



Center for Plant Conservation's Best Practice Guidelines for the reintroduction of rare plants



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ABSTRACT

Recent estimates indicate that one-fifth of botanical species worldwide are considered at risk of becoming extinct in the wild. One available strategy for conserving many rare plant species is reintroduction, which holds much promise especially when carefully planned by following guidelines and when monitored long-term. We review the *Center for Plant Conservation Best Reintroduction Practice Guidelines* and highlight important components for planning plant reintroductions. Before attempting reintroductions practitioners should justify them, should consider alternative conservation strategies, understand threats, and ensure that these threats are absent from any recipient site. Planning a reintroduction requires considering legal and logistic parameters as well as target species and recipient site attributes. Carefully selecting the genetic composition of founders, founder population size, and recipient site will influence establishment and population growth. Whenever possible practitioners should conduct reintroductions as experiments and publish results. To document whether populations are sustainable will require long-term monitoring for decades, therefore planning an appropriate monitoring technique for the taxon must consider current and future needs. Botanical gardens can play a leading role in developing the science and practice of plant reintroduction.

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1. Introduction

Recent estimates indicate that one-fifth of botanical species worldwide are considered at risk of becoming extinct in the wild (Pimm and Raven, 2017). Given that the emerging impacts of climate change forebode unprecedented risk and rates of endangerment, traditional conservation measures (e.g., habitat protection and management) alone will not likely be able to prevent species extinction. Touted worldwide as a valuable conservation strategy, in the next century plant reintroduction will certainly be a solution to stabilize and restore declining plant populations that face global change (Kennedy et al., 2012). As repositories of rare plant germplasm and horticultural expertise, botanical gardens can play a vital role in the reintroduction and recovery of threatened plant species in the wild (Guerrant et al., 2004).

The process of plant reintroduction combines the art and science of horticulture, ecology, and evolution. Although early efforts

to reintroduce plants to the wild often suffered from failure (Falk et al., 1996), more recently practitioners have refined the practice of reintroduction and have shared their experiences of success and failure for the sake of improving future practice (Maschinski and Haskins, 2012). Founded in 1984, the Center for Plant Conservation (CPC) is a consortium of over 40 botanical gardens and conservation partners in the United States that are dedicated to safeguarding imperiled native plants from extinction. Collectively our participating institutions hold over one-third of the globally rare plant species in North America in seed banks and garden collections; we advance science-based best practices and share these around the world to advocate for plants and their value for humankind (www.saveplants.org). Our early guidelines provided a foundation for making genetically diverse conservation collections of rare plant species in a manner that does not harm wild populations (CPC, 1991). These informed the next series of guidelines for reintroduction planning and implementation (Falk et al., 1996). Because ex situ collections in botanical gardens and other institutions provide critical support to species survival in the wild, including a majority of plant material used in rare plant reintroductions around the world (Dalrymple et al., 2012), we provided

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updated guidelines for genetic sampling, seed storage, and ex situ collection management of rare plants (Guerrant et al., 2004). Working with botanical gardens and conservation practitioners within and beyond our network, the CPC reviewed the process of reintroducing rare plants to the wild and developed an updated *Center for Plant Conservation Best Reintroduction Practice Guidelines* (Maschinski et al., 2012a).

The intention of plant reintroduction is to ensure the conservation of a unique species in a natural context where it can undergo evolution within its historical indigenous range. The *Center for Plant Conservation Best Reintroduction Practice Guidelines* built upon previous work of other plant conservation colleagues and other published guidelines (Falk et al., 1996; IUCN, 1998; SER, 2002; Vallee et al., 2004; Maschinski and Haskins, 2012.) Our refined guidelines considered and incorporated comprehensive literature reviews about key topics in plant reintroduction that were conducted by authors of chapters in *Plant Reintroduction in a Changing Climate: Promises and Perils* (Albrecht and Maschinski, 2012; Dalrymple et al., 2012; Guerrant, 2012; Neale, 2012; Maschinski et al., 2012b), and peer-review comments from CPC conservation officers, who have conducted over 100 reintroductions over the past 30 years. A majority of these reintroductions use propagules (seeds & whole plants) that have been wild-collected, maintained in ex situ collections, and then propagated at botanical gardens using CPC's best-practice genetic and reintroduction guidelines (Maschinski and Haskins, 2012).

2. The Center for Plant Conservation Best Reintroduction Practice Guidelines

The *Center for Plant Conservation Best Reintroduction Practice Guidelines* suggest following a logical framework when considering a reintroduction. The framework's six components – justification, preparation, public involvement, implementation, aftercare, and monitoring – cover all aspects of a reintroduction program and are generalizable to most plant species, geographic locations, and ecosystems. Here our goal is to highlight key components of the guidelines and use case studies to emphasize important concepts, drawing primarily on work conducted within the CPC network. For a comprehensive review of the international reintroduction literature and for the complete *Center for Plant Conservation Best Reintroduction Practice Guidelines*, we refer readers to Maschinski and Haskins (2012).

2.1. Review species status and threats to justify the reintroduction

Before committing to the time, expense and labor of a reintroduction, it is important to verify that it is justified. A reintroduction may be justified if the species is extinct in the wild or its distribution is known and there are few, small, declining populations. It is also important to consider alternative conservation strategies, to know what is threatening the species, and to ensure that these threats are absent from any recipient site. Reintroduction is never the first step or only recovery action for an endangered species, but rather is integrated wholly with ex situ conservation (Guerrant et al., 2004) and other in situ conservation measures (e.g., natural area protection and management and threat abatement).

Also important is evaluating when reintroduction should be avoided. A reintroduction is *not* justified if any of the following conditions exist: 1) It will undermine the imperative to protect existing sites; 2) Previous tests indicate that it is infeasible to propagate plants or germinate seeds; 3) High quality diverse source material is not available; 4) Existing threats have not been minimized or managed; 5) The reintroduced species or its management has potential to impact other species at the recipient site

negatively; 6) There is a possibility of collateral impact via competition, hybridization, or invasion; 7) There is evidence that the reintroduced taxon would harm other threatened and endangered species or conflict with their management; 8) The reintroduction is not legally, administratively or socially supported; or 9) Suitable habitat is not available or is not understood.

It is important to use this justification framework for traditional as well as conservation introductions. Recognizing that some species have threats within their range that may cause extinction, existing reintroduction guidelines condone conservation introductions only if there are no viable sites within a species' range (Maunder, 1992; Falk et al., 1996; IUCN, 2013; Vallee et al., 2004). Conservation introductions outside of a species' known range have been called managed relocation, assisted migration and assisted colonization (Haskins and Keel, 2012; IUCN, 2013). The major objections levied against them have been the ecological risks of relocated species becoming invasive or introducing new pathogens into the recipient community (Ricciardi and Simberloff, 2009; but see Schlaepfer et al., 2009; Reichard et al., 2012). In a review of conservation options for endemic plants and animals from the Florida Keys, an archipelago that is gravely threatened by sea level rise, the authors considered the proposition of conservation introductions and found that the greatest obstacle was the threat of hybridization with congeners in any nearby suitable habitat (Maschinski et al., 2011).

2.2. Develop a reintroduction plan. Whenever possible design the reintroduction as an experiment and seek peer review

The reintroduction plan should consider the legal, logistic and land management issues related to the recipient site and the target species. A series of questions provide guidance for each aspect of the planning process. The questions help practitioners consider alternatives, apply best practices, and keep all stakeholders informed. Because the intention of a reintroduction is to create a self-sustaining population, which is a process that may take decades, there must be consideration of long-term consequences of the reintroduction including the cost of monitoring and land management required to sustain the habitat. Failure to account for these fundamental tenets can make the difference between a failed or thriving reintroduction.

Processes operating at large and small scales can influence the reintroduction plan. At large scales community and ecosystem level disturbance regimes should be known and understood. Are you expecting floods or fire to sweep through your recipient site? What steps can you take to enhance the survival of the reintroduced population even with large-scale or frequent disturbance? At smaller scales, do you understand how competition with other plants or abiotic conditions at the recipient site will influence your species? How well do you understand the existing and potential threats?

Reintroductions, when conducted as experiments, can fill gaps in knowledge about the species' biology or ecology and contribute to the science (Seddon et al., 2007; Abeli and Dixon, 2016; Maschinski and Quintana-Ascencio, 2016). The more that is known about the species' habitat requirements and biology, the greater the probability of success. Researching aspects of species' biology can help guide choices for genetic composition of the reintroduction (Maschinski et al., 2013), spacing individuals to maximize pollen exchange (e.g., Pinto-Torres and Koptur, 2009), or attributes of the recipient site that can optimize plant growth, survival, and reproduction of different life stages (Wendelberger and Maschinski, 2016). They may also contribute to understanding of community as a whole (Lindenmayer et al., 2007; Naem, 2016).

Reintroduction plans can build upon existing information or they can utilize what is unknown as a springboard for an experimental design. Sample questions encompassing horticultural and demographic aspects include: For your taxon, is there an advantage to using large container plants versus seeds? What survival rates can you anticipate for using one versus the other? What kind of aftercare will be needed and for how long (Guerrant, 2012; Fenu et al., 2016)? What microsite is needed for best growth and recruitment (Maschinski et al., 2012b)?

To test how microhabitat, fire, and propagule type affected demographic performance of the U.S. endangered shrub, *Pseudoziziphus celata*, Menges et al. (2016) used experimental introductions. They found that transplants out-performed seeds, shaded sites supported higher survival and seed germination than open sites, and they showed that the species has broader microhabitat preferences than they had hypothesized.

Reintroduction plans should also account for a species life-history in the experimental design and when setting success benchmarks. For example, reintroductions of the root-holoparasite, *Dactylanthus taylorii*, to four sites in New Zealand revealed that sowing method, canopy type, and the dominant host influenced establishment and flowering (Holzapfel et al., 2016). Because flowering maturation required four years, different conclusions could have been drawn if the reintroduction had not been tracked for a decade and accounted for this species' growth rates.

Reintroduction science has expanded to a point where we can use reintroductions to test ecological or evolutionary theory (Seddon et al., 2007; Abeli and Dixon, 2016). Sample questions include: How does breeding history influence population persistence (Maschinski et al., 2013)? What will the genetic composition of your founding population be and how many individuals will you use (Albrecht and Maschinski, 2012; Maschinski et al., 2013)? Does proximity to extant populations influence reintroduced population persistence (Hanski, 1998)? How important is lack of genetic variation for adaptation and establishment of small populations (Koko et al., 2017).

2.3. Ascertain whether genetic studies are needed before conducting the reintroduction and if possible, conduct studies to measure genetic structure of the focal species

Genetic information is essential before doing a reintroduction if any of the following conditions exist: 1) The population has less than 50 individuals setting fruit; 2) Populations are highly fragmented and isolated; 3) There are no pollinators present; 4) No viable seed set is occurring; 5) High herbivory is occurring; 6) Plants have different morphology in different locales; 7) Some populations have distinct ecology; 8) The species is difficult to distinguish from a congener; 9) The species has unclear taxonomy or 10) There is fear of hybridization at the recipient site.

Knowing the genetic structure of the target species' populations will help guide decisions about the appropriate source material to use. Ideally the genetic composition of a reintroduced population should be as diverse as possible while representing the local gene pool. Using genetically heterogeneous founders will improve the ability of plants to cope with varying environments (Colas et al., 2008; Falk et al., 1996; Guerrant et al., 2004; Neale, 2012). Conventional practice has been to maintain genetically distinct populations separate for reintroductions. If genetic analyses reveal that populations are similar genetically, then it is safe to mix populations. For example, genetic analysis of eight known US endangered *Jacquemontia reclinata* populations revealed that seven of populations were similar, while one from a site with unique ecology was distinct (Thornton et al., 2008). The two largest populations were not genetically differentiated, therefore mixing these

in a breeding study was possible. Using plants generated from controlled crosses that ranged from selfed to mixed-population crosses, authors tracked the demographic consequences of breeding history on reintroduced population success and found that survival and next generation recruitment was significantly greater for the mixed-population cross group (Maschinski et al., 2013). In another example, genetic analysis revealed that the most appropriate male plants to use as suitors for Italian female populations of the dioecious aquatic, *Stratiotes aloides*, were not the most geographically proximal, but were from the more distant Rhine basin (Orsenigo et al., 2017). Thus, pre-reintroduction genetic studies illuminated the genetic consequences of significant long-distance seed dispersal in this species.

When genetic information is unavailable, we recommend noting source population performance and size and following the decision tree recommended by Frankham et al. (2011). When founding populations numbers are declining and/or are very small (<100 individuals), they may suffer from inbreeding depression and may not be the most suitable source for reintroductions (Armbruster and Reed, 2005). Seed size and germination behavior may provide additional information about the genetic health of a source population (Godefroid et al., 2016). Experimentally mixing populations with similar ecogeography can increase genetic diversity and may increase the likelihood of population persistence in a changing climate (Broadhurst et al., 2008).

2.4. Select appropriate source material

Guidelines recommend choosing source material from a location that has similar climatic and environmental conditions to the reintroduction site. This recommendation is supported by studies that show plant community and habitat type matching of the source material to the reintroduction site is often more important than other factors (e.g., geographic distance) for long-term survival of rare plant reintroductions (Noël et al., 2011). In the CPC network, many reintroductions rely upon source material that is collected from wild populations and then stored and grown in botanical gardens. Although genetic issues can arise in ex situ settings that affect long-term viability, Dalrymple et al. (2012) found similar survival rates when comparing reintroduced individuals derived from ex situ sourced material to those sourced directly from wild populations.

When using plants that are derived from ex situ collections, several steps can be taken to minimize artificial selection and other genetic complications. Maintaining even family line representation in cultivation can reduce selection risk and maintain genetically diverse material. To accomplish this, CPC guidelines recommend that when making seed collections, maintain maternal lines in separate envelopes to allow the tracking of maternal line accessions carefully over time. When cultivating material, horticulturists may have to resist the temptation of favoring propagation of the most vigorous plants.

2.5. For long-lived species, reintroduce plants of varying sizes and life-stages to account for variable success of stages in different microsites

Using diverse founders will improve the probability of survival for several reasons. Context-specific factors within the recipient site play a key role in determining whether the reintroduced population will persist and these may be subtle and changing over time. For example, not unlike other species, the light conditions supporting the highest densities of recruited seedlings of Florida state endangered *Tephrosia angustissima* var. *corallicola* distinctly differed from the light conditions supporting whole adult plants

introduced to a South Florida preserve (Wendelberger and Maschinski, 2016). Introducing different aged plants can capture opportunities for growth in all light conditions present at the recipient site.

Using diverse age stages can also play an important role in reducing extinction risk. For example, prior to our reintroduction, the long-lived Florida state endangered palm, *Pseudophoenix sargentii*, had few live individuals. Although the palm can achieve reproductive maturity in cultivation within 14 years, a seed collected in 1982, cultivated at Fairchild Tropical Botanic Garden and reintroduced to the wild in 1992 required an additional 25 years to produce flowers and fruit in the wild (Lange et al., 2017). By introducing the largest plants we could feasibly manage, we improved the age structure of the population and increased the number of plants that may be able to gain reproductive maturity in a shorter timeframe than the majority of the plants growing naturally in the wild (Maschinski and Duquesnel, 2007).

2.6. Choose a suitable recipient site

There is mounting evidence from reintroduction studies that an appropriate recipient site can make the difference between reintroduced population persistence or extinction (Dalrymple et al., 2012; Maschinski et al., 2012b). Most desired reintroduction sites have biological and physical features (community composition, topography, soil type, climate, etc.) similar to those of extant populations and are protected areas within the species' historic range. We recommend conducting a recipient site assessment that allows one to compare potential recipient sites quantitatively (Maschinski et al., 2012b). An important caveat is that threats must be alleviated or eliminated prior to introduction. Using the extant population site conditions as a reference is valid only if the population is not declining there (Knight, 2012).

2.7. Use at least 50 plants for a reintroduction

Evidence from a review of over 174 plant reintroductions supports population dynamic theory that large populations, founded with greater than 50 whole plants, will have greater probability of survival than small populations founded with less than 50 whole plants (Albrecht and Maschinski, 2012). Whenever possible, Guerrant (1996) advised to use as large a founding population as is practical to overcome the demographic and genetic constraints associated with small population size. For example, in reintroduced populations of the perennial herb, *Leucojum aestivum*, Abeli et al. (2015) observed greater seed production and seedling recruitment over a four-year period when initial planting densities were high relative to when they were low. Extremely rare species may require managed breeding and extensive propagation to prepare for eventual reintroduction. Robichaux et al. (2017) outplanted 10,212 Ka'u silversword seedlings (*Argyroxiphium kauense*) between 2004 and 2009 and monitored survival and growth for a decade. By 2014, 5894 plants were alive and 46 plants had flowered.

If using seeds, far more will be required as researchers should realize that germination rates of 1% are not uncommon. For an extremely rare species or one that has low seed set, bulking seeds and propagating plants to build significant numbers in a nursery prior to reintroduction is advised.

2.8. Use good horticultural practice

Prior to and while conducting the reintroduction, it is necessary to use good horticultural practice. Making sure that plants are healthy, acclimated, and are not carrying weeds, pests, or pathogens to the recipient site is critical. While planting at the site,

ensuring proper spacing for root growth and adequate water are important to give transplants the greatest probability of survival. Be sure that all participants who will plant your species are trained and supervised to follow the protocols necessary for plant health (Maschinski et al., 2012a,c).

2.9. Develop a monitoring plan

Our reviews of plant reintroductions revealed a disturbing yet understandable fact. Most reintroductions are monitored for less than 5 years, yet may require decades to evaluate whether the reintroduced population is sustainable (Albrecht et al., 2011; Dalrymple et al., 2012). Knowing that reintroductions are a long-term commitment makes it extremely important to develop a monitoring plan that is feasible, practical, and appropriate for the life cycle of the reintroduced taxon. It may not be possible to conduct detailed demographic monitoring of individuals, but this intense level of monitoring will provide the best information about population growth and extinction risk (Morris and Doak, 2002).

Duquesnel et al. (2017) conducted 31 seed introductions of *Phoradendron rubrum* in the South Florida habitats where it is regionally rare and threatened by hurricane events and sea level rise. Monthly monitoring revealed that 38% of seeds germinated and that next generation recruitment occurred nearly eight years after seed sowing. Because portions of the species' life cycle are hidden, it was difficult to determine the success or failure of the introductions at various times. It has taken over a decade to achieve successfully established populations.

Comparing demographic data collected synchronously for wild and reintroduced populations will be the true reference for growth. For example, caged reintroduced seedlings of the US endangered *Purshia subintegra* had higher survival than wild seedlings in drought years even though they suffered serious loss (Maschinski and Quintana-Ascencio, 2016). Similarly, Laguna et al. (2016) compared nine safety neopopulations of *Limonium perplexum* to the single wild population and found parallel oscillations. Without the wild reference, the perception of reintroduced group successes would have been quite different.

After more than a decade and ten introductions, the US endangered long-lived perennial shrub *P. celata* has only produced fruit in a single augmented population (Menges et al., 2016). In comparison to the wild plants, introduced plants had slower growth, more delayed flowering, and no clonal spread, indicating that assessing whether the introductions have improved recovery of the species will require much more time.

In reintroduced populations of the cliff-dwelling, Mediterranean endemic, *Centaurea corymbosa*, Colas et al. (2008) found population dynamics differed from natural populations, but long-term growth rates were similar. Reintroduced individuals exhibited higher survival but lower fecundity than individuals in natural populations. Successful reintroduction therefore required multiple outplanting years and techniques to maximize fecundity of reintroduced individuals.

3. Discussion

Worldwide botanical gardens, including participating institutions of the Center for Plant Conservation, are advancing the science and practice of plant conservation (Mauder, 1992; Guerrant et al., 2004). As guardians of rare plant germplasm that may be extinct in the wild, botanical gardens have personnel with horticultural expertise that maintain careful records and healthy collections of plants that are potentially available for plant reintroductions (Miller et al., 2016). With increasing habitat loss, degradation, fragmentation and rapid climate change,

reintroduction will become an increasingly important component of endangered species recovery and one that relies upon genetically appropriate and diverse *ex situ* collections (Cochrane et al., 2007). To improve the design and outcomes of plant reintroduction programs, we developed the CPC guidelines using the best-available science from the international community and years of practical application within the CPC network of botanical gardens. Determining whether a reintroduction is sustainable may take decades therefore, it requires institutional rather than individual persistence and dedication to evaluate the success of the reintroduction (Albrecht et al., 2011). Institutional recordkeeping is one of the great strengths of botanical gardens. It is important that current researchers conducting reintroductions maintain their records in such a way that 10, 25, and 50 years from now, successors at the garden can relocate the reintroduction, can know details of what was done, and can monitor the progress. We encourage independent or academic researchers to collaborate with botanical gardens on long-term studies.

As reintroduced populations build to levels that would be considered sustainable, they benefit rare species by increasing the total number of individuals living in nature, increasing the spatial occupancy of the species, and thereby reducing extinction risk. Because reintroduction is a conservation strategy that works only in tandem with *ex situ* conservation, community involvement, and species' biological research, botanical gardens can be the world leaders in plant reintroduction practice. Improving our information sharing will support efforts worldwide, allowing all practitioners to learn from successes and failures (Volis, 2016).

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