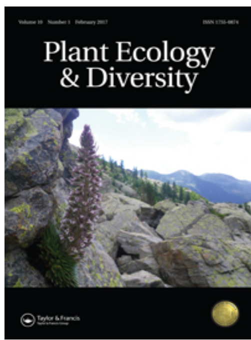

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Genetic variation and clonal diversity in introduced populations of *Mimulus guttatus* assessed by genotyping at 62 single nucleotide polymorphism loci

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Background: 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.
Aims: 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).
Methods: 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.
Results: 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.
Conclusions: 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.

Keywords: clonal diversity; clonal growth; genetic diversity; *Mimulus*; non-native species; SNP genotyping

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 2011), 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

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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 (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, 2008). Individual plants may produce several thousand small seeds (P. Pantoja and M. Vallejo-Marín, *unpublished*), 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 in 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).

Materials and methods

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.

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, our unpublished results 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 (V. Simon-Porcar, J. Silva, S. Meeus, J. Higgins and M. Vallejo-Marin, unpublished). 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

Table 1. Characteristics of the 14 introduced and 10 native populations analysed in this study. Life history: Perennial (P), annual (A).

Population code	Life history	Location	Latitude	Longitude	Source	Sample size	*N*	# of loci
United Kingdom								
HAM	P	Hamnavoe, Isle of Yell, Shetland	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

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.

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).

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.

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.

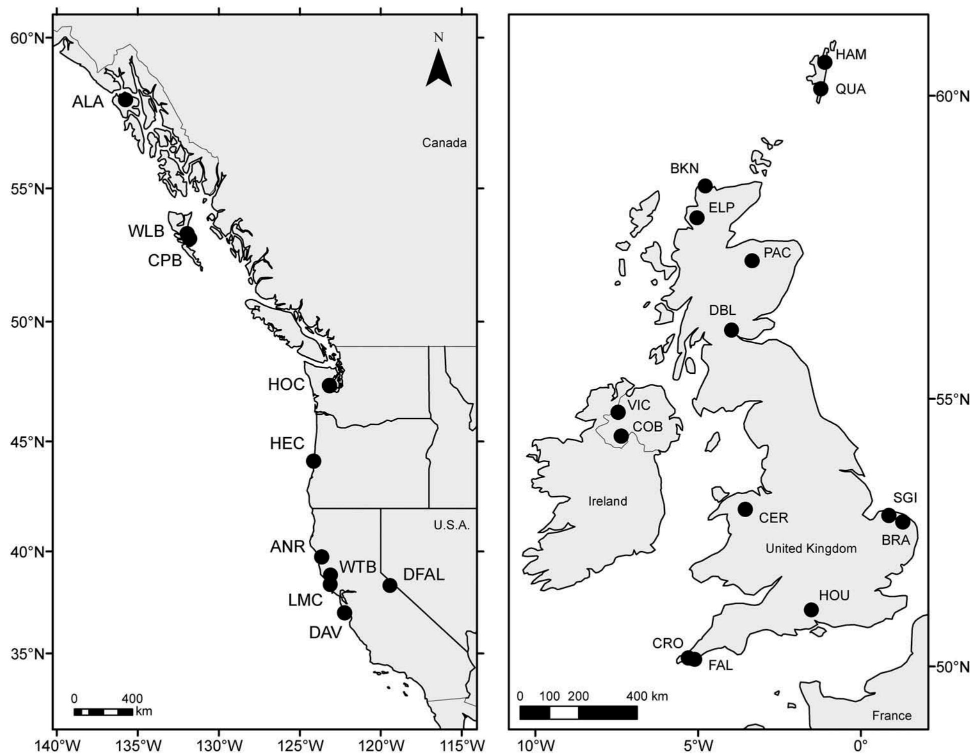

Figure 1. Populations of *M. guttatus* sampled for this study in North America (left) and the UK (right). Population codes as in Table 1.

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, 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).

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 (H_o), expected heterozygosity (H_e), and the inbreeding coefficient (F_{is}). Confidence intervals for F_{is} 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, H_o, and H_e 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.

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 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.

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).

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 H_o = 0.31 ± 0.03 (H_o = 0.26 ± 0.02, when calculated for unique MLGs only). Average gene diversity (H_e) across UK populations was 0.32 ± 0.02 (range 0.14–0.39; Table 3). The mean inbreeding coefficient (F_{is}) calculated using all UK individuals was 0.09 ± 0.01 (F_{is} = 0.13 ± 0.02, when only unique MLGs are included). Individual populations showed significant deviations from Hardy–Weinberg in F_{is} values, from heterozygote excess (BKN, BRA, and ELP) to heterozygote deficit (CRO, DBL, HOU, and QUA; Table 3). Negative F_{is} 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 F_{is} 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; H_e = 0.31 ± 0.01), while, as expected, observed heterozygosity was lower (H_o = 0.13 ± 0.01, range 0.08–0.18).

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 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 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. 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.

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).

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

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 F_{is} 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	(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

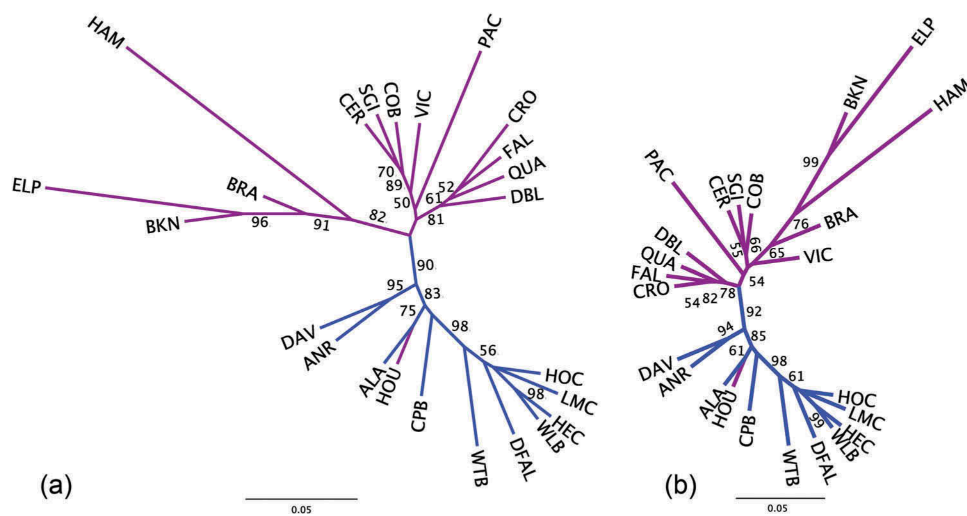

Figure 2. Neighbour Joining (NJ) trees depicting the relationships among 10 native North American populations and 14 introduced populations in the United Kingdom. The cladogram was built using pairwise genetic distances (Provesti's distance) between populations. (a) Tree estimated using all 375 individuals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

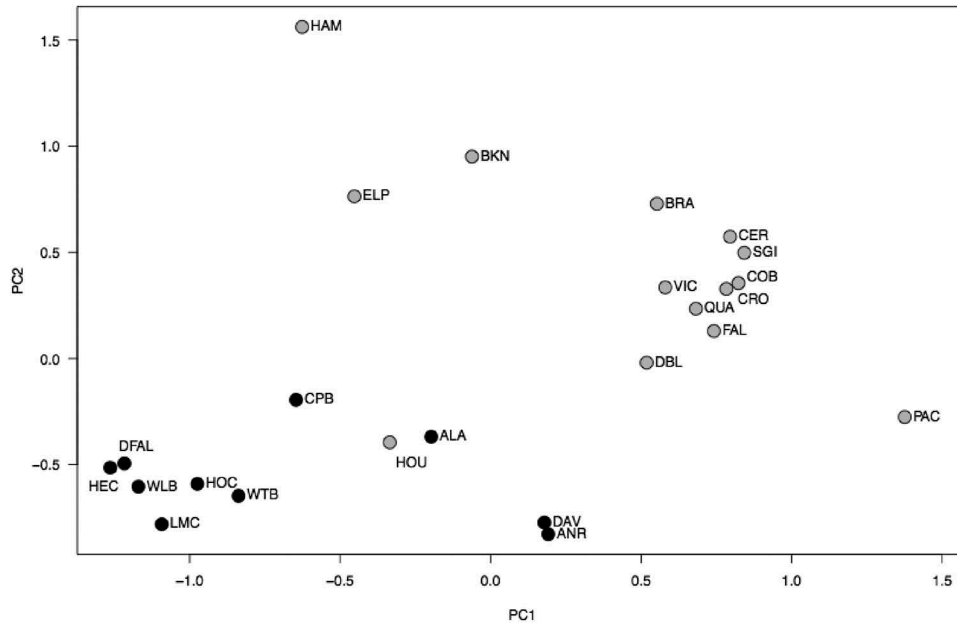

Figure 3. Relationship among *M. guttatus* populations in both native (black symbols) and introduced ranges (grey symbols) as inferred from principal component analysis (PCA) of the clone-corrected data set. Data points represent population averages of the first two principal components (PC1, PC2).

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
United Kingdom and North America			
Among regions	1 (1)	1.681 (1.656)	11% (11%)
Among populations	22 (21)	1.638 (0.949)	10% (6%)
Among individuals	351 (286)	4.993 (6.994)	32% (45%)
Within individuals	375 (309)	7.443 (6.036)	47% (39%)
United Kingdom only			
Among populations	13 (12)	2.192 (1.340)	15% (9%)
Among individuals	247 (182)	3.156 (5.670)	22% (39%)
Within individuals	261 (195)	9.155 (7.505)	63% (52%)
North America only			
Among populations	9	0.239	2%
Among individuals	104	9.282	27%
Within individuals	114	3.522	71%

populations, but is insufficient to detect finer patterns of genetic structure within regions. At a broader scale, our genetic analysis supports 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 have been derived from 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 in 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 and 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 F_{st} (average pairwise F_{st} 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: F_{st} = 0.05; New Zealand: F_{st} = 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; Φ_{ST} = 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.

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, the increasing access to new tools, such as RADseq and Genotype by Sequencing (Narum et al. 2013), is a promising avenue to genotype both large

numbers of SNP markers and individuals. 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.

Data availability

Genotype data was submitted to Dryad: doi:10.5061/dryad.91v3n.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here.

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