproduce more sexually. For a drought intolerant species such as *M. guttatus*, low precipitation may limit the survival of stolons and increase plant mortality, and consequently seeds that have the benefit of dormancy could be a better alternative for successful reproduction. In contrast, clonal reproduction is favoured in cold, wet and climatically stable places as demonstrated for many other plants (Ye et al. 2016). Climatic variables associated with soil water content are important selective pressures for *M. guttatus* native populations (Oneal et al. 2014) and this study demonstrates that climate can also influence the investment in sexual and clonal reproduction among non-native populations. Although in chapter 4 we do not indicate whether the relative investment in both reproductive traits is a result of divergent selection in different places, given the genetic variation for clonal and sexual reproduction (van Kleunen 2007b), and the importance of clonal and sexual reproduction for native and non-native population fitness (Peterson et al. 2016; Chapter 5), is it possible that shifts in reproductive traits result in local adaptation to different climatic conditions. Future reciprocal transplants among populations from different climates, combined with phenotypic selection analysis, are necessary to confirm local adaptation.

The common garden experiment of Chapter 3 showed that the expression of the trade-off between stolon length (estimated as the length of the longest stolon multiplied by the number of stolons) and number of flowers is stronger under limited conditions of space. This result suggests that, in the field, plants exposed to restricted space such as in high density populations would be limited to invest in flower production and clonal expansion with the same intensity. In conditions of high available space (e.g., low-density population), in contrast, populations would invest more in sexual reproduction than in clonality. Density, however, does not influence the proportion or number of stolons relative to flowers in the field (Chapter 4), which indicates that space in populations with different densities seems to affect clonal expansion rather than number of clones. Truscott et al. (2008a) have shown that availability of bare sediment influences the occurrence and number of patches of *M. guttatus* in the UK. This study suggests that space is an important environmental driver that influences not only plant establishment, but also the relative investment in sexual reproduction and clonal lateral spread.

The reduced allocation to sexual investment compared to stolon length found in Chapter 3, could be an adaptive strategy of plasticity to limited space availability. Phenotypic plasticity enables the expression of advantageous traits in different environments and therefore expands the species' ecological breadth (i.e., set of environmental conditions and resources necessary for reproduction and survival) (e.g., Sultan 2001). Moreover, greater plasticity of invasive species over co-occurring native species may be an advantage for invasion (Molina-Montenegro et al. 2012; Hou et al. 2015). For instance, in an experiment using substrates with different soil heterogeneity, the invasive clonal species *Alternanthera philoxeroides* showed higher stolon length, total biomass and growth rate than the native congener *A. sessilis* in heterogeneous soil. *A. philoxeroides* also exhibited higher plasticity by maintaining consistent values of these traits across homogeneous and heterogeneous substrates than the non-invasive species *Myriophyllum aquaticum* and *Jussiaea repens,* suggesting that higher trait values and plasticity may help the invasion of *A. philoxeroides* when competing with others species (Wang et al. 2016). A previous study with introduced *M. guttatus* showed that native plant species richness decreased as *M. guttatus* cover increased (Truscott et al. 2008b). It is possible that the negative effect of *M. guttatus* on native species is a consequence of greater plasticity of sexual and clonal reproduction in *M. guttatus* relative to other

co-occurring species. Higher allocation to stolon length than to flowers under limited space could maintain population cover and, therefore increase competitive ability of *M. guttatus* under high density. In addition, selection for intense clonal spread (Table 4, Chapter 5) could increase *M. guttatus* cover, resulting in the loss of native community richness.

6.3 Final remarks and recommendations for future studies

Empirical evidence of adaptive evolution in non-native plant species is key to understanding the processes that allow populations to deal with novel environmental challenges (Colautti and Lau 2015). Our results reveal outbreeding depression by showing that fitness of admixed introduced-introduced individuals is higher than admixed native-introduced ones. Although the mechanism of outbreeding depression is still unknown, the disruption of local adaptation can be the potential cause of the observed outbreeding depression. Moreover, flowering time, clonal reproduction, floral display and plant height are under selection in the non-native range. Together, the evidence for selection in the invasive range, and outbreeding depression in admixed introduced populations, suggest adaptive evolution of *M. guttatus* in the UK. In addition, the results show that considering multiple fitness components, clonal and sexual reproduction are important integrated traits that affect population growth rate (λ), particularly in individuals derived from non-native population admixtures, and there is an environmental dependency on the antagonistic relationship between clonal and sexual reproduction that may result in populations using alternative reproductive strategies in different environments. Analysis of phenotypic divergence among populations, and the role of natural selection can give insights into the importance of adaptive evolution for the increased performance of non-native plants, which can amplify the negative effects on native communities.

Although a single species was investigated, the combination of clonal and sexual reproduction is often found in other invasive plants (Dong et al. 2006; Jesse et al. 2010; Kettenring et al. 2016) and the high levels of genetic diversity held within some invasive plant populations (Genton et al. 2005; Erfmeier and Bruelheide 2011) can potentially serve as material for natural selection, suggesting that the findings of this research may be applied to a wider number of invasive plant

species. Thus our results inform our understanding of traits and evolutionary process that may contribute to invasion of other species. This research with *M. guttatus* provided evidence that sexual and clonal reproduction can contribute to population growth rate and suggest that both modes of reproduction can be adaptive strategies in different environments, which may also be applied to other partial clonal invasive species. The evidence of natural selection (Chapter 5) may indicate that adaptive evolution can assist other genetically and phenotypically diverse invasive species, like *M. guttatus,* to spread over different environmental conditions (e.g, Colautti and Barrett 2013). More generally, the potential of rapid adaptation can have implications for management of invasive species as natural selection can result in individuals with high tolerance to biological controls (Müller-Schärer et al. 2004).

Demonstrating and characterising natural selection in the non-native range allows us to identify specific traits that may be involved in adaptation to the introduced environment. Next, studies should focus on determining the agents of selection of *M. guttatus* in the UK and, as introduced *M. guttatus* is widespread across the UK (Vallejo-Marin and Lye 2013), whether there are different patterns of selection and local adaptation in populations under different biotic and abiotic conditions. For instance, in an elegant field experiment, Colautti and Barrett (2013) demonstrated that rapid adaptation contributed to invasion of *Lythrum salicaria*. They presented four lines of evidence: latitudinal clines in growth and flowering times; reciprocal transplants of populations to test for local adaptation across latitudinal gradients; measurements of divergent selection on growth and flowering time among populations; and comparison of fitness effects by local adaptation and other factors such as enemy release and competitive advantage.

Our work identifying traits under selection represents a first but important step in the study of adaptation to new environments using *Mimulus* as a study system and will allow researchers to pursue other lines of investigation, e.g., understanding the genomic basis of rapid evolution during invasions. For instance, using the QTL (quantitative trait loci) approach it is possible to link traits important for successful invasion to regions of the genome affecting these traits in a segregating population, usually an F2, generated from a cross between native and invasive populations (Prentis et al. 2008). In another approach, Hodgins et al. (2013) using genome-wide gene expression data of the common ragweed *Ambrosia*

artemisiifolia identified many genes that are expressed differently between native and invasive ranges; and that most of these genes have potential functions related to stress responses. The authors suggested that gene expressions at these candidate genes are related to the rapid growth and increased reproduction of introduced populations, particularly in the light stress conditions found previously in Hodgins and Rieseberg (2011). In line with these studies, a follow-up study could investigate the genetic architecture of introduced *M. guttatus* underlying the traits under selection found in chapter 5 (e.g., number QTLs or candidate genes, interaction among genes and gene expression differences between native and invasive ranges of *M. guttatus*). Identifying the genetic basis of adaptive evolution in non-native species will provide a broader understanding of the mechanisms that underlie successful plant invasions (Lee 2002).

6.4 References

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