ON THE NATURE OF THINGS: ESSAYS

New Ideas and Directions in Botany

What to do when we can't bank on seeds: What botanic gardens can learn from the zoo community about conserving plants in living collections[1]

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Species loss due to human activities is occurring at an unprecedented pace (Barnosky et al., 2011), and over the last few decades, botanic gardens have responded by expanding their conservation activities (CBD, 2010). Botanic gardens play a particularly critical role in the development of ex situ (off-site) collections of threatened plant species, and the botanic garden community has helped develop current best practices for ex situ conservation and reintroduction (e.g., via the Center for Plant Conservation; Guerrant et al., 2004). The majority of plant species are amenable to seed banking or at least vegetative propagation (horticultural or tissue culture), which allows them to be preserved for decades or even centuries before regeneration is necessary and before genetic and demographic viability become an issue (Havens et al., 2004). Consequently, much of the research geared to support ex situ plant conservation has focused on understanding how to efficiently capture wild genetic diversity to enable successful future reintroduction efforts (Guerrant et al., 2004). However for "exceptional" plant species that either do not produce seeds or produce seeds that are recalcitrant (i.e., desiccation intolerant so they cannot be dried and frozen), maintaining demographic viability and genetic diversity ex situ can be particularly challenging (Pence, 2014). To date, less effort has been dedicated to ensuring that these living collections, once brought into ex situ cultivation, remain genetically diverse and demographically viable over the long term to support reintroduction efforts (but see Havens et al., 2004).

For many exceptional species, living plant collections are the only currently available ex situ conservation option, and the maintenance of these living collections introduces numerous genetic and demographic challenges associated with small, isolated populations. If not curated correctly, these small populations are subject to founder effects, genetic drift, and inbreeding, and can experience selective pressure from biotic and abiotic conditions in the ex situ environment. These factors could compromise future reintroduction efforts and ultimately lead to loss of the species from ex situ collections altogether. In the future, cryopreservation and/or tissue culture may be viable ex situ approaches for exceptional species, thus minimizing immediate concerns about genetic and demographic losses. However, the techniques required for these alternative germplasm conservation approaches are often species-specific and currently unavailable for many threatened species (Pence, 2014). In addition, capacity and resources to develop and maintain cryopreservation protocols is limited or lacking in many regions. Until research and resources reach a point where all exceptional species can be cryopreserved, living collection management will continue to be critical.

Despite the value of ex situ collections, some threatened plant species and valuable genetic resources have already been lost from botanical collections (Govaerts, 2010). Of 844 plant taxa identified as extinct in the wild in 2010, 9% were curated in collections, while another 5% had been in collections but subsequently lost (Govaerts, 2010). While the cause of these losses was not reported, it is likely that at least some were the result of genetic or demographic collapse. It is clear that an integrated and more collaborative approach is needed to effectively conserve threatened species ex situ. For example, *Brighamia insignis* (Campanulaceae), an endemic Hawaiian succulent species, is functionally extinct in the wild with only one remaining extant individual. It is cultivated ex situ in at least 57 botanical collections around the world, but in need of an integrated management plan. This species can be seed banked, but seeds lose

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viability within a decade, and plants are relatively short-lived (10–20 yr), necessitating the continual production of new plants and seeds. Currently, management efforts have focused on propagating individuals rather than on maintaining genetic diversity. This situation is particularly concerning because recent research showed that genetic diversity of *B. insignis* is not equally distributed among ex situ collections (Walsh, 2015). Given these results, and reports of poor seed set, increasing gene flow among ex situ collections was recommended. Unfortunately, there is no system in place to identify appropriate pollen donors, facilitate inter-institutional crosses, or plan for how seed produced should be distributed to improve the genetic and demographic prospects of this species globally. This approach is clearly not a sustainable solution to managing the thousands of threatened exceptional plant species (Pence, 2013) held in ex situ plant collections around the world.

These challenges closely parallel those faced by the zoological community for maintaining living collections of captive animal populations (Lees and Wilcken, 2009). They have developed tools and protocols to minimize the risk of genetic and demographic decline across the entire ex situ population (Ballou et al., 2010) and are beginning to incorporate cryopreserved gamete and embryo banks (e.g., for whooping cranes, black-footed ferrets, and giant pandas) in these approaches. Achieving these genetic and demographic management goals requires the maintenance of multi-institutional studbooks that track pedigrees of all captive individuals while enabling cooperatively managed breeding programs to make scientifically informed breeding decisions (Lacy, 1994). This includes equalizing contributions from unique genetic lines (or founders) to maximize the retention of genetic diversity, which is beneficial for ex situ population health and robustness of future reintroductions (Lacy, 2013). The zoological community used this approach for the black-footed ferret (*Mustela nigripes*), which was thought extinct until in 1987 when 18 individuals were found in the wild and brought into captivity (Shoemaker et al., 2014). To manage this captive population, the Association of Zoos and Aquariums (AZA) used an electronic studbook and population management software (e.g., PMx; Lacy et al., 2012) to guide the distribution of individuals to multiple breeding facilities to minimize inbreeding and maximize retention of genetic diversity. As of 2013, more than 3500 black-footed ferrets have been reintroduced into the wild (Shoemaker et al., 2014). This example provides an effective model to manage ex situ collections for ultimate reintroduction that could be emulated by many botanic gardens for threatened plant species.

As new technology, data, and challenges arise, metapopulation management approaches and tools, like those used for the black-footed ferret, are under continual evolution (Lacy, 2012, 2013), and many are transferable or adaptable to the management of ex situ plant collections (Price et al., 2004). Although versions of these approaches have been used for some agronomically important crops including the maintenance of genetically diverse germplasm for species with recalcitrant seeds such as coconuts (Reed et al., 2004), there are few, if any, examples of ex situ metapopulation management being applied to threatened plants. This is, in part, because few of the many hundreds of rare plant reintroductions have required ex situ seed increase because adequate seed has been available in the wild. As plants become rarer in the wild and as restoration efforts are scaled up, there will be greater congruence with methodologies employed in other disciplines. Here we outline seven steps that illustrate how the botanical community can build from the approaches developed by the zoological community to maintain ex situ genetic diversity of threatened plant species and directly support in situ conservation, particularly for exceptional species currently managed as living plant collections rather than as banked seed.

Step 1: Identify which plant species will most benefit from the development of cooperatively managed breeding programs (including working with cooperators to understand in situ threats, reintroduction potential, and ex situ needs). Individual institutions can contribute to this step by nominating candidate species. Botanic garden networks, such as Botanic Gardens Conservation International (BGCI), can compile information on threatened species that fit these criteria to provide guidance.

Step 2: Establish a sponsor institution(s) with the capacity to build and manage a multi-institution ex situ collection for a species. Ideally, the sponsor institution would be within the range of the target species (to facilitate the use of ex situ collections to support in situ conservation efforts) and have on-site expertise in propagating the particular plant species.

Step 3: Inventory ex situ collections via the BGCI PlantSearch database, including any material reported in tissue culture or cryopreserved collections as well as living plant collections, to identify all institutions with accessions of the species and gather details to assess the potential genetic diversity of the entire multisite collection. This information will include the original source of the material and any other institutions it has been shared with, its pedigree, number of generations in cultivation, and when possible, genetic data. Information on the health and reproductive status of each individual in collections will also be necessary. The sponsoring institution will need to compile this data into a database similar to the Zoological Information Management System (ZIMS: Species360 [formerly International Species Information System], Bloomington, Minnesota, USA) or PopLink (Faust et al., 2012) programs. BGCI is currently working on an extension of PlantSearch to support this function (Smith, 2016).

Step 4: If populations remain in the wild, work with landowners and managers to conduct a gap analysis to determine how effectively genetic diversity in ex situ collections represents remaining in situ populations. These efforts should be coordinated by the sponsor institution(s), working in partnership with other collaborators as needed.

Step 5: Coordinate the acquisition of new wild material and the distribution of that material to ex situ collections. At this stage, the best unit of conservation should also be identified (e.g., ecotypes, varieties, subspecies, species), taking into account taxonomic uncertainty (e.g., ploidy, cryptic species). While zoos almost always manage all captive animals as a single population within each region of the world or even globally, there are many reasons why it might be appropriate for botanic gardens to manage groups/populations separately (e.g., if there is significant ecotypic variation within the species).

Step 6: Develop and implement an ex situ conservation management plan for the species. It may be possible to hold and maintain the majority of genetic diversity for small herbaceous species at one institution (e.g., *B. insignis* at the National Tropical Botanical

Garden), and distribute material to other institutions as backups. Alternatively, a metapopulation management approach will be needed for large, long-lived species with recalcitrant seeds (e.g., oak trees [*Quercus* spp.]). Genetic and demographic management software such as PMx will be required to determine ideal breeding and allocation plans. As ex situ management proceeds, the sponsor and all participating institutions will need to ensure updates of demographic and/or genetic changes are made to their accession information and be able to provide material for breeding among institutions as needed.

Step 7: Work with collaborators to use ex situ material to support in situ conservation efforts. This support may include providing germplasm for reintroduction efforts, collecting, and sharing data from ex situ collections to address in situ threats, and working with land managers to overcome genetic or demographic issues in situ based on what has been learned from maintaining plants ex situ.

In summary, there are strikingly similar genetic management needs for plants and animals in ex situ conservation collections. To date, ex situ conservation programs in the botanic garden community have concentrated largely on banking of seeds and pollen, while the zoo community has focused largely on living collections of animals. We have much to learn from one another; zoos could adapt botanic garden approaches for their embryo and gamete banking programs, and botanic gardens could adapt zoo-developed tools and practices to better manage their living collections of threatened, exceptional species. By integrating management of breeding programs with in situ conservation efforts from the start, the botanic garden community may be able to improve upon the experience of zoos. We are currently testing this approach with *Brighamia insignis* and *Quercus oglethorpensis*, with the goal of developing a system that could be applied much more broadly within the botanic garden community.

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