Biological Conservation 203 (2016) 134–142

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/bioc

Consequences of *in-situ* strategies for the conservation of plant genetic diversity

R. Whitlock [a,*,1], H. Hipperson [a,1], D.B.A. Thompson [b], R.K. Butlin [a], T. Burke [a]

[a] Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK
[b] Scottish Natural Heritage, Silvan House, 3rd Floor East, 231 Corstorphine Road, Edinburgh EH12 7AT, UK

ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 29 July 2016
Accepted 2 August 2016
Available online xxxx

Keywords:
AFLP
Biogeography
Conservation genetics
Genetic diversity
In-situ conservation
Plants

ABSTRACT

Conservation biologists have drawn up a range of guidelines for the conservation of genetic diversity—to maximise the chances that populations of threatened species persist, and to conserve this variation for its potential utility. However, our understanding of the effectiveness of conservation guidelines for maintaining genetic diversity *in situ* is limited. Furthermore, we lack information on how species-level variation in mating system affects these genetic conservation strategies. We used the British geographical ranges of eight widespread but declining plant species, varying in breeding system, as a model to assess the effectiveness of guidelines for the *in-situ* conservation of neutral genetic diversity. By applying simulated *in-situ* conservation scenarios to amplified fragment length polymorphism data, we show that the conservation of one population (the "minimum-set" approach) would retain ~70% of common allelic variation, but few or no rare alleles (alleles with frequency ≤ 0.05). Our results indicate that the conservation of >35% of populations would be needed to reach the Convention on Biological Diversity's recommendation to conserve 70% of genetic diversity *in situ*, as applied to rare alleles (~10 populations within each species' British range). The capture of genetic variation in simulated conservation networks was insensitive to breeding system. However, a spatially stratified approach to population selection led to significantly greater capture rates for common alleles in two of our study species, relative to a spatially random strategy. Our study highlights the challenges of conserving genetic variation, and emphasises the vulnerability of genetic biodiversity to reductions in the extent of species' ranges.

1. Introduction

Conservation practitioners have limited resources to carry out their work, and must mitigate extinction threats to species and populations against a background of activities that compete with conservation for land use. Hence they often need to make, either explicitly or implicitly, decisions regarding how many and which populations in a species' range should be conserved (Margules and Pressey, 2000; Prendergast et al., 1999). The populations comprising species' ranges often differ genetically from one another. For instance, levels of genetic diversity can vary in response to local population size and habitat fragmentation, and populations also differ in the expression of inbreeding depression and in their environmental adaptations (Aguilar et al., 2008; Angeloni et al., 2011; Ellstrand and Elam, 1993; Frankham, 1996; Franks et al., 2014; Leimu and Fischer, 2008). Thus, the decision to protect a subset of populations is likely to carry immediate consequences for the conservation of genetic biodiversity (Neel and Cummings, 2003a), and may also alter the demographic sustainability of populations through habitat fragmentation and responses to environmental change.

Conservation and agricultural biologists have used theoretical and empirical approaches to understand how genetic diversity is captured under different conservation scenarios, and to formulate guidelines for the conservation of genetic diversity (summarised in Table 1). Initially, these dealt with the capture of allelic diversity within *ex-situ* collections, and were derived from sampling theory for neutral alleles (Marshall and Brown, 1975). Recent *ex-situ* guidelines range from relatively small targets (*e.g.* collection of seed from 10 individuals in each of five populations; Centre for Plant Conservation (CPC, 1991) to comprehensive collections of germplasm (Brown and Marshall, 1995). However, these *ex-situ* guidelines are also relevant to, and have been extended to include, the conservation of genetic diversity *in situ* (Dulloo et al., 2008; Neel and Cummings, 2003a). This development is important, because only 28–38% of threatened plants have five populations in *ex-situ* collections (Godefroid et al., 2011). Furthermore, *ex-situ* populations can rapidly become genetically diverged from their source populations (Lauterbach et al., 2012), may lose adaptation to their source environment, and may become inbred (Schoen and Brown, 2001), highlighting the need for complementary *in-situ* conservation.

* Corresponding author at: Institute of Integrative Biology, University of Liverpool, The Biosciences Building, Crown Street, Liverpool L69 7ZB, UK.
E-mail address: r.whitlock@liverpool.ac.uk (R. Whitlock).
[1] Joint first authors/these authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biocon.2016.08.006

Table 1
Summary of conservation guidelines relevant to the conservation of genetic diversity.

Guideline	Intended scope	Description
Margules et al. (1988) minimum set approach	Representation of species within protected area networks	Aims to represent each species at least once, *i.e.* at least one population per species
Marshall and Brown (1975) target	*Ex-situ* collections of crops and their wild relatives	Aims to capture each of a species' common alleles (those present at frequency ≥ 0.05 in any individual population) with 90–95% probability; 50–100 individuals from each population
Brown and Briggs (1991) guideline	*Ex-situ* collections of endangered plant species	Recommends collection of a minimum of 10 individuals from each of five populations
Centre for Plant Conservation (1991) original guideline	*Ex-situ* collections of endangered plant species	Recommends collection of 10–50 individuals from each of five populations
Dulloo et al. (2008) guideline	*In-situ* networks of genetic reserves for crop wild relatives	Recommends conservation of a minimum of five populations *in situ* within genetic reserves (protected areas)
Lawrence et al. (1995) guideline	*Ex-situ* germplasm collection for natural or agricultural plant populations	Aims to conserve at high probability all of the common alleles (frequency > 0.05) present in a species; collect seed or vegetative tissue from 172 plants
Brown and Marshall (1995) guideline	*Ex-situ* seed collection	Recommends collection of seed from 50 individuals from each of 50 populations per ecogeographical region of each species
Centre for Plant Conservation updated guideline (Guerrant et al., 2004)	*Ex-situ* seed collection for endangered plant species	Recommends collection of seed from 50 individuals from each of 50 populations per ecogeographical region of each species
Updated global strategy for plant conservation (CBD, 2010)	Crops, their wild relatives and other socio-economically important plant species	Recommends conservation of 70% of genetic diversity

An understanding of the effects of population sampling on the conservation of genetic diversity is also needed to guide policy. The Convention on Biological Diversity (CBD) provides an international policy framework for the conservation of plant genetic diversity, which applies particularly to its uses in crop breeding and to its human utility value (*e.g.* for crop improvement; Castañeda-Álvarez et al., 2016; CBD, 1992). Furthermore, the IUCN states that there is a need for 'the maintenance of existing genetic diversity and viable populations of all taxa in the wild in order to maintain biological interactions, ecological processes and function' (Maunder and Byers, 2005). Recent revisions to this general framework (CBD, 2010) recommend the conservation of 70% of genetic diversity (Table 1). However, this recommendation was accompanied by little specific guidance as to what sort of genetic diversity should be targeted, or how many populations should be conserved *in situ* to achieve this, especially for wild species with low potential utility value (*e.g.* species that are not wild relatives of crop plants).

The impacts of genetic guidelines (Table 1) on the conservation of genetic diversity and demographical sustainability have not been assessed thoroughly. Assuming that the genetically effective population size (N_E) is 10% of the census population size (N_C), the larger guideline census sample sizes listed in Table 1 would imply N_E exceeding 50, on average (Palstra and Ruzzante, 2008). These effective population sizes may be sufficient for the maintenance of fitness in the short term (Franklin, 1980; Jamieson and Allendorf, 2012). However, such approximations remain highly controversial, and are not guaranteed to hold in individual cases, due to wide variation in the ratio of N_E/N_C among species (Frankham et al., 2014; Franklin et al., 2014).

It also remains unclear as to how effectively these sampling strategies would conserve the quantitative genetic variation that underpins evolutionary potential and adaptation (Hamilton, 1994; Schoen and Brown, 2001). In principle, variation at neutral molecular markers could be used as a proxy (Brown and Briggs, 1991). Conclusions regarding quantitative variation would then rest on the assumption that neutral and quantitative genetic variation share similar sampling properties (Hamilton, 1994). However, neutral genetic structure is only weakly correlated with quantitative genetic structure (Leinonen et al., 2008; Reed and Frankham, 2001; Willi et al., 2006), limiting its utility as a general indicator in conservation genetics. Ultimately, genomics approaches are likely to greatly enhance our understanding of the distribution of quantitative and detrimental genetic variation in species of conservation concern, resolving these uncertainties (Savolainen et al., 2013; Shafer et al., 2015). In the meantime, neutral molecular markers continue offer a valid method for assessing the genetic consequences of conservation guidelines and strategies.

Previous studies have shown that the conservation of neutral genetic variation depends strongly on the numbers of populations conserved (Neel and Cummings, 2003a), and that ecological criteria and reserve guidelines might lead to poor representation of genetic biodiversity in conservation networks (Neel and Cummings, 2003b). This early work investigated the effectiveness of genetic conservation strategies using four rare outbreeding plant species (Neel and Cummings, 2003a). Inbreeding (selfing) plant species were not included in these studies, but their genetic responses to habitat fragmentation and inbreeding differ in important ways from those of outcrossing species. For example, habitat fragmentation leads to stronger reductions in molecular variation in outcrossing species than in selfing species (Aguilar et al., 2008). Furthermore, common and recently rare plant species are at greater risk of losses of genetic biodiversity following fragmentation than naturally rare plant species (Aguilar et al., 2008). Thus, there is a need to assess the effectiveness of genetic conservation guidelines in a broader set of species, incorporating both inbreeding, and relatively more widespread taxa.

Here, we consider the effectiveness of conservation sampling guidelines for capturing species' genetic diversity *in situ*, using amplified fragment length polymorphism (AFLP) datasets gathered from natural populations of eight currently widespread, but declining plant species. Our study species spanned a range of mating systems from highly inbreeding to obligate outcrossing. We simulated a range of *in-situ* conservation strategies by sampling populations from each genetic data set, using both randomised and spatially stratified sampling approaches, and measured the effects on the retention of common and rare alleles. We also measured the influence of conservation scenarios on levels of expected heterozygosity and genetic differentiation. Our results confirm that much common allelic diversity may be readily conserved in relatively few populations (~five), but also suggest that a substantially greater number of populations (≥10) would be required to capture rare allelic variation efficiently.

2. Material and methods

2.1. Study species

We studied eight herbaceous plant species native to the British Isles: *Arabis glabra* (L.) Bernh., *Cirsium eriophorum* (L.) Scop., *Cirsium heterophyllum* (L.) Hill, *Dianthus deltoides* (L.), *Gentianella campestris* (L.) Börner, *Iberis amara* (L.), *Pinguicula vulgaris* (L.) and *Trollius europaeus* (L.); nomenclature follows Stace (1997). These species were selected through consultation with UK conservation agencies. In addition, they were selected to be representative of species that have suffered recent reductions in their geographical ranges. All except *C.*

eriophorum have experienced retraction in range extent in the UK (Preston et al., 2002). Furthermore, they were chosen to include a balance of geographical distributions, including species distributed predominantly within the north and predominantly within the south of the British Isles (Fig. A1, Supplementary materials; available as an online appendix). Finally, species were selected so that their mating systems typified the span present in herbaceous plants, from highly inbreeding to obligate outbreeding (Table 2). All the species are diploids, except *G. campestris* and *P. vulgaris*, which are tetraploid and octoploid, respectively, relative to the basic chromosome numbers within their genera (Casper and Stimper, 2009; Löve and Löve, 1975).

2.2. Study populations and sampling

We selected study populations of each study species based on a spatially hierarchical design. First, we created maps of the local density of presence/absence records for each species (Fig. A1), and used these to choose up to six geographical sampling regions per species (Fig. 1; Table A2; Fig. A3). Sampling regions were spread across the British range of each species and, where possible, each occupied a local gradient in density (a local range edge). Next, we selected up to eight populations per region (where possible), and approached landowners for site access permissions. Almost all study populations had distinct boundaries, often reflecting the patchy distribution of the species' preferred habitats (Table A4). The mean size of the sampling regions (maximum distance between any pair of populations within a region) was 35.1 km. The mean distance among pairs of sampled populations within regions was 20.2 km.

We visited our study populations over two field seasons (2005 and 2006) and recovered ~57 leaf tissue samples per population on average (each approximately 1 cm^2), applying additional *a priori* sampling rules within populations (Text A5, Table A6). These samples were placed immediately on silica gel and stored at room temperature until needed.

2.3. DNA genotyping

DNA was extracted from leaf samples using a high-throughput plate-based method (Whitlock et al., 2008a), and genotyped using AFLP markers (Vos et al., 1995). AFLP chromatograms were scored using the software GENEMAPPER V3.7 (Applied Biosystems) and AFLPSCORE version 1.4b (Whitlock et al., 2008b). Genotyping error was controlled by genotyping replicate tissue samples and through error rate analysis within AFLPSCORE. We present full details, including methods for analysis of genetic diversity and structure, in Text A7 and Table A8.

2.4. Assessment of allelic variation

We considered only AFLP fragment presence alleles, since identification of null alleles is ambiguous when they are present at low frequency. We restricted our assessment of allelic variation to loci that were polymorphic globally across all populations within species. Allele frequency was measured using Zhivotovsky's Bayesian approach (Zhivotovsky, 1999), and we classed fragment presence alleles as either common ($p > 0.05$ in at least one population) or rare ($p \leq 0.05$ in all populations). Rare alleles could not be identified in *Cirsium eriophorum*, since all population sample sizes were <20.

Information on the level of within-population inbreeding (F_{IS}) is needed to be able to calculate allele frequencies using AFLP markers, and this information was not available for all of our species. Therefore we estimated allele frequencies and identified common and rare alleles

Table 2
Mating system, sampling design, and a summary of genetic diversity and structure inferred from AFLP data, for each of eight plant study species.

Species (family)	Mating system[a]	Sampling design				Genetic diversity[b]			Population genetic differentiation F_{ST}[c] (S.E.)	Isolation by distance[d]	
		No. regions	No. populations	No. individuals (Mean; range per population)	No. loci	Number loci polymorphic (range per population)	H_T	H_W (S.E.; range of H_j)		Mantel Z-statistic	Slope and intercept of IBD
Arabis glabra (Brassicaceae)	Inbreeding	3	16	724 (44.3; 17–80)	83	72 (6–20)	0.165	0.057 (0.005; 0.025–0.082)	0.657** (0.036)	2832***	0.480, −0.036
Cirsium eriophorum (Asteraceae)	Possibly inbreeding	5	25	333 (13.4; 10–16)	79	38 (2–17)	0.114	0.058 (0.006; 0.016–0.122)	0.499** (0.101)	4220	−0.016, 1.291
Cirsium heterophyllum (Asteraceae)	Outbreeding, can reproduce vegetatively	5	37	1782 (48.7; 16–136)	111	110 (41–87)	0.238	0.163 (0.008; 0.035–0.260)	0.315** (0.102)	4868***	0.037, 0.422
Dianthus deltoides (Caryophyllaceae)	Mixed mating, self-compatible	3	18	827 (45.8; 16–124)	95	79 (9–44)	0.218	0.135 (0.009; 0.056–0.199)	0.383** (0.087)	1238***	0.095, 0.242
Gentianella campestris (Gentianaceae)	Mixed mating, self-compatible	6	26	1475 (40.7; 18–186)	109	103 (4–71)	0.137	0.078 (0.013; 0.016–0.253)	0.429** (0.099)	4244***	0.158, 0.264
Iberis amara (Brassicaceae)	Self-incompatible	4	17	1313 (77.2; 21–183)	256	244 (81–160)	0.199	0.172 (0.005; 0.133–0.216)	0.135** (0.109)	248***	0.051, −0.020
Pinguicula vulgaris (Lentibulariaceae)	Mixed mating, self-compatible, can reproduce vegetatively	6	24	775 (32.3; 18–47)	139	108 (11–28)	0.095	0.065 (0.003; 0.045–0.097)	0.318** (0.046)	1637***	0.047, 0.227
Trollius europaeus (Ranunculaceae)	Obligate outbreeder	5	42	1530 (36.4; 15–49)	119	119 (48–76)	0.191	0.176 (0.002; 0.154–0.216)	0.080** (0.062)	200***	0.002, 0.011

[a] "Mixed mating" refers to situations in which plants set seed when non-self-pollen has been excluded, but where outcrossed pollen is thought to be produced in the presence of pollinators. "Outbreeding" refers to the situation where plants set little or no seed when pollinators are excluded, but where the precise mechanism behind this reduced seed set is not known. Further details and references can be found in Text A7.

[b] H_T and H_W are, respectively, the total gene diversity of the entire sample of individuals and the mean of gene diversities for individual populations.

[c] Calculation of F_{ST} follows Lynch and Milligan (1994), and its significance has been tested by 5000 permutations of individuals among populations.

[d] Isolation by distance calculations were done using $F_{ST} / (1 - F_{ST})$ as the measure of genetic distance, and log of distance measured in km as the measure of physical distance.

** $p < 0.01$.

*** $p < 0.001$.

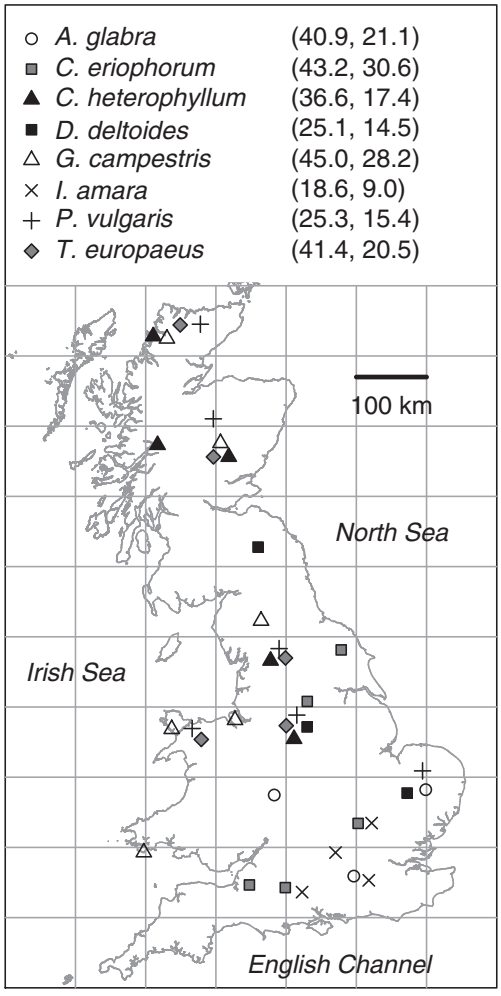

Fig. 1. Map of mainland Britain showing location of sampling region centroids for eight plant species, within which study populations were selected for recovery of leaf tissue samples. Values in parentheses appearing beside species names are mean of maximum breadth of sampling regions, and mean distance between pairs of sampled populations within regions, respectively, both in km. Gridlines are spaced at 100 km. Vertical gridlines are aligned with grid north in the British (Ordnance Survey) national grid reference system. Location markers for region centroids have been jittered in space where necessary to improve their visibility relative to other markers.

first assuming complete inbreeding at equilibrium ($F_{IS} = 1$), and then assuming Hardy-Weinberg equilibrium (HWE; $F_{IS} = 0$). However, the results of the analyses described below were very similar irrespective of the value of F_{IS} chosen, so we present the results for the analysis assuming inbreeding. Additionally, we considered fragment frequency thresholds of $1 - q^4 = 0.186$ and $1 - q^8 = 0.337$ for the polyploid species (*G. campestris* and *P. vulgaris*, respectively) in order to identify common and rare alleles in these species. These correspond approximately to allelic thresholds of 0.05 for each species, assuming polysomic inheritance and HWE. The outcomes of analyses using these thresholds were qualitatively similar to those assuming diploidy. In addition, we note that the assumption of diploidy is conservative in terms of the quantity of genetic variation captured by conservation sampling designs. Therefore, we present only the diploid analysis for these polyploid species.

2.5. Population sampling

We adopted the Monte Carlo population sampling procedure described by Neel and Cummings (2003a) to estimate the sampling distribution for capture of allelic diversity, within-population heterozygosity (H_W) and genetic differentiation (F_{ST}). In short, we drew samples of populations, without replacement, ranging in size from 1 to $n - 1$ of the total number of sampled populations, to simulate the *in-situ* conservation of subsets of populations for a given species. Sampling of populations was random with respect to the region membership of the populations (hereafter, the "fully randomised" sampling design). We drew 1000 replicate Monte Carlo samples for each number of populations considered. Individual Monte Carlo samples specified the identity of populations, including all sampled individuals, to be "conserved" *in situ*. We then used data from polymorphic AFLP loci to determine the proportion of common, rare and all (both common and rare) alleles retained in each sample of populations. This approach assumes that sampled populations conserved the genetic variation they contained perfectly, while variation from non-sampled populations was assumed to be lost. The effects of population sampling on genetic structure were assessed for each sample, by calculating H_W and F_{ST}, following the methods of Lynch and Milligan (1994). To assess the consequences of a spatially representative strategy for the *in-situ* conservation of genetic variation, we performed a second set of sampling simulations. Here, we sampled randomly, as before, but constrained population selection to maintain balance in the representation of the original sampling regions as the number of conserved populations was increased (hereafter, the "spatially stratified" sampling design).

We used the results from the Monte Carlo analyses to determine the genetic consequences of the minimum set approach and the Centre for Plant Conservation's's (1991) five-population guideline for the *in-situ* conservation of genetic variation. Although the original CPC (1991) guidelines have been superseded (Guerrant et al., 2004), they provide a useful benchmark to allow comparison of our results with those of Neel and Cummings (2003a). We also assessed how many populations would be required to achieve the CBD's recommendation to conserve 70% of genetic variation in plant species (which we interpreted as a proportion of alleles) and the Marshall and Brown (1975) target of conserving all common alleles with >90% confidence. Data analyses were carried out in R (version 3.2.2; R Development Core Team, 2008), except where specified otherwise.

3. Results

3.1. Allelic diversity

The proportion of allelic diversity captured (measured as either all, common or rare alleles) rose rapidly with the first few populations conserved by our simulated *in-situ* sampling designs. Beyond this, the rate of capture of additional variation decelerated towards an asymptote (at 100% of alleles captured). In some species and simulation runs this asymptote could be reached before all populations had been sampled (Fig. 2).

Conservation of only one population per species (the minimum set approach) captured 63.7% of all alleles on average across eight species (range 43.1–78.4%), and 69.8% of common alleles (range 49.8–91.0%). No rare alleles were detected in any populations of *C. eriophorum*, *C. heterophyllum* or *D. deltoides*. In the remaining five species, rare alleles were considerably more difficult to capture than common alleles. Samples of single populations recovered 3.7% of rare allelic variation (mean across five species; range <0.1%–10.0%).

The CPC recommended conserving material *ex situ* from at least five populations (Brown and Briggs, 1991; CPC, 1991). Conserving five populations under our fully randomised *in-situ* sampling design captured 86.4% of all alleles (mean across eight species; range 81.0–92.1%) and 91.1% of common alleles (range 81.0–95.5%). As with the minimum-set approach, rare alleles were more difficult to capture when only five populations were sampled (43.6% on average, range 14.3–70.5%; five species). However, rare alleles showed the greatest relative increase in capture between the minimum-set (one-population) strategy and the CPC's five-population approach, indicating that these are most sensitive to choice of conservation sampling strategy. Use of a spatially

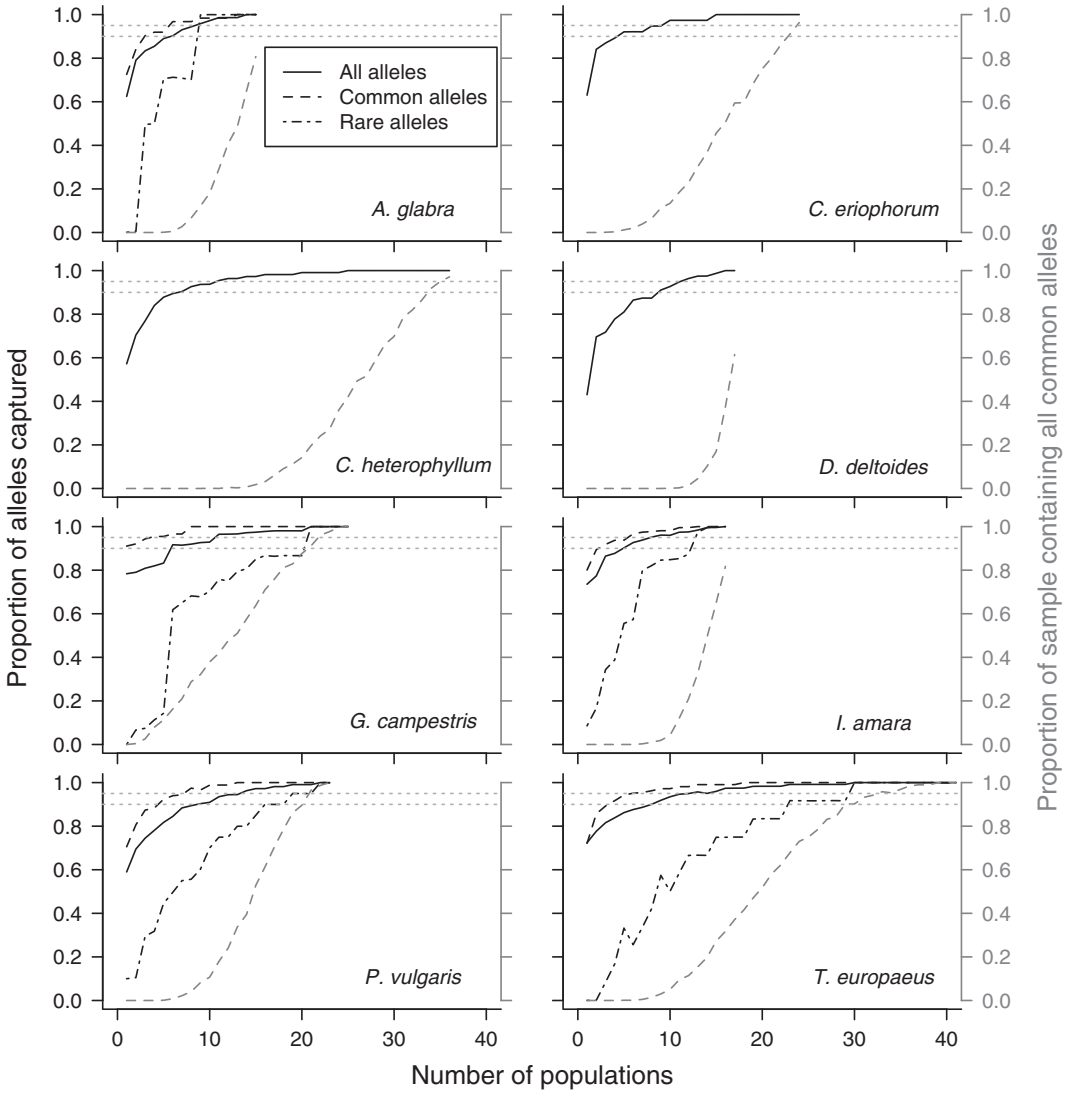

Fig. 2. Proportion of all, common (AFLP fragment presence allele frequency > 0.05) and rare (allele frequency ≤ 0.05) alleles captured when different numbers of populations are selected at random for inclusion in *in-situ* conservation networks. Black curves indicate the modal proportion of alleles conserved for each number of sampled populations. Dashed grey curves indicate the proportion of 1000 resampled datasets that conserved all common alleles (axis for this curve is to the right of each graph). Dashing on the black curves indicates allele subset (all, common or rare). The horizontal dotted grey lines indicate the proportions 0.90 and 0.95.

stratified design to sample five populations significantly increased the capture of common allelic variation in *C. heterophyllum* (5.7% increase) and *D. deltoides* (7.6% increase), relative to a fully randomised design ($p = 0.014$ and $p = 0.002$, respectively). Neither the capture of common allelic variation in the remaining species, nor the capture of rare allelic variation in any of the species differed significantly between fully randomised and spatially stratified sampling designs.

The CBD recommend the conservation of 70% of genetic diversity (Table 1, CBD, 2010). Seventy per cent of all, or common, allelic variation in our genetic datasets could be captured in three or fewer populations on average (<16% of all populations), for all species, regardless of sampling design. However, the capture of 70% of rare allelic variation required 5–15 (mean 9.4) populations per species (31–42% of all populations of each species) when using a fully randomised sampling design, and 6–16 (mean 11.2) populations per species when using the spatially stratified sampling design.

In order to meet the Marshall and Brown (1975) target for conserving all common alleles with 90% confidence using a fully randomised sampling design, it was necessary to conserve, on average, 87.8% of the total number of populations (range 69–100%, equivalent to 16–29 populations per species; Fig. 2, grey curves). This target could be achieved in a comparable number of populations using spatially stratified sampling (13–31 populations, 82.9% of the total population number on average across species).

3.2. Genetic structure

Sampling subsets of populations for conservation lead to a pattern of regression to the mean in within-population heterozygosity (H_W), with most variability among replicate samples when few populations were sampled (Fig. 3). This pattern was most pronounced for species with the highest range of within-population genetic diversity (*e.g. G. campestris*, *C. heterophyllum*; Table 2, Fig. 3). When only one population was selected, H_W varied by 19.9% on average across species from the values observed when all populations were selected (range 0.4–54.3%), and by 2.5% where five populations were selected using the fully randomised sampling design (range 1.4–4.3%). We observed similar, but more pronounced effects when we considered the extent of genetic differentiation (F_{ST}) resulting from sampling different numbers of populations for conservation (Fig. 3). For example, when two

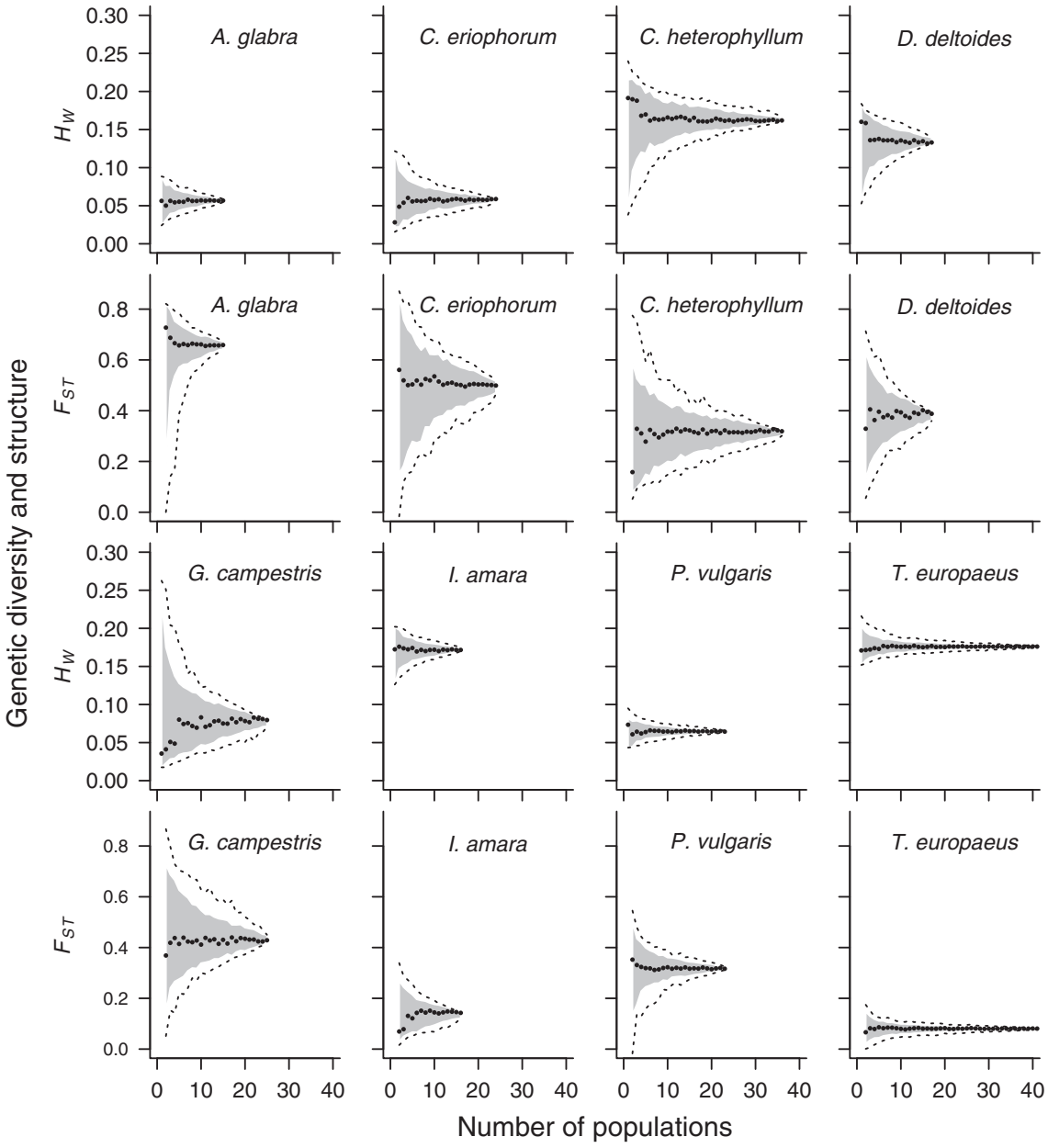

Fig. 3. Effects of population number on within-population gene diversity (H_W) and genetic differentiation (F_{ST}) within simulated *in-situ* conservation networks. Populations were drawn randomly from the total set for each species. Points indicate modal diversity or differentiation for each number of sampled populations. The shaded areas indicate 95% confidence zones for diversity or differentiation, constructed from highest posterior density intervals (HPDIs) for each number of sampled populations. Dotted black lines show the minimum and maximum diversity or differentiation for each number of sampled populations.

populations were selected at random, F_{ST} spanned almost the full range of possible values; the resulting population "network" could be either very highly differentiated ($F_{ST} > 0.8$) or close to undifferentiated ($F_{ST} \approx 0$; Fig. 3). On average, the random selection of any one pair of populations for conservation resulted in F_{ST} deviating from the value obtained when all populations were considered by 8.8% (range 2.2–43.5%), while conserving five populations at random resulted in a mean deviation of F_{ST} by 4.9% (range <0.1–12.5%). Genetic structures resulting from the spatially stratified population sampling design closely paralleled those for the fully randomised design (Fig. A9).

3.3. Breeding system

Genetic diversity within, and differentiation among, populations of our study species were broadly as expected, given what is known about their breeding systems; values for each species were consistent with mean values for studies on plants with similar breeding systems (Table 2; Hamrick et al., 1991; Nybom, 2004). However, any effect of breeding system on the capture of allelic variation when different numbers of populations were sampled was limited. There was some indication that breeding system could influence the capture rate of allelic variation within the first few populations conserved. For example, conservation of only one population (the minimum-set approach) captured on average 62.5% and 63.1% of all alleles for the inbreeding species *A. glabra* and *C. eriophorum*, respectively. The same sampling intensity conserved a greater quantity (73.6% and 72.4%) of the diversity present in the obligate outbreeders *I. amara* and *T. europaeus*. However, *C. heterophyllum* and the species with mixed mating systems showed a range of values bracketing those for other species (43.1% in *D. deltoides* to 78.4% in *G. campestris*).

4. Discussion

We used AFLP data obtained from eight plant species to simulate the effects of different conservation sampling designs proposed to capture and conserve genetic diversity. Our study was novel in its focus on widespread (but declining) species with a range of breeding systems, and in investigating the sampling of rare as well as common alleles. In agreement with previous studies, our results show that many of the common alleles present within species ranges can readily be included in relatively small networks of conserved populations. However, the capture of rare allelic variation is likely to be much more sensitive to the choice of conservation sampling design; our data indicate that greater numbers of protected areas may be necessary for the *in-situ* conservation of total range-wide genetic biodiversity in line with recent guideline targets (CBD, 2010).

4.1. Effects of plant mating system

Our study was novel in considering the effects of *in-situ* conservation strategies in eight plant species that differ markedly in mating system. Levels of genetic diversity within individual populations broadly reflected the expectation given each species mating system (Table 2; Fig. 3). However, the capture of allelic variation within samples comprising multiple populations was not related to species' mating system. Therefore, our results suggest that the effects of *in-situ* strategies for conserving genetic variation that incorporate multiple populations (*e.g.* CPC, 1991) might be relatively insensitive to mating system variation, and of general utility across plant species.

4.2. Random vs. stratified sampling strategies

Our study simulated the effects of conservation guidelines for genetic diversity by sampling populations from a total set either at random, or using a spatially stratified design. Use of a spatially stratified strategy to select populations for conservation led to a significant improvement in the capture of common allelic variation for two of our eight study species. This finding supports previous suggestions for *ex-situ* conservation that material should be collected from geographically or ecologically divergent populations to maximise the conservation of genetic diversity (Farnsworth et al., 2006; Kell et al., 2012). It is likely that improvements in the capture of genetic diversity under a spatially stratified sampling design were due to range-wide patterns of genetic structure. Our study species displayed significant genetic isolation by geographical distance (present in 7 species; Table 2) and significant differentiation among and within regions (Fig. A10, Table A11). Such genetic structure is likely to be common in plant species (Nybom, 2004). Therefore, stratified designs are likely to be of general applicability in establishing *in-situ* conservation networks that effectively capture genetic biodiversity.

Spatially stratified approaches to population selection for *in-situ* conservation may also more fully represent the range of quantitative variation present within species, strengthening evolutionary potential. Levels of neutral diversity in individual populations (measured in this study using AFLP) are not likely to be a reliable indicator for corresponding levels of quantitative variation (Reed and Frankham, 2001). However, many plant populations are adapted to their local environment (Leimu and Fischer, 2008), implying that populations often contain the genetic variants required for fitness in the environments that they occupy. Thus, it may be sensible to select a set of populations for conservation that represent the full range of environments occupied by a species, in order to facilitate the capture of this variation (Dulloo et al., 2008). The use of a spatially stratified sampling design, combined with the inclusion of additional ecologically divergent populations (Farnsworth et al., 2006; Kell et al., 2012), represents one valid approach for establishing such a conservation network.

There are likely to be additional non-spatial criteria that conservation practitioners will consider before selecting populations for conservation. From a genetic perspective, these may include the selection of individual populations that have a large genetically effective population size (N_E; Gregory et al., 2006; Jamieson and Allendorf, 2012). Choosing populations with high N_E is desirable because the risk of inbreeding decreases as N_E increases. Inbreeding is likely to lead to the expression of inbreeding depression in plant populations (Angeloni et al., 2011) and controls the loss of variation through drift (Hedrick, 2005). Natural populations with large N_E are most likely to be genetically diverse (Frankham, 1996). Thus, the inclusion of a subset of populations with large effective size will enhance the likelihood that protected area networks contain a core of genetically diverse and demographically sustainable populations.

4.3. The conservation of rare allelic variation

We found that rare alleles (≤0.05 in frequency range-wide) were challenging to capture within simulated *in-situ* conservation networks. On average across species, only 43.6% of rare allelic variants could be conserved in random samples of five populations. Use of spatially stratified population selection did not improve capture rates for rare alleles. These results underscore the potential difficulties in designing conservation networks that would efficiently capture these variants. However, it should be noted that our analyses may overstate the difficulties in capturing rare alleles, since not all populations were sampled exhaustively; 36% of the individuals present in each population were sampled, on average, and some rare alleles may have gone undetected. Nonetheless, the use of our results to guide population selection for *in-situ* conservation will be conservative, because a greater proportion of rare alleles will be captured than our results imply.

Guidelines for the conservation of genetic diversity could, in principle, be adjusted to accommodate rare variants. Any decision to do so, however, must be justified on the basis of value added to conservation outcomes. Rare alleles can have beneficial, deleterious or neutral effects on fitness. Evidence from mutation accumulation experiments suggests that most novel (initially rare) alleles arising through mutation are deleterious (Keightley and Lynch, 2003), and hence could have negative impacts on population growth and should not be targets for conservation. However, other rare alleles can be beneficial, increasing fitness, for example, by facilitating adaptation to changing ecological conditions, or offering increased resistance to parasites (Loewe and Hill, 2010; Piertney and Oliver, 2005). The distribution of fitness effects for the rare alleles detected in our study is unknown. Thus, in the following section we consider the implications of genetic conservation guidelines for both common and rare alleles.

4.4. Implications of current guidelines

The tenth conference of the parties to the Convention of Biological Diversity (CBD, 2010) has recommended the conservation of 70% of genetic diversity for crops, their wild relatives and other socio-economically important species. The target could be reached easily for common allelic variation within three or fewer populations, but was much more difficult to reach for rare allelic variation, requiring 5–16 populations per species. Thus, the CBD target implies the need for a significant investment in protected areas, if genetic biodiversity is to be conserved *in situ*.

The CPC (1991) suggested that *ex-situ* collections should be made from five populations per species, in order to adequately conserve common allelic variation. Our results confirm earlier findings (Neel and Cummings, 2003a) that the application of this guideline for *in-situ* conservation leads to the effective representation of common allelic diversity. In contrast, however, our findings also indicate that this strategy would be of limited value in capturing rare alleles; less than half of all rare alleles were captured in samples of five populations. Thus, the CPC guideline represents a “minimum standard” for genetic conservation, but may not be appropriate when it is desirable to maximise the

conservation of the genetic diversity (*e.g.* in the conservation of crop genetic resources).

We found two further guidelines to be poorly suited for the conservation of genetic biodiversity *in situ*. First, Marshall and Brown (1975) recommended that all of a species' common alleles should be conserved with >90% probability. Our observations support earlier findings (Neel and Cummings, 2003a), indicating that it would be necessary to sample a very high proportion of populations (69–100%) to achieve this target, rendering it impractical. Second, we found that application of the minimum-set approach (conservation of one population per species; Margules et al., 1988; Margules and Pressey, 2000) is likely to lead to conservation networks with only poor coverage of range-wide genetic biodiversity.

4.5. In-situ conservation for widespread but declining species

Very rare plant species rightly have a high priority for conservation. However, currently widespread but rapidly declining species can also be considered threatened (*e.g. Gentianella campestris*; Cheffings et al., 2005; IUCN, 2001), and retain significant range-wide genetic biodiversity that is at risk of loss. These species of intermediate conservation concern may be more vulnerable to population loss than rare species with stable distributions, as their decline from a formerly widespread distribution may lead to local inbreeding and, potentially, inbreeding depression, through loss of connectivity (Habel and Schmitt, 2012). Our results suggest that more stringent conservation targets for genetic diversity might be appropriate for these species, especially if rare allelic variation is to be maintained. For example, the conservation of ten populations of each of our study species *in situ* would be a reasonable minimum target in order to protect genetic diversity within their British ranges. If ten populations were selected, as opposed to five (the CPC target), the average proportion of rare allelic variation captured could be increased markedly (from 43.6% to 75.1%). Furthermore, this approach would have satisfied the CBD, 2010 target (70%), if applied to rare allelic variation, for four of the five species that had rare alleles.

4.6. Conclusions

Our results show that *in-situ* conservation networks should easily capture a large proportion of the common allelic variation present within species of conservation concern, regardless of their mating system. In contrast, rare allelic variation is likely to be captured much less efficiently, and our results suggest that the genetic consequences of *in-situ* conservation strategies will differ most in their effects on these rare variants. For species that are currently relatively widespread, an *in-situ* conservation guideline of a minimum of 10 populations, selected using a spatially stratified design, could prove a useful complement to CPC's five-population guideline for *ex-situ* conservation. Such an approach would likely enhance the conservation of rare allelic variation *in situ*, and assist in reaching the most recent CBD guidelines on the conservation of genetic biodiversity.

Acknowledgements

We gratefully acknowledge the assistance of M. E. Mannarelli, A. M. J. Skinner, B. Green, J. Morrison, and R. Dale during field and lab work. We thank the Botanical Society of the British Isles and its Vice-County Recorders for granting access to location records for our study populations. We also thank land managers and owners who granted us permission to access study sites, and J. Hodgson, P. Hollingsworth and three anonymous reviewers for constructive comments on earlier versions of this paper. The work was supported by a Natural Environment Research Council (NERC) grant to the UK Population Biology Network, a NERC/Scottish Natural Heritage CASE studentship to H. H., and a NERC grant to T. Burke (NE/H001824/1). The funders played no role in the collection, analysis and interpretation of data or in the writing of the report.

Appendix A. Supplementary materials

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.biocon.2016.08.006.

References

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., Lobo, J., 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol. Ecol. 17, 5177–5188.
Angeloni, F., Ouborg, N.J., Leimu, R., 2011. Meta-analysis on the association of population size and life history with inbreeding depression in plants. Biol. Conserv. 144, 35–43.
Brown, A.H.D., Briggs, J.D., 1991. Sampling strategies for genetic variation in *ex situ* collections of endangered plant species. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, New York, pp. 99–119.
Brown, A.H.D., Marshall, D.R., 1995. In: Guarino, L., Rao, V.R., Reid, R. (Eds.), A Basic Sampling Strategy: Theory and Practice, in Collecting Plant Genetic Diversity: Technical Guidelines. Wallingford, CAB International.
Casper, S.J., Stimper, R., 2009. Chromosome numbers in *Pinguicula* (Lentibulariaceae): survey, atlas, and taxonomic conclusions. Plant Syst. Evol. 277, 21–60.
Castañeda-Álvarez, N.P., Khoury, C.K., Achicanoy, H.A., Bernau, V., Dempewolf, H., Eastwood, R.J., Guarino, L., Harker, R.H., Jarvis, A., Maxted, N., Müller, J.V., Ramirez-Villegas, J., Sosa, C.C., Struik, P.C., Vincent, H., Toll, J., 2016. Global conservation priorities for crop wild relatives. Nature Plants 2, 16022.
CBD, 1992. Convention on Biological Diversity. Janeiro, Rio de.
CBD, 2010. Global strategy for plant conservation: technical rationale, justification for updating and suggested milestones and indicators. Conference of the Parties to the Convention on Biological Diversity, Tenth Meeting. UNEP/CBD/COP, Nagoya, Japan.
Cheffings, C.M., Farrell, L., Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. (Eds.), 2005. The Vascular Plant Red Data List for Great Britain. Joint Nature Conservation Committee, Peterborough.
CPC, 1991. Genetic sampling guidelines for conservation collections of endangered plants. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, pp. 225–238.
Dulloo, M.E., Labokas, J., Iriondo, J.M., Maxted, N., Lane, A., Laguna, E., Jarvis, A., Kell, S.P., 2008. Genetic reserve location and design. In: Iriondo, J.M., Maxted, N., Dulloo, M.E. (Eds.), Conserving Plant Genetic Diversity in Protected Areas: Population Management of Crop Wild Relatives. CABI, Wallingford, Oxfordshire, pp. 23–64.
Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Syst. 24, 217–242.
Farnsworth, E.J., Klionsky, S., Brumback, W.E., Havens, K., 2006. A set of simple decision matrices for prioritizing collection of rare plant species for *ex situ* conservation. Biol. Conserv. 128, 1–12.
Frankham, R., 1996. Relationship of genetic variation to population size in wildlife. Conserv. Biol. 10, 1500–1508.
Frankham, R., Bradshaw, C.J.A., Brook, B.W., 2014. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol. Conserv. 170, 56–63.
Franklin, I.R., 1980. Evolutionary changes in small populations. In: Soulé, M.E., Wilcox, B.A. (Eds.), Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer, Sunderland, pp. 135–149.
Franklin, I.R., Allendorf, F.W., Jamieson, I.G., 2014. The 50/500 rule is still valid – reply to Frankham et al. Biol. Conserv. 176, 284–285.
Franks, S.J., Weber, J.J., Aitken, S.N., 2014. Evolutionary and plastic responses to climate change in terrestrial plant populations. Evol. Appl. 7, 123–139.
Godefroid, S., Rivière, S., Waldren, S., Boretos, N., Eastwood, R., Vanderborght, T., 2011. To what extent are threatened European plant species conserved in seed banks? Biol. Conserv. 144, 1494–1498.
Gregory, A., Burke, T., Ferris, R., Robson, J., Smithers, R., Whitlock, R., 2006. The conservation of genetic diversity: science and policy needs in a changing world. JNCC Report, No. 383. Joint Nature Conservation Committee, Peterborough, UK.
Guerrant, E.O., Fiedler, P.L., Havens, K., Maunder, M., 2004. Revised genetic sampling guidelines for conservation collections of rare and endangered plants. In: Guerrant, E.O., Havens, K., Maunder, M. (Eds.), *Ex Situ* Plant Conservation: Supporting Species Survival in the Wild. Island Press, Washington, USA, pp. 419–441.
Habel, J.C., Schmitt, T., 2012. The burden of genetic diversity. Biol. Conserv. 147, 270–274.
Hamilton, M.B., 1994. *Ex situ* conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks. Conserv. Biol. 8, 39–49.
Hamrick, J.L., Godt, M.J.W., Murawski, D.A., Loveless, M.D., 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, pp. 75–86.
Hedrick, P.W., 2005. Genetics of Populations. third ed. Jones and Bartlett, Boston.
IUCN, 2001. IUCN red list categories and criteria: version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.
Jamieson, I.G., Allendorf, F.W., 2012. How does the 50/500 rule apply to MVPs? Trends Ecol. Evol. 27, 578–584.
Keightley, P.D., Lynch, M., 2003. Toward a realistic model of mutations affecting fitness. Evolution 57, 683–685.
Kell, S., Maxted, N., Frese, L., Iriondo, J.M., Ford-Lloyd, B., Kristiansen, K., Katsiosis, A., Teeling, C., Branca, F., 2012. *In situ* conservation of crop wild relatives: a methodology for identifying priority genetic reserve sites. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M., de Carvalho, M.A.A.P. (Eds.), Agrobiodiversity

Conservation: Securing the Diversity of Crop Wild Relatives and Landraces. CABI Publishing, Wallingford, pp. 7–19.
Lauterbach, D., Burkart, M., Gemeinholzer, B., 2012. Rapid genetic differentiation between *ex situ* and their *in situ* source populations: an example of the endangered *Silene otites* (Caryophyllaceae). Bot. J. Linn. Soc. 168, 64–75.
Lawrence, M.J., Marshall, D.F., Davies, P., 1995. Genetics of genetic conservation. I. Sample size when collecting germplasm. Euphytica 84, 89–99.
Leimu, R., Fischer, M., 2008. A meta-analysis of local adaptation in plants. PLoS One 3, e4010.
Leinonen, T., O'Hara, R.B., Cano, J.M., Merilä, J., 2008. Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. J. Evol. Biol. 21, 1–17.
Loewe, L., Hill, W.G., 2010. The population genetics of mutations: good, bad and indifferent. Philos. Trans. R. Soc., B 365, 1153–1167.
Löve, A., Löve, D., 1975. The Spanish gentians. Anales del Instituto Botánico. 32, pp. 221–232.
Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3, 91–99.
Margules, C.R., Pressey, R.L., 2000. Systematic conservation planning. Nature 405, 243–253.
Margules, C.R., Nicholls, A.O., Pressey, R.L., 1988. Selecting networks of reserves to maximise biological diversity. Biol. Conserv. 43, 63–76.
Marshall, D.R., Brown, A.H.D., 1975. Optimum sampling strategies in genetic conservation. In: Frankel, O.H., Hawkes, J.H. (Eds.), Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge, UK, pp. 53–80.
Maunder, M., Byers, O., 2005. The IUCN Technical Guidelines on the Management of *Ex Situ* Populations for Conservation: reflecting major changes in the application of *ex situ* conservation. Oryx 39, 95–98.
Neel, M.C., Cummings, M.P., 2003a. Effectiveness of conservation targets in capturing genetic diversity. Conserv. Biol. 17, 219–229.
Neel, M.C., Cummings, M.P., 2003b. Genetic consequences of ecological reserve design guidelines: an empirical investigation. Conserv. Genet. 4, 427–439.
Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific diversity in plants. Mol. Ecol. 13, 1143–1155.
Palstra, F.P., Ruzzante, D.E., 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Mol. Ecol. 17, 3428–3447.
Piertney, S.B., Oliver, M.K., 2005. The evolutionary ecology of the major histocompatibility complex. Heredity 96, 7–21.
Prendergast, J.R., Quinn, R.M., Lawton, J.H., 1999. The gaps between theory and practice in selecting nature reserves. Conserv. Biol. 13, 484–492.
Preston, C.D., Pearman, D.A., Dines, T.D., 2002. New Atlas of the British and Irish Flora. Oxford University Press, Oxford.
R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Reed, D.H., Frankham, R., 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55, 1095–1103.
Savolainen, O., Lascoux, M., Merila, J., 2013. Ecological genomics of local adaptation. Nat. Rev. Genet. 14, 807–820.
Schoen, D.J., Brown, A.H.D., 2001. The conservation of wild plant species in seed banks. Bioscience 51, 960–966.
Shafer, A.B.A., Wolf, J.B.W., Alves, P.C., Bergström, L., Bruford, M.W., Brännström, I., Colling, G., Dalén, L., De Meester, L., Ekblom, R., Fawcett, K.D., Fior, S., Hajibabaei, M., Hill, J.A., Hoezel, A.R., Höglund, J., Jensen, E.L., Krause, J., Kristensen, T.N., Krützen, M., McKay, J.K., Norman, A.J., Ogden, R., Österling, E.M., Ouborg, N.J., Piccolo, J., Popović, D., Primmer, C.R., Reed, F.A., Roumet, M., Salmona, J., Schenekar, T., Schwartz, M.K., Segelbacher, G., Senn, H., Thaulow, J., Valtonen, M., Veale, A., Vergeer, P., Vijay, N., Vilà, C., Weissensteiner, M., Wennerström, L., Wheat, C.W., Zieliński, P., 2015. Genomics and the challenging translation into conservation practice. Trends Ecol. Evol. 30, 78–87.
Stace, C., 1997. New Flora of the British Isles. second ed. Cambridge University Press, Cambridge, UK.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407–4414.
Whitlock, R., Hipperson, H., Mannarelli, M., Burke, T., 2008a. A high-throughput protocol for extracting high-purity DNA from plants and animals. Mol. Ecol. Resour. 8, 736–741.
Whitlock, R., Hipperson, H., Mannarelli, M., Butlin, R.K., Burke, T., 2008b. An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. Mol. Ecol. Resour. 8, 725–735.
Willi, Y., Van Buskirk, J., Hoffmann, A.A., 2006. Limits to the adaptive potential of small populations. Annu. Rev. Ecol. Syst. 37, 433–458.
Zhivotovsky, L.A., 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Mol. Ecol. 8, 907–913.