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Abeli, T, Dalrymple, SE, Godefroid, S, Mondoni, A, Muller, JV, Rossi, G and Orsenigo, S (2019) Ex situ collections and their potential for the restoration of extinct plants. Conservation Biology. ISSN 0888-8892

Ex situ collections and their potential for the restoration of extinct plants

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Keywords: botanical gardens; conservation translocations; de-extinction; herbaria; introduction; reintroduction; resurrection biology; seed banks

Article impact statement: Ex situ collections avoid loss of plant diversity, but recovering lost genetic diversity from ex situ material only is highly challenging.

Abstract

The alarming current and predicted species extinction rates have galvanized conservationists in their efforts to avoid future biodiversity losses but for species extinct in the wild, few options exist. We posed the question, can these be restored, and if so, what role can e*x situ* plant collections (i.e. botanic gardens, germplasm banks and herbaria) play in the recovery of plant genetic diversity? We reviewed the relevant literature to assess the feasibility of recovering lost plant genetic diversity using *ex situ* material and the chances of survival of subsequent translocations. Thirteen attempts of recovering species extinct in the wild were found, most of which from material preserved in botanic gardens (12) and seed banks (2). A single case of a locally extirpated population was recovered from herbarium material. Eight (60%) of these cases were successful or partially successful translocations of the focal species or population, while the other five failed or was too early to judge. Our review exposes the many constraints of using *ex situ* source material for the restoration of plant genetic diversity to the wild, but also highlight the opportunities that modern collecting practices present for plant conservation. Limiting factors are the scarcity of available source material stored *ex situ*, low viability and reduced longevity of the material, low genetic variation, lack of evolution (especially for material stored in germplasm banks and herbaria) and socio-economic constraints. However, our review suggests that all types of *ex situ* collections may effectively contribute to plant species conservation, if their use is informed by a thorough understanding of the aforementioned issues. For these reasons, we conclude that the recovery of plant species currently classed as extinct in the wild is not 100% successful and the possibility to achieve this should not be used as a justification for insufficient *in situ* conservation efforts.

INTRODUCTION

According to the most recent scenarios, global biodiversity is predicted to decline over the 21st century, at alarming rates (Pereira et al. 2010). Techniques to halt the loss of biodiversity include intentionally moving organisms for conservation purposes, in other words conservation translocations (IUCN 2013; see Table 1 for terminology). Translocations such as reintroduction and reinforcement assume that the focal species can be restored to an *in situ* habitat. More interventionist translocations such as ecological replacement and de-extinction, or more accurately, the introduction of proxies of extinct species, have raised concerns that organisms being moved and released into near-natural ecosystems will carry too many risks due to the inability of the released organisms to exactly fulfil the ecological place of the extirpated species (Seddon et al. 2014; Seddon 2017). These terms are important to the communication and evaluation of conservation management but distinguishing the interventions can sometimes obscure commonalities that are useful to improving future practice. In this review we draw together a body of work that evaluates *ex situ* contributions to plant conservation and highlights the many considerations that are relevant to well-established interventions such as reintroduction, and the implications this has for actions that might have much in common with the controversial debate around de-extinction. Our main aim is to assess the feasibility of recovering lost plant genetic diversity from *ex situ* plant material by evaluating the role of *ex situ* collections in cases where a final translocation of a species extinct in the wild was achieved. In addition to cases from the peer-review literature, we identified unpublished examples of species formerly declared extinct in the wild at the global level and reintroduced via a survey distributed to staff and affiliates of the European Native Seed Conservation Network (ENSCONET), the IUCN Conservation Translocation Specialist Group and through the authors' contact network of 174 conservation biologists in 38 countries.

De-extinction has been made possible by the technological advances in many fields of biology but the concept has developed within the zoological sciences as a tool to reverse animal extinctions. It is the creation of a *proxy* of an extinct species (IUCN 2016), whereby the term '*proxy*' acknowledges that the resurrected individuals are materially different to the focal species of the attempted de-extinction. Animal de-extinction techniques can be categorised as back-breeding, cloning via somatic cell nuclear transfer (SCNT) and genetic engineering (Shapiro 2017). However, because of the demanding technological requirements for animal de-extinction, the most advanced examples of recovery of ancient genotypes lost from the wild actually involve plants. In contrast to animal de-extinction, the recovery of plant genetic diversity lost from the wild can be achieved relatively easily by propagating seeds and spores, and culturing plant tissue.

The analysis of de-extinction is relevant because many of the criticisms levelled at it can also be aimed at restoration from *ex situ* collections, an action which is one's only resort for many species. We can use the known opportunities and constraints in this field of conservation to explore de-extinction and lead to pragmatic recommendations for furthering the de-extinction debate.

The recent growth of a date palm (*Phoenix dactylifera* L.) from seeds found in a Roman archeological site in Israel and dating back to the first century A.D., suggests that genotypes lost long ago can be successfully recovered (Sallon et al. 2008). *Phoenix dactylifera* exceeds the previous records for viable seeds of *Canna compacta* Rosc. (550 years old; Lerman & Cigliano 1971) and *Nelumbo nucifera* Gaernt. (1288 years old; Shen-Miller et al. 1995). However, these are overshadowed by *Silene stenophylla* Ledeb. recovered from ovary plant tissues preserved in the Siberian permafrost for 30,000 years (Yashina et al. 2012). The longevity of these plant materials makes a compelling case for the possibility of the recovery of extinct species.

Whilst these examples highlight the potential for genetic recovery where the species in question is still extant, it raises the issue that the reinstatement of their genetic material might introduce strains that are substantively different to currently extant populations. They therefore serve to illustrate the point that the use of long-preserved genetic material such as seeds in historical *ex situ* collections, might be akin to introducing a proxy of the existing species in line with definitions of de-extinction.

O'Donnell and Sharrock (2017) state that there are about 500 plant species which are currently preserved *ex situ* which are either extinct in the wild or locally extirpated. Therefore, an analysis of opportunities and constraints resulting from the availability of propagules in *ex situ* plant collections is essential to evaluate their real potential in recovering lost genetic diversity and for translocation in general.

RESTORATION OF GENETIC DIVERSITY FROM LIVING *EX SITU* COLLECTIONS

Restoration of genetic diversity from botanic gardens *sensu lato*

Collections of living specimens include those in public botanic gardens, private gardens, community gardens, arboreta, nurseries and zoos (botanic garden hereafter; From 2016; Bird et al. 2017).

Botanic gardens are very effective in increasing plant stocks through *ex situ* propagation and while a large proportion of plants in botanic gardens are common ornamentals, the cultivation of rare and threatened species for conservation purposes (including conservation translocations; Heywood et al. 2017; see Table 2 for examples) has become increasingly important (Mounce et al. 2017).

Despite the growing role of *ex situ* living plants in conservation, the use of material propagated in botanic gardens presents significant constraints that may jeopardise the success of future interventions. Firstly, genetic variation of *ex situ* populations may decline after several generations of cultivation due to high inbreeding rates, genetic drift and/or small numbers of founders originally collected in the wild, especially for very rare species (Table 3; Maunder et al. 2001a; Wang et al. 2016; Wilson et al. 2017). The most recent collection and management strategies aim to minimize some of these problems by adding specimens to living collections to achieve genetic diversity comparable with that of wild populations (Cibrian-Jaramillo et al., 2013; Christe et al. 2014; Griffith et al. 2015; Table 3), but there may be a trade-off between improved genetic diversity and increased costs of maintenance of additional plants. Advanced tools developed for managing the genetic variation in captive animal populations are increasingly applied to *ex situ* plant collections (e.g. PMx software; Lacy et al. 2011) and strategic material exchange between botanic gardens worldwide ensures back-up collections and a high number of individual plants that can be used for propagation. Swapping material for cross-fertilisation effectively maintains genetic diversity *ex situ* (e.g. Cibrian-Jaramillo et al. 2013), unlike the exchange of clones or inbred individuals that might result in genetically similar stocks (Theaker & Briggs 1993). The second major constraint in the use of cultivated material affects even the most carefully managed collections: cultivation and horticultural care are known to affect both the evolution of *ex situ* plant populations and the individual ability to tolerate stress, with poorly understood consequences for translocation (Ensslin et al. 2015). These cultivation techniques become selective forces affecting genotypes and life traits in garden populations of different species, with greater effects on annual and short-lived plants, whilst long-lived perennials as trees are less affected, or show no effects at all (Ensslin et al. 2011; Lauterbach et al. 2012). Selective forces can be positive but are likely to be maladaptive when plants are subsequently released into natural habitat (Ensslin et al. 2011). Recent cultivation guidelines aim to reduce the effects of *ex situ* cultivation cares (Basey et al. 2015). Nursery conditions can reduce plant viability and vigor after several generations, especially in short-lived plants and although *ex situ* stocks can be revitalized with new propagules from wild or *ex situ* sources (Navarro et al. 2016), this is of course impossible with species which are already extinct in the wild. Proper manipulations of the growing conditions have recently proved effective in improving *ex situ* plant quality via plasticity, preconditioning or transgenerational maternal effects (Brancaleoni et al. 2018).

There have now been many successful translocations of endangered plant species, where plants have been collected from the wild, multiplied in *ex situ* conditions and restored back to the wild (e.g. Ramsay 1998). Despite this, the potential of botanic gardens to contribute to translocation is not fully realised (Cibrian-Jaramillo et al. 2013). Constraints associated with using living specimens from botanic gardens (Table 3) limit the possibility to reintroduce species (or subspecific taxa) extinct in the wild from botanic garden material only, especially for material collected before modern protocols and collection management strategies and tools were adopted (Cavender et al. 2015). It is therefore not surprising that of 13 species globally extinct in the wild or locally extirpated which have been reintroduced from botanic gardens only three (23%) were successfully reintroduced while 31% of cases failed, and (38%) were considered only partially successful (Table 2).

Restoration of genetic diversity from germplasm banks

Germplasm banks for plants are mainly represented by seed and spore banks specifically developed to store plant material for conservation and research purposes in the long term (Schoen & Brown 2001). There are more than 1750 germplasm banks in the world, most of them represented by agricultural gene banks (storing crop diversity) and several represented by wild plant gene banks (the most relevant for this study) storing wild species diversity. Germplasm banks store in total about 4.6 million accessions (Hay & Probert 2013).

One of the targets of the Global Strategy for Plant Conservation 2011-2020 is to make 20% of the germplasm bank collections available for *in situ* conservation translocation actions (http://www.plants2020.net/gspc-targets/). Therefore, it is important to evaluate the potential of germplasm bank accessions to contribute to the recovery of lost genetic diversity. Germplasm banks can contribute to plant recovery in two ways, 1) directly through their collections and propagation facilities and 2) through the conservation expertise of the researchers who curate the collections. This expertise can help to drive necessary research on longevity in storage, dormancy-breaking and germination requirements of rare wild species to improve effective seed use (Merritt & Dixon 2011). Stored seed stocks were used as source material for several reintroductions of threatened species (Cochrane et al. 2007 and reference therein). However, we found only two cases of species extinct in the wild recovered using seed bank material (*Diplotaxis settiana* and *Erica verticillata*; Table 2), both of which were successful.

Similar to natural systems, high seedling mortality can negatively affect a conservation translocation by rapidly depleting the plant stocks. To overcome this, several reintroduction guidelines suggest growing plants in a controlled environment before their release as adult or sub-adult plants (Godefroid et al. 2011b; Maschinski & Albrecht 2017). In this case, propagation and ongoing care should be undertaken in such a way as to minimise the detrimental impacts of cultivation discussed in the previous section.

The key target of germplasm banks is to keep seeds and spores alive by preserving their inherent longevity. About 75 to 80% of seed-bearing species produce orthodox seeds that can survive a drying process under standard conditions (i.e. drying at 15% eRH, 15°C) and prolonged storage at − 20°C (Walters et al. 2013). Under these conditions, seed germinability might take decades, perhaps centuries, to decline (see Walters et al. 2005). Nevertheless, even under optimal storage conditions loss of seed viability due to seed aging over time is inevitable (Bewley et al. 2013), and this in turn affects seedling emergence and survival. Longevity varies between species and different populations of the same species depending partly on climate, with plants from hot, dry sites generally lasting longer than those from cool, wet climates (Probert et al. 2009; Mondoni et al. 2011). Other important correlates of seed longevity include embryo size and maturity (Probert et al. 2009) and seed dispersal syndrome (Merrit et al. 2014). Additionally, seed longevity has shown transgenerational changes associated with environmentally induced parental effects (Kochanek et al. 2010).

One of the significant advantages of seed banks is the ability to store many species with orthodox seeds in a limited space, reducing collection maintenance costs. Seed banks can flexibly accommodate seeds when they are produced in quantity (in response to unpredictable masting events for example) and multi-year accessions reduce pressures on small wild populations (Cochrane et al. 2007). An issue with seed stocks of rare species is the quantity that may be available for translocation, which is usually very low (Cochrane et al. 2007). Moreover, up to 10% of all angiosperms produce recalcitrant seeds, i.e. seeds that are not desiccationtolerant and which therefore cannot be stored using standard seed banking protocols (Berjak & Pammenter 2008), and this percentage increases to 36% if *critically endangered* plant species are considered (Wyse et al. 2018). *Ex situ* conservation of recalcitrant seeds is sometimes possible with cryogenic technologies, whereby seeds are rapidly cooled at ultralow temperatures, often in liquid nitrogen (–196°C; Walters et al. 2008). Cryopreservation may be used for tissues other than seeds (e.g. meristems). However, cryogenic storage is costly and requires specialized infrastructure and highly trained personnel. In addition, recalcitrant seeds require rigorous preparations before being exposed to cryogenic storage (e.g. surgical dissection of embryonic axis; Engelmann 2011). Consequently, the conservation of species with recalcitrant seeds for large-scale plant translocation is technically possible, but may not be feasible from a practical or financial point of view.

RESTORATION OF GENETIC DIVERSITY FROM HERBARIUM SPECIMENS

As sources of genetic material for translocation, herbaria compare poorly with living collections, especially germplasm banks, where high-quality storage conditions are applied to promote seed/spore longevity. Nevertheless, the sheer number of preserved specimens, more than 387 million specimens in about 3000 herbaria (Thiers 2018) means that their potential to provide genetic material should be considered (Bowles et al. 1993). Indeed, considering that species not occurring in the wild and preserved in seed banks should not be considered as extinct *sensu* IUCN (Dalrymple & Abeli 2019), herbaria represent the sole possibility to resurrect true extinct species.

So far, there have been few attempts to use herbarium specimens in translocation and most research to date has only explored their potential as a propagule source. Several authors have obtained viable spores and seeds from herbarium vouchers up to 237 years old, which indicates that spores and seeds may remain viable in an herbarium for a long time (see Molnár et al. 2015 and references therein). In ferns, chlorophyllous spores decrease their viability more rapidly than non-green spores. Studies on angiosperms suggest that *Fabaceae* have some of the most long-lived seeds surviving in herbarium sheets followed by *Poaceae* and *Apiaceae* (for more details, see Molnár et al. 2015). However, storage conditions seem more important than taxonomic or ecological characteristics in determining seed viability preserved in herbarium specimens (Godefroid et al. 2011b).

Only one attempt has progressed towards the establishment of viable plant populations from propagules gained from herbarium specimens (*Crepis foetida* L. subsp. *foetida*; Table 2; Sears 2011).

Some critical issues limit the use of herbarium spores or seeds (Table 3). Herbarium-sourced translocation material is generally scarce in terms of the number of specimens for rare species and number of spores/seeds preserved within each specimen (Godefroid et al. 2011b). Moreover, spores and seeds typically show low viability and in old specimens the DNA is often degraded (Leino & Edqvist 2010). Godefroid et al. (2011a) explored the feasibility of propagating 26 extinct taxa from the Belgian flora from old herbarium vouchers that had been stored for 23 to 158 years. Of the 2,672 seeds tested, only 8 seeds from a single species germinated and these did not produce viable seedlings.

Several studies reported germination as a percentage of the sown seeds that germinated, without considering that an unknown proportion of those seeds were already dead at the beginning of germination tests (Smith et al. 2003). This prevents the accurate assessment of seed viability. Germination is often reported at radicle emergence, i.e. the tip of the root tissue has penetrated the seed coat. However, Godefroid et al. (2011b) observed radicle emergence in > 100 year old viable seeds of *Bupleurum tenuissimum* L., without any further development of the shoots further complicating the accurate reporting of germination from herbarium-sourced propagules.

Low seed germination percentages of seeds from herbarium specimens might also be due to a deep secondary dormancy status induced by unfavourable storage conditions (Merritt et al. 2014). Seed dormancy works as an ecological mechanism that allows seeds to germinate only when conditions are suitable for supporting seedling growth (Finch-Savage & Leubner-Metzger 2006), but it is a constraint when seeds have to be used for plant regeneration (Ladouceur et al. 2017). Proper dormancy breaking techniques should be applied to induce germination, such as cold and/or warm stratification and dry after-ripening in the case of physiological dormant seeds (see for a review, Baskin & Baskin 2014). However, when working with rare species, the required information is often not available, and experimentation is therefore necessary (Godefroid et al. 2016), which may rapidly deplete the already limited stock of seeds available from herbarium specimens.

Low viability of herbarium propagules may also be due to pest control treatments, including the application of chemicals and heat treatments (Godefroid et al. 2011b; Godefroid et al. 2017). Modern protocols for the care of herbarium specimens avoid the use of chemicals where possible, by using sealed containers and periodic freezing (RBGE 2017).

The final, but very important caveat associated with using herbarium specimens is that delivering conservation benefit might undermine the primary use of herbaria collections; sampling spores or seeds from herbaria may destroy or irreparably damage the specimens which significantly limits the use of voucher specimens of historical importance for taxonomic descriptions (Graves & Braun 1992; Shiga 2013). The assessment as to whether that risk is worth taking is something that needs consideration at species level and taking into account the availability of specimens for both systematics and conservation.

CONSTRAINTS OF *EX SITU* PLANT COLLECTIONS FOR THE RESTORATION OF LOST GENETIC DIVERSITY AND POSSIBLE SOLUTIONS

General obstacles to the recovery of plant genetic diversity lost from the wild

Volis & Blechner (2010) clearly identify the main roles of *ex situ* collections in conservation: 1) creating a backup of genetic material should *in situ* conservation actions fail; 2) preserving a significant portion of the genetic diversity of a species and; 3) propagating species for restoration. Botanic gardens and seed banks can be very effective in achieving these goals, while the potential of herbaria still needs further investigation. Botanic gardens play a valuable role in propagating plants for translocation using natural populations as source material (e.g. Baker et al. 2014; Makowski et al. 2016). However, this approach is not possible when a species or other taxon is extinct in the wild and there are additional constraints that make the recovery and release of genetic diversity lost from the wild difficult (Tables 1 and 3). Below we summarise these constraints to stimulate a scientific debate on possible solutions.

1) Unintentional selection after several generations in *ex situ* cultivation of short-lived species make propagules unsuitable for their reintroduction.

2) The tendency to have low quantities of seed/plant stocks preserved *ex situ* is often reflected in reduced genetic diversity of *ex situ* collections (Sarasan et al. 2016) and recovery attempts based on only a very small number of founders (e.g. *Normania triphylla* (Lowe) Lowe one individual; Delmail et al. 2012).

3) From an evolutionary point of view, material stored in germplasm banks and herbaria is only representative of the time at which it was collected, whereas environmental factors impose evolutionary changes in extant plant populations (Lowe et al. 2000). When material from old *ex situ* collections is propagated, it may no longer be adapted to current abiotic conditions and biotic interactions (e.g. with symbionts, pests and pollinators; Schoen & Brown 2001). This is particularly relevant for species that have long been missing from their natural habitat - after the rearrangement of the ecosystem following their extinction, they may no longer find a suitable niche. For example, Yashina et al. (2012) found significant differences in the flower morphology of ancient and extant *Silene stenophylla* plants that may reflect different reproductive strategies.

4) Despite the fact that most lost genetic variation and extinct species are preserved as herbarium specimens, the value of this material is doubtful because of the unsuitable conditions for the long-term viability of seeds and spores and pest-control treatments.

5) Spontaneous hybridization between morphologically similar congenerics can also occur in *ex situ* collections because of overlapping flowering period and spatial proximity (Maunder et al. 2004). Such a risk of hybridization may considerably restrict the conservation value of botanic garden collections (Volis 2017). Interspecific hybridization of some *ex situ* collections intended for reintroduction have already been raised previously in the case of the extinct species *Sinojackia xylocarpa* Hu (Zhang et al. 2009) and *Sophora toromiro* Skottsb. (Püschel et al. 2014).

Of course, there are further obstacles to the recovery of species extinct from the wild which are not directly connected to the type of source material (see Sandler 2013):

i) lack of suitable habitats. Before re-introducing a species extinct in the wild, one must first ensure that its habitat still exists or that it has been appropriately restored. In some cases, this is difficult if not impossible, because we lack even a description of the original native habitat, e.g. in the case of *Tulipa sprengeri* Baker (Maunder et al. 2001b);

ii) support from the local community. The case of *Bromus bromoideus* (Lej.) Crep. illustrates the complexity of recovery programmes from a social perspective. Endemic to southern Belgium and northern France, *B. bromoideus* has been extinct in the wild since 1935. Today it exists in six *ex situ* collections, but no translocations are currently planned. The species is associated with a crop that is nowadays rarely used in Western Europe (*Triticum spelta* L.) and a survey among farmers had shown that most of them were not in favour of its translocation (Godefroid et al. 2010).

Lessons for practitioners

In recent years, botanic gardens and seed banks have made significant progress towards the conservation of endangered species. In Europe, a recent assessment showed that 63% of European threatened species are already conserved *ex situ* in seed banks (Rivière et al. 2018). However, it appears that relatively few of these are used for translocation actions in the wild. According to the European seed bank database ENSCOBASE (as of 20/06/2018), of the 67620 seed accessions of native plants stored in European seed banks, only 64 accessions (= 0.09%) were used in translocation programmes (http://enscobase.maich.gr/).

In order to promote the use of *ex situ* accessions, facilities such as botanic gardens and germplasm banks could modify collection strategies to ensure that the harvested material can be used for translocations in the wild (Walck & Dixon 2009). For high priority species, seed, spore and plant collections should follow the most recent protocols to optimise the genetic diversity captured with reference to the global genetic population structure of the target species (Hoban & Schlarbaum 2014). Ideally, material collections should focus not only on amassing numbers of species when many of these species will be poorly represented, but also on improving the quality of the collections. Collecting should therefore include infra-specific taxonomic levels (e.g. subspecies, ecotypes), different ploidy levels (different chromosome numbers within a species), several populations from across a species' distribution (Akeroyd & Wise Jackson 1995; Griffith et al. 2015), both sexes in dioecious species, and should represent the interannual variability of seed/spore performance with multiple-year collections (Table 4). Alternative techniques such as cryopreservation and tissue cultures should be improved to allow recalcitrant-seeded species to be maintained *ex situ* (Wyse et al. 2018).

Our review highlights the role of herbaria in supporting species conservation, particularly in the recovery of species extinct in the wild. However, survival of plant propagules in herbaria is often accidental. Moreover, herbaria would have difficulties to conform to these new collecting policies; collecting many individuals for an herbarium would seriously jeopardize the chances of survival of endangered species. However, herbaria might play a more valuable conservation role by ensuring the availability of flowering and fruiting plants among their vouchers from which pollen and seeds may be collected and adopting pest treatments with no or limited effects on spore, seed and pollen viability. Such recommendations are of importance considering the recent debate on synthetic biology applied to conservation (including DNA synthesis; Piaggio et al. 2017) as advancements in molecular engineering will make herbaria possible sources of genetic material. Herbaria may also provide important information to support restoration programmes such as dates of occurrence, distribution and habitat of a focal species.

The final phase of genetic restoration is translocation of propagules or plants to the wild and subsequent management to promote establishment and regeneration. However, the relatively low success rate of translocation has prompted recommendations for improved practice in many aspects of the intervention (Godefroid et al. 2011a; Dalrymple et al. 2012). Our review has suggested how our *ex situ* facilities might be better employed but we also recommend the integration of conservation biology with competencies known to agronomists (e.g. genome editing, marker assisted breeding) which are as yet, unused or even unknown in the field of wild plant conservation.

In conclusion, despite recent advancements in technology, the recovery of plants that are extinct in the wild, and their subsequent translocation, is still a little-used conservation approach due to the logistical and ecological complexity in undertaking such interventions. Whilst we have detailed the growing role and overlooked potential of *ex situ* plant collections, we have also deliberately articulated the limitations of the various modes of storage and the implications there are for genetic restoration. These observations lead us to conclude that the recovery of some threatened species may rely on *ex situ* plant conservation in the future, but successful intervention will not depend on this alone. With plant material secured *ex situ*, more time is available for engagement with stakeholders, habitat rehabilitation or the development of suitable propagation techniques. However, the infrastructure, policy and practice of threatened species management must continue to prioritise *in situ* species protection with *ex situ* interventions taking a supporting role and under no circumstance should the existence of botanic gardens, seed banks and herbaria be used as a justification against effective *in situ* species protection through other means.

Acknowledgments: We are grateful to Stephane Buord (Conservatoire Botanique National de Brest, France), David A. Burney (National Tropical Botanical Garden, Kalaheo, Hawaii), Carly Cowell (South African National Parks), Ren Hai (Chinese Academy of Science, China), Thomas Heller (Royal Botanic Gardens, Kew, UK), Hillary K. Kawelo (Natural Resource Manager, U.S. Army Garrison, Hawaii), Sierra McDaniel (National Park Service, US), and Norio Tanaka (Tsukuba Botanical Garden, National Museum of Nature and Science, Japan), Dr. Marko Hyvärinen (Finnish Museum of Natural History, Finland) for the valuable information on the species reported in table 2 and other species. Authors thank all the colleagues that have replied to the survey, in particular: Doug Bickerton (Policy Officer, Threatened Species, Department for Environment and Water, South Australia), Dr. Mike Maunder (The Eden Project), Priptal Soorae (IUCN Conservation Translocation Specialist Group); Paul Smith (Botanic Garden Conservation International). We also thank the three anonymous reviewers for their useful suggestions. The Grant of Excellence Departments, MIUR-Italy (ARTICOLO 1, COMMI 314 – 337 LEGGE 232/2016) is gratefully acknowledged for the support to one of the authors (Prof. Thomas Abeli).

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Terminology	**Meaning**	**Source**
Conservation translocation	Intentional movement and release of a living organism where the primary objective is a conservation benefit. It includes population reinforcement, reintroduction and conservation introduction.	IUCN, 2013
De-Extinction	Term used in a limited sense to	IUCN, 2016

	apply to any attempt to create some proxy of an extinct species, not an exact replica of any extinct species.	
Recovery	Term used here to indicate the process of re-establishing species or populations, that were lost from the wild. It does not include the release of the material in a recipient site.	This article

Table 1 – Explanation of the terminology used in the review.

Species	**Status**	**Source collection**	**Success**	**Generation length /growth form**	**Reference**
Bromus interruptus (Hack.) Druce	Extinct in the wild, UK	Private garden	Successful	Annual/Herb	Marren 2005
Cyanea superba Cham.	Extinct in the wild, Hawaii, US	Botanic garden	Too early to judge	Ca. 10 years/Tree	Hillary Kawelo, pers. comm.
Cylindrocline lorencei A.J.Scott	Extinct in the wild, Mauritius	Botanic garden	Unsuccessful	Unknown/Tree	Stéphane Buord, pers. comm.
Diplotaxis siettiana Maire	Extinct in the wild, Spain	Seed bank	Successful	Annual or Biennal/Herb	Pérez Latorre et al. 2011
Erica turgida Salisb.	Extinct in the wild, South Africa	Botanic Garden	Unsuccessful	Unknown/Shrub	Carly Cowell, pers. comm.
Erica verticillata P.J. Bergius	Extinct in the wild, South Africa	Botanic garden; Private garden; Seed	Successful	< 10 years/Shrub	Hitchcock & Rebelo 2017

		bank			
Eriocaulon heleocharioides Satake	Extinct in the wild, Japan	Botanic garden	Partially successful	Annual/Herb	Tanaka et al. 2015; Norio Tanaka, pers. comm.
Hibiscadelphus giffardianus Rock	Extinct in the wild, Hawaii, US	Botanic garden	Partially successful	Not found/Tree	Belfield et al. 2011
Lachanodes arborea (Roxb.) B.Nord.	Extinct in the wild, St. Helena, UKOTs	Local plant nursery	Partially successful	Unknown/Tree	Thomas Heller pers. comm.
Lysimachia minoricensis J.J. Rodr.	Extinct in the wild, Minorca, Spain	Botanic garden	Partially successful	Biennal/Herb	Galicia Herbada & Fraga Arquimbau 2011
Normania triphylla (Lowe) Lowe	Extinct in the wild, Madera, Portugal	Botanic garden	Unsuccessful	Unknown	Delmail et al. 2012
Sophora toromiro Skottsb.	Extinct in the wild, Easter island, Chile	Botanic garden	Unsuccessful	Not found/Shrub	World Conservation Monitoring Centre 1998; Maunder et al. 2000
Trochetiopsis erythroxylon (Forst.) Marais	Extinct in the wild, St. Helena, UKOTs	Nursery	Partially successful	< 20 years/Tree	Lambdon & Ellick 2016; Thomas Heller, pers. comm.

Table 2 – Species extinct in the wild recovered from *ex situ* source material; ‘successful’ refers to a reintroduced population that is self-sustainable, resulting in a new generation; ‘partially successful’ refers to translocation in which released plants are still alive but have not produced a second generation; ‘unsuccessful’ refers to reintroduced populations that disappeared without a new generation. Indications of success or failure reported in table 2 were derived directly from the literature cited or from personal communications from the authors of the reintroductions. Affiliations of the colleagues that provided personal communications are given in the acknowledgments.

Strengths/Weaknesses	**Botanic gardens *s.l.***	**Seed banks**	**Herbaria**
Strengths			
Propagation facilities	x	x	
Improved collecting strategies ensuring that genetic wild diversity is represented *ex situ*	x	x	
Improved cultivation strategies avoiding adaptation to *ex situ* conditions	x		
Low cost of maintenance		x	x
Safe long-term storage		x	

Historic collections (old material preserved)			x
Large number of specimens		x	x
Weaknesses			
Reduced genetic variation in old accessions	x	x	x
Founder effect	x	x	
Unintentional selection (e.g. larger seeds, faster germination, dormancy loss, bigger plants, etc)	x	x	
Adaptation to garden conditions	x		
Hybridization	x		
Genetic drift through recurrent propagation	x		
Material susceptible to pests and diseases	x		x
Freezing of evolution		x	x
Historical importance (that limits usability)			x
Accidental propagule survival			x
Opportunities			
Application of advanced technologies (e.g. molecular engineering and synthetic biology)	x	x	x
Maternal effects	x		
Pre-conditioning	x		

Table 3 – Strengths, weaknesses and opportunities of *ex situ* collections as source material for recovery of plant lost genetic diversity and translocation.

Obstacles	Possible solutions
Reluctance from land management authorities, government agencies, local stakeholders	Outreach and educational programmes
Low success rate of translocation	Further research and training of specialized professional figures with multidisciplinary competences
Reason for extinction unknown	Further studies should be encouraged in the lab or on the field to identify specific threats
Low genetic diversity	If possible, accessions of different origin should be included in order to maximize the original genetic diversity. Genetic studies of *ex situ* collections could help in assessing the conservation

	value of this material
Challenging propagation	Further research needed on the development of efficient propagation protocols
Habitat degraded or no longer existing	Habitat must first be restored to a state that ensures the viability of the target species

Table 4 – Summary of obstacles to the recovery of plant extinct in the wild and possible solutions