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Implementing a new approach to effective conservation of genetic diversity, with ash (*Fraxinus excelsior*) in the UK as a case study

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ABSTRACT

Gene conservation programs help safeguard species and tangibly benefit ecological restoration, agriculture, forestry, and horticulture. Here we describe a new method for deciding which and how many populations and individuals to conserve ex situ, and we demonstrate the method by evaluating collections of European Ash (*Fraxinus excelsior*) for an ongoing seed-banking project, the UK National Tree Seed Project (NTSP). The method uses simulations and geographic distribution data, and does not require (but can utilize) genetic data. We estimate that NTSP collections have captured > 90% of all alleles and of locally common alleles. We identified optimal sampling solutions at large and small spatial scales, and for northern isolated vs. southern core populations. We also quantified genetic "points of diminishing returns" with a more precise method than previous studies. This analysis revealed that (for European ash, for a goal of capturing one copy of each allele) an optimal "stopping point" is approximately 35 populations, 10 to 30 trees per population, and 30 seeds per tree. Overall, we conclude that the NTSP protocol of random sampling of at least 15 trees per population from two populations per seed zone is effective. We demonstrated how collectors can adjust the number of populations, individuals and seeds sampled using the concept of "genetic equivalence", allowing projects to accommodate practical or ecological constraints. Lastly we showed that for a conservation goal of 50 allele copies rather than one copy, a much larger sampling effort is needed (> 150 populations). This new approach can be tailored to any species. It is applicable to any seed collection seeking to capture genetic diversity, as well as in situ gene conservation approaches. We emphasize that the ability to quantitatively estimate the outcome of gene conservation activities can help design, justify, or evaluate future programs.

1. Introduction

A challenge for biodiversity conservation practitioners is to be both effective and efficient- to devise and implement plans that have a high probability of achieving defined outcomes within the constraints of limited resources. Computational modeling, informed by real data and knowledge of natural history, can help predict whether a conservation action is likely to be successful and help choose among possible options. For example, for several decades, population viability analysis has been performed using modeling approaches to help plan optimal conservation actions like supplementation (Boyce, 1992; Hoban, 2014), while conservation planning has been used to design optimal reserve networks (Carwardine et al., 2009). However, methodologies for ensuring effective conservation of a species' evolutionary potential (i.e. genetic diversity, the variability and distribution of genes) are relatively undeveloped in spite of the known importance of genetic variation for species, ecosystems, and human society (Brown and Marshall, 1995, Klein et al., 2009, though see Richards et al., 2007, Schlottfeldt et al., 2015).

Genetic diversity is important because it allows species to adapt and survive under changing conditions, and provides resilience to stresses such as drought, pests and diseases. Genetic variation in foundation species, including trees, can stabilize ecosystems during environmental fluctuations (Hughes et al., 2008). Where genetic diversity is lost, problems such as inbreeding may occur. Furthermore, genetic diversity underlies traits which are valued by human societies such as timber quality or disease resistance. Genetic diversity is therefore vital for artificial selection or breeding of plants. The need to conserve genetic resources is well recognized in the Convention on Biological Diversity (e.g. Aichi Target 13, https://www.cbd.int/sp/targets/). It is

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particularly vital to understand, conserve, and make accessible forest genetic resources, as recognized in international commitments like the Global Plan of Action on Forest Genetic Resources (FAO, 2014), the Montreal Process on sustainable forestry (www.montrealprocess.org/), the Global Strategy for Plant Conservation (https://www.cbd.int/gspc/), and a 2016 IUCN Resolution on autochtonous forest genetic diversity (https://portals.iucn.org/library/node/46521).

Ex situ conservation (such as collecting seeds or plant parts for long-term storage and eventual propagation) is an important component of strategies to conserve genetic diversity because in situ populations experience a wide range of threats such as habitat change, over-exploitation, climate change, invasive species and new pests (Cochrane et al., 2007; Li and Pritchard, 2009; Cavender et al., 2015). Well-planned seed bank collections play a vital complementary role to in situ conservation by: preventing extinctions, providing seeds for species or habitat recovery, and providing accessible, well-documented germplasm for research and experimentation. Seed banks often prioritize the conservation of rare and threatened species, species required for habitat restoration, or economically important crops and their relatives. It is particularly urgent to bank seed of forestry species given their economic value, the ecological value of woodland habitats, and the significant risk of catastrophic loss to disease or pests (FAO, 2014).

The Millennium Seed Bank Partnership (MSBP), led by the Royal Botanic Gardens, Kew is the largest ex situ conservation project in the world. The MSBP comprises a range of national and international seed collecting projects that emphasize taxonomically verified, genetically representative, geolocated seed of high quality and quantity, obtained with full consent and associated data (Liu et al., 2018). The MSBP UK National Tree Seed Project (NTSP), initiated in 2013, focuses on capturing infra-specific genetic diversity of target species within a politically-defined geographic area. UK trees and woodlands are threatened by an increasing number of exotic, problematic pests and pathogens, in addition to environmental uncertainties such as climate change. Many woodlands are small and isolated and have been modified or managed for centuries. In this context, the NTSP was developed to conserve the genetic diversity of UK tree species and facilitate their future survival. The project seeks to make genetically representative collections of UK native trees and shrubs, and to maintain these collections as a long term conservation strategy, but also to make them accessible for conservation and research activities. Collecting seed from a large number of mother trees, spread across a range of biogeographic zones, is expected to capture a high proportion of the genetic diversity present in the UK. A coordinated network (over 30 partner organizations, comprising over 600 professionals and citizen science volunteers) made collections from 2013 to 2017 (Trivedi and Kallow, 2017; Kallow and Trivedi, 2017; Trivedi, 2016).

The NTSP provides a useful case study for common challenges to capture genetic diversity, whether for long-term conservation or more immediate use. Ex situ collections usually aim to best represent the genetic variation of the targeted population or species in order to be of the highest value to researchers or conservationists, though some collections target specific phenotypes or traits such as disease resistance rather than broad representation (such as whitebark pine in the USA, Mahalovich and Dickerson, 2004). Genetically diverse collections will contribute to greater reintroduction success and provide a wide base for breeding or selection (Brown and Marshall, 1995; Guerrant Jr et al., 2004).

Genetically-based variation in form, phenology, pathogen resistance, environmental tolerance, and other traits are distributed heterogeneously among regions, populations and individuals. Thus a careful collection plan is needed that includes: where to collect, from how many individuals and/or populations to collect, and how much seed to collect. Existing guidelines (summarized in Hoban and Strand, 2015) are based on simple mathematical equations (i.e. 50 individuals per population) first developed in the 1970s (Marshall and Brown, 1975; Brown and Marshall, 1995; Lawrence et al., 1995; Lockwood et al., 2007), or best guesses based on intuition (50 populations, Brown and Hardner, 2000, or 5 populations, Falk and Holsinger, 1991). A further recommendation is to select populations throughout the species' range, because range edge populations could have unique genetic variation (Lesica and Allendorf, 1995). Some recommendations also include practical considerations, for example, to collect 10 times as much seed as the anticipated need, to allow for duplication of the collection for safeguarding, use of the collection for research, germination testing over time, sharing with other users, and other losses (Way, 2003). The commonness of a species and the purpose of the ex situ collection are also used to determine the collection plan (Guerrant Jr. et al., 2013). For common species it is useful to have a larger collection (i.e. to use for restoration, research or distribution). Ideal recommendations must also be adjusted to practical limitations, such as when a population does not have 50 fruiting individuals at the time of collection, or when species are locally uncommon.

Ideally seed collections should be tailored to species' traits, their geographic distribution, and the planned end use of the seed, but quantitative guidance (e.g., numbers of populations, trees or seeds) is lacking (Falk and Holsinger, 1991; Guerrant Jr et al., 2004; Guerrant Jr. et al., 2013, Hoban et al., 2015). Evidence for the benefits of different sampling strategies is sparse and underdeveloped; as a consequence, most collecting protocols follow the "50 individuals per population minimum" but show wide variation in total number of seed, spatial sampling advice, and other considerations (Hoban and Strand, 2015). Therefore, conservation collections may not be effectively or efficiently conserving genetic variation.

Recent progress has been made on developing two main quantitative approaches to designing seed collections for a target species (or evaluating the genetic diversity of seed collections which are already made): genetic surveys and simulation studies. (1) A genetic survey uses DNA markers to assess genetic diversity across a species' range. Computer algorithms are used to subsample this dataset to "try out" different sampling strategies and produce very specific advice for a target species (Griffith et al., 2014, 2017; Mcglaughlin et al., 2015). This approach has the downside of requiring thorough collections of DNA from tissue samples across the species' range, in order to quantify existing in situ genetic diversity. This is not feasible for common species and is challenging and expensive even for rare species. (2) Mathematical or simulation models based on a species' traits such as dispersal characteristics or population structure can be used to produce broad guidelines for species having a given trait (Hoban and Strand, 2015; Kashimshetty et al., 2017) and to identify general principles (Hoban and Schlarbaum, 2014). The downside of this approach is that models used so far have been simple and the guidelines are not species-specific. Both approaches have been applied to relatively few cases.

We demonstrate here a new approach for designing seed collection strategies for target species, which combines these two approaches. It uses demographic-genetic models tailored to the target species: a model at fine-scale including a realistic simulation of the species' dispersal biology and a model at large scale including realistic spatial distribution of populations. The models are used to produce a synthetic estimate of a species' extant genetic diversity, and then to test different sampling strategies in order to identify effective strategies. The model can be checked against real genetic data from previous studies. We demonstrate that this approach can be used to produce sampling guidelines for a target species, without requiring an intensive genetic survey covering the whole range. This approach therefore solves the disadvantages of the two approaches mentioned above (expense and lack of specificity).

We focus our new approach on the case of European ash (*Fraxinus excelsior*), a common tree across Europe that contributes important ecosystem functions (Mitchell et al., 2014). The effective ex situ conservation of UK ash genetic diversity is a high priority for the NTSP because ash is the second most common native hardwood tree (126 million ash trees in the UK, making up 14% of forest standing volume) but is threatened by the devastating disease ash dieback (caused by a