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

2

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20

21 **Keywords:** botanical gardens; conservation translocations; de-extinction; herbaria;

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23

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

26

27 **Abstract**

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

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51 INTRODUCTION

52 According to the most recent scenarios, global biodiversity is predicted to decline over the 21st century, at
53 alarming rates (Pereira et al. 2010). Techniques to halt the loss of biodiversity include intentionally moving
54 organisms for conservation purposes, in other words conservation translocations (IUCN 2013; see Table 1
55 for terminology). Translocations such as reintroduction and reinforcement assume that the focal species can
56 be restored to an *in situ* habitat. More interventionist translocations such as ecological replacement and de-
57 extinction, or more accurately, the introduction of proxies of extinct species, have raised concerns that
58 organisms being moved and released into near-natural ecosystems will carry too many risks due to the
59 inability of the released organisms to exactly fulfil the ecological place of the extirpated species (Seddon et
60 al. 2014; Seddon 2017). These terms are important to the communication and evaluation of conservation
61 management but distinguishing the interventions can sometimes obscure commonalities that are useful to
62 improving future practice. In this review we draw together a body of work that evaluates *ex situ*
63 contributions to plant conservation and highlights the many considerations that are relevant to well-
64 established interventions such as reintroduction, and the implications this has for actions that might have
65 much in common with the controversial debate around de-extinction. Our main aim is to assess the feasibility
66 of recovering lost plant genetic diversity from *ex situ* plant material by evaluating the role of *ex situ*
67 collections in cases where a final translocation of a species extinct in the wild was achieved. In addition to
68 cases from the peer-review literature, we identified unpublished examples of species formerly declared
69 extinct in the wild at the global level and reintroduced via a survey distributed to staff and affiliates of the
70 European Native Seed Conservation Network (ENSCONET), the IUCN Conservation Translocation
71 Specialist Group and through the authors' contact network of 174 conservation biologists in 38 countries.
72 De-extinction has been made possible by the technological advances in many fields of biology but the
73 concept has developed within the zoological sciences as a tool to reverse animal extinctions. It is the creation
74 of a *proxy* of an extinct species (IUCN 2016), whereby the term '*proxy*' acknowledges that the resurrected
75 individuals are materially different to the focal species of the attempted de-extinction. Animal de-extinction
76 techniques can be categorised as back-breeding, cloning via somatic cell nuclear transfer (SCNT) and genetic
77 engineering (Shapiro 2017). However, because of the demanding technological requirements for animal de-

78 extinction, the most advanced examples of recovery of ancient genotypes lost from the wild actually involve
79 plants. In contrast to animal de-extinction, the recovery of plant genetic diversity lost from the wild can be
80 achieved relatively easily by propagating seeds and spores, and culturing plant tissue.

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

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

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

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

101

102 **RESTORATION OF GENETIC DIVERSITY FROM LIVING *EX SITU* COLLECTIONS**

103 **Restoration of genetic diversity from botanic gardens *sensu lato***

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

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

110 Despite the growing role of *ex situ* living plants in conservation, the use of material propagated in botanic
111 gardens presents significant constraints that may jeopardise the success of future interventions. Firstly,
112 genetic variation of *ex situ* populations may decline after several generations of cultivation due to high
113 inbreeding rates, genetic drift and/or small numbers of founders originally collected in the wild, especially
114 for very rare species (Table 3; Maunder et al. 2001a; Wang et al. 2016; Wilson et al. 2017). The most recent
115 collection and management strategies aim to minimize some of these problems by adding specimens to living
116 collections to achieve genetic diversity comparable with that of wild populations (Cibrian-Jaramillo et al.,
117 2013; Christe et al. 2014; Griffith et al. 2015; Table 3), but there may be a trade-off between improved
118 genetic diversity and increased costs of maintenance of additional plants. Advanced tools developed for
119 managing the genetic variation in captive animal populations are increasingly applied to *ex situ* plant
120 collections (e.g. PMx software; Lacy et al. 2011) and strategic material exchange between botanic gardens
121 worldwide ensures back-up collections and a high number of individual plants that can be used for
122 propagation. Swapping material for cross-fertilisation effectively maintains genetic diversity *ex situ* (e.g.
123 Cibrian-Jaramillo et al. 2013), unlike the exchange of clones or inbred individuals that might result in
124 genetically similar stocks (Theaker & Briggs 1993). The second major constraint in the use of cultivated
125 material affects even the most carefully managed collections: cultivation and horticultural care are known to
126 affect both the evolution of *ex situ* plant populations and the individual ability to tolerate stress, with poorly
127 understood consequences for translocation (Ensslin et al. 2015). These cultivation techniques become
128 selective forces affecting genotypes and life traits in garden populations of different species, with greater
129 effects on annual and short-lived plants, whilst long-lived perennials as trees are less affected, or show no
130 effects at all (Ensslin et al. 2011; Lauterbach et al. 2012). Selective forces can be positive but are likely to be
131 maladaptive when plants are subsequently released into natural habitat (Ensslin et al. 2011). Recent

132 cultivation guidelines aim to reduce the effects of *ex situ* cultivation cares (Basey et al. 2015). Nursery
133 conditions can reduce plant viability and vigor after several generations, especially in short-lived plants and
134 although *ex situ* stocks can be revitalized with new propagules from wild or *ex situ* sources (Navarro et al.
135 2016), this is of course impossible with species which are already extinct in the wild. Proper manipulations
136 of the growing conditions have recently proved effective in improving *ex situ* plant quality via plasticity,
137 preconditioning or transgenerational maternal effects (Brancaleoni et al. 2018).

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

148

149 **Restoration of genetic diversity from germplasm banks**

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

155 One of the targets of the Global Strategy for Plant Conservation 2011-2020 is to make 20% of the
156 germplasm bank collections available for *in situ* conservation translocation actions
157 (<http://www.plants2020.net/gspc-targets/>). Therefore, it is important to evaluate the potential of germplasm
158 bank accessions to contribute to the recovery of lost genetic diversity. Germplasm banks can contribute to
159 plant recovery in two ways, 1) directly through their collections and propagation facilities and 2) through the

160 conservation expertise of the researchers who curate the collections. This expertise can help to drive
161 necessary research on longevity in storage, dormancy-breaking and germination requirements of rare wild
162 species to improve effective seed use (Merritt & Dixon 2011). Stored seed stocks were used as source
163 material for several reintroductions of threatened species (Cochrane et al. 2007 and reference therein).
164 However, we found only two cases of species extinct in the wild recovered using seed bank material
165 (*Diplotaxis settiana* and *Erica verticillata*; Table 2), both of which were successful.

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

171 The key target of germplasm banks is to keep seeds and spores alive by preserving their inherent longevity.
172 About 75 to 80% of seed-bearing species produce orthodox seeds that can survive a drying process under
173 standard conditions (i.e. drying at 15% eRH, 15°C) and prolonged storage at – 20°C (Walters et al. 2013).
174 Under these conditions, seed germinability might take decades, perhaps centuries, to decline (see Walters et
175 al. 2005). Nevertheless, even under optimal storage conditions loss of seed viability due to seed aging over
176 time is inevitable (Bewley et al. 2013), and this in turn affects seedling emergence and survival. Longevity
177 varies between species and different populations of the same species depending partly on climate, with plants
178 from hot, dry sites generally lasting longer than those from cool, wet climates (Probert et al. 2009; Mondoni
179 et al. 2011). Other important correlates of seed longevity include embryo size and maturity (Probert et al.
180 2009) and seed dispersal syndrome (Merritt et al. 2014). Additionally, seed longevity has shown
181 transgenerational changes associated with environmentally induced parental effects (Kochanek et al. 2010).

182 One of the significant advantages of seed banks is the ability to store many species with orthodox seeds in a
183 limited space, reducing collection maintenance costs. Seed banks can flexibly accommodate seeds when they
184 are produced in quantity (in response to unpredictable masting events for example) and multi-year accessions
185 reduce pressures on small wild populations (Cochrane et al. 2007). An issue with seed stocks of rare species
186 is the quantity that may be available for translocation, which is usually very low (Cochrane et al. 2007).
187 Moreover, up to 10% of all angiosperms produce recalcitrant seeds, i.e. seeds that are not desiccation-

188 tolerant and which therefore cannot be stored using standard seed banking protocols (Berjak & Pammenter
189 2008), and this percentage increases to 36% if *critically endangered* plant species are considered (Wyse et al.
190 2018). *Ex situ* conservation of recalcitrant seeds is sometimes possible with cryogenic technologies, whereby
191 seeds are rapidly cooled at ultralow temperatures, often in liquid nitrogen (−196°C; Walters et al. 2008).
192 Cryopreservation may be used for tissues other than seeds (e.g. meristems). However, cryogenic storage is
193 costly and requires specialized infrastructure and highly trained personnel. In addition, recalcitrant seeds
194 require rigorous preparations before being exposed to cryogenic storage (e.g. surgical dissection of
195 embryonic axis; Engelmann 2011). Consequently, the conservation of species with recalcitrant seeds for
196 large-scale plant translocation is technically possible, but may not be feasible from a practical or financial
197 point of view.

198

199 **RESTORATION OF GENETIC DIVERSITY FROM HERBARIUM SPECIMENS**

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

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

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

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

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

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

242 Low viability of herbarium propagules may also be due to pest control treatments, including the application
243 of chemicals and heat treatments (Godefroid et al. 2011b; Godefroid et al. 2017). Modern protocols for the

244 care of herbarium specimens avoid the use of chemicals where possible, by using sealed containers and
245 periodic freezing (RBGE 2017).

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

252

253

254 **CONSTRAINTS OF *EX SITU* PLANT COLLECTIONS FOR THE RESTORATION OF LOST** 255 **GENETIC DIVERSITY AND POSSIBLE SOLUTIONS**

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

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

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

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

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

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

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

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

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

295 ii) support from the local community. The case of *Bromus bromoides* (Lej.) Crep. illustrates the complexity
296 of recovery programmes from a social perspective. Endemic to southern Belgium and northern France, *B.*
297 *bromoides* has been extinct in the wild since 1935. Today it exists in six *ex situ* collections, but no

298 translocations are currently planned. The species is associated with a crop that is nowadays rarely used in
299 Western Europe (*Triticum spelta* L.) and a survey among farmers had shown that most of them were not in
300 favour of its translocation (Godefroid et al. 2010).

301

302 **Lessons for practitioners**

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

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

321 Our review highlights the role of herbaria in supporting species conservation, particularly in the recovery of
322 species extinct in the wild. However, survival of plant propagules in herbaria is often accidental. Moreover,
323 herbaria would have difficulties to conform to these new collecting policies; collecting many individuals for
324 an herbarium would seriously jeopardize the chances of survival of endangered species. However, herbaria

325 might play a more valuable conservation role by ensuring the availability of flowering and fruiting plants
326 among their vouchers from which pollen and seeds may be collected and adopting pest treatments with no or
327 limited effects on spore, seed and pollen viability. Such recommendations are of importance considering the
328 recent debate on synthetic biology applied to conservation (including DNA synthesis; Piaggio et al. 2017) as
329 advancements in molecular engineering will make herbaria possible sources of genetic material. Herbaria
330 may also provide important information to support restoration programmes such as dates of occurrence,
331 distribution and habitat of a focal species.

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

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

351

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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

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

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

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

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

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Strengths/Weaknesses	Botanic gardens	Seed banks	Herbaria
	<i>s.l.</i>		
Strengths			
Propagation facilities	x	x	
Improved collecting strategies ensuring that genetic wild diversity is represented <i>ex situ</i>	x	x	
Improved cultivation strategies avoiding adaptation to <i>ex situ</i> 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		

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

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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 <i>ex situ</i> 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

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