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

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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.

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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.*