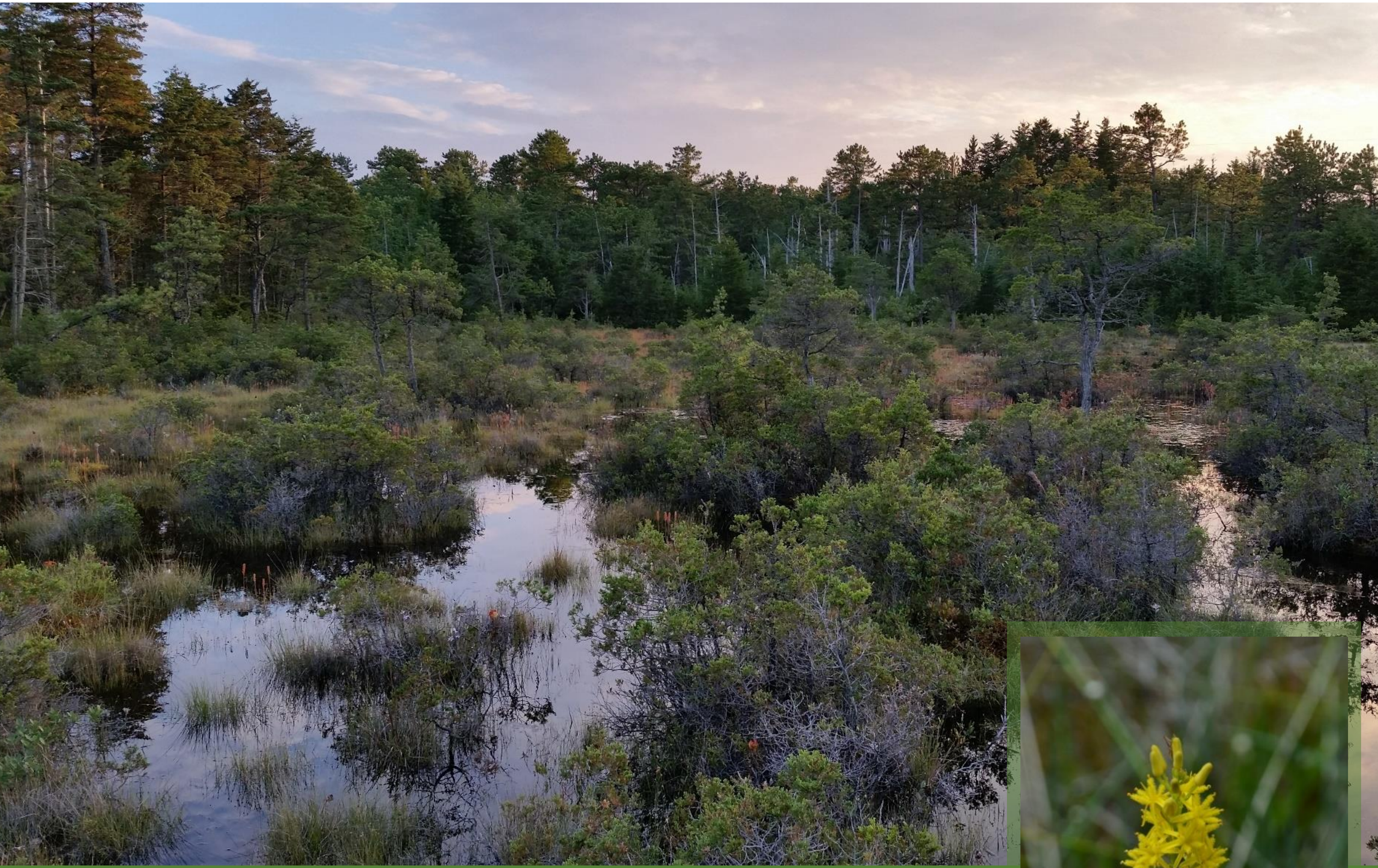

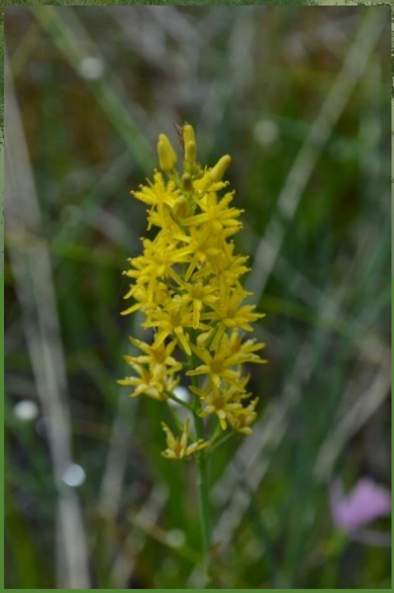

Rare Plant Propagation and Reintroduction

Questions and Considerations for Natural and Historic Resources Lands in New Jersey

New Jersey Department of Environmental Protection
Division of Parks and Forestry
Office of Natural Lands Management

May 2021

Rare Plant Propagation and Reintroduction: Questions and Considerations for Natural and Historic Resources Lands in New Jersey

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The author wishes to acknowledge the following persons who contributed to this manuscript: Elena Williams, Robert J. Cartica, Dr. Jay F. Kelly, Jason Hafstad, Roman Senyk, Lee Minicuci.

This report should be cited as follows: New Jersey Department of Environmental Protection. 2021. Rare Plant Propagation and Reintroduction: Questions and Considerations for Natural and Historic Resources Lands in New Jersey. New Jersey Forest Service, Office of Natural Lands Management. 77 p.

Cover Landscape Photo: Webbs Mill Bog by Kathleen Walz
Cover Inset Photo: Bog Asphodel (*Narthecium americanum*) by Jason Hafstad

Contents

1. Executive Summary

New Jersey is home to many rare and endangered plant species. The management and conservation of these species is essential to the maintenance of healthy, functioning ecosystems here and elsewhere. Practitioners of rare species management agree that the *in situ* conservation of rare species and their habitats is the priority for conservation and the best way to avoid local or global species extinctions. Although reintroducing plants has a low success rate and is not an alternative to conserving plants in their natural habitats, it remains an important tool for the recovery of rare, threatened, and endangered plants in certain circumstances. For some species on the brink of extinction, it may be the only option left.

Given the significant potential for negative consequences involved in rare species reintroductions, this report attempts to summarize some of the major concerns and considerations identified in the current scientific literature in order to avoid the potential for harm that may result from such activities.

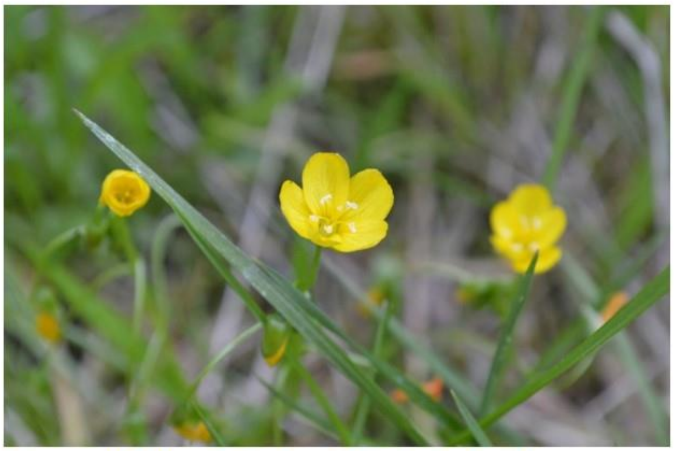
There are many types of reintroductions, but reintroduction is most generally defined as the intentional movement of a species into habitats or areas it previously occupied or where it has become extinct or nearly extinct. The term, however, is used to describe many other actions, including but not limited to augmentation of existing populations, reintroduction of a species to sites where the species was known to previously occur, or establishment of new populations in suitable habitat at undocumented sites within a species' historical range.

There are many potential pitfalls associated with plant reintroductions, requiring due diligence of researchers both before and after reintroductions are conducted in order to avoid them. First and foremost, researchers should keep in mind that rare plant reintroductions have a low long-term success rate and are often not the appropriate first course of action. Reintroduction projects also have the potential to do harm to the recipient site and cause loss of the species' genetic fitness. Furthermore, reintroductions often require considerable knowledge about the species' biology and ecology, and the time-consuming nature and expenses involved in developing and implementing such projects must be considered.

Justified reintroduction projects are characterized by lower risk to the species, extensive prior research on the biology of the species, community analysis between known occurrences and potential recipient sites, use of experimental design and adaptive management, collaboration among multidisciplinary experts, long-term monitoring and documentation of each stage of the project, and publishing or disseminating all results, even if the reintroduction fails. Conversely, projects that are riskier may remove adult plants from natural populations, plant outside of the species' known historical range, involve intensive site disturbance, or may have proceeded without understanding the basic biology and ecology of the species.

A key consideration is to carefully choose species that are appropriate for reintroduction efforts. Some rare plants are naturally rare. They occupy extremely narrow ecological niches, sometimes at the edge of their geographical range. It is entirely possible that a rare species in New Jersey is currently occupying the full extent of suitable habitat in the state and does not require any artificial intervention. Understanding the extent of both current and potential suitable habitat in New Jersey is an important first step toward proper management of any rare species.

Historical context is also an important consideration. New Jersey has experienced dramatic changes in land use and resource extraction patterns over time, especially during the 19th and early 20th centuries. Some of these land practices may have increased habitat for certain rare species. If this is the case, population declines today may reflect a return to historically consistent patterns of distribution.



Hammond's Yellow Spring Beauty (*Claytonia virginica* var. *hammondiae*). This yellow variety of Spring Beauty is found at only one location in New Jersey and has never been documented anywhere else.

Many of the multitude of challenges involved with rare plant reintroduction projects must be considered and addressed during the planning stages. Proposed rare plant reintroduction projects may or may not be justified based on a variety of criteria. If justified, a rare plant reintroduction proposal must thoroughly address the conservation status, biology and ecology, and reason for rarity of the species. The project must have current and future resources secured in terms of funding, personnel with subject matter expertise, and the manpower to conduct the work. Researchers should make sure they are in compliance with laws and policies regarding rare plant species. Sourcing and collecting plant material must be done in a way that balances preventing harm to the donor population with collecting sufficient quantity and appropriate genetic diversity of seed for a successful reintroduction. Choosing a recipient site must be a careful and deliberate process, and include analysis of edaphic conditions, a comprehensive species and community inventory, and consideration of associated species found in natural populations. The project proposal must address the outplanting process in terms of timing, amount, density, and spatial pattern. Plans for site management and long-term monitoring are critical elements for ensuring project success. Proposals should include specific goals and objectives, ensure the ability to be replicated by others, and define the short- and long-term metrics needed to measure success. It is important to report the outcome of rare plant reintroduction projects; even failed reintroductions have value in sharing the methods used, experimental results, and the known or speculated reasons for failure.

Other considerations arise after reintroductions are completed. If not documented properly, for example, they can obscure the patterns of biogeography and autecology of natural populations. There is also the risk of complications involving administrative, legal, and regulatory procedures. Legal protections for rare plant populations may or may not be extended to created populations, or may be rescinded if introductions artificially increase the number of populations. Rare plant reintroduction projects run the risk of giving the impression that creating populations is easy and inexpensive, and that intact natural habitat is unnecessary for the preservation of species. Another possible unintended consequence of rare species reintroductions is shifting the focus of conservation work away from the root cause of the species' decline.

The body of this document provides a detailed overview of the latest research concerning the risks and benefits of propagation and reintroduction of rare plant species. Readers are referred to the appendices for resources to assist in the development and review of reintroduction proposals on lands managed by the Department of Environmental Protection's Natural and Historic Resources group. In particular, Section 5.1 consists of a comprehensive checklist for reviewing reintroduction proposals. Written as a series of questions and considerations, it will enable managers and reviewers to gauge the completeness and potential for harm or benefit of any given project. Incorporating these guidelines during the planning process will help to assess if a rare plant reintroduction is warranted and raise the likelihood of a successful outcome.

2. Introduction

2.1. Purpose of this Document

Over the years, the New Jersey Department of Environmental Protection, Office of Natural Lands Management (ONLM), has been asked to review and advise the Department on various proposals to collect rare plant material from state lands for the purpose of propagation and later reintroduction to wild habitat. These proposals come from professionals, students, and amateur rare plant enthusiasts who are looking to be involved in these kinds of conservation projects as a means to save rare plant species, and as an alternative to ongoing challenges with successful management of rare plant species *in situ*. Developers have also proposed relocating rare plants on private lands as a way to mitigate habitat destruction incurred in the process of land conversion. Additionally, as climate change progresses, ONLM is preparing for the potential that future proposals may suggest assisted migration be utilized in New Jersey.

Recognizing that rare species management is complicated, and that the science is still in its infancy (Godefroid et al. 2016) and often ends in failure, the ONLM views rare plant reintroductions as a last-resort measure that must not be undertaken without first considering other options. However, there are situations where reintroduction may be the appropriate response for specific species.

This document addresses the need for a deeper understanding of the issue and provides a review process for rare plant reintroduction proposals. It provides a summary of the scientific literature on rare plant reintroductions, including examples of both successful and unsuccessful reintroduction projects, key findings, and lessons learned. We provide guidelines for components of rare plant reintroduction proposals, a checklist for reviewers, and reputable contacts for further information. This document is not a how-to guide for those interested in planning a rare plant reintroduction; many such resources exist, and we provide some links and information for organizations in the Appendix (Section 5.2). A glossary of specialized terms is provided in Section 5.3.

We use the term reintroduction throughout for consistency, although the literature often uses other related terms, sometimes as synonyms, sometimes to achieve either a broader or more precise meaning. See the Glossary (Section 5.3) for terms such as augmentation, translocation, and conservation introduction.

Species names are written as published by the authors of the original paper which may not be the currently accepted Latin name. The Latin name is given only the first time a species is mentioned; thereafter, only the common name is used. If a species does not have an English common name, then only the Latin name is used.

2.2. Protecting Natural Populations is the Priority

All practitioners of rare species management agree that the *in situ* conservation of rare species and their habitats is the best insurance against losing those species in the wild; this consensus comes from publications in scientific journals (Enßlin et al. 2011; Fahselt 2007; Falk and Olwell 1992; Haase and Rose 2001; Kaye 2009), state and federal policy (Maryland Plant Reintroduction Task Force [MD PRTF] 1999; North Carolina Plant Conservation Program [NC PCP] 2005; U.S. Fish and Wildlife Service [USFWS] 2000; Virginia Division of Natural Heritage [VA DNH] 2008; Washington Natural Heritage Program [WA NHP] 2012), and non-governmental organization (NGO) guidelines (Canadian Botanical Association [CBA] 2014; Center for Plant Conservation [CPC] 2019; New England Wild Flower Society [NEWFS] 2002).

The Center for Plant Conservation – an organization that has worked for decades on the science of rare plant conservation and whose scientists have implemented many reintroduction projects – states that they "do not support or promote reintroduction as an alternative to *in situ* ecosystem protection" (CPC 2019). The U.S Endangered Species Act recognizes that habitat protection is crucial. For federally listed species, there are legal protections for designated critical habitat thereby prohibiting activities that can damage, destroy, or modify the habitat (Volis 2015). These protections are based on the best scientific evidence and although state listed plant species are not afforded the same legal protection, the same ecological principles apply.

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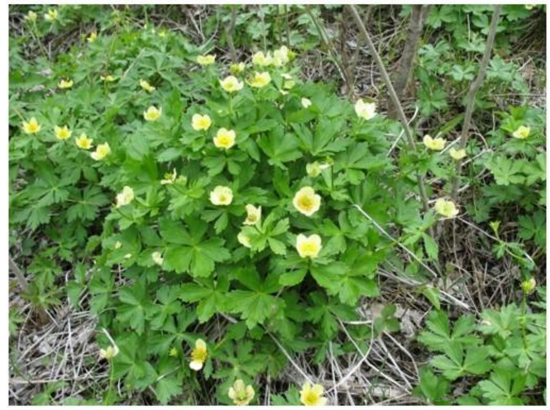
In order to conserve *in situ*, we must protect habitat by addressing the threats that rare plants face and the factors responsible for species' decline (USFWS 2000). The presence of rare species indicates that the habitat itself is significant as well – it may contain specific microclimatic conditions, unusual soil, lack of negative human influence, or other ecological conditions that can't be replicated (CBA 2014). "The habitat is as important to scientific knowledge and our cultural heritage as the rare species itself" (CBA 2014).

Plant reintroductions are not a replacement for *in situ* habitat protection (Reiter et al. 2016). The safest conservation method is to keep plants in their native wild habitats where they can adapt to changing environmental conditions (Baker et al. 2014), contribute to “the full range of interactions with other organisms, and where the natural process of evolution can continue" (Enßlin et al. 2011). Species conservation requires *in situ* protection of a species’ natural habitat in order to maintain its evolutionary potential (Volis 2015). Each plant species plays a particular role in the ecosystem. A rare species introduced into a new site may not function as it did in its natural habitat.



The globally rare Spreading Globe Flower (*Trollius laxus* ssp. *laxus*) is confined to distinct wetland habitats fed by highly alkaline groundwater.

Currently no alternatives to *in situ* protection of rare species can guarantee success; we can only ensure a high probability of a species’ persistence by conserving natural habitats (Volis 2015). The probability of survival of reintroduced species is too low and the risks are too great to allow plant reintroductions to be among our primary strategies (Godefroid et al. 2011). Ecologists acknowledge that reintroductions are sometimes warranted but should be conducted as experiments that allow us to better understand the species’ biology and what reintroduction methods work (Guerrant and Kaye 2007). Rare plant propagation and reintroduction work is sometimes necessary to save species, but it should be undertaken by scientific organizations (WA NHP 2012).

“The habitat is as important to scientific knowledge and our cultural heritage as the rare species itself” (CBA 2014).

2.3. Potential Negative Implications

Many researchers have commented on the potential negative implications of reintroductions (Falk and Olwell 1992; Griffith et al. 2017; Wells 2012), including ecological, social, and economic considerations (International Union for the Conservation of Nature/Species Survival Commission [IUCN/SSC] 2013; USFWS 2000).

2.3.1. Obscures biogeography and autecology

Biologists have valid concerns about being able to discern natural populations of rare species from those that are planted (Fahselt 1988). Creating new plant populations obscures the natural distribution and range, and the ecological and biogeographical patterns, of plant species (Kaplan and The Czech Botanical Society 2007; New York Natural Heritage Program [NY NHP] 2008; Parkin 2005; USFWS 2017). Undocumented plant introductions could lead to “spurious results in studies of nutritional and moisture requirements, allelopathic interactions, or factors controlling distribution" (Fahselt 1988). This has occurred in the real world and the two following examples illustrate this problem.

In a 1996 publication in the journal *Castanea*, botanists from the Virginia Natural Heritage Program published a state record for the rare Plymouth gentian (*Sabatia kennedyana*) (Fleming and Ludwig 1996). They believed it to be a significant discovery, as it had not been found in mid-Atlantic states where there is an over 600-mile gap in its distribution (NatureServe 2020; Sorrie and Weakley 2001; Fleming and Ludwig 1996). Later they found out that it was not a natural population, but had been planted there (VA DNH 2008).

Similarly, a botanist studying the hard shield fern (*Polystichum aculeatum*) accidentally learned that some populations he had discovered were not actually within its natural range, but instead had been transplanted by another botanist (Fahselt 1988). In both cases, experienced and knowledgeable professional botanists wasted time and energy and were led to false conclusions by others having planted species into wild habitats without first communicating or consulting with the local research community.


Hard Shield Fern (*Polystichum aculeatum*)

2.3.2. Regulatory and administrative Issues

Outplanting rare plant species presents a suite of challenges and complications to administrative, legal, and regulatory procedures. Distinguishing natural and created rare plant populations is important for assigning conservatism or rarity ranks (Farnsworth 2004), tracking the conservation status of rare plants, issuing wetland and other land use permits, and enforcing state and federal restrictions on the sale of listed plants (USFWS 2017). Conservation planners typically intend to prioritize funding towards management and protection of natural populations and want to avoid allocating funds to introduced populations (Farnsworth 2004; NY NHP 2008; VA DNH 2008).

More troublesome is the fact that legal protections for natural rare plant populations may or may not be extended to created populations (Reinartz 1995). Other states don't distinguish between natural and created populations (NY NHP 2008), so if introductions artificially increase the number of populations, the species could abruptly lose legal protections (NEWFS 2002; Parkin 2005; Reinartz 1995). This can also affect ecosystems since "protection of listed species is often our only powerful tool for habitat protection" (Reinartz 1995). For federally listed species, there is concern about delisting species based on the abundance of created populations without assurance of their long-term survival (USFWS 2017).

2.3.3. Misconstrues complex natural systems

Engaging in rare plant reintroduction projects runs the risk of giving the mistaken impression that successfully moving plants is easy and inexpensive, and that intact natural habitat is unnecessary for the preservation of species. Although biologists are well aware that plant reintroduction projects are incredibly complicated and often unsuccessful, they are also wary that moving rare plants could wrongly be seen as a substitute for proper conservation action, or be used to achieve regulatory compliance with environmental rules and regulations. Protecting plant populations in their natural habitats "may be seen as an unnecessary hindrance to development" (Wells 2012). Reintroduction has been used to justify the destruction of natural populations. For example, in Western Australia a state-funded research project successfully developed propagation techniques for a rare orchid, which was then used to justify destruction of one of this orchid's few remnant populations (Falk and Olwell 1992). The messaging around rare plant reintroductions should be that natural habitats are essential for survival of rare plants, but reintroduction can be attempted as a last resort when the damage has already been done, not as a way to rationalize the destruction of natural habitat (Falk and Olwell 1992).

> "The best place to conserve plant biodiversity is in the wild, where a large number of species present in viable populations can persist in their natural habitats with their associated ecological interactions" (Godefroid et al. 2011).

2.3.4. Does not address the root cause of species decline

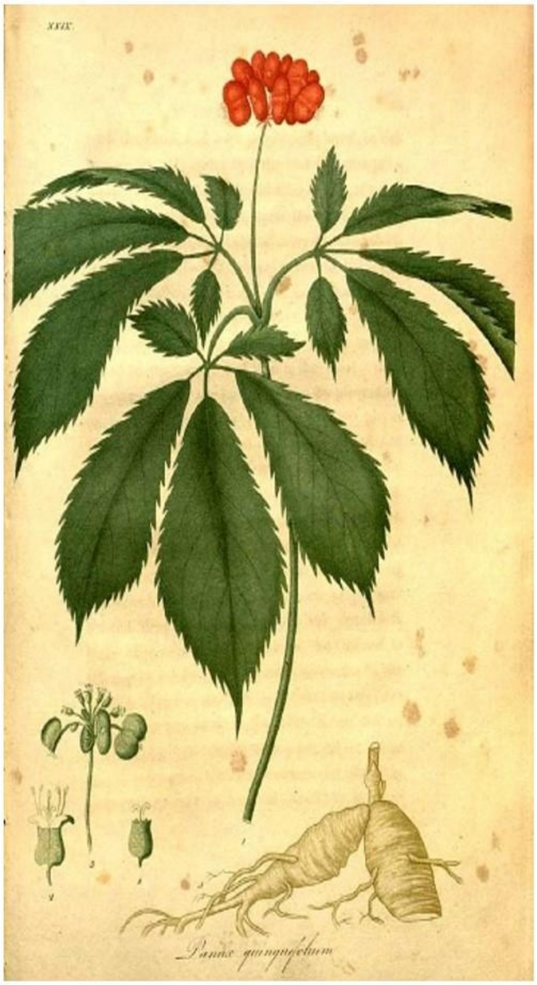
A possible unintended consequence of rare species reintroductions is shifting the focus of conservation work away from the root cause of the species' decline (NY NHP 2008). Habitat destruction is a major threat to rare plants, and often their habitats are also rare or becoming so due to lack of appropriate management (USFWS 2017). Rare species and those in decline are often responding to a negative shift in their habitat. The decline should be viewed as a canary in the coal mine – an indication that the root cause is an issue with the habitat, not with the species itself (VA DNH 2008). The use of reintroductions could undermine the primary goal to protect natural remnant populations, communities, and ecosystems (Parkin 2005), especially if the species is moved elsewhere as in the case of rescue or mitigation.

3. Overview of Rare Plant Reintroductions

3.1. Purpose, Goals, and Benefits

3.1.1. Problem

Worldwide, plant species populations are declining, and the extinction rate has been increasing (Heywood and Iriondo 2003; Noël et al. 2011). Humans have increased the extinction rate by 100 to 1000 times the natural rate (Godefroid et al. 2011). Although extinction estimates have varied (Maschinski and Albrecht 2017; Ren et al. 2014), a new calculation, which accounts for several known and quantifiable biases, indicates that 39% of all vascular plant species worldwide may be threatened with extinction (Nic Lughadha et al. 2020). In addition to species-level extinctions, population-level extinctions are of concern; they are much more frequent and widespread than species extinctions and there is growing evidence that these extinctions will degrade ecosystems with negative impacts to human society (Paris et al. 2018). The primary cause is loss or degradation of habitat, which includes habitat fragmentation, land-use change, and development (Bontrager et al. 2014; Botanic Gardens Conservation International [BGCI] 2015; Ensslin et al. 2015; Maschinski et al. 2013; Noël et al. 2011). Climate change is also a dominant cause (Grewell et al. 2013; Maschinski and Albrecht 2017). Other causes include over-collection and over-exploitation (Vasseur 2013), competition from non-native invasive species (Maschinski and Haskins 2012), and narrow range distributions (Ren et al. 2014).



Collection of wild plants is one of the threats to populations of American Ginseng (*Panax quinquefolius*) in New Jersey.

The threats plants face mean that *in situ* conservation is not always possible, especially in extreme cases of habitat destruction or land-use change (Ensslin et al. 2015). Altered and degraded habitats are so common now that traditional conservation measures (e.g., habitat protection and management) are not always enough for all populations to recover, especially in the absence of naturally occurring propagules (Godefroid et al. 2011). A wide range of approaches will be needed to address the global biodiversity crisis (Paris et al. 2018). A suite of *ex situ* practices such as botanical gardens, seed banks, and reintroduction efforts can be used in species restoration programs (Volis 2015).

Restoration ecology and reintroduction biology have advanced in recent decades to combat the dramatic loss of plant species (Maschinski and Haskins 2012; Noël et al. 2011). Practitioners of reintroductions view them as a viable and important tool for the recovery of rare, threatened, and endangered plants (Haskins 2015; MD PRTF 1999), and for some species on the brink of extinction, it may be the only option left (Haskins 2015). Although increasingly common, this strategy is controversial due to the significant potential for negative consequences (Vasseur 2013).

3.1.2. Goals of reintroductions

The primary goal for the majority of species is to establish resilient, self-sustaining populations that contain the appropriate amount of genetic diversity needed to adapt to changing environmental conditions (Guerrant and Kaye 2007; MD PRTF 1999). Ultimately, the goal is to prevent regional extirpation and global extinction (Fenu et al. 2016; Grewell et al. 2013; Heywood and Iriondo 2003; Maschinski and Albrecht 2017; Parkin 2005). Reintroduction projects can work towards this by improving the genetic diversity of species (Fenu et al. 2016;

Maschinski et al. 2013), increasing the size of populations (Fenu et al. 2016) and overall abundance and distribution on the landscape (Albrecht and Long 2019). The intention is to improve the capacity of rare species to reproduce and survive in the long-term in the wild (Albrecht and Long 2019; Guerrant and Kaye 2007), and to endure natural stresses and disturbances (Reinartz 1995).

3.1.3. Benefits of reintroductions

Many proponents have espoused the benefits of rare plant reintroductions. These benefits fit into three categories: benefits to the target species, to natural communities, and to habitat management. When reintroduction projects make use of experimental trials, researchers are able to add to the body of scientific knowledge about the biology, genetics, and ecology of the target species (Clements 2013). Successful reintroductions can enlarge dwindling populations and restore the historical range of species that have been declining or extirpated from some locations (Clements 2013). Across a landscape, reintroductions may decrease the fragmentation of populations (Clements 2013), alleviating the risks associated with limited genetic diversity (Clements 2013) by restoring meta-population dynamics (Heywood and Iriondo 2003) and promoting genetic exchange among populations (Clements 2013). At the community level, reintroductions will increase biological diversity to a site or landscape (Parkin 2005; MD PRTF 1999) and can help return plant communities to their former composition (Clements 2013). Reestablishing keystone or foundation species to their habitat will also work to restore vital ecosystem processes (Grewell et al. 2013; Heywood and Iriondo 2003). Finally, biologists can use the new populations of rare species to test alternative techniques to tackle the threats faced by the extant populations (Clements 2013).

3.2. Risks and Challenges

Rare plant reintroduction projects have a multitude of inherent risks and challenges which must be considered during the planning stages. Researchers must keep in mind that rare plant reintroductions have a low long-term success rate (Abeli et al. 2014) and are often not the appropriate course of action (Allen 1994).

3.2.1. Harm to the source population and site

The process of collecting rare plants can be detrimental to the source population and site (Maschinski and Haskins 2012). Removing plant material – whether seed, cuttings, or whole plants – can result in harm in various ways. With natural populations of rare species, the removal of plants or seeds from their natural habitat reduces current abundance, potential future abundance, and/or some amount of genetic variability, possibly resulting in an increased risk of extinction (USFWS 2000). Any material collected may not survive to produce another generation and so constitutes a loss of valuable DNA to the already depauperate source populations (Parkin 2005). There is also potential that the removal of propagules or individuals can threaten critical ecological function (IUCN/SSC 2013).

3.2.2. Harm to the recipient site

Damage to the collection site (Clements 2013; Farnsworth 2005) and recipient site can occur through trampling and physical damage to the native flora (CBA 2014; Clements 2013). The recipient site may also be damaged through soil disturbance (Clements 2013) which can affect the local seed bank and soil biota. Depending on the extent and intensity of the outplanting process and sensitivity of the site, there is the possibility for unintentional alteration of ecological processes (Clements 2013). Extreme care must be taken to not introduce invasive plant seeds which are easily transported on boots and equipment from site to site (CBA 2014; CPC 2019; NY NHP 2008; Reiter et al. 2016; Vinge-Mazer 2017). Pathogens or disease can be transferred to the new site in potting soil or on roots, stems, or foliage of plants (Abeli et al. 2014; CPC 2019; Clements 2013; NY NHP 2008; Reiter et al. 2016; Rigg et al. 2017; USFWS 2000; Vinge-Mazer 2017).

3.2.3. Loss of genetic fitness

The genetic structure of natural populations often includes locally adaptive genotypes that evolved over very long timeframes (Reinartz 1995) and are critical to the species' survival. Each stage in the reintroduction process holds potential to lose genetic diversity or negatively change the genetic structure of rare plant populations. In the short-term, seed quality is inadvertently decreased, (Basey et al. 2015; Vitt et al. 2016), and in the long-term, the ability to adapt to environmental change is reduced (IUCN/SSC 2013). For these reasons, rare plant propagation and restoration activities must consider the role of genetic diversity and fitness (Maschinski and Haskins 2012).

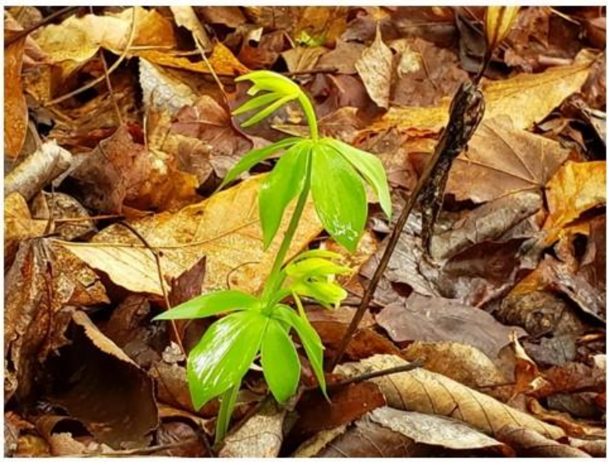
Additionally, the source populations of rare species may themselves have reduced gene flow and loss of genetic diversity inherent in small isolated populations or resulting from habitat fragmentation (Ottewell et al. 2016; Volis 2015). This lack of genetic diversity may not be apparent except under certain situations such as drought or disease (Basey et al. 2015). If genetic inbreeding or outbreeding depression need to be corrected for, a specialized program and genetic testing must be part of the recovery process. In propagule collecting, some genetic diversity will be missed (Haase and Rose 2001), resulting in a reduced amount of genetic variability available in the propagated population (USFWS 2000). During propagation in greenhouses and other controlled environments, the plant material is exposed to new selection pressures that could reduce its ability to survive and reproduce in its natural habitat (Ensslin et al. 2015; USFWS 2000).

Finally, propagating and outplanting rare plants is risky because greenhouse or garden grown genotypes may cross with wild genotypes, potentially reducing fitness of wild populations (Farnsworth 2004).

3.2.4. Unknown biology and ecology

A reintroduction should not only place the target species back into a site, but also make sure its ecosystem function is restored; this will require taking into account pollinators, mycorrhizal symbionts, seed dispersers, nutrient cycles, and hydrology (Allen 1994). Rare plant reintroductions are inherently challenging because not enough is known about many rare plant species and the ecosystems they inhabit, their ecological requirements, pollination systems, propagation methods or germination requirements (Allen 1994; Clements 2013; Godefroid et al. 2016), and specific microhabitat needs (Albrecht and Long 2019; MD PRTF 1999).

Failing to make the appropriate habitat match is one of the main reasons reintroductions don't succeed (Godefroid et al. 2011). Fragmentation and habitat degradation make it difficult to locate recipient sites that are large enough and have the ecological conditions necessary to sustain the species and its important biotic associates (Godefroid et al. 2016; Questad et al. 2014; Reiter et al. 2016). Even simply defining the species' distribution and its core range versus edge range is problematic in altered landscapes with populations impacted by habitat fragmentation and climate change (Vitt et al. 2016), thus complicating decisions around sourcing material and choosing recipient sites. Further complicating the issue, rare species often have declining populations and are in degraded areas (Godefroid et al. 2016), so those remnant populations are often not in ecologically optimal habitat (Bontrager et al. 2014). Attempting to define suitable habitat using the habitat attributes of populations that are declining will probably lead to a failed reintroduction (Albrecht and Long 2019; Maschinski and Haskins 2012).



Despite its high profile as a Federally Threatened orchid species, there is still much that is unknown about the mycorrhizal associates and ecological requirements of the Small Whorled Pogonia (*Isotria medeoloides*).

Reintroductions are also risky in that, due to climate change, we cannot assume that species' historical ranges will continue to have suitable habitat (IUCN/SSC 2013). In fact, the effects of climate change will likely decrease suitable habitat within species' historic ranges, forcing researchers to look beyond historic ranges for acceptable habitat (Maschinski and Haskins 2012; Ren et al. 2014), an approach called assisted migration that has its own controversies and is largely untested for rare plants (Maschinski and Haskins 2012; Vitt et al. 2016).

3.2.5. Limited resources

Reintroduction projects can be expensive and require funding for each step in the process (e.g., background research and planning, propagation and outplanting, site management operations, monitoring) (Clements 2013; Fenu et al. 2016; Ottewell et al. 2016). The long timeframes necessary to implement each project and monitor the results introduces uncertainty around maintaining long-term funding and sourcing additional funding if unplanned-for issues arise (Clements 2013). Funding sources may lose interest or prioritize other projects in the future (Ottewell et al. 2016), leaving half-finished projects high and dry.

3.2.6. Long time commitment

Conservation reintroduction projects are time consuming (Fenu et al. 2016). A reintroduction project may take years of planning and preparation to lay the groundwork for a well-designed project (Allen 1994; Godefroid et al. 2016). Outplanted populations of rare plants may take years, or even decades, to stabilize after the initial establishment (Godefroid et al. 2016) as well as requiring regular long-term maintenance (Clements 2013). Even then, success is not guaranteed. The substantial time commitment without guarantee of successful outcomes requires a level of dedication few may be willing to take on. Researchers must also ask if the project will have long term funding and higher-level support over time even as people come and go with staff turnover (Ottewell et al. 2016).

3.2.7. Unforeseen threats

Success is dependent on some factors that cannot be controlled like natural but damaging weather events that could decimate an outplanting, such as tornado, drought, flood, hurricanes, and wildfire (Fenu et al. 2016; Reiter et al. 2016). Vandalism and illegal collection of plants may also be valid concerns in some areas or for some species like orchids which are especially prone to theft (Reiter et al. 2016).

3.3. Elements of Successful Projects

Designing, installing, and managing new plant populations is among the most challenging aspects of rare plant conservation (Heywood and Iriondo 2003). Regardless of whether the reintroduction itself is successful, there are some common elements that help to make the project successful.

3.3.1. Extensive prior research

A major complication for rare plant reintroductions is that extensive and detailed knowledge about the species, its community, and the larger ecosystem is often lacking (Parkin 2005; MD PRTF 1999). For many rare species, basic biology, reproductive systems, life history, and microhabitat requirements may be unknown or poorly understood (MD PRTF 1999). Researchers should also consider the species' historical abundance and how that may have changed with human land use and changing habitat availability. It takes considerable time and resources to investigate and gather this information (Maschinski et al. 2013). Although it may be impossible to know all details of the target species prior to attempting a reintroduction, the early planning phase must include a comprehensive review of the literature (MD PRTF 1999). Practitioners can use knowledge gaps as an opportunity to test hypotheses throughout the reintroduction process (CPC 2019; Parkin 2005).

3.3.2. Experimental design and adaptive management

There are often important aspects of rare species' biology, ecology, or causes of decline that are unknown. While some things can be learned from an intensive literature review or from observational studies, important characteristics will likely remain unknown. A reintroduction project can still move forward provided researchers conduct reintroduction projects as scientific experiments (Guerrant and Kaye 2007) by developing a sound experimental design and implementing careful record-keeping that allows for reasonable inferences about the results. Such studies are best undertaken after careful monitoring and observations in the field in order to better inform our understanding of what factors may be limiting the species and/or causing its decline (Heywood and Iriondo 2003). The practical aspects of reintroduction methodologies themselves may also be tested (Guerrant and Kaye 2007) including propagation methods, season of outplanting, or microhabitat differences. A well-designed experiment will incorporate replication and control replicates. Researchers can opt to use a pilot study to test hypotheses on a small scale (IUCN/SSC 2013). A benefit of small-scale pilot studies or trial introductions is that any “unacceptable impacts can be mitigated or reversed" (IUCN/SSC 2013).

> “Reintroduction projects should be planned in such a way so as to collect as much scientific data as possible to help expand the knowledge base for the rare species.” (MD PRTF 1999)

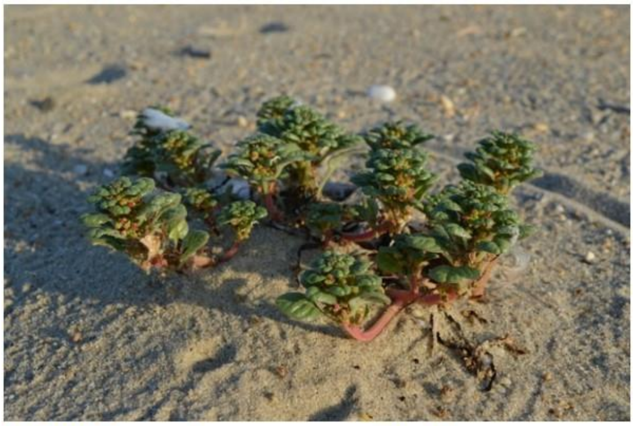
Testing multiple methods will increase the chances that some portion of the outplanted individuals will establish successfully (Guerrant and Kaye 2007). Regardless of the outcome, reintroduction attempts structured as experiments produce results that are better able to pinpoint precisely which methods led to success or failure and why that was the case (Guerrant and Kaye 2007; Maschinski and Haskins 2012). Without experimental procedures, however, reintroduction results are no better than guesswork based on weak evidence.

Adaptive management is a process of flexible decision making in which the outcomes of experiments, management actions, and natural events are carefully monitored and used to improve scientific understanding and adjust policies or methods (Williams 2011). It is distinguished from a trial and error approach by stating clear objectives, identifying uncertainties, predicting consequences, and monitoring (Williams 2011). The iterative process of doing, learning, and adjusting leads to a better understanding of the subject and therefore better resource management (Williams 2011).

Adaptive resource management is an excellent framework around which to build a rare plant reintroduction project since there is so much uncertainty and risk in rare plant management. Successful projects incorporate uncertainty and adaptation into the project design (Parkin 2005). Practitioners must take advantage of every opportunity to test management-relevant hypotheses, be willing to refine methods, and allow experimental results to guide the reintroduction process (IUCN/SSC 2013; Noe et al. 2019; Pavlik 1997).

3.3.3. Collaboration

Most natural resources endeavors are by their nature multidisciplinary, and as such are much more successful when approached as a team effort with biologists in relevant fields bringing together a mix of knowledge and skills to work towards a common goal. Rare plant reintroduction projects fit this characterization and are the perfect opportunity to gather input from botanists, ecologists, geneticists, horticulturalists, conservation practitioners, and other restorationists. It’s impossible for even the most intelligent and prolific naturalists to know everything, so collaboration is the key to building a better project with greater likelihood of success.



The ONLM is one of many governmental and non-governmental organizations working with the US Fish and Wildlife Service to study the Federally Threatened Seabeach Amaranth (*Amaranthus pumilus*) and manage its beach habitat.

If the target species is a federally listed species, then there may be an official recovery team organized by the U.S. Fish and Wildlife Service. For state listed rare plants, governmental agencies, conservation organizations, and other local institutions are all invested in protecting the local natural communities and rare plant species; coordinating with conservation practitioners from these groups on reintroduction projects ensures that "individual projects provide benefits that are in keeping with the broader conservation context for these species" (Parkin 2005).

Joining forces with organizations, universities, communities, businesses, tribes, and other stakeholders has a multitude of benefits. They can help access scientific expertise and equipment (Pain 2018) and improve protocols (Clements 2013). Teaming up with others can make it easier to get grants (Pain 2018) or at least spread out the financial risk. "Interacting with people with different perspectives or approaches also prevents you from getting tunnel vision about your research" (Pain 2018). Collaborators can share roles and responsibilities, and balance out each other's strengths and weaknesses, talents, and time availability. Ultimately, assembling a group with the right mix of specialties and abilities will serve to increase the quality, creativity, and enthusiasm generated towards the reintroduction project, and improve access to the resources and funding necessary to keep it going.

3.3.4. Long-term monitoring

"In the best circumstances, conservation practitioners work in tandem with land managers and the public to restore healthy, wild habitats and populations of rare species" (Maschinski and Haskins 2012).

A successful rare plant reintroduction project depends on extended monitoring of the new population and the outplanting site (CPC 2019; Fenu et al. 2016; Heywood and Iriondo 2003; Maschinski and Haskins 2012; Vitt et al. 2016). The average time of monitoring reintroductions has typically been 2-3 years, but it can take over a decade to know if a reintroduction is successful – whether the plants will survive, reproduce, and become a viable self-sustaining population (Allen 1994; CPC 2019; Ren et al. 2014; Vitt et al. 2016). Early successes do not guarantee long-term success, so researchers need to commit to their investment with a strategy for long-term monitoring.

As with the collection and introduction of seeds and propagules, the monitoring process itself has the potential to damage the site. Care should be taken for monitors to avoid trampling, soil disturbance and introduction of invasive species and pathogens during site visits. Through intensive monitoring in the initial few years after outplanting, practitioners can take note of many indicators such as general health status, flowering, fruiting, and recruitment (Clements 2013; Reiter et al. 2016). The plants may need supplemental watering or protection from herbivores until they fully establish. Researchers can also use monitoring to investigate the broader community ecology of the species, such as presence of pollinators or herbivores that can provide valuable information to better understand the reasons for positive or negative project outcomes (Reiter et al. 2016). Regular monitoring leads to better stewardship of the recipient site which may need ongoing management to provide the best habitat conditions for the target species (Grewell et al. 2013; Lloyd et al. 2018). Prescribed fire, non-native invasive plant removal, and canopy thinning are all commonly performed management activities to enhance habitat for rare plant species. A detained long-term monitoring plan will also provide important data about the species' response to infrequent events like drought, flooding, or insect outbreak, as well as broader-scale changes in climate (CPC 2019; Haskins 2015).

Rare plant reintroductions are often seemingly successful in the early years but end in failure years or decades later (Fenu et al. 2016; Haskins 2015). Even if the reintroduction does not succeed, the data gathered can help to inform researchers as to why the plants did not survive and may indicate how to better proceed in the future. Finally, long-term monitoring data can help evaluate the project's "overall success in terms of logistics, economics, and community involvement" (Clements 2013).

3.3.5. Disseminate results

Detailed, full, and honest reporting is an important part of the scientific method and is especially worthwhile in new and developing fields such as rare plant reintroduction and recovery (Parkin 2005). There is a strong bias in scientific literature to not publish negative experimental results, although detailed description of methods, and results – including failures – helps others learn and advance the science (CPC 2019; Fenu et al. 2016; NY NHP 2008). As long as the project is well-conceived and executed, any outcome should yield useful ecological information (Parkin 2005). Unsuccessful outcomes may be as valuable as successful reintroductions (Drayton and Primack 2012). Some researchers feel so strongly about this they recommend failure analysis to delve deep into understanding how and why the project was unsuccessful (Drayton and Primack 2012).

Seabluff Catchfly (*Silene douglasii* var. *oraria*)

In the restoration of Piper's daisy (*Erigeron piperianus*) in Washington state, researchers adjusted their analyses to account for unexpected insect herbivory and a freeze event that occurred soon after outplanting (Link and Cruz 2015). The authors state, "we felt these factors should be reported because of the unique opportunity for instruction that they represent" (Link and Cruz 2015). They recognized these unplanned events as object lessons not abject failures, and reported honestly how their experiment took an unforeseen path but still added value to the science.

Researchers reintroducing seabluff catchfly (*Silene douglasii* var. *oraria*) in Oregon were caught off guard when a wildfire burned through their research site within weeks of outplanting seeds. Instead of abandoning the study, they took advantage of the disturbance to analyze seedling emergence among burned, unburned, and smoke affected plots (Lofflin and Kephart 2005).

3.4. Review of the Scientific Literature

3.4.1. Summary of reintroduction reviews

We summarize four meta-analyses of plant introduction attempts (Albrecht and Maschinski 2012; Dalrymple et al. 2012; Godefroid et al. 2011; Guerrant 2012) and one additional paper that reviews three of the meta-analysis papers (Guerrant 2013). Results of each vary and give differing impressions of the success of reintroduction as a conservation tool. Lack of overlap in the source studies is one reason why meta-analysis results differed. All authors recognize the issue of bias in the literature towards publishing reintroduction successes as opposed to reporting on the failures, and the paucity of long-term monitoring data in reintroduction research.

3.4.1.1. Albrecht and Maschinski 2012

The authors conducted a meta-analysis of 174 reintroduction attempts drawn from the Center for Plant Conservation (CPC) International Reintroduction Registry, a database of reintroduction attempts derived from published studies (peer-reviewed and gray literature) on plant reintroduction projects. Propagules planted multiple times at the same location were considered as one reintroduction attempt. This analysis focused on factors related to population persistence. The goal was “to determine the role of founder population size and propagule stage on the outcome of reintroduction projects” (Albrecht and Maschinski 2012). Attempts were considered a failure if they failed to establish a persistent population (according to the authors of the original study) or when population size fell below ten individuals. Attempts were considered a success if the population was persistent at the last known monitoring period. They did not use an absolute or consistent time frame for survival; consequently, it was beyond the scope of the study to say what percentage of reintroductions were successful.

Results showed that the age of the founders was an important factor; for woody and herbaceous perennials, if founders were more than one year old when planted, they typically survived better, grew faster, and attained reproductive maturity sooner than plants from seed founders. They also looked at the effect of the size of the founding population and found that, for reintroductions using seeds, the size of the founding population did not affect survival. However, when seedlings (<1 year old) and older plants (>1 year old) were used, larger founder populations tended to survive more often than smaller founder populations.

The authors provide a few key lessons learned from the 174 reintroduction attempts they analyzed. When using seedlings and older plants, founder sizes larger than 50 individuals generally increased the probability of establishing a persistent population. Using seedlings and older plants increased the chances of establishing a persistent population over the use of seeds. Reintroductions using seed had low establishment rates regardless of the total number of seeds used. Ultimately many environmental factors influence plant survival, so using a large founding population cannot guarantee population persistence.

3.4.1.2. Dalrymple et al. 2012

The authors searched scientific journals and national natural history databases to compile research on 128 species, representing 304 attempts to introduce plant populations (species and sites were duplicated in their analysis). Analyses included the number of propagules used, the number that survived, the length of the monitoring period, and whether next-generation recruitment occurred.

Of the 72 recent projects (five years since installation), 46 reintroduced populations were still surviving and 26 had perished. Of the 50 projects installed 10 years prior, 20 reintroduced populations were still surviving and 30 had perished. There were few projects with over 10 years of data and the status of many was unknown, probably due to a lack of monitoring.

Reintroductions using seeds had the lowest average propagule survival (5% survival out of 47 attempts) compared to juvenile or adult plant reintroductions. Despite this, using seed resulted in a higher percentage of propagules reaching reproductive maturity (49%) and producing offspring (47%) than projects using juvenile or adult plants. However, these were primarily annual species, which naturally produce offspring faster than perennials. A short life cycle doesn't necessarily translate to better survival over the long term. The average monitoring time for projects using seed was 34 months.

Reintroductions using juvenile plants (not at reproductive maturity) had a 65% propagule survival rate, and the projects had the lowest population mortality rate (9%). Monitoring times were the longest at 41 months, which is still too short a time frame to get an accurate measure of reproductive success for many perennial species. Out of 134 reintroduction attempts using juvenile plants, only 19% produced flowers or fruits and only 5% produced offspring.

Reintroductions using adult plants seem to be more successful than reintroductions using juvenile plants, but monitoring times were only about 36 months on average. They had the highest mean propagule survival rate (85%), but there was a lot of variation in survival among the projects (+/- 24%). The population survival rate was 84%, 35% reached reproductive maturity, and 21% recruited offspring. Although adult plants were used, the plants need time to acclimate to the site so they may not flower, fruit, and produce offspring for quite some time.

Unfavorable habitat was a common reason cited for reintroduction failure; specific causes included drought in the first few years after outplanting, too much and not enough disturbance, unsuitable substrate texture, and competition from invasive plant species. For reintroductions using juvenile plants, species with larger range sizes had higher mortality than narrowly endemic species, illustrating the fact that site selection for reintroduction is not simple, even for species that seem to have broad habitat tolerances. There was no difference in mortality of wild versus *ex situ* sourced reintroduced populations, but wild-sourced propagules may have higher recruitment than *ex situ* propagules, making them more successful by that standard. Removing the threats faced by the target species did not ensure or improve survival of the reintroduced population. Reintroduced rare plant species face significant causes of mortality other than the specific threats identified by the researchers. Removing the obvious threats is important but cannot guarantee survival. Key elements of the species' biology or habitat needs may yet be unknown.

3.4.1.3. Godefroid et al. 2011

The authors reviewed 26 published papers and 55 scientist questionnaire surveys; a total of 249 reintroductions from North America, Europe, Africa, and Australia were included in the meta-analysis. Reintroduction success was based on metrics of the survival, flowering, and fruiting rates of the reintroduced plants. They did not include recruitment as a measure of success because that data was rarely available in the studies they reviewed. Their literature review contained recruitment data from 39% of studies, while 32% of the researcher surveys had recruitment data. When it was reported, recruitment in reintroduced populations was sporadic or nonexistent.

Looking at all data combined, the average survival rate of reintroduced species was 52%, the average flowering rate was 19%, and the average fruiting rate was 16%. When broken down by year after reintroduction, survival and flowering rates declined strongly over the four years. Survival rates reported in the literature were much higher than reported in questionnaire surveys.

In the first year after reintroduction, survival was greater when seedlings were used than if seeds were used. Increasing the number of reintroduced individuals positively influenced survival rates. Bare-root seedlings survived more often than seedlings rooted in potting soil. Using at least one type of site management in the reintroduction improved survival rates. Making use of known genetic variation in the reintroduced species improved survival rates.

Reintroduction success was most positively influenced by using plants from diverse source populations, reducing competition by removing surrounding plants, and placing the reintroduction into a protected area. Fencing the site also affected success to a lesser degree.

Monitoring data was beyond 10 years for some studies, but statistical analyses only used data from studies that were four years post-reintroduction. Godefroid et al. (2011) acknowledge that although the short timeframe in their analysis is not ideal, the declining trends in the first few years suggest that most of the reintroductions were unlikely to survive and, in the majority of these cases, reintroduction was not a successful conservation strategy. Survey questionnaire respondents were somewhat more optimistic: 29% of all survey respondents reported that the reintroduction attempt was successful; this increased to 34% when only considering reintroductions that were at least 10 years old. Forty-two percent of all survey respondents reported that it was too early to judge whether the reintroduction was successful or not; this decreased to 31% when only considering reintroductions that were at least 10 years old.

Several weaknesses in reintroduction programs were identified: "(1) Insufficient monitoring following reintroduction (usually ceasing after four years); (2) Inadequate documentation, which is especially acute for reintroductions that are regarded as failures; (3) Lack of understanding of the underlying reasons for decline in existing plant populations; (4) Overly optimistic evaluation of success based on short-term results; and (5) Poorly defined success criteria for reintroduction projects" (Godefroid et al. 2011).

Godefroid et al. (2011) give suggestions for improving the value of plant reintroductions as a conservation tool: "(1) an increased focus on species biology; (2) using a higher number of transplants (preferring seedlings rather than seeds); (3) taking better account of seed production and recruitment when assessing the success of reintroductions; (4) a consistent long-term monitoring after reintroduction."

3.4.1.4. Guerrant 2012

The author compiled data from the CPC International Reintroduction Registry (CPC 2019), a database of reintroduction projects. Out of 145 projects in the registry, the author used 89 projects in his analysis because they included detailed information about the experimental design and results. This is a descriptive paper (not a statistical analysis) comparing methods and outcomes of the reintroduction attempts.

Guerrant (2012) looked for patterns in the projects' methods, but he doesn't link these patterns to project outcomes. About half of the projects in the registry attempted reintroduction or augmentation of the species into previously or currently occupied sites, while the rest attempted introductions into unoccupied sites either within or outside of the historic range. Most projects (77%) collected propagules from a single source population. Vegetative adults were most commonly used as founders, followed by seedlings, seeds, juveniles, and reproductive adults. Most projects (67%) consisted of only a single outplanting attempt; 28% had two or three outplantings.

Guerrant (2012) also described patterns in the hypotheses tested. Seventy percent of projects tested hypotheses of experimental factors, 15% tested genetic factors, and 14% tested demographic factors. Most projects (69%) used survival as their metric to evaluate success. A few commonalities in results were described. Of four projects that explicitly compared plants and seeds, plants were more successful founders than seeds. Of two projects that compared survival rates by the age of plant, both found that larger plants had better survival. The size of the founding population is also important to success – larger founding populations have a better chance of surviving and growing than smaller founding populations.

As of 2009, the fate of 49 projects was known – 45 projects had surviving plants while only four projects had failed. The survival rate of these projects was 90%, although most of the projects were only a few years old, so only short-term results were available. Further metrics of success for the reintroduced populations include the following mileposts: 76% had reached reproductive adulthood, 33% produced a second generation, and 16% had reproductive adults in the next generation. The author concluded that long-term monitoring is essential to reintroduction projects. Plant species with long life histories may not show signs of failure for many years, and only by continued monitoring can any signs of decline be detected and managed.

3.4.1.5. Guerrant 2013

Guerrant (2013) compares and contrasts three meta-analyses of plant reintroduction projects: Dalrymple et al. (2012), Godefroid et al. (2011), and Guerrant (2012). Although they share some common ground, these three reviews used mostly non-overlapping datasets, different definitions of success, and different time scales, thus they came to very different conclusions about the state of and prospects for rare plant reintroduction as a conservation tool.

Dalrymple et al. (2012) were conservative in their assessment of reintroduction projects, stating that “this review cannot conclusively comment on the effectiveness of reintroductions.” While they were thorough in their literature search and provided good insights and suggestions, they reviewed many projects in which the outcome was unknown, so they were unable to determine if those reintroductions were successful or not.

Godefroid et al. (2011) put forth a negative assessment of reintroduction projects. They conclude that “reintroduction is generally unlikely to be a successful conservation strategy as currently conducted.” However, Guerrant (2013) points out that they limited their analysis to projects with short time frames. The short-term survival of the founding individuals is not a reliable measure of success because it's known and expected that there will be an initial decline after outplanting. This well-known phenomenon should be planned for, but the judgement concerning the overall fate of the project should not be based on it (Guerrant 2013). Flowering and fruiting were other metrics of success, but it’s unlikely that species will attain reproductive maturity in the short timeframes that this analysis considered (Guerrant 2013). Finally, Guerrant (2013) took exception to the way Godefroid et al. (2011) drew conclusions from analysis of very small subsets of data.

Guerrant (2012) had the most positive assessment of reintroduction projects, finding that there is “strong evidence in support of the notion that reintroduction, especially in combination with *ex situ* conservation, is a tool that can go a long way toward meeting the need it was intended to address.” Guerrant (2013) attributes this to his review having used data on the reproductive status of founders and the production of a second generation, so the longer-term outcomes of the projects he reviewed were known, whereas many project outcomes were unknown in Dalrymple et al. (2012) (Guerrant 2013).

All three reviews suffered from two issues: bias in the scientific literature to publish successful reintroductions over failed projects, and the typical short period of monitoring after the reintroduction was initiated (Guerrant 2013). Defining reintroductions as a success is often done too early in the process, although Dalrymple et al. (2012) and Godefroid et al. (2011) both did so. Moving forward, reintroduction projects should consider reporting on benchmarks of “initial establishment, reproductive maturity, the production of a next generation, and whether or not any founders or their descendants were extant” (Guerrant 2013). The lack of overlap seen in these three comprehensive reviews led the authors to different conclusions, but also “suggests that there are probably a great many more projects yet to be found, and from which we can all learn” (Guerrant 2013).

3.4.2. High and low risk reintroduction projects

3.4.2.1. Higher risk reintroduction projects

The defining features of higher risk reintroduction projects include:

- Removing adult plants from natural populations
- Planting outside of known historical range (assisted migration)
- Not using an experimental design to properly test hypotheses
- Intensive site disturbance
- Lacking biological and ecological understanding of the target species

Three examples of higher risk rare plant reintroduction projects are summarized below. It is not our intention to harshly criticize other projects and we are aware that we don't know all the issues and limitations experienced by the researchers. However, the projects summarized below represent examples of higher risk situations that should be avoided. Awareness of the risks, proper planning, and learning from previous research will help make for more efficient and cost-effective rare plant management and avoid damage to our rare plant populations in New Jersey.

3.4.2.1.1. Sand Jurinea (Jurinea cyanoides)

Sand jurinea (*Jurinea cyanoides*) is a pre-boreal relict species found in Central Europe. It is an herbaceous perennial in the composite family that is usually found in xeric sand grasslands on inland dunes (or similar sand-dominated habitats) that have nutrient-poor soil, moderate sand drifts, and an open vegetation structure. It is listed as an endangered species in Germany. Populations are threatened by pine-afforestation, sand mining, and inappropriate management or lack of management (grazing, mowing) that results in grass encroachment. It is also limited by lack of a persistent seed bank and not having adaptations for long-distance dispersal (Tischew et al. 2017).

Sand Jurinea (*Jurinea cyanoides*)

Researchers planted sand jurinea seeds in test plots using three site prep methods. They found that an inversion treatment led to successful seed germination, growth, and survival after seven years. The inversion site prep method involved removing the upper soil layer to a depth of 30 cm and refilling the area with sandy soil from nearby sites, originating from layers below 30 cm depth. The inversion treatment was significantly more successful than other treatments; in the control and mown treatments, dense competing vegetation caused high below-ground competition and low light availability resulting in low survival of sand jurinea seedlings (Tischew et al. 2017).

Results of this experiment seem to show that the successful reintroduction of sand jurinea necessitates the creation of extremely nitrogen-poor soils and the complete elimination of all competing species. The authors recommend that this soil inversion treatment be implemented on areas of at least 100 m^2 in order to avoid competition from nearby clonal plant species (Tischew et al. 2017).

What are the risks?

Although the inversion treatment resulted in the best growth and survival of sand jurinea in this experiment, this site prep method is extremely labor intensive and destructive to both the recipient site and the nearby site from which the new soil is extracted. Removal of 30 cm of topsoil is an example of the drastic methods to be avoided in plans to restore rare plant species in New Jersey.

3.4.2.1.2. Mudflat quillplant (Lilaeopsis masonii)

Mudflat quillplant (*Lilaeopsis masonii*) is a rare perennial dicot endemic to California where it is known from the Sacramento-San Joaquin River Delta and nearby shores of San Francisco Bay. It is a plant of freshwater marshes, brackish marshes, and other estuary habitats. It is threatened by development, flood control, and agriculture (Constance and Wetherwax 2012). In November 1979, only seven occurrences were known, and it was listed as a rare species under the California Endangered Species Act. Millions of dollars have been spent on mitigation and recovery projects for mudflat quillplant. Several mitigation projects to restore it across its range have failed because transplantation of this species requires disturbance of the habitat, leaving the newly established plants susceptible to being dislodged by flowing water.

Mudflat quillplant is able to colonize and persist on a wide range of substrates, from pure sand to riprap, and new populations can establish naturally when their inherently unstable bank habitat is left undisturbed. Due to these qualities, mudflat quillplant populations rebounded across the landscape in areas where its habitat was left alone. For example, across a six-mile part of the river that was monitored, its occurrences increased from 69 to 118, and its spatial extent more than doubled (Grewell et al. 2013).

In addition to not understanding its habitat requirements, researchers had not accurately documented occurrences of mudflat quillplant because its population ecology makes it tricky to monitor. Conventionally, known occurrences are recorded as present or absent and relocated in later years. However, closely spaced patches of mudflat quillplant "frequently merge through vigorous rhizomatous growth, turning two or more small occurrences into a single large patch" (Grewell et al. 2013) which were interpreted as fewer patches and thus a loss to the population. These inaccuracies confounded data analysis and led to the wrong conclusions as to mudflat quillplant population decline. Ultimately, the total area occupied is more important than the number of patch occurrences (Grewell et al. 2013). Only after adjusting the data collection protocol to fit the species' population dynamics could researchers obtain an accurate assessment of its status.

What are the risks?

Mudflat quillplant is currently known from seven California counties and occupies more than

1800 km^2 of estuarine habitat across the San Francisco Estuary. Yet millions of dollars were wasted in mitigation and restoration projects because researchers lacked both a complete understanding of its habitat (undisturbed yet naturally unstable riverbanks) and an appreciation for its population ecology (the merging of small patch occurrences). This example shows that a thorough scientific foundation must underpin reintroduction projects (Grewell et al. 2013).

3.4.2.1.3. Simplicia laxa

Simplicia laxa is a grass species that is critically threatened in New Zealand. It is currently known from fewer than 15 populations, several of which are in decline, and most populations are small. Many of the known populations occur on unprotected private land or in places threatened by invasive weeds and habitat loss. It easily propagated from rooted pieces and can also grow from node cuttings (de Lange 2020).

In 2010, mature plants were collected from the wild, divided, and vegetatively propagated in a shade-house "to generate sufficient individuals for replication across different microhabitats and sites" (Lloyd et al. 2018). *S. laxa* was sourced from one site, propagated in 2010, and outplanted in 2011. Because it grows slowly in cultivation, there were only 36 plants available for planting, so it was only outplanted to one site. *S. laxa* was planted in each of three microhabitats at the chosen site: beneath rock overhangs, beside (directly adjacent to) rocks but not under overhangs, and in the open at least 1 meter away from rocks. Researchers collected five years of monitoring data from 2011 through 2017 (Lloyd et al. 2018).

At the same time, the researchers collected, propagated, and outplanted another rare species, *Carex inopinata*. They were experimentally testing new micro-habitats for both *S. laxa* and *C. inopinata*. *Simplicia laxa* did not successfully establish; all plants died after several years. Researchers speculate that the habitat at the recipient site may not have been a good match for the genetic ecotype of the source material. However, they were more successful with *C. inopinata* at all stages of the experiment. *C. inopinata* was collected from two source populations; it was allowed more time to propagate and it grew faster, so there were 74 individuals available to outplant, and each founder population was comprised of individuals from the two source populations (Lloyd et al. 2018).

What are the risks?

In rare and declining populations, removing whole mature plants seems like an unnecessary risk to remnant populations; seeds or cuttings would have been less detrimental. Not enough time was allotted to the propagation process; *S. laxa* grew slowly in cultivation and there was limited material to work with, yet the decision was made to proceed with the outplanting. Additionally, sourcing material from more populations and waiting to have enough to outplant at more than one site would have provided an opportunity to tease out the cause of the reintroduction failure. Instead, it appears that the successful process implemented for *C. inopinata* was not used for *S. laxa*, potentially because of a desire to plant and monitor *S. laxa* within the same timeframe as *C. inopinata*.

3.4.2.2. Lower risk reintroduction projects

The defining features of lower risk reintroduction projects include:

- Scientific and experimental frameworks
- Collaborative partnerships
- Site management and threat removal
- Long-term monitoring commitment

Lower risk projects are not always successful at establishing the new rare plant population, but they are thoughtfully planned, make efficient use of resources, and report on the process and the outcome.

3.4.2.2.1 Limestone glade milkvetch (Astragalus bibullatus)

Limestone glade milkvetch (*Astragalus bibullatus*) is a federally endangered, slow-growing, perennial forb that occurs on limestone cedar glades in Tennessee. It is known from eight sites with fewer than 5000 individuals in total (Albrecht and Long 2019). It is threatened by habitat destruction and fragmentation caused by commercial and residential development (Albrecht and McCue 2010). Most natural populations are small with declining growth rates (Albrecht and Long 2019).

Albrecht and McCue (2010) report on the first reintroduction attempt. They experimentally reintroduced seedlings to test how survival and reproduction was affected by the use of three different source populations, five recipient sites, and seasonality of the outplanting. Monitoring of the plantings took place over seven years. While source population was not influential, outplanting in the fall led to greater survival and plants were more likely to reach reproductive maturity. Despite apparent success in the early years, all individuals ultimately died. Possible causes for this failed attempt were poor habitat suitability, herbivores, or small founder size (Albrecht and McCue 2010).

Limestone Glade Milkvetch (*Astragalus bibullatus*)

Building upon the information learned from the first reintroduction attempts, Albrecht and Long (2019) designed a second experiment that included protection from herbivores, thinning woody plant canopy, outplanting in a wider range of microhabitats, and increasing the founder population. Monitoring took place for five years after planting and is ongoing. Herbivore exclusion and microhabitat were the most influential to the limestone glade milkvetch; survival rates and fruit production were greater in the xeric barren ecotones than in the mesic ecotones. This second reintroduction attempt will continue to be monitored.

These two rare plant reintroduction projects for the limestone glade milkvetch were lower risk because they:

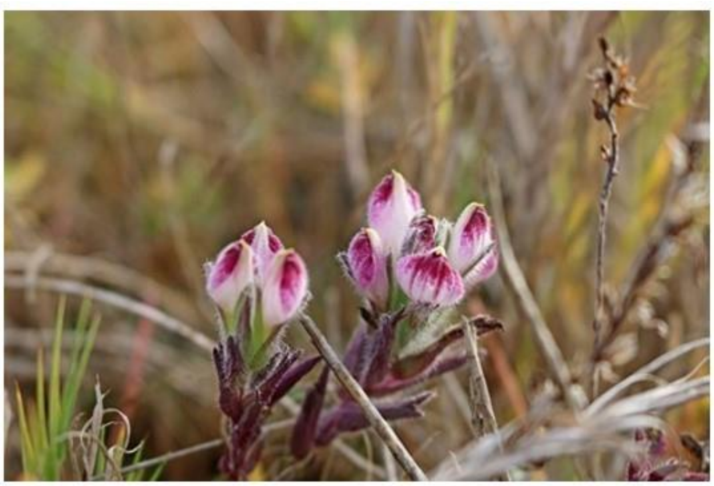
- Used well-planned experimental designs and tested hypotheses
- Were monitored long-term
- Used adaptive management (built upon information learned from previous reintroduction attempts)
- Thoroughly reported on the first attempt even though it resulted in failure

3.4.2.2.2 Salt marsh bird's beak (Chloropyron maritimum **subsp.** *maritimum)*

Salt marsh bird's beak (*Chloropyron maritimum* subsp. *maritimum*), is a hemiparasitic annual forb native to southern California and Mexico. It occurs in coastal saltmarshes (Calflora 2020). As a hemiparasite, it needs host plant roots to parasitize.

It was listed as endangered under the Endangered Species Act in 1978 and the California State Endangered Species Act in 1979 (Wallace 2009). Salt marsh bird's beak was extirpated from the Sweetwater Marsh, San Diego Bay National Wildlife Refuge in California, in 1988. A reintroduction attempt began that same year (Noe et al. 2019). Seeds were collected from a nearby site and sown in 30 separate patches of high marsh with canopy openings each year from 1990 to 1993. Ten surveys (total counts) were conducted during peak flowering over a 26-year period (Noe et al. 2019).

Over time, experimental data and adaptive management helped researchers get a better understanding of the biology and ecology of salt marsh bird's beak. They discovered that pollination was a limiting factor, so seeding locations were moved to topographically variable remnant salt marshes to accommodate for the habitat needs of the preferred pollinator, solitary burrowing bees. Salt marsh bird's beak needs a short and open canopy of native perennial plants; thinning the canopy of competing vegetation increased its density and flower production. The species also requires native perennial plant roots as hosts to parasitize; non-native annual grasses were found to be inadequate hosts because they die before salt marsh bird's beak sets seed. Finally, boom and bust population cycles of salt marsh bird's beak can be dramatic and worrisome, but the long-term monitoring dataset showed they are explained by changes in rainfall, temperature, salinity, and tidal amplitude associated with an 18.6-year lunar event cycle. (Noe et al. 2019).



Salt Marsh Bird's Beak (*Chloropyron maritimum* subsp. *maritimum*)

In 2016, the reintroduction site had a resilient, genetically diverse population. "Boom" years of peak abundance can have upwards of 14,000 individuals, while in the cyclical "bust" years the population may be reduced to fewer than 100 plants. However, its narrow ecological requirements make salt marsh bird's beak vulnerable to the effects of climate change and specifically sea level rise (Noe et al. 2019).

This project is a great example of using an adaptive management approach; they used monitoring and experimentation to test management-relevant hypotheses. Annual meetings among biologists and collaborators took place to decide on how to proceed throughout the science-based iterative process to restore salt marsh bird's beak, and decisions were based on research findings (Noe et al. 2019).

Reasons this was a lower risk reintroduction project:

- Used a well-designed experimental framework to test hypotheses
- Collaboration among experts from different fields of biology
- Incorporated principles of adaptive management to create better habitat
- Commitment to long-term monitoring and continued investment in the project's success

3.4.2.2.3 Tennessee purple coneflower (Echinacea tennesseensis)

Tennessee purple coneflower (*Echinacea tennesseensis*) is a perennial forb endemic to central Tennessee. It is most abundant along the ecotone of glade/barrens complexes in soil depths between 5-12 cm. It is shade intolerant, so it's less abundant in deeper barren soils where it must compete for sunlight with woody species (Bowen 2011). It has always been rare due in part to its specialized habitat and short distance seed dispersal (Mosby 2014). Threats to the species are over-collection, succession of the glade habitat, and loss of habitat due to development (Mosby 2014). In 1979, Tennessee purple coneflower became one of the first plants to be listed as federally endangered (Walck et al. 2002).

Tennessee Purple Coneflower (*Echinacea tennesseensis*)

A reintroduction project established 15 subpopulations in glades close to and within natural subpopulations. These plantings took place on state park, state forest, and US Army Corps of Engineers properties. Researchers found that the species has naturally low levels of genetic diversity so the seed can be mixed from multiple subpopulations without fear of genetic depression. Additionally, an experiment found that survival was not dependent on the site from which the seed was sourced (Walck et al. 2002).

As of 2011, there were 920,279 plants in both natural and introduced subpopulations (Bowen 2011). Reintroduced populations are successful in terms of viable seed production and they have the same or greater seed set as the natural colonies (Mosby 2014). Tennessee purple coneflower has been delisted thanks to the efforts of many organizations in conducting population monitoring, seed collection and preservation, implementing the reintroduction project, and continued site management (Walck et al. 2002).

Collaboration among government agencies, private companies, and non-profit organizations was integral in saving Tennessee purple coneflower from extinction. Many of the natural coneflower sites occurred on privately owned property and long-term protection was not likely unless the land could be acquired by government agencies or conservation organizations. The Tennessee Natural Areas Program (TNAP) and the Tennessee Chapter of The Nature Conservancy partnered to purchase four sites which became Designated State Natural Areas (DSNAs). The Nashville Super Speedway Inc. provided TNAP with a conservation easement to protect another natural area. Assistance in purchasing natural areas also came from American Airlines, Bell South, and the USFWS Recovery Land Fund. The Missouri Botanical Garden and other plant nurseries conducted *ex situ* seed propagation. The Tennessee Natural Heritage Program monitors the plant populations. TNAP manages the glades and barrens by conducting prescribed burns, bushhogging, and controlling invasive species. The U.S. Army Corps of Engineers, the National Park Service, and the Tennessee Division of Forestry assist with managing Tennessee purple coneflower on the public lands that are also DSNAs (Bowen 2011).

Reasons this was a lower risk reintroduction project:

- Prior research addressed questions about genetic diversity
- The project was designed as an experiment to test a hypothesis about source sites
- Collaboration among many groups assisted with funding, land acquisition, and logistics of continued land management

4. Rare Plant Reintroduction Proposals

Proposals to reintroduce rare plant species should adhere to a standard set of criteria. These criteria may be updated in the future based on new information as the science progresses. Each case will first be considered justified or not and depending on whether other viable options exist (Section 4.1 below). If justified, each proposal should address each of the points in Section 4.2.

4.1. Justifying Rare Plant Reintroductions

4.1.1. Situations where it may be justified

While it should be the option of last resort (Georgia Plant Conservation Alliance [GPCA] 2008), there are situations that call for rare plant species propagation and reintroduction. For example, *in situ* conservation can't be guaranteed at sites where the effects of climate change are too severe, as in the case of rising sea levels (Ensslin et al. 2015).

In cases where habitat destruction or land-use change cannot be prevented (Fahselt 1988) due to development, the project is referred to as a rescue or a mitigation (IUCN/SSC 2014). A rescue is different from a mitigation: in rescue loss of the population cannot be avoided or is inevitable (no legal protections prevent the development from destroying a population), ; mitigation is mandated to occur and the developer pays for the mitigation project in exchange for being allowed to proceed with their development plan.

The CPC (2019) provides a guide to situations in which a rare plant reintroduction program may be justified. A reintroduction may be justified if the species is already extinct in the wild, but genetic material has been saved *ex situ* and suitable habitat is available for outplanting. If there are remaining extant populations, a reintroduction project may be justified if the following conditions are met:

- The distribution of the species is known and there are few, small, and declining populations, and;
- Alternative management options have been considered and conducted, yet have been judged to be inadequate for long-term conservation of the species, and;
- Threats have been identified, and;
- There is high risk of extinction if *in situ* management is the only method used to restore the species (CPC 2019).

Hawaii has many examples of rare plant reintroduction projects that were justified because extinction was imminent. For example, alani (*Melicope knudsenii*) is an endemic species reduced to one wild individual and one in cultivation; it produces fruit, but insects predate the seeds preventing them from reaching maturity. Another example is Hawaiian pricklyash (*Zanthoxylum hawaiiense*). The only three remaining individuals of this species were isolated from each other and not within the range of natural pollinators. Recovery efforts for these two rare plant species have seen early successes (Luna 2018).

Hawaiian Pricklyash (*Zanthoxylum hawaiiense*)

4.1.2. Situations where it may not be justified

The CPC (2019) also provides a guide to situations in which a rare plant reintroduction program may not be justified.

A reintroduction may not be justified if:

- Reintroduction will undermine the imperative to protect existing sites
- High-quality, diverse source material is not available
- Previous tests indicate that it has not been possible to propagate plants or germinate seeds
- Existing threats have not been minimized or managed
- Suitable habitat is not available, or sufficiently understood
- The reintroduced species may potentially negatively impact species in the recipient site via competition, hybridization, or contamination
- There is evidence that the reintroduced taxon would harm other threatened and

endangered species or conflict with their management
- The reintroduction is not supported legally, administratively, or socially

Additional guidelines from the IUCN/SSC (2013) state that "any source population should be able to sustain removal of individuals/propagules, and removal should not jeopardize any critical ecological function, except in the case of an emergency or rescue removal."

4.2. Topics a Proposal Must Address

This section briefly reviews the topics that must be addressed in a rare plant reintroduction proposal. We describe the ecological underpinnings of why each point is important to consider and give examples from real-world reintroductions. This is not intended as a how-to for reintroductions or proposals; please refer to the Resources (Section 5.2) and References (Section 6) for guidance on reintroduction project design.

4.2.1. Conservation status

Rare plant reintroductions begin with gathering information on the target species' distribution and population demographics to obtain a clear and thorough picture of its conservation status (Fenu et al. 2016; Volis 2015). Documenting the current distribution of the species entails obtaining or creating current maps of the present and historical populations and how they relate to ecoregions and patterns in geology, soils, hydrology, and other habitat features (CPC 2019; Maschinski and Haskins 2012; Volis 2015). An updated inventory of the existing populations tells researchers if the populations are increasing, decreasing, or stable (Schemske et al. 1994; Volis 2015). Detailed inventories of each population include counts or estimates of the percentage of reproductive, juvenile, and seedling stages, and measures growth and reproduction (CPC 2019). These details are important because recovery efforts will be most efficient if they focus on the life history stages that have the greatest impact on population growth (Schemske et al. 1994).

> "For even the most intensively studied rare species, we often lack the detailed understanding of their biology and microenvironmental requirements that is needed for successful reintroduction" (Albrecht and Long 2019).

4.2.2. Biology and ecology

Autecological research – the biology, habitat needs, and the biotic interactions of a species – is critical to sound conservation science (Schemske et al. 1994), as is an understanding of the species' genetic structure (Ouborg et al. 2006). Rare plant species reintroduction projects especially require a deep and sophisticated understanding of the biology and ecology of the species involved (Falk et al. 1996) and must be preceded by thoroughly researching the species' biology (Maschinski and Haskins 2012). For instance, an understanding of life history traits will determine the minimum viable population size needed for reintroductions (Godefroid et al.

2016), while interspecific interactions may be important in establishing or maintaining populations (Ehrenfeld 2000). However, the lack of data on key biological and ecological requirements is the primary cause for most of the failures in species recovery plans (Heywood and Iriondo 2003; Rigg et al. 2017). Therefore, researchers must do a thorough review early in the planning process. We often don't have comprehensive knowledge of rare plant species, although it may not be essential that absolutely every detail is understood prior to attempting a reintroduction (MD PRTF 1999). Where important biological information is lacking, researchers should attempt to incorporate what is known into the experimental design of the reintroduction plan (CPC 2019). If important parts of the species' biology, ecology, or causes of decline or extinction are unknown, these can be tested experimentally as part of the reintroduction project. Researchers should consider multiple hypotheses, test them, and use the results to guide the project towards success (IUCN/SSC 2013).

The following sections are four broad categories to research:

- basic biology, including population biology and reproductive biology
- genetic structure
- habitat requirements
- interspecific interactions

4.2.2.1. Reproductive and population biology

Understanding the target species' basic biology is vital to recovery work (IUCN/SSC 2013; MD PRTF 1999). However, too often there is insufficient knowledge about the reproductive biology of rare plants to develop successful propagation methods (Kunz et al. 2014). Specific topics of study can include growth form (Fenu et al. 2016), short- and long-term survival (Albrecht et al. 2019), reproductive biology (Ehrenfeld 2000; MD PRTF 1999), clonal propagation ability (Godefroid et al. 2016), fertility (Albrecht et al. 2019; Fenu et al. 2016), seed production (Godefroid et al. 2016), dispersal mechanisms (Fenu et al. 2016; Godefroid et al. 2016), germination (Fenu et al. 2016), recruitment (Albrecht et al. 2019), and population establishment (Albrecht et al. 2019).

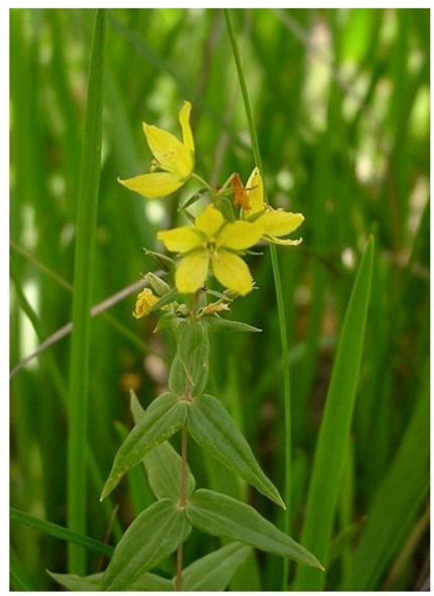
Rare species may have atypical reproduction biology which will be important to understand (Brumback et al. 2004). Seedling recruitment can take over a decade for some long-lived perennials (Albrecht et al. 2019); if unaware of these long time-lags between episodic seedling establishment, researchers might decide a reintroduction project is a failure too early in the process. The lack of knowledge about the morphological characteristics of different age classes or life stages may lead to an inability to accurately discriminate between seedlings versus mature plants or plants emerging from prolonged dormancy in the wild, resulting in incorrect inferences about the extent of seedling recruitment and/or the need for interventions (Kelly 2006).

4.2.2.2. Genetic structure

A primary goal for restored rare plant populations is for them to be adapted to local conditions and "resilient to environmental change" (Albrecht et al. 2019). In general, genetically diverse populations are better able to adapt and persist through yearly fluctuations in weather, competition, soil conditions, and disease than less diverse populations (Basey et al. 2015). Some research has shown that more genetically diverse populations can also provide benefits to the wider ecosystem by increasing nutrient retention and supporting more abundant and diverse animal communities (Basey et al. 2015). As important as population-level genetic diversity is, it may not be readily apparent. Clues to genetic diversity can be seen in the variation of plant size, early or late flowering times, and in the differing number of flowers and seeds produced. Other genetic variations may only be visible when stressful conditions, such as drought or disease outbreak, lead to death or survivorship of individuals within a population (Basey et al. 2015).

However, while genetic diversity on average may confer benefits for adapting to changing environments, this is not always the case. Plant species inhabiting island environments, fugitive species, and others capable of self-pollination frequently have lower genetic diversity than continental or other populations without exhibiting clear detrimental effects. Equally important to genetic diversity, moreover, is the significance of locally-adapted genotypes for conservation and reintroduction. The genetic composition of local populations typically develops through long-standing interactions with local environments, yielding genetic and phenotypic characteristics that are better adapted to them. In either case it is important to consider the genetic structure of local populations and to reflect and integrate these conditions into the selection of propagules for reintroduction.

Roughleaf yellow loosestrife (*Lysimachia asperulifolia*) is a federally endangered species found in North and South Carolina and is threatened by fire suppression, drainage and conversion of habitat, and development. Rhizomes were harvested from two populations, including one that was to be destroyed by a highway expansion project. Researchers at the North Carolina Botanical Garden tested propagation methods in a greenhouse and later outplanted some of the material to new sites. Previously thought to be a perennial (lives more than 2 years), this study found that it actually has a pseudoannual lifecycle, in which the above and belowground structures only live for one year, but the rhizomes split off underground to form new plants (ramets) the next season. Recognizing this uncommon life cycle has helped researchers to understand the observed patterns of population growth despite the lack of sexual reproduction (Kunz et al. 2014).



Roughleaf Yellow Loosestrife (*Lysimachia asperulifolia*)

The U.S. Fish and Wildlife Service (USFWS 2000) does not require – but strongly recommends – having genetic studies done whenever possible, recognizing that there are situations when it is unrealistic, such as when "acute conservation needs may legitimately outweigh delays that would be incurred by such a requirement." The CPC (2019) recommends conducting genetic studies if there could be issues with hybridization or if it is difficult to distinguish the target species from a congener, if the species looks different in different locations, or if one or more populations occupies a distinct habitat from the majority of populations. Important facets of the target species' genetics can include the amount and distribution of genetic variation (Fenu et al. 2016; Godefroid et al. 2016) within and among collection sites, subpopulations, populations, and metapopulations (CPC 2019; Ehrenfeld 2000; Godefroid et al. 2016).

4.2.2.3. Habitat requirements

Identifying suitable habitat is critical to the successful establishment of sustainable populations (Maschinski and Haskins 2012; Noël et al. 2011). Inappropriate habitat is a primary cause of rare plant reintroduction failure (Godefroid et al. 2011; Noël et al. 2011), so proposed recipient sites must be thoroughly evaluated to determine if they meet requirements of the target species (IUCN/SSC 2013). However, insufficient time is often devoted to conducting a detailed evaluation due to tight project deadlines (Fahselt 2007). There are a multitude of abiotic habitat features that may be important, such as the amount and quality of sunlight, moisture, type of soils and bedrock, slope and aspect, temperature extremes in summer and winter, and disturbance regime.

Additional complications arise when attempting to characterize the niche of a rare species if it is only known from a few sites that differ in community composition and vegetation structure (Albrecht and Long 2019). Researchers must question if any of the current habitats are ideal or if they have been so degraded it has led to the species' decline.

Habitat requirements may be extremely subtle and difficult to quantify (Albrecht and Long 2019). For example, the type of substrate can be very influential, as it is for the rare limestone glade milkvetch which can only grow on well-drained soil and needs a specific type of limestone, even though there are many other types of limestone in its range in Tennessee (CPC 2019). Ruth's golden aster (*Pityopsis ruthii*) – another rare plant in Tennessee – is most successful when planted in rock crevices 10–15mm wide and greater than 1 cm deep (Wadl et al. 2018); these precise conditions closely match those where Ruth's golden aster naturally establishes. Results from a project in Switzerland attempting to reintroduce 25 rare wetland plant species found that success was primarily determined by habitat similarity between the source and recipient sites, outweighing the effects of founding population size and species traits (Noël et al. 2011). Insufficient knowledge about the microhabitat preferences also contributed to the repeated failures of reintroduction efforts by different researchers for chaffseed (*Schwalbea americana*) in New Jersey. Subsequent research into the fine-scale soil, water, and plant community associations of the species in the wild led to the first successful propagation and reintroduction of chaffseed more than 10 years after recovery efforts began (Kelly 2006).

Natural and human created disturbance regimes play an important role in creating habitat for rare species. “In order to meet a variety of restoration goals, it is sometimes necessary to harness human activities and presence” to perpetuate habitats (Ehrenfeld 2000). Examples include the integration of grazing, prescribed burning, and/or mowing to maintain the open, treeless habitats required by prairie species, or the use of active timber management to provide the structure and light environment that many early-successional species need (Porneluzi et al. 2014).

The degree to which these activities are sufficient to provide or maintain suitable habitat for all aspects of the target species’ life cycle is not necessarily clear and should not be assumed or taken for granted. Additionally, the timing, frequency, intensity, and extent of these practices and/or initial conditions of the site may yield variable results and effectiveness in meeting project goals. Such interventions may also result in significant collateral damage to other species, or even counterproductive results to those intended. Careful study, monitoring and adaptive management are therefore needed for responsible implementation of these types of management practices.

4.2.2.4. Interspecific interactions

Other species play a role in the growth and survival of reintroduced plants (Guerrant and Kaye 2007), so rare plant reintroduction proposals must take into account the plethora of interactions that occur between rare plant species and the other organisms they rely upon. These interactions include pollinators, seed dispersers, symbiotic mycorrhizal fungi, hemiparasitic and parasitic plants, competition between plants, and plant consumers (herbivores).

Insect partners provide important functions, so pollinator baiting is used to verify the presence of pollinators at a particular site before outplanting (Reiter et al. 2016). If the obligate pollinators or seed dispersers are not present at the recipient site, the reintroduced species is at risk of losing reproductive fitness or ability to spread effectively and sustain itself at the new site (CPC 2019; Parkin 2005). For example, the rare Willamette daisy (*Erigeron decumbens*) is thought to be limited by two pollination issues. First, it occurs in small habitat fragments where pollinator abundance tends to be lower. It also has very small populations of fewer than 40 individuals; when the plant population size is small there is increased chance of generalist pollinators transferring pollen from different plant species (Thorpe and Kaye 2011).

Other plants are important players in rare plant reintroductions. Competition with other plants is often an issue in restoration projects; for example, Piper’s daisy reintroductions were more successful where competition was reduced by thinning native bunchgrasses and controlling invasive plants (Link and Cruz 2015). Restoration of parasitic and hemiparasitic rare plants is tricky as it requires presence of the host plants in the right structure and composition to benefit the target species (Grewell et al. 2013). Golden Indian paintbrush (*Castilleja levisecta*) is an example of a federally listed rare species that is a root parasite that gains advantage from proximity to its host plants (Haase and Rose 2001). Chaffseed prefers species with abundant roots near the surface (Kelly 2006; Helton et al. 2000). For some rare parasitic species, the specific host plant species may need to be companion-planted along with the target species (Guerrant and Kaye 2007).

Soil organisms can be a cryptic yet important piece of the specialized habitat requirements of rare species (Albrecht and Long 2019). Soil microorganisms such as beneficial microbial partners and mycorrhizal fungi are important in shaping plant communities, and proper attention to soil communities has been shown to benefit restorations and reintroductions (Michaelis and Diekmann 2018; Rigg et al. 2017). The benefits are numerous and include increased establishment, growth, nutrient and water uptake, protection from herbivores, resistance to root pathogens, resilience to environmental stress such as drought, improved photosynthetic efficiency, and overall better survival (CPC 2019; Maschinski and Haskins 2012; Rigg et al. 2017). For example, in a reintroduction of Kincaid's lupine (*Lupinus sulphureus* var. *kincaidii*), individuals inoculated with nitrogen-fixing symbiotic bacteria were more likely to flower than uninoculated controls (Guerrant and Kaye 2007).

It is worth examining the history of the reintroduction and propagation of American chaffseed in New Jersey in more detail, because it illustrates how important knowledge of all phases of a species' biology and ecology are to a successful reintroduction effort. This hemiparasitic species in the Orobanchaceae (broomrape) family is listed as endangered in both the United States and the state. The New Jersey population of the species is the last of approximately 19 historic occurrences once known from the state, and one of only two remaining in the northern half of the species' range from Cape Cod, Massachusetts, to Fort Bragg, North Carolina. Systematic monitoring and management of the population has been conducted each year since 1993 by the New Jersey Department of Environmental Protection, Office of Natural Lands Management, through a cooperative agreement with the U.S. Fish and Wildlife Service (NJDEPE 1993) and consistent with a federal Recovery Plan for the species (USFWS 1995). These activities consisted primarily of an annual summer census to determine the status of the population, habitat management to maintain suitable habitat conditions, and biological research, propagation and reintroduction activities aimed at advancing its recovery.

American Chaffseed (*Schwalbea americana*)

From 1993 to 2000, this last New Jersey population of chaffseed numbered only 58 to 144 individuals, and extensive field and greenhouse propagation efforts were conducted seeking both to augment the population and reintroduce it to known historic sites (Obee 1995, Obee and Cartica 1997, Yurlina 1998, Cartica et al. 1999, Van Clef 2000, Determann 2001, Van Clef 2001). These efforts were ultimately unsuccessful at producing even a single viable plant for reintroduction. Although the seeds tended to germinate without difficulty, most seedlings reached no more than a few centimeters in height, and few survived beyond their first growing season. A variety of potential host species, soil types, seed storage conditions, and other treatments were used (Obee 1995, Yurlina 1998). Similar problems plagued efforts to propagate chaffseed from seed in the field. In the largest of the seed addition experiments (Van Clef 2000), only 35 of 9,000 seeds planted adjacent to an existing chaffseed colony germinated, and none of these survived. Similarly, no germination was observed in two other experiments at this location (Obee 1995, Yurlina 1998), in seed plots placed adjacent to existing colonies (Yurlina 1998), or at two other historic chaffseed sites in Wharton State Forest, where a total of 800 seeds were planted (Cartica et al. 1999). In another case, only five of 155 seedlings potted with and without little bluestem (*Schizachyrium scoparium*) host plants in the field survived until the end of the growing season and none returned the following year (Obee and Cartica 1997).

In 2001, however, the chaffseed population suddenly increased to over 600 individuals, which it maintained for the next four years. While the increase itself was likely due to a delayed response to prescribed burning which took place in 1999, it also offered a unique opportunity to identify habitat conditions that were favorable to the species (Kelly 2006). After the increase, the population exhibited a distinctly clustered distribution that was not noticed in previous years, and examination of the soil, water and plant community conditions present found significant differences in the chaffseed clusters compared to adjacent sites. Subsequent experiments found that soil outside the clusters inhibited chaffseed germination, for example, and certain herbaceous plant species in the Asteraceae (especially *Chrysopsis mariana,* Maryland golden aster) were found to be preferred hosts for chaffseed compared to other woody or grass species. By incorporating these conditions into greenhouse propagation efforts, researchers were able to successfully grow the plant for the first time in more than 10 years, and they also proved to be useful as indicators for identifying suitable locations for outplanting of chaffseed at historic and other sites. These efforts, first conducted in 2006 and 2008, resulted in approximately 50% survival of initial plantings after 10 years, as well as successful growth, flowering and seedling recruitment at two out of three colonies, achieving all the biological benchmarks for successful reintroduction. Efforts are now underway to expand these activities to establish additional populations at other sites across its historic range in New Jersey.

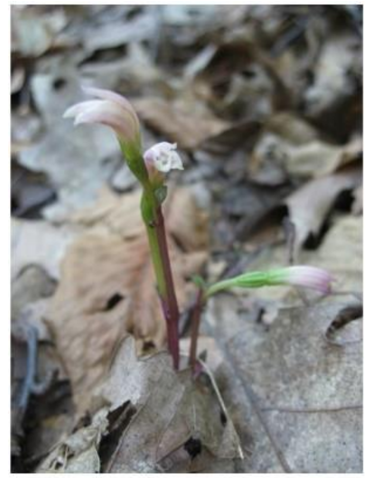
Maryland Golden Aster (*Chrysopsis mariana*)

4.2.3. Reason for rarity

Researching threats to species is another task for the early planning stages of plant reintroduction (Maschinski and Haskins 2012). In order to successfully restore a species, we must understand the factors at play in its decline (Maschinski and Haskins 2012). The biological traits and life history of the species should be taken into account when assessing threats (IUCN/SSC 2013). If the species has been absent from a site, additional threats may be present that were not there before and did not lead to the species' rarity, so researchers need to consider what caused extirpation in the past and what present threats may exist at the reintroduction site (IUCN/SSC 2013). Threats to the target species may differ at different geographic scales and throughout the year in different seasons (IUCN/SSC 2013).

Human-caused rarity is sadly all too common. Changes to the habitat are often a cause of decline, for example fire suppression, wetland draining, introduced invasive species or pathogens, conversion of habitat, development, and climate change (Gray et al. 2019; Kunz et

Orchids are especially prized. Species native to North America were featured in this collection of stamps recently issued by the US Postal Service.



Three Birds Orchid (Triphora trianthophora), a State Endangered Species, was one of the species highlighted in the Postal Service's wild orchid collection.

al. 2014; VA DNH 2008). Species also become rare when they are loved to death by humans – when we prize a species for what it gives us socially, aesthetically and economically, and by over collecting for the horticulture trade (BGCI 2015). Robbins' cinquefoil (*Potentilla robbinsiana*) became rare when it was over collected for herbaria specimens and hikers trampled its main habitat on Mount Washington in New Hampshire (Brumback et al. 2004).

Other types of rarity are inherent or artificial rarity. Inherently rare species are rare because they occupy rare habitats or microhabitats (Volis 2015) like sinkhole ponds, seepage bogs, or very specific substrates such as serpentine soils (VA DNH 2008). Artificially rare species may be classified as rare at the state-level simply because a political boundary captures the edge of its range. For many species with artificial rarity, it's acceptable to be rare within a political boundary provided their populations and habitat are secure, stable, and protected. Researchers must determine if the species and/or its habitat is truly declining or in danger.

4.2.4. Resources

In natural resources management, there is often a struggle for the resources necessary to research, plan, implement, monitor and complete projects. With limited funds and personnel, administrators are forced to make hard decisions about how to prioritize the many worthwhile goals and projects presented to them (USFWS 2017). Funding, expertise, and manpower are three often limited resources that are needed in long-term conservation planning.

4.2.4.1. Funding

Long-term funding is necessary for a reintroduction project, and planners must estimate the costs and secure adequate funding to support the project through the phases of planning, propagation, outplanting, monitoring, and management of the restored population, possibly for decades after the initial establishment (CPC 2019; IUCN/SSC 2013). Because of the long timeframe of reintroduction projects, it's expected that the plan will need to be revised over time, and budgets should be flexible enough to accommodate reasonable changes (IUCN/SSC 2013), especially in the context of an adaptive management plan.

An economic analysis is usually not included in publications on reintroduction projects, but federally endangered species recovery project plans do provide the estimated costs for each fiscal year of the project (USFWS 2020). Although authors don't typically include the costs associated with their projects, Fenu et al. (2016) detailed the costs of reintroducing the rare *Dianthus morisianus* on coastal dunes in south-west Sardinia. They report that 80% of funds in the first five years went to erect fencing to protect the plants as they established at the new site to protect them from herbivores and human disturbance (Fenu et al. 2016). Dunwiddie and Martin (2016) mention the cost comparison of using seed (about $0.30 per 1000 seeds) versus seedlings (about $3.00 each) in restoring golden paintbrush. Balancing costs against survivorship and long-term success is a calculation that cannot be ignored for any project.

4.2.4.2. Expertise

For rare species reintroduction to be effective it must be a collaborative effort among experts in multi-disciplinary biological fields (Allen 1994; IUCN/SSC 2013). If the target species doesn't have an official recovery team, then the principal investigators of the reintroduction project should consult with experts in relevant fields with biological and technical expertise (Allen 1994; Clements 2013; IUCN/SSC 2013). Experts in population ecology, genetics, and propagation (Reiter et al. 2016) can often provide information about the target species, plant associates, and conspecifics (MD PRTF 1999) and offer input to improve protocols (Clements 2013). For example, an entomologist would be a valuable collaborator when reintroducing a species that is dispersed by a particular ant species; a mycologist should be consulted for a species that requires fungal symbionts (Allen 1994).

In addition to biological subject matter experts, it's important to include land managers, restorationists, and other stakeholders from various local conservation organizations, NGOs, institutions, and government agencies (Clements 2013; Maschinski and Haskins 2012; Parkin 2005; Reiter et al. 2016). With open communication, and perhaps direct involvement in implementation, these connections help ensure that individual projects fit seamlessly within the broader conservation objectives of the region (Parkin 2005).

4.2.4.3. Laborers/manpower

One resource that cannot be underestimated is having a labor force that is willing and able to conduct the work. Seed collecting, outplanting, and monitoring are often very labor intensive and time consuming. Students, volunteers, land managers, and other stakeholders can be enlisted to assist if they are properly supervised and trained in all protocols (CPC 2019). It can be both strategic and rewarding to involve the public and volunteers from local environmental groups in order to foster shared goals and encourage their investment in the project's success (Hanson and Nelson 2015).

4.2.5. Legality

Rare plant reintroductions should have the approval, when required, of the government regulatory agencies in the region as well as the landowners in charge of the property where the seed is collected from and where the outplanting will take place (Haase and Rose 2001). Researchers should make sure they are in compliance with all laws and policies regarding rare plant species and obtain the required permits (IUCN/SSC 2013).

Few authors put such details in their publications, but Gilser and Duncan (2011) were transparent about the legal hurdles they faced in their efforts to reintroduce Nelson's checkermallow (*Sidalcea nelsoniana*) in Oregon. They faced numerous roadblocks such as gaining permits to use specific sites for rare plant reintroductions and to use prescribed fire, but eventually were able to fulfill all the legal requirements and implement the project (Gilser and Duncan 2011).

4.2.6. Sourcing plant material

Sourcing rare plant material for a reintroduction project takes thoughtful research and planning and a thorough understanding of the origin and type of material (Fenu et al. 2016). Whether propagules are sourced from remnant wild populations or *ex situ* collections, there are many issues to consider. Decisions around selecting the source population(s) and collecting seed should not be taken lightly as each decision can affect the outcome of the reintroduction (Godefroid et al. 2016; Maschinski and Haskins 2012). Researchers must consider: 1) for a species, which populations to sample from; 2) within each population, how many and which individuals to collect seed from; and 3) for each individual, how many seeds to collect (Volis 2015).

Ultimately, many of the choices come down to the need to balance preventing harm to the donor population with collecting enough material to ensure sufficient quantity of seed and genetic diversity for a successful reintroduction. Larger founding populations are more likely to survive, but this is limited by the availability of propagules and the impact of collection on the source population (IUCN/SSC 2013; Maschinski and Haskins 2012).

Decisions made when choosing the source individuals or propagules for reintroduction projects will define the new population's ability to adapt (Maschinski and Haskins 2012). Researchers may choose to mimic the genetic diversity seen in natural populations or infuse greater diversity into the new populations (Maschinski and Haskins 2012). Rare plant populations often suffer from low genetic diversity and fitness because of small population size and fragmented habitats (Ottewell et al. 2016; Volis and Blecher 2010). Reintroductions that use genetically diverse plant material tend to establish better and recover more quickly after disturbance (Basey et al. 2015). Species with high genetic diversity are better able to adapt and survive when faced with environmental changes and threats (Basey et al. 2015; BCGI 2015). A genetically healthy population is both large enough in number of individuals and has an adequate amount of gene flow to minimize the effects of genetic drift (Ottewell et al. 2016). Some research has shown that more genetically diverse populations can also provide benefits to the wider ecosystem by increasing nutrient retention and supporting more abundant and diverse animal communities (Basey et al. 2015).

Understanding the pattern of genetic diversity in the source population(s) is very important because if the source of propagules is not chosen carefully, the reintroduced population could end up suffering from inbreeding or outbreeding depression (Maschinski and Haskins 2012; Volis 2015). Inbreeding and outbreeding depression are primarily issues that affect outcrossing species, especially species that are self-incompatible, but rarely affect species that usually self-pollinate (Volis and Blecher 2010).

Inbreeding depression affects plant species whose populations have become isolated due to habitat degradation and fragmentation because they have a “high probability of mating between genetically identical or closely related genotypes” (Maschinski and Haskins 2012; IUCN/SSC 2013; Volis and Blecher 2010). As a result, the offspring suffer from a reduction in vigor, reproductive output, survival, and ultimately, they are less able to adapt to environmental change (Maschinski and Haskins 2012; IUCN/SSC 2013). These are the species that are “likely to benefit from enhanced gene flow opportunities” with distant populations (Reinartz 1995). Outbreeding depression is most often exhibited in species that are naturally rare and have historically occurred in isolated populations (Reinartz 1995). It is caused by the mating of individuals that originate from dissimilar or isolated habitats but have been planted in close proximity (Volis and Blecher 2010). Outbreeding depression is essentially the interbreeding of distantly related individuals that results in a generation with lower fitness than the parents (Maschinski and Haskins 2012; Volis and Blecher 2010).

At each step in the process – from sourcing, collecting, and cleaning seed to propagation and outplanting – there exists the potential for inadvertent selection that may reduce genetic diversity (Basey et al. 2015; Haase and Rose 2001; Heywood and Iriondo 2003; Vitt et al. 2016). Experts recommend that all rare plant recovery plans explicitly state how they will account for plant genetic concerns (Pierson et al. 2016). Rare plant genetics is often not included in reintroduction plans because of the perceived costs, underestimated benefits, and the genetic principles and processes may not be understood by the planners (Pierson et al. 2016). Genetic lab testing has become more accessible and affordable but is not always necessary; insight into locally adapted traits such as viability and fecundity can be gleaned from common garden or reciprocal transplant studies (Maschinski and Haskins 2012).

Each reintroduction project must decide whether to use seeds from one or more source populations, and there is no one rule of thumb to apply that will fit all cases (Guerrant and Kaye 2007; Maschinski and Haskins 2012). Using a single source population may be the best option when the goal is to maintain genetic integrity of specific adaptations to local conditions, as with inherently rare and isolated species (Maschinski and Haskins 2012). Sometimes only one source population has sufficient seed production to support a harvest (Guerrant and Kaye 2007). Alternatively, sourcing from multiple populations can mimic natural long-distance gene flow that may no longer occur due to fragmented habitats; it can increase genetic diversity and relieve inbreeding depression (IUCN/SSC 2013).

Sourcing material from the edge of the species' range versus the core could also affect the fitness of new populations. Populations at range limits may have "unique solutions to evolutionary pressures" such as increased seed dispersal (Guerrant et al. 2014). Additionally, sampling along an environmental gradient such as elevation may be important to capturing genetic variation (Guerrant et al. 2014; Volis 2015). Unique genetic diversity that confers competitive advantages may be found at degraded sites, or during stressful conditions such as a drought or disease outbreak (Basey et al. 2015).

How much seed to collect must depend on the size of the donor population and care must be taken to not over collect especially from small populations or those that produce few seeds (Parkin 2005). A common guideline is to collect from fewer than 10% of the individuals of a rare plant population (Farnsworth 2005); a more conservative guideline to collect from fewer than 5% of the population is used in cases where more caution is warranted. The amount of seed collected also has implications for conserving genetic diversity. A previous recommended rule-of-thumb was to collect 30 to 60 (~50) individuals per population as a minimum sample size to capture non-rare alleles (Hoban 2019). However, recent genetic surveys and computer modeling shows that this recommendation is rarely optimal; the ideal sample size depends on the species' traits and is often significantly different from ~50 per population (Hoban 2019). Hoban (2019) used simulations to provide guidance about the number of individuals from which to collect in order to preserve genetic diversity while accounting for the inevitable losses due to germination failure and disease.

Researchers must also decide when and how often to collect seed for rare plant reintroductions. They may opt to sample over the course of a season if flowering time is variable among individuals across a season (Volis 2015). Or they can choose to sample over the course of a few years to avoid hitting a population hard in one year; this is a good strategy for small donor populations with infrequent or limited seed production (Volis 2015). Both of these approaches will likely capture more genetic diversity than a single collection event (Guerrant et al. 2014). In narrow-leaved coneflower (*Echinacea angustifolia*), for example, researchers noticed genetic differences between seeds harvested from late-flowering plants and seeds from plants harvested earlier in the season (Guerrant et al. 2014).

Climate change introduces another wrinkle into the issue of appropriately sourcing rare plant material (Vitt et al. 2016). Increases in average temperature may cause the timing of seed maturation to become less predictable, and extreme drought or flooding may reduce seed viability (Vitt et al. 2016). For rare plants that are vulnerable to climate change, the practice of "predictive sourcing" attempts to balance using propagules adapted to current conditions and propagules that also hold the genetic diversity to adapt to the predicted future environmental conditions (IUCN/SSC 2013). Thus, planning for climate change must allow for sourcing seed outside of the predetermined 'local' area (Vitt et al. 2016).

With rare plant rescue or mitigation efforts, the phenology of the plant species and the timeline of the development project (e.g., highway expansion; utility right-of-way) will dictate what can be saved and in what form. If possible, mature seed should be collected and preserved. This may require requesting to delay the development schedule to wait for the plants to produce seed. Multiple years of seed collection is ideal but may not be realistic. The next priority is to rescue the whole plants, but again, the specifics will depend on the species' unique biology. Researchers must investigate details about transplanting methods and soil bacterial or mycorrhizal symbionts for the best chances of the plants' survival. Having prior agreements with any and all local agency, business, or organization that has nursery space and horticultural expertise would be good planning for rare plant rescues; botanical gardens and universities are often good options to partner with, as well.

4.2.7. Propagules and propagation

Reintroduction projects need team members that have expertise in propagation and cultivation procedures (Fenu et al. 2016). The specific propagation methods used will depend on the species' life history, characteristics of the recipient site, and costs and other logistics (CPC 2019). Most projects are able to use direct seeding, nursery grown seedlings, or older plants as opposed to requiring *in vitro* propagation methods. *In vitro* propagation is the vegetative propagation of plants using tissue cuttings. It is less invasive than removing whole plants (Reed et al. 2011), and might be appropriate for some species that produce no seeds or only a small amount of seed, have seeds with deep dormancy, or extremely slow seedling growth (Bunn et al. 2011; Pence 2011). Use of *in vitro* methodology has been successful in propagating several endangered ferns (Pence 2011).

4.2.7.1. Choosing seed or whole plants

Rare plant reintroduction projects must choose whether to use whole plants grown in a greenhouse or to broadcast seed at the new site (Halsey et al. 2017). Albrecht and Maschinski (2012) reviewed 174 plant reintroductions and found that older plants were significantly more likely to survive than seed or seedlings less than a year old. Whole plants tend to have higher survival, especially in stressful ecosystems like dunes (Godefroid et al. 2011; Albrecht and Maschinski 2012).

Ultimately, when seed is used to reintroduce a species, its germination success at the recipient site answers any question of germination being possible, while a project using seedlings or older plants as founders may require years to determine if seed germination is possible on site (CPC 2019).

Using seed is often most appropriate for annual and short-lived species (CPC 2019). Seeds are relatively inexpensive and usually fairly easy to store and transport (CPC 2019; IUCN/SSC 2013). However, dormancy-breaking treatments may be necessary (CPC 2019) and seed germination rates are usually 1-10% in the wild, so projects may require thousands of seeds (CPC 2019). Seeds have lower survival than seedlings (Kaye 2009), but using seed can be cheaper than seedlings, so the low establishment rate may be acceptable when taking overall costs into consideration (Kaye 2009). Ultimately, when seed is used to reintroduce a species, its germination success at the recipient site answers any question of germination being possible, while a project using seedlings or older plants as founders may require years to determine if seed germination is possible on site (CPC 2019).

Whole plants are more successful for species with intermediate lifespans such as herbaceous perennials (CPC 2019). If the species is long-lived, a mix of juveniles and mature plants of varying sizes can increase the probability of successful establishment (CPC 2019). Larger plants tend to do better in reintroductions (Kaye 2009) because the establishment rate is higher than with seed (Dunwiddie and Martin 2016; Guerrant and Kaye 2007). However, there is often a higher mortality rate for nursery-grown plants after outplanting in the wild because of a tendency for individual plants to acclimate and thrive in greenhouse conditions at the expense of other individuals that are better adapted to survival in a natural setting (Haase and Rose 2001). To counter this effect the pampered nursery-grown survivors must be carefully acclimated to the outdoor environment to prevent further loss after returning them to the wild.

Sandhills milkvetch (*Astragalus michauxii*) is a rare species of the Sandhills region of the Atlantic Coastal Plain of the southeastern U.S. Habitat loss, fire suppression, and land conversion are the primary reasons for its decline. It was thought to have both physical dormancy and chemical inhibitors to germination, so early propagation attempts used a multistep process of sulfuric acid scarification, soaking in water for 24 hours, mechanical scarification to break physical dormancy, and finally a leaching process to remove chemical inhibitors. Seeking to simplify the process and make it more cost-effective for large-scale recovery projects, researchers tested new methods and discovered that it germinates well with mechanical scarification of the outer and inner seedcoat, and that no chemical inhibitors were present. Other factors complicated the propagation process though, including a sensitivity to soil moisture and planting depth, susceptibility to fungal pathogens, and need for regular fertilizing. Sandhills milkvetch also forms associations with nitrogen-fixing bacteria and mycorrhizae, so the soil media must be inoculated with native soils. The new protocols successfully produced a large number of plants for reintroduction projects (Kunz et al. 2016).

4.2.7.2. Breaking seed dormancy

Breaking dormancy in seeds can be a very complicated and involved process, but the germination requirements for wild species are rarely available and even closely related species may differ enough so that propagation methods have to be adjusted (Gray et al. 2019).

Seedcoats may require cold stratification or other actions to stimulate germination such as fire, heat, smoke, water, or passage through an animal's intestines. Blackberry (*Rubus* spp.) seeds can inhibit germination through both physical and physiological dormancy; the thick seedcoat is a physical barrier and biochemical processes control internal dormancy (Gray et al. 2019). When artificially propagated, researchers must find creative ways to mimic these natural processes. For example, seeds of Ruth's golden aster were first dried, then washed with alcohol for one minute, passed through a flame, washed with bleach for 20 minutes, and finally rinsed three times with water before being placed into soil medium (Wadl et al. 2018). Even when a propagation method is found to work, the germination rate may still be low. For Florida prairie clover (*Dalea carthagenensis* var. *floridana*), freezing seeds only increased germination from 4% to 9%, although that can be enough to improve establishment rates (Maschinski et al. 2018).

4.2.8. Outplanting

4.2.8.1. Site selection

Understanding what habitat is best for the species and finding or creating that habitat is a primary challenge when reintroducing plant species (Halsey et al. 2017; Maschinski and Haskins 2012). Survival and mortality are directly associated with selecting the proper outplanting site (Luna 2018), and poor quality or unsuitable habitat is one of the main causes of failure in reintroductions (Albrecht et al. 2019; Questad et al. 2014). Finding optimal sites is not easy as it requires thorough consideration of physical, biological, historic, and logistical factors, the details of which may not be available (Grewell et al. 2013; Maschinski and Haskins 2012).

Modern technology is improving our ability to find the right habitat for rare plant reintroductions. Questad et al. (2014) used LiDAR (light detection and ranging) and a topographic habitat-suitability model to examine habitat suitability for 11 species of threatened and endangered plants on the Island of Hawaii. Lidar is a high-resolution remote sensing method that can map small landscape features like soil depressions that may signal important habitat for rare species. This, along with habitat suitability models, can improve reintroduction success by directing planting activities towards areas with the most favorable habitat (Questad et al. 2014).

> "Choosing a recipient site should be done with great care and intention." (CPC 2019)

Rare plant reintroduction projects should occur on property that is legally protected from development and other land use changes (Guerrant and Kaye 2007). Land managed with conservation goals won't be threatened with development (Kaye 2009); however, if recipient sites are unprotected from human activities, the reintroduced populations may be impacted (Volis 2015).

The choice of recipient site must consider if the site can support a population of the target species that is large enough to be ecologically functional (Clements 2013). Not only size, but also the connectivity of the site at the landscape scale can be important (Wolf et al. 2015). New populations can serve to connect existing populations and serve to facilitate dispersal among populations, thereby improving the health of the metapopulation (CPC 2019).

Knowing the history of the proposed recipient site can help to both explain the existing site conditions and understand how that may change in the future. The reintroduced population may face different future site conditions based on climate change or choices made in management (CPC 2019; Guerrant and Kaye 2007). Similarly, historical presence of the target species is important to know (Guerrant and Kaye 2007) because often rare plants do not successfully establish in sites if they have no history of occurrence there, regardless of nearby historical presence (Volis and Blecher 2010). If there are natural populations of the target species, the best option may be enhancement of those populations, as long as the populations are not in decline or the factors that caused decline are removed (Volis 2015). Suitable habitat outside known locations may not have been colonized due to fragmentation or dispersal limitations (Volis 2015). For example, a rare endemic aster in southern France (*Centaurea corymbosa*) had greater survival in introduced vs. natural populations partly because intentionally planted microsites were more favorable than microsites reached by chance after seed dispersal in natural populations (Colas et al. 2008).

The ecological similarity between the source population site and the reintroduction site is a good predictor for rare plant establishment success and is often the primary factor influencing establishment success (Kaye 2009; Noël et al. 2011). However, this may not be the case in the face of climate change or other disruptions to natural ecosystem processes if the effects are not immediately visible (Volis 2015).

Long-term population persistence depends on matching the target species' niche requirements with the broad- and fine-scale environmental attributes of the recipient site (Maschinski and Haskins 2012; Vitt et al. 2016). If the reintroduction site selection is based only on coarse indicators like the general habitat type, then the reintroduction may fail if the site is not a close enough match for the species' needs. (Michaelis and Diekmann 2018). Likewise, seed and seedlings may not germinate, grow, or reproduce if the source material is not adapted to the conditions at the recipient site (Basey et al. 2015).

When habitats are very similar to sites where the species has thriving extant populations, the reintroductions are more likely to establish (CPC 2019, Noël et al. 2011, Volis 2015). For example, researchers saw short-term success when they introduced Piper's daisy to a new location based on it having similar soil, plant community composition, and slope to an existing habitat (Link and Cruz 2015). Reintroductions have failed on sites that differ from historic habitat, as was the case for the Florida prairie clover which did not establish well in a novel habitat a mere 100 meters from the historical habitat (Maschinski et al. 2018). Similarly, efforts to reintroduce chaffseed to historic sites in NJ were unsuccessful until research into the microhabitat preferences of the species were conducted. These studies found suitability of habitat conditions to vary within a scale of only few meters (Kelly 2006).

Functional ecosystem processes such as nutrient and moisture cycling, competition, herbivory, and natural disturbance regime are all factors that influence the ability of species to colonize a site (Albrecht et al. 2019; CPC 2019).

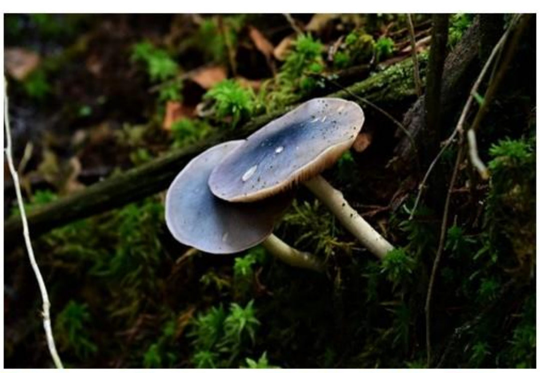
For many rare species, the appropriate substrate type is crucial. This can include parent soil type, texture, and moisture holding capacity (Vitt et al. 2016). The reintroduction of Ruth's golden aster depended on very specific substrate requirements – bedrock crevices of a specific minimum depth and width were necessary to its survival (Wadl et al. 2018).

Using indicator species can be a tool to help identify the proper habitat. The reintroduction project for marsh sandwort (*Arenaria paludicola*) found that water parsley (*Oenanthe sarmentosa*) in high abundance was an indicator of habitats where marsh sandwort had better success as long as the canopy was relatively open and other habitat conditions were met (Bontrager et al. 2014). Indicator species are especially important for parasitic plant species that depend upon specific host plants or plant communities for their survival (Kelly 2006).

Golden Indian Paintbrush (*Castilleja levisecta*)

Other plant species can assist the target species establish and assimilate into its new environment (CPC 2019). As opposed to competition, facilitation may be the primary interaction among plants, especially in stressful environments (Bontrager et al. 2014). Companion planting is a technique whereby a secondary species (nurse species) is planted along with the target species to facilitate its growth and development (Garnett 2003; Ren et al. 2014). As hemiparasitic plants, the reintroduction of both golden Indian paintbrush (Guerrant and Kaye 2007) and salt marsh bird's-beak (*Chloropyron maritimum subsp. maritimum*) (Noe et al. 2019) required attention to the presence of appropriate host plants. Other symbiotic relationships can affect the growth and survival of reintroduced plants. When reintroducing Kincaid's lupine, plants were more likely to flower when inoculated with nodulating bacteria (Guerrant and Kaye 2007).

Mycorrhizal fungi form symbiotic relationships with plant roots, assisting many plant species in root and shoot development and improving reproductive capacity (Maschinski and Haskins 2012; Reiter et al. 2016). Despite being cryptic organisms, researchers have developed methods to detect the presence of mycorrhizal fungi and baiting methods to harness them in restoration plantings (Reiter et al. 2016), methods to produce mycorrhizal fungal inoculum on a large scale (Maschinski and Haskins 2012), and dispersing mycorrhizae-infused soil pellets (Kapoor et al. 2008). Although necessary to some species' success, introducing microbes to a site is not without potential negative consequences. Maschinski et al. (2012) describe three scenarios: the introduced microbes could become invasive at the outplanting site, changing the composition of the existing soil microbe community; the introduced microbes could coexist with the native microbes; or the most likely outcome is that the introduced microbes could be outcompeted by the local native soil microbes and disappear from the site. It's also important to keep in mind that the fungal species involved in early life stages of germination and seedling development may be different from the fungal species in symbiosis with the plants during later adult and reproductive stages (Fay et al. 2018).



Calliderma indigofera was first described in 1876 from New Jersey, and then not reported again for over 100 years.

Reintroduction of the critically endangered Wollemi pine (*Wollemia nobilis*) provides an example of the importance of the soil microbiota. Individuals that did not have a species-specific fungal community one year after planting were unhealthy and not growing (Rigg et al. 2017). In the second year, species-specific bacterial communities had formed in their roots and surrounding soil (Rigg et al. 2017). The role of microbial communities is not often considered but this research highlighted its influence on restoration success.

Plant conservation and restoration depends on the positive interactions between restored plant populations and their animal pollinators and seed dispersers (Ruhren and Handel 2003). For insect pollinated plants, the appropriate generalist or specialist pollinator species must be present at the recipient site and interact with the new populations of the target species. This was investigated in restoring mallee honeymyrtle (*Melaleuca acuminata*) in southwest Australia; researchers used genetic evaluation of reintroduced and natural reference populations along with comparisons of mating systems and reproductive output to show that pollinators were as effective in new populations as they were in natural populations (Millar et al. 2019).

4.2.8.2. Planting process and design

The planting process itself can influence outcomes. It is important to pay attention to the timing of planting, as well as the number of outplanting sites and attempts. The timing of outplanting or seeding can influence population establishment and persistence (Albrecht and McCue 2010, Luna 2018), and it may be necessary to experiment with timing to discover a successful protocol (Guerrant and Kaye 2007). Reintroduction projects that outplant on multiple sites increase the chances of success because the ideal microsite habitat is more likely to be selected and because a localized disturbance event won't necessarily destroy all subpopulations if they are scattered over some distance (IUCN/SSC 2013).

Multiple outplantings attempted per project over many years should increase the likelihood of establishing a viable population (CPC 2019; Guerrant and Kaye 2007; Maschinski and Haskins 2012). This strategy may be needed so that over time the population can develop in size and structure to maintain itself and expand (CPC 2019). Additionally, using large founding population sizes and spreading out the release over several years may act as a bet-hedging strategy against unpredictable natural disturbances (IUCN/SSC 2013) whether acute like a tornado, more long lasting such as a drought, or in highly stochastic environments like dunes or floodplains (Albrecht and McCue 2010).

The amount, density, and spatial pattern of planting are important to consider because they influence the future genetic health of the new population. The density and spatial pattern affect the variation of selfing or outcrossing among plants (CPC 2019). When there is low density for those species that are obligate outcrossers, Allee effects can be a negative result (Colas et al. 2008). In this case, planting in clusters would be more beneficial than a spread out planting design (CPC 2019). The species' competitive ability is also important to take into account. For example, if it does not compete well with neighboring vegetation then planting in open spaces would be more beneficial (CPC 2019). Ultimately the number of founding individuals used will impact the outcome of the project. Planting more propagules – whether seed or seedlings – improves the chances of success since unanticipated environmental factors often cause mortality in a great percentage of the propagules (CPC 2019; IUCN/SSC 2013; Maschinski and Haskins 2012; Vitt et al. 2016).

In the restoration of *Centaurea corymbosa*, researchers created a simulation model of

Harperella (*Harperella nodosum*) is a federally endangered species found in the southeastern U.S. The species is threatened by severe flooding – a direct result of deforestation and development which has increased runoff from impervious surfaces in areas of high population density along the

Harperella (*Harperella nodosum*)

Potomac River in Maryland and West Virginia. Harperella is semiaquatic, grows on cobble bars in streams, and is benefitted by frequent low magnitude floods which scour the cobble bars removing algae and other plant competitors. A major challenge in its restoration was finding appropriate habitat for reintroduction. Through experimental reintroductions, researchers discovered a 'Goldilocks' suite of conditions that allowed Harperella to successfully establish in western Maryland and northeastern Virginia: 1) at least 6 hours of full sun; 2) constantly damp cobble bars; 3) nearby flowing water with minor periodic flooding; and 4) protection from severe flooding. Harperella is tolerant of brief flooding events up to 60-90 cm deep, but not during flowering (prevents pollination) or before seed development (prevent seed maturing). It is intolerant of prolonged exposure to deep water, but low intensity flooding that knocks the stem over promotes vegetative reproduction via node-rooting (Wells 2012).

population dynamics using parameters such as its fecundity, self-incompatibility, intraspecific competition, and demographic factors. They found that the extinction probability for a new population was highly influenced by the density of sown seeds because it later determined the density of compatible mates and, therefore, the fecundity of the population (Colas et al. 2008).

Reintroduction projects that use seedlings must consider acclimatization to conditions in the wild. The conditions in which they were produced are less stressful than they will be at the recipient site (Maschinski and Haskins 2012). A period of acclimatization allows for the seedlings to adapt to their new environment (Michaelis and Diekmann 2018). Some steps toward acclimatizing can be taken before outplanting through horticultural management by modifying light levels, irrigation, and available nutrients (IUCN/SSC 2013). Plant leaves can be fairly easily acclimated to ambient sunlight simply by moving the seedlings to outside beds during cultivation (Michaelis and Diekmann 2018).

After planting, supplementary watering is often used to increase germination rate and seedling survival (Maschinski et al. 2018) and is common in orchid reintroductions (Reiter et al. 2016). Watering needs may be unknown until some experimenting is done. For example, Bontrager et al. (2014) suspected that too-dry soil caused mortality of marsh sandwort on some sites; a greenhouse test for desiccation found that the seedlings' tolerance threshold was 20 days of desiccation after the wilting point.

For some species, adaptation of the root system to natural soil conditions can also be important; soil attributes such as nutrient availability, water content, pH, organic matter, compaction, and microbial community influence root growth, development, and morphology, and the quality of root exudates (Michaelis and Diekmann 2018; Rigg et al. 2017).

Beyond considerations of timing and design, practitioners must consider the fact that the outplanting process can introduce negative impacts to the site. Thorough risk assessments can ascertain if the planting operation itself may negatively affect the recipient site through physical damage to the resident vegetation (CBA 2014; Clements 2013) or negative interactions between the target species and the resident community (Abeli et al. 2014; Clements 2013). Accidental introduction of nonnative invasive plants to the outplanting site is also of great concern; seed can unintentionally be moved about on equipment, vehicles, and boots (CBA 2014). Care must be taken to prevent introducing pests and pathogens from greenhouse potting soil which may be detrimental to the recipient site (IUCN/SSC 2013; NY NHP 2008; USFWS 2017; VA DNH 2008). Prior to outplanting Wollemi pine, researchers tested the potting soil for the presence of *Phytophthora cinnamomi*, a soil-borne root pathogen (Hardham and Blackman 2018; Rigg et al. 2017). In an orchid reintroduction project, researchers cleaned all footwear and equipment with the antiseptic Phytoclean or methylated spirit to prevent the spread of fungal-borne diseases (Reiter et al. 2016).

4.2.8.3. Site management

Site management activities are often needed prior to the planting so the site meets the requirements of the target species and to reduce or remove habitat-related threats, especially those that hinder important life history events (Kaye 2009; IUCN/SSC 2013; Tischew et al. 2017). Ensuring the site receives long-term protection and ongoing management is also crucial for success in terms of both short-term establishment and long-term survival (CPC 2019; Fenu et al. 2016). Management activities required often include soil preparation, fencing or caging to protect from herbivores, invasive plant removal, reducing competing vegetation through grass cover reduction, improving the light environment via canopy thinning, and ensuring the appropriate disturbance regime (Albrecht and Long 2019; Bontrager et al. 2014; CPC 2019; Colas et al. 2008; Fenu et al. 2016; Kaye 2009; Link and Cruz 2015; Ruhren and Handel 2003; Vitt et al. 2016).

In California, reintroduced populations of soft bird's beak (*Chloropyron molle* ssp. *molle*) significantly increased after management activities to control the invasive broadleaved pepperweed (*Lepidium latifolium*) (Grewell et al. 2013). In New Jersey it is essential to protect plants from herbivory by overabundant white-tailed deer. In an experimental restoration of common native herbaceous plants, survival and reproduction of plants was heavily impacted by deer unless fencing excluded the deer from plantings (Ruhren and Handel 2003). In Florida, cages were used to protect reintroductions of the rare semaphore pricklypear (*Consolea corallicola*) from the cactus moth (*Cactoblastis cactorum*), an invasive South American cactus-eating moth (Stiling 2010).

4.2.9. Monitoring

Extended monitoring of the outplanting site is critical to the project success (Fenu et al. 2016; Maschinski and Haskins 2012; Vitt et al. 2016). The average monitoring time frame is 2-3 years after planting, but this is not long enough to determine if the project will be successful (Ren et al. 2014; Vitt et al. 2016). Practitioners should develop a plan for long-term monitoring of the reintroduction (CPC 2019) and keep in mind that reintroduction success in the early years usually doesn't reflect longer-term performance (Fenu et al. 2016). It's important to monitor over time to capture all developmental stages (Albrecht et al. 2019); however, the timeframe needed depends on the target species' life-history strategy because longer-lived species tend to take longer to reach reproductive maturity and recruitment of the next generation (Albrecht et al. 2019). Reintroductions may seem successful but then fail years or decades later due to changing conditions. The timeframe to evaluate projects will vary, and it will often take over a decade to determine whether a reintroduction is going to work (Allen 1994). Even after a successful reintroduction and subsequent delisting, species "will still require careful monitoring in our changing climate" (Haskins 2015).

Monitoring has many purposes. Extended monitoring is necessary to ascertain if the new population is self-sustaining and to predict other long-term trends (CPC 2019), capture fluctuations in population demographics and habitat quality (Lloyd et al. 2018), predict population trajectories, and develop population viability models (CPC 2019). Long-term monitoring also produces the data needed to evaluate how the target species responds to infrequent events such as drought, and can expose genetic concerns like inbreeding that may not play out for multiple generations after outplanting (CPC 2019). The data gathered with long-term monitoring helps to produce better stewardship and scientifically guided management actions (Grewell et al. 2013); it is integral within an experimental and adaptive management framework to be able to document successes and failures, change course before it's too late, and report findings to the scientific community (CPC 2019).

The types of data to be collected during monitoring must take into consideration the target species' life history, such as age when it first reproduces, longevity, trajectory of population growth, as well as what monitoring resources are available and the objectives for the experimental components of the project (CPC 2019; IUCN/SSC 2013). If a goal is to achieve a specific population size or structure, then specific demographic data must be collected (CPC 2019). Seedling recruitment is an important benchmark to demonstrate that a self-sustaining population is possible (Albrecht et al. 2019), and production of the third generation may be even more telling, as it shows that the site and founding individuals produced seed capable of continuing the reproductive process. In addition to monitoring the target species, practitioners should monitor the site for ecological changes in the habitat (IUCN/SSC 2013). Over time the intensity of monitoring effort may be reduced, increased, or may adjust from experimental to observational data collection as was the case for the reintroduction of Virginia sneezeweed (*Helenium virginicum*) in the Missouri Ozarks (CPC 2019). After the short-term goals of the experiment were accomplished and reintroduced populations were growing rapidly with successful recruitment, the monitoring protocol switched from a full-scale demographic monitoring of individuals to count estimates and surveys for new threats (CPC 2019).

4.2.10. Defining success

Plant conservation biologists have had varying definitions of success for reintroduction projects (Volis 2015), but the overarching components are similar. Still, determining that a reintroduction is successful is not simple because it’s not always easy to judge whether a project will “produce self-sustaining populations that are resilient to disturbance” (Guerrant and Kaye 2007).

The primary goal of rare plant reintroductions is to produce a sustainable population of the target species (Drayton and Primack 2012). Defining other objectives – or benchmarks of biological success (Guerrant and Kaye 2007) – helps the practitioners identify other parameters along the path towards the final goal (Pavlik 1997). It may be tempting to simply set the reintroduction goal as the survival of a specific number of plants, but a more important goal is restoring the important demographic processes that ensure population growth and persistence (Grewell et al. 2013). Additionally, reporting on project milestones in status reports at multiple intervals after outplanting is helpful for the restoration community to learn from each project (Grewell et al. 2013; Guerrant 2013). These milestones may include the degree of success of the initial establishment, reaching reproductive maturity, producing a second generation, significant mortality of founders and later generations, and the formation of satellite populations (Guerrant 2013).

> "Desired outcomes would be resilient, self-sustaining, genetically diverse populations, with multiple patches in multiple sites" (Noe et al. 2019).

While both short- and long-term criteria are useful to evaluate reintroduction projects (Ren et al. 2014), ultimately, long-term monitoring is needed to fully evaluate reintroduction projects (Ren et al. 2014; Volis 2015). Even 10 years post reintroduction, it can still be too early to know if a reintroduced population will become self-sustaining and resilient to stressors/disturbances (Guerrant and Kaye 2007). The four main categories of reintroduction success are abundance, geographic extent, persistence, and resilience (Pavlik 1996; Pavlik 1997). Most criteria to evaluate success fit into these four categories.

Metrics of abundance include the number of individuals that initially establish and survive, their vegetative growth, and rates of emergence after the first dormant season (Godefroid et al. 2011; Guerrant and Kaye 2007; Haskins 2015; Kaye 2009; Pavlik 1996; Reiter et al. 2016).

Abundance also includes metrics of reproductive capacity or fertility such as flowering, fruiting, and seed production, with the objective that the species is able to complete its life cycle and produce more seeds than were planted during the reintroduction (Drayton and Primack 2012; Godefroid et al. 2011; Guerrant and Kaye 2007; Pavlik 1996; Reiter et al. 2016; Ren et al. 2014; Volis 2015).

Persistence refers to its ability to be self-sustaining via natural reproduction (without human assistance) and the production of viable offspring (CPC 2019; Colas et al. 2008; Drayton and Primack 2012; Godefroid et al. 2011; Guerrant and Kaye 2007; Haskins 2015; Kaye 2009; Noe et al. 2019; Pavlik 1996; Reiter et al. 2016; Ren et al. 2014; Volis 2015). Persistence also involves the target species being integrated into the ecosystem (Ren et al. 2014) by fulfilling its role or regaining its function in the natural community (Guerrant and Kaye 2007; Pavlik 1996; Ren et al. 2014; Wolf et al. 2015). An example is establishing links with other species such as using local pollinators to complete its reproductive process (Ren et al. 2014).

The geographic extent refers to the distribution of populations (Haskins 2015) or subpopulations across multiple sites (Noe et al. 2019). A successful reintroduction objective is to see the new population occupying a stable area or expanding in area (Drayton and Primack 2012; Reiter et al. 2016) via propagule dispersal and potentially producing satellite populations (Drayton and Primack 2012; Ren et al. 2014) and/or connections to remnant populations (Pavlik 1996).

Resilience speaks to the species' ability to adapt to changing environmental conditions (Ren et al. 2014; Volis 2015) through having appropriate genetic diversity (Haskins 2015; Noe et al.2019; Pavlik 1996), vegetative dormancy, forming a seed bank, resprouting after fire, or other adaptations to stressors and disturbances (Guerrant and Kaye 2007; Pavlik 1996; Ren et al. 2014). Some authors recommend genetic assessments to evaluate the resilience of reintroduced species (Millar et al. 2019; Reiter et al. 2016), such as comparing the genetic diversity and differentiation of reintroduced populations to remnant populations.

A tool to evaluate the potential success of reintroduction projects is population viability analysis (PVA) – a quantitative model-based approach that uses demographic and abundance data to evaluate the population growth rate, probability of extinction, and likelihood of population persistence for given conditions at a specific site (Volis 2015; Wolf et al. 2015). It can also be used to rank the importance of threats or management actions and identify gaps in monitoring data that could be used to improve the results of the recovery project (Wolf et al. 2015). However, intensive datasets are needed for PVA to deliver reliable predictions (Volis 2015), and few reintroduction projects have lasted long enough to have enough data to build PVA models (Maschinski et al. 2013).

It's important for rare plant reintroduction projects to report on the outcome, whether the reintroduced plants live or die (Grewell et al. 2013). Failed reintroductions have value in that reporting on the experimental results and the known or speculated reasons for failure will benefit other researchers and help them improve their methods for similar projects in the future (Parkin 2005; Ren et al. 2014).

5. Appendices

5.1. Checklist for Proposal Reviews

This section includes a checklist of questions and considerations for developers and reviewers of reintroduction proposals. The content is primarily from CPC (2019), with adjustments made to fit the needs of this document.

5.1.1. Justification (See Section 4.1 for more information)

A reintroduction may be justified based on consideration of the following:

- Is the species extinct in the wild? OR:
- Are the populations few, small, and declining? AND
- Have all other management options been considered and conducted, yet have been judged to be inadequate for long-term conservation of the species? AND
- Have the threats been identified? AND
- Is the species at high risk of extinction if it's only managed *in situ*?

If the species meets any one of the following criteria, then do NOT proceed with reintroduction.

- Field survey has been inadequate in determining the true status of rarity of the species throughout its range.
- Reintroduction will undermine the imperative to protect existing sites.
- High quality, diverse source material is not available.
- Previous tests indicate that it has not been possible to propagate plants or germinate seeds.
- Suitable habitat is not available, nor understood.
- Existing threats have not been minimized or managed.
- The reintroduced species may potentially negatively impact species in the recipient site via competition, hybridization, or contamination.
- There is evidence that the reintroduced taxon would harm other rare species or conflict with their management.
- The reintroduction is not supported legally, administratively, or socially.

5.1.2. Planning (See Section 4.2 for more information)

Goals and Consequences

- Long-term goals of the proposed project are clearly defined.
- Potential negative consequences are addressed.
- Results will be reported even if the project is unsuccessful.

Conservation status (See Section 4.2.1 for more information)

- Are there recent survey and status updates?
    - Specific information on the number of populations has been collated within the last 18 months.
    - Counts or estimates of the number of individuals in each population have been done.
    - The age structure of the populations is known.
    - The relationship of populations in a metapopulation context is known.
- What degree of protection does the target species have?
- What are the threats to the target species?

Reason for decline or rarity (**See Section 4.2.3 for more information**)

- Why is the target species rare?
- Is the cause of decline known for wild populations?
- Are there management options other than reintroduction that can address the decline of extant populations?

Biology and ecology of target species (**See Section 4.2.2 for more information**)

- Is the biology and ecology of the species understood?
- Has available ecological knowledge regarding the species and its community been reviewed?
- If there are important unknowns of the species' biology or ecology, does the proposal consider them and/or include them as part of the experimental design?
    - What is the genetic structure of the wild populations?
    - If hybridization is a concern, what are the ploidy levels of the wild populations?
    - Does the species suffer symptoms of inbreeding depression?
    - Is there evidence of outbreeding depression?
    - Based upon special ecology, unique morphology or spatial disconnection from other populations, do you suspect that a population has local adaptation?
    - Based upon the presence of a congener in the wild population and/or variable morphology, do you suspect that the species is hybridizing with a congener?
    - Are genetic studies needed to understand the source/remnant populations? Genetic studies are needed if the following conditions are met for the source/remnant populations:
        - Population has fewer than 50 individuals flowering and setting fruit.
        - The species is clonal.
        - Little or no viable seed is being set.
        - There are potential taxonomic concerns, taxonomic ambiguity, hybridization, or variation in ploidy.
        - The species is declining, and little is known about the biology or life history of the species.
        - The species has highly fragmented and isolated populations.

    - The species looks different in different locations.
    - One or more populations of the species has distinct ecology from the majority of populations.
- Are specific habitat and microhabitat details known?
- Are interspecific interactions relevant to survival and reproduction known (pollinators, seed dispersers, herbivore pressure, symbiotic partners, and parasitic hosts)?
- What additional knowledge is needed to conduct the project well?

Resources (**See Section 4.2.4 for more information**)

- Does the proposal provide a budget for implementing, monitoring, and management of the restored population?
- Is sufficient funding secured to conduct the reintroduction?
- Are sufficient funds available for aftercare and required monitoring?
- Are collaborators with relevant subject matter expertise involved?

Legal/regulatory issues (**See Section 4.2.5 for more information**)

- Do proposed experiments meet all regulatory requirements?
- What legal or regulatory requirements are needed for the reintroduction?
- Have permits been acquired and are they up to date?
- Do permits cover aftercare activities?

Miscellaneous

- Have all potential logistical issues been considered?
- Is there a large enough and committed workforce to conduct each stage of the project?

5.1.3. Implementation

Experimental Design (**See Section 3.3 for more information**)

- What additional knowledge is needed about the species biology or other factors?
- How can the reintroductions be planned as experiments to address these unknowns?
- What is the experimental design? How much replication is needed for adequate statistical analyses? How will the study be analyzed?
- Will you examine natural variation in survival, mortality, and recruitment and tie these to environmental factors?
- Will the reintroduction consider key habitat factors including light, moisture, elevation, or temperature?
- Will the underlying environmental drivers of population growth be measured?
- Will genetic factors be part of the experimental design?
- What data will be collected during monitoring and how will it be analyzed?

- Will the reintroduction further our knowledge of the rare species' ability to cope with climate change?
- Are you testing factors within a single site or across multiple sites?
- Have you sought statistical assistance for the experimental design and analysis?
- If plants are susceptible to herbivory, will their response be included in the design or should the plants be protected?

Sourcing Propagules (See Section 4.2.6 for more information)

- Should material come from an *ex situ* source, only one wild source population, or mixed population sources?
- Will propagule collection harm the donor population?
- How much seed will be collected to ensure the donor population is not harmed?
- From which wild population(s) will the material be collected for use in the reintroduction?
- What is the basis for collecting source material from a particular location(s)?
- Does the proposal include good reasoning for how many propagules to collect, when to collect propagules, and how often to collect propagules?
- Was the process for collecting source material designed to benefit the genetics for the founding population?
- How will the propagules be stored and handled?
- What is the genetic composition of the material to be reintroduced, and is it an appropriate match for the recipient site?

Propagation (See Section 4.2.7 for more information)

- Have the germination protocol and/or propagation methods been determined?
- Has enough time been allotted to generate the source material?
- How many plants or seeds are available and how many are needed?
- What founder population size will be used?
- What size and age structure of plants will be used?
- Will plants need to be acclimatized to the recipient site?

Site Selection (See Section 4.2.8.1 for more information)

- Is there still suitable habitat left within the species' range?
- Have you ranked several potential suitable recipient sites to determine the best location for the reintroduction to occur?
- Have abiotic conditions (e.g., soil, precipitation, temperature) and biotic conditions (for example, predators, mutualists, invasive species) at the reintroduction site that are associated with plant performance and population growth been measured?
- Has the land use history of the recipient site been researched?
- Is current and future land use of the recipient site and surrounding sites compatible with

sustainability of the reintroduced population?

- Are potentially hybridizing congeners present at recipient site? Which ones? Are they present at nearby sites? Are they present within the target species' range?
- Is the recipient site within the species' climate envelope now? Are there models suggesting the location will be safely within the climate envelope in the future?
- Do the recipient sites have connectivity to increase the probability of dispersal?
- Are there adequate areas for population expansion (microsites within the recipient site and adjacent suitable habitat outside of the recipient site)?
- What are the distances between the proposed reintroduction and nearby wild populations?
- Are essential ecological processes intact at the site? If not, how will they be established?
- Who owns the reintroduction site, and how will the site be managed over the long term?
- Is the taxon already extant at the recipient site, was it historically present there, or is this a completely new location?
- Are long-term protection and management plans documented for suitable recipient sites?
- Do potential recipient sites have additional threats to the target species that are not present in sites with wild populations? If so, can these threats be ameliorated prior to any planned reintroduction?
- Are pollinators known and present?

Outplanting (See Section 4.2.8 for more information)

- What site preparation is required before the plants can be installed (for example, canopy thinning, invasive species removal, etc.)?
- What is the best season to transplant or sow seeds?
- How will you transport plants to the recipient site?
- Do you have the necessary equipment and staffing?
- What is the planting layout design (density, spatial pattern, etc.)?
- How many propagules will be outplanted?
- How will personnel avoid damage to the recipient site?

Site Management (See Section 4.2.8.3 for more information)

- What were the previous threats that may have caused the species to become extirpated from the site?
- Are the threats absent or now adequately managed at the site? Have the threats been reduced or eliminated?
- What is the potential for future threats?
- Will continued habitat management be required after the plants are reintroduced?
- Can the appropriate disturbance regime be ensured? Does the species require habitat conditions that no longer exist on site (e.g., fire, periodic inundation, etc.)? Can those

conditions be mimicked?

- Can competition from invasive plant species be eliminated?
- Are the plants susceptible to herbivory? Will they be protected?
- What kind of aftercare for the plants (e.g., watering) will be needed and how frequently should it be performed?
- Is the land manager/owner of the site supportive and involved?
- Who will maintain and manage the population?

Monitoring (**See Section 4.2.9 for more information**)

- Has a monitoring plan been prepared and reviewed?
- How will you ensure that monitoring will be performed for many years in the future? How will the plants be mapped and marked/numbered?
- How long will monitoring be conducted?
- Have you considered an adaptive monitoring plan?
- Have you developed a clear unambiguous datasheet to collect monitoring data? If the monitoring persists for decades, will your successors be able to obtain and interpret the data you have collected?

Reporting Outcomes (**See Section 4.2.10 for more information**)

- How is success defined and how will it be measured?
- What is the plan for reporting results?

5.2. Resources

- The Center for Plant Conservation provides a resource to learn about reintroductions that have been done and is a source for potential peer reviewers (info@saveplants.org) (CPC 2019).
- The North Carolina Plant Conservation Program requests and evaluates reintroduction plans as part of the process of granting legal permission to proceed with a plant reintroduction in the state of North Carolina: (http://www.ncagr.gov/plantindustry/plant/plantconserve/index.htm) (CPC 2019)
- The International Union for Conservation of Nature Re-introduction Specialist Group published the *Re-introduction Practitioner's Directory 1998* intended to facilitate communication between individuals and institutions undertaking animal and plant reintroductions: (http://www.iucnsscrsg.org/) (CPC 2019)
- The Global Restoration Network provides a web-based information hub linking research, projects, and practitioners: (http://www.globalrestorationnetwork. org/) (CPC 2019).
- The Center for Plant Conservation provides a site selection evaluation form that can be used to evaluate a single site or to prioritize among multiple sites (CPC 2019).

- The Native Plant Network provides a searchable database of propagation protocols with information about collecting, cleaning, sowing, dormancy, special treatment. You can register as a propagator and submit protocols: (nativeplantnetwork.org) (Haase and Rose 2001).
- The Montgomery Botanical Center provides detailed protocols for collecting genetic diversity from wild populations: https://www.montgomerybotanical.org/research/collections-genetics/ (BGCI 2015).
- Frankham et al. (2011) provide a decision tree for determining the probability of outbreeding depression between two populations; this would be useful for making decisions when collecting source material (Volis 2015).
- Hoban (2019) provides advice for seed collecting to conserve genetic diversity.

5.3. Glossary

"Population reintroduction is a field still searching for a consistent vocabulary" (Kaye 2009).

Adaptive management – a process of flexible decision making in which the outcomes of experiments, management actions, and natural events are carefully monitored and used to improve scientific understanding and adjust policies or methods of natural resources management (Williams 2011).

Allee effects – the correlation between small population size or density and decreased fitness of a population or species (Drake and Kramer 2011).

Allele – DNA found on one location on a chromosome that corresponds to a trait. Depending upon the plant and the number of paired chromosomes it has, one-to-many alleles may be responsible for traits related to appearance, chemistry, or growth. In genetic tests, the number of unique alleles is one measure of genetic diversity (CPC 2019).

Assisted migration (aka assisted colonization) – translocation of a species to favorable habitat beyond their native range to protect the species from human-induced threats, such as climate change (Ricciardi and Simberloff 2008, as cited in Volis 2015).

Augmentations (aka enhancement/enrichment/replenishment) – adding seeds or individual plants to a population that were propagated from that same population, with the aim of increasing population size or genetic diversity and thereby improving viability; re-creating a recently extirpated population with individuals propagated from that population. Often an *ex situ* facility is the intermediary between the original collection from a population to propagation in a nursery setting before the propagules are placed back into the wild population (CPC 2019).

Autecology – Autecology concerns the study of interactions between an individual, a population, or a species and its total environment including all the physical and biotic factors directly influencing a given organism or organismic unit as well as anything affected by it. Autecology has sometimes been defined as ‘species ecology’ and it has also been equated with physiological ecology (Pianka 2008).

Congener – a member of the same taxonomic genus as another plant or animal; for example, white oak (*Quercus alba*) and post oak (*Quercus stellata*) are congeners (Merriam-Webster.com 2020).

Conspecific – of the same species; for example, red maple (*Acer rubrum* var. *rubrum*) and trident red maple (*Acer rubrum* var. *trilobum*) are conspecifics (Merriam-Webster.com 2020).

Conservation translocation – a definition coined by IUCN to describe intentional movements of organisms within the species' indigenous range (reinforcement or augmentation of existing population and reintroduction into an area once but not currently occupied by the species) and movements outside of its indigenous range, including conservation introductions, comprising assisted colonization and ecological replacement (CPC 2019).

Controlled propagation – the human-induced propagation of plants from seeds, spores, callus tissue, divisions, cuttings, or other plant tissue, or through pollination in a controlled environment. Defined in the context of this policy, controlled propagation refers to the production of individuals, generally within a managed environment, for the purpose of supplementing or augmenting a wild population(s), or reintroduction to the wild to establish new populations (USFWS 2000).

Critical habitat – the habitat required to ensure the persistence of a species, which is evaluated in terms of the population size, acceptable probability of extinction risk, or number of patches needed to achieve population viability over a specified time period (Hall et al. 1997 and Reed et al. 2006, as cited in Volis 2015).

De-extinction – the reversal of extinction, via cloning or genetic engineering or other technologies. Some authors may use it to describe reviving plant species extinct in the wild but extant in seed banks or other living collections (Jørgensen 2013, as cited in Vitt et al. 2016).

Donor population (aka source population) – wild populations that are the source for propagules for reintroductions (Maschinski and Haskins 2012).

Element occurrences – generally refer to populations and can be represented by a single individual, contiguous group of individuals, or several discrete patches or subpopulations that are within 2 km of each other and not separated by unsuitable habitat greater than 1 km wide (Kunz et al. 2014).

Enhancement (aka reinforcement/augmentation) – a type of restoration for endangered plants that occurs at sites with extant populations (Falk et al. 1996, as cited in Wadl et al. 2018).

Ex situ – offsite, away from the wild population, usually referring to collection held in nursery or botanic garden (CPC 2019).

Ex situ institutions – botanical gardens, seedbanks, and horticultural nurseries where seeds or whole plants are propagated before being outplanted into recipient sites (Maschinski and Haskins 2012).

Extant – still in existence; surviving; not extinct.

Fitness – the ability to survive and reproduce in a given environment (Ottewell et al. 2016).

Founding population – the population that is outplanted as part of a reintroduction project (CPC 2019).

Founder(s) – the individual(s) that starts a new population (CPC 2019).

Gene flow or gene migration – any movement of individuals, and/or the genes from one population to another. It can be described as limited, meaning that the individuals living near one another are closely related (e.g., monkshood), or extensive, meaning that it is possible to find traces of genes in an individual that lives very far away (e.g., wind-pollinated plants) (CPC 2019).

> "...biodiversity management is…complicated by inconsistent and rapidly changing terminology. The (rare plant) literature includes references to introduction, reintroduction, relocation, translocation, transplantation, revegetation, restoration, rehabilitation, augmentation, enhancement, and other terms. Many of these carry specific meanings, while others are used variously or interchangeably" (Falk and Olwell 1992).

Genetic drift – variation in the relative frequency of different genotypes in a small population, owing to the chance disappearance of particular genes as individuals die or do not reproduce (CPC 2019); increased genetic differentiation between populations (Ottewell et al. 2016).

Genetic load and purging – genetic load is the amount of deleterious mutations in a population due to inbreeding. Inbred individuals can be purged from populations through selection, resulting in decreased genetic load (Ottewell et al. 2016).

Genotype – the genetic constitution of an individual (genotyping is determining the genetic constitution of an individual) (CPC 2019).

Gray literature – reports, working papers, government documents, white papers, and evaluations produced by organizations outside of the traditional commercial or academic publishing and distribution channels; organizations that produce gray literature include government departments and agencies, non-governmental organizations, private companies, and consultants (Wikipedia contributors 2020).

Habitat enhancement – improving the suitability of sites to sustain populations by removing competitors and predators (Dunwiddie and Martin 2016). The manipulation of the physical, chemical, or biological characteristics of a habitat to change a specific function or seral stage of the habitat for the purpose of benefitting species. Habitat enhancement includes activities conducted to shift a native plant community successional stage such as prescribed burning (USLegal.com 2020)

Historic range – the suitable habitat within the physiogeographic range of the taxa since the 1600s, but not necessarily documented from the specific site (MD PRTF 1999).

In situ – in wild habitat (CPC 2019).

In situ conservation – conservation that aims at either enhancement of existing populations or creation of self-supporting new populations via reintroductions and translocations, using sampled or propagated material (Volis and Blecher 2010).

Inbreeding – mating between closely related individuals. Over many generations, genetic disorders may arise (CPC 2019).

Inbreeding depression – reduced fitness of progeny resulting from breeding of related individuals (CPC 2019).

Introduction – the intentional or accidental human dispersal of a living organism outside its historically known native range (IUCN 1998, as cited in Vitt et al. 2016); a type of restoration for endangered plants that occurs at previously unoccupied sites (Falk et al. 1996, as cited in Wadl et al. 2018).

Managed relocation – the movement of species, populations, or genotypes to places outside the areas of their historical distributions to maintain biological diversity or ecosystem functioning with changing climate (Schwartz et al. 2012, as cited in Vitt et al. 2016).

Metapopulation – a group of spatially separated populations of the same species that interact (e.g., the interaction can be pollen or seeds moving between plant populations) (CPC 2019).

Mitigation – a general term used to describe a wide variety of actions taken to avoid, reduce or compensate for adverse impacts of development (Berg 1996, as cited in Vitt et al. 2016); a legal term for an action that is taken to offset the adverse impacts of development on species. E.g., a parcel of land where a species occurs may be preserved as mitigation for developing a portion of the species' habitat (CPC 2019).

Non-governmental organization (NGO) – organizations founded by citizens, which include clubs and associations, which provide services to its members and others; they are usually nonprofit organizations (Wikipedia contributors 2020).

Outbreeding – a condition where flowers of one plant receives pollen from another plant of the same species (CPC 2019).

Outbreeding depression – low fitness of progeny resulting from mating between two genetically distant (and usually physically distant) plants (CPC 2019).

Outcrossing – the form of plant reproduction that requires pollen from another plant of the same species to form seeds (CPC 2019).

Outplanting – transplanting from a botanical garden nursery (*ex situ* setting) to a wild setting for purposes of reducing the extinction risk of a species or population and allowing persistence in a natural setting (CPC 2019).

Phenotype – the measurable appearance of a trait (CPC 2019).

Recipient site – location chosen to outplant individuals of a rare plant species in order to create a population in a reintroduction project.

Recruitment – the process by which new individuals are added to an existing population through vegetative reproduction or seedlings; seedling recruitment includes seed germination, seedling survivorship, and seedling growth (Eriksson and Ehrlén 2008).

Reinforcement (aka supplementation/enhancement/augmentation) – an effort to increase population size or diversity by adding individuals to an existing population (Akeroyd & Wyse Jackson 1995 and IUCN 1998, as cited in Godefroid et al. 2011).

Reintroduction – the intentional movement of species into habitat it previously occupied (CPC 2019); the deliberate establishment of individuals of a species in an area where it has become extinct or nearly extinct. The aim is to establish a viable, self-sustaining population that has sufficient genetic resources to adapt to environmental change (Ren et al. 2014); the movement of seed or plants propagated *ex situ* to the wild either within the species' natural range or within its home range (Reiter et al. 2016).

Rescue – refers to the collection of whole plants or plant parts from a site where they will otherwise be destroyed.

Restoration – the process of repairing damage caused by humans to the diversity and dynamics of indigenous ecosystems (SER 2002, as cited in Vitt et al. 2016).

Selfing (aka self-fertilize) – the process by which pollen from one flower fertilizes the same flower and successfully sets seed (CPC 2019).

Self-compatibility – for plants with both male and female organs located on the same plant, the ability to self-fertilize (Ottewell et al. 2016).

Source population (aka donor population) – wild populations that are the source of propagules for reintroductions.

Species recovery – the process whereby native species or populations within their indigenous range that have become endangered as a result of habitat loss, decrease in population size or loss of genetic variability, are recovered to a state where they are able to maintain themselves without further human intervention (Heywood 2019).

Stochastic event – an unpredictable or chance event (CPC 2019).

Succession – the process of change in the species composition and structure of an ecological community over time (Wikipedia contributors 2020).

Target species (aka candidate species/priority species) – plant species that have been selected for particular conservation attention or action (Volis 2015).

Taxon – a taxonomic group of any rank, such as a species, family, or class. Sometimes this term is used rather than species, because it will encompass varieties and subspecies (CPC 2019).

Translocation – defined very broadly, is the deliberate transfer of plant material from one area to another. Plant material can include seeds, cuttings, propagated seedlings, or whole plants (Vinge-Mazer 2017).

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