

## *Ex situ* conservation in botanical gardens – challenges and scientific potential preserving plant biodiversity

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### Abstract

In the Anthropocene, the world's plant diversity is threatened with extinction and the erosion of the genetic diversity of natural populations. According to the State of the World's Plants and Fungi 2020 of the Royal Botanic Gardens, Kew, two out of five of the ~350,000 known vascular plant species are at risk of extinction. Despite the considerable toolkit of biodiversity conservation practices, usually it is hard to choose the best option to stop biodiversity loss. *Ex situ* conservation has seen massive development due to radical losses of natural ecosystems, and its incrementing necessity has been underscored by Target 8 of the 2011-2020 Global Strategy for Plant Conservation. As we crossed the finish line of this strategy in 2020, a review of the accumulated knowledge on the *ex situ* living collections has become particularly important. Despite the increasing attention received by *ex situ* conservation, studies on the sustainability, quality, and usability of the plant material prior to establishing the garden collections are few, leaving major gaps unfilled in terms of best *ex situ* conservation practices. Here we present an overview of the results and experiences in *ex situ* conservation focusing on living plant collections, with the aim of guiding conservation practitioners towards the most efficient working methods. We evaluate the future needs and perspectives of this conservation technique, based on case studies on both woody and herb species. Possible conservation applications and priorities suggested for future works are summarized.

**Keywords:** biodiversity conservation; *ex situ*; functional traits; genetic diversity; living collections; review

### Introduction

*Ex situ* (off-site) methods are the “Noah’s Ark” approaches that complement *in situ* plant conservation in alleviating the negative consequences of habitat loss (Falk, 1987; Given, 1987; Brown and Briggs, 1991; Volis and Blecher, 2010). *Ex situ* conservation mitigates the extinction risk of species and populations by displacing the genetic resource to a new place such as seed banks, field gene banks, or to living collections located mainly in botanical gardens. As a result, *ex situ* conservation can fulfil a wide range of purposes and roles: (1) preserve

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a representative sample from a species' genetic material, i.e., represent the germplasm backup against extinction (2) have an important educational and demonstration role, (3) offer a viable alternative to commercial harvesting from natural populations, (4) propagated individuals can be used to re-establish or enrich wild source populations, (5) represent long-term gene reserves and preserve functional traits for future use (Brown and Briggs, 1991; Maunder *et al.*, 2001; Guerrant *et al.*, 2004; Volis and Blecher, 2010). Further, *ex situ* collections provide study opportunities on a species' genetic material without harming the original wild source (Li and Pritchard, 2009).

*Ex situ* methods include a wide variety of techniques depending on the source material, intensity of maintenance and financial possibilities, e.g. cryopreservation (seeds, pollen or tissue stored in liquid nitrogen), tissue culture (storage or propagation), field gene banks, living collection, DNA reserves and seed banking (Maunder *et al.*, 2004; Hawkes *et al.*, 2012).

Combined methods better secure the long-term conservation of a species against unexpected events. *Ex situ* management of germplasm starts with the collection of propagules (seeds or planting material), which after a short-term conservation period undergo a regeneration and/or multiplication phase (depending on the initial material). The subsequent conditioning/harvesting and cultivation phase provides opportunities to evaluate the germplasm' viability, vigour, vitality and health, as well as to evaluate the success of the *ex situ* conservation method used. Propagule collection and conditioning are followed by the final step, the repatriation (Pineda *et al.*, 2007).

*Ex situ* conservation has undoubtedly limitations, including the erosion of the original genetic material due to genetic drift, inbreeding, outbreeding, accumulation of deleterious alleles, hybridization and introgression (Volis, 2017; Ensslin and Godefroid, 2019; Nonić and Šijačić-Nikolić, 2019). Phenotypic changes such as loss of seed dormancy, increased germination rate, phenological and growth shifts, as well as changes in reproduction and competitive ability are typical signs of the altered original, wild material. Moreover, novel garden conditions can induce evolutionary shifts leading to e.g., loss of adaptation to drought stress, loss of interspecific competition ability or profound changes to phenotypic traits, and thus to unconscious selection (as consequences of horticultural assistance) and domestication syndrome (trait changes associated with genetic changes due to domestication) (Hammer, 1984).

Seed banking, cryopreservation and *in vitro* conservation are alternative, mostly cost-effective *ex situ* methods (Maunder *et al.*, 2004). Plant species can be preserved in seed-banks for many years without serious deterioration, therefore many of the problems can be avoided like the genetic erosion or selective change in genetic constitution of species (Thompson, 1974). The tendency of *ex situ* conservation via seed banking is increasing, there are at least 350 seed banking botanical gardens in 74 countries preserving almost 57,000 taxa (O'Donnell and Sharrock, 2017). Millenium Seed Bank Partnership (MSBP) is the largest wild plant conservation program and has developed seed conservation standards which characterize recent best practices for long term conservation of orthodox seeds (MSBP, 2015; O'Donnell and Sharrock, 2017; Liu *et al.*, 2020). Living collections of the global botanical garden network preserve at least 105,634 plant species, which represent 59% of all plant genera (Mounce *et al.*, 2017). Because seed banking, cryopreservation and *in vitro* conservation need totally different preservation premises and conditions, these methods are not discussed within the frame of this paper.

Studies preceding the establishment of *ex situ* collections are few, and those following the establishment of *ex situ* collections are heavily imbalanced towards the investigations of the variability of cultivated populations ("cultivated varieties") used for ecological restoration. Phenotypic changes incurred by the *ex situ* populations relative to the wild source populations are even less frequently reported (Ensslin and Godefroid, 2019). In efforts to improve *ex situ* preservation practices, several general recommendations have emerged. For example, preserving larger population sizes is generally preferable to prevent genetic erosion and decrease the consequences of genetic drift and inbreeding (Volis, 2017; Ensslin and Godefroid, 2019). It is not easy to design a clear and exact roadmap for ideal population sizes due to species' different mating types and reproductive strategies in plants (Basey *et al.*, 2015; Ensslin *et al.*, 2015), therefore 100 to 1000 individuals are thought to be

sufficient in maintaining adaptive genetic diversity (Frankham *et al.*, 2014; Basey *et al.*, 2015). Further recommendations intended to limit genetic drift and inbreeding include periodically repeated introduction of new genetic material, controlled pollination, and pollinator placement in the vicinity of the collections. To avoid hybridization and introgression, closely related accessions should be planted at a minimum of a few hundred meters apart (Basey *et al.*, 2015). To prevent changes and shifts in traits it is highly recommended to collect even numbers of female and male individuals and to sample from all mother plants that will produce the next generation. Unconscious selection should be prevented by planting both the early and late germinating seedlings in the *ex situ* population (Volis, 2017; Ensslin and Godefroid, 2019). To prevent evolutionary changes and adaptation to garden condition the number of *ex situ* generations should be limited to 2-5 generations and adjusted to the species' lifespan and life form (short-lived species with fast changes over a few generations are more affected) (Schoen and Brown, 2001; Havens *et al.*, 2004; Prasse *et al.*, 2010). Simulating the original habitat or planting the plants to a more natural site which is maintained by the botanical garden (*quasi in situ*) can as well prevent genetic changes and adaptation processes (Volis and Blecher, 2010).

It is thus clear that establishing a representative germplasm requires careful planning because genetically non-representative samples or their poor documentation can jeopardize conservation attempts (Maunder *et al.*, 2001; Maunder *et al.*, 2004). Without studying the wild population structure and species history, optimal sampling designs are difficult to envisage (Hoban and Schlarbaum, 2014; Hoban and Strand, 2015; Hoban, 2019). Following the establishment of novel populations, it is critical to evaluate the effectiveness of the different *ex situ*, and alternative *quasi in situ* techniques used (Ensslin and Godefroid, 2019).

As many taxa have already been preserved in *ex situ* collections, and the number of studies related to wild species *ex situ* conservation are also rapidly growing, reviewing current knowledge and assembling experiences of the *ex situ* collections has become particularly important. Target no. 8 of the 2011-2020 Global Strategy for Plant Conservation recommends that "At least 75 percent of threatened plant species should be preserved in *ex situ* collections, preferably in the country of origin, and at least 20 percent available for recovery and restoration programmes" (GSPC). Expiring target goals bring up questions about the sustainability, quality, and usability of the collections for future successful conservation activities. In this review we focus on the problems and drawbacks of the *ex situ* methods, as these have a great impact on the living collections, as well as on the field gene banks. Hereafter we use the term *ex situ* related to these conservation methods. Furthermore, we synthesize the state-of-the-art of *ex situ* conservation and we present a summary of the key aspects (e.g. environmental, genetic, demographic) important for an efficient, long-term *ex situ* conservation, highlighting the study gaps where necessary. We focus on two major study groups recommended for simultaneous and realistic evaluations of *ex situ* conservation success (Wei and Jiang, 2020): genetic studies and morphological trait measurements and examinations. We conducted an extensive search through the available literature using ScienceDirect Core Collection, Google Scholar and Web of Science. We used the following keywords: 'ex situ', 'wild plant' and 'conservation' and selected the articles related to living plant collections at a global scale.

## Genetic studies in *ex situ* populations

### *The challenge of preserving genetic diversity*

One of the long-term goals of *ex situ* conservation is to preserve the maximum level of genetic diversity of a species, and its source populations. *Ex situ* living collections like seed banks or tissue culture, because of the limited space, can be interpreted as small, isolated populations of a species (Maunder *et al.*, 2001), which are inherently exposed to genetic risks such as genetic drift, inbreeding depression or founder effect (Lande, 1994; Husband and Campbell, 2004). Genetic drift is the main acting force against the Hardy-Weinberg equilibrium (i.e., the optimal state of a population, when allele and genotype frequencies are equilibrated and remain stable over the next generations), causing random change in allele frequencies from generation to generation due to

stochastic events (Allendorf *et al.*, 2013). As a consequence, genetic drift may reduce the fitness of individuals and limit the evolutionary potential of *ex situ* populations (Ensslin and Godefroid, 2019). Such forces acting on the genetic composition of populations usually do not affect hard populations which are in Hardy-Weinberg equilibrium (Allendorf *et al.*, 2013) but hit non-equilibrated populations such as the ones established *ex situ*. Inbreeding depression occurs in small populations when related individuals' mate with each other, causing increased homozygosity and loss of alleles. Mating strategy and pollination mode greatly influence the possible inbreeding rate. However, we know little about the natural inbreeding rates of most wild plants, making *a priori* field studies necessary to evaluate possible consequences of inbreeding under *ex situ* conditions (Allendorf *et al.*, 2013; Ensslin and Godefroid, 2019). The founder effect is another great challenge to populations established *ex situ* with a small number of individuals, because rare and unique alleles can be left out from these founder populations, resulting in underrepresented genetic variability. Moreover, a small *ex situ* population can preserve deleterious alleles at higher frequencies compared to natural populations, which can lead to population extinction on the long run (Allendorf *et al.*, 2013). In the last decades, new molecular markers such as AFLP (amplified fragment length polymorphism), ISSR (inter simple sequence repeat), SSR (simple sequence repeat) and SNP (single-nucleotide polymorphism) can highlight population genetic traits at increasingly finer resolution, continuously improving the chances of better population sampling for *ex situ* conservation.

*The importance of preserving unique alleles and sampling from the whole distribution area of the target species*

It is increasingly recognized that the full breadth of genetic variation of a species is the additive genetic makeup of all populations across the distribution range. Narrow endemic, rare and endangered species' populations in different ecological environments across the range may undergo local adaptation or experience genetic drift, due to which they may hold unique alleles or allele combinations that need to be captured under *ex situ* (Lesica and Allendorf, 1995; Vitt and Havens, 2004; Hampe and Petit, 2005; Allendorf *et al.*, 2013). Unique alleles can carry information on important functional traits that could be key for a species' survival under special scenarios such as rapid climate change (Reyes-Valdés *et al.*, 2018). If some populations carrying important genes are omitted from *ex situ* collection, the long-term evolutionary potential of the species will be altered, the viability of its *ex situ* or reintroduced populations can decrease and the extinction risk of the species increases under environmental change (Etisham-Ul-Haq *et al.*, 2001; Silva *et al.*, 2020). Therefore, evaluating population variability across the species' entire area of distribution becomes critical for guiding the sampling strategies. For example, if there is a higher variability between populations than within populations, a proper strategy for any taxon would be to collect only a few individuals per population but from many populations throughout the whole range to preserve the integrity of the original species genome (Cochrane, 2004). However, capturing and preserving unique alleles is not an easy task, and *ex situ* collections report mixed success. For example, *Vatica guangxiensis* S.L. Mo is an endangered plant species of China with a low level of genetic variation in the natural populations. While the *ex situ* collection had intermediate genetic variation, exclusive alleles from the natural populations were not retrieved in the garden collection, and more extensive sampling was needed before future restoration and repatriation (Li *et al.*, 2002). Conversely, other *ex situ* conservation attempts managed to preserve very high rates of private alleles. In *Leucothrinax morrisii* (H.Wendl.) C. Lewis & Zona, an endangered taxon of Florida, USA, the mean diversity ( $H_e$ ) was similar between the *ex situ* collection and wild populations, and the allelic capture was greater than 94% (Namoff *et al.*, 2010).

*Ex situ* collections may even preserve unique alleles that have already been lost from the natural sites. In a study of *Taxus yunnanensis* W. C. Cheng & L. K. Fu based on microsatellite markers, Miao *et al.* (2015) detected 23 unique alleles in cultivated populations and just 14 unique alleles in natural populations. Such alleles may hold important attributes for a species' future survival, and the importance of their preservation and reintroduction in the wild is outstanding. Similarly, in a study on *Zelkova carpinifolia* (Pall.) K. Koch five new

haplotypes were identified in *ex situ* collections that were not matched in the wild populations (Christe *et al.*, 2014).

Despite the widespread knowledge of the theoretical underpinnings, the geographic representation of populations reported in former *ex situ* collections is often poor, but there are outstanding exceptions. A case study on *Berberidopsis corallina* Hook.f., a threatened and endemic vine of Chile showed a high degree of genetic differentiation between Northern and Southern wild populations that likely represented two distinct refugia. The genetic diversity of *ex situ* collections was similar to the rate recorded in small natural populations, but it only encompassed the genetic material of the Northern populations, and the species ran the risk of losing important alleles. To fix this shortcoming the authors called for the establishment of a new *ex situ* collection that would include the Southern region (Etisham-Ul-Haq *et al.*, 2001). In a comparative molecular analysis of *Zelkova abelicea* (Lam.) Boiss and *Zelkova carpinifolia* (Pall.) K. Koch using cpDNA markers, the *ex situ* collections of the two species represented unequally the variability of the corresponding natural populations (Christe *et al.*, 2014). While the stands of both species had lower variability than in the wild, *Z. abelicea* preserved only two out of 33 haplotypes in the wild populations, which most probably originated from a single region. *Zelkova carpinifolia* (Pall.) K. Koch on the other hand successfully preserved 12 out of the 15 wild haplotypes, which belonged to two genetically distant phylogeographic regions (Eastern and Western cluster).

Sampling from across species' whole area of distribution often safeguards unique and important alleles if the geographic distribution subsequently shrinks or the *in situ* populations become extinct. Wilson *et al.* (2017) evaluated and compared the genetic diversity of the endangered *Zizania texana* Hitchc. *in situ* populations from the San Marcos River and *ex situ* population in the San Marcos Aquatic Resources Center, using six microsatellite markers. Overall, the *ex situ* populations exhibited lower allelic diversity but some natural population "segments" have decreased in size, therefore the conservation value of the *ex situ* stand that holded unique and important individuals has increased. In concert with studies that revealed poor geographic representation in *ex situ* stands, Wilson *et al.* (2017) underscored the necessity of capturing all unique alleles to secure the genetic diversity of the wild populations, which can be achieved by a more extensive sampling and further addition of individuals.

Establishing a functional seed bank collection requires an effective sampling design to capture local and rare allelic variants (Petit *et al.*, 1998; Schoen and Brown, 2001). Gargiulo *et al.* (2019) investigated the genetic diversity of germplasm collections of the Millennium Seed Bank and wild-provenance populations of *Taxus baccata* L. using nuclear microsatellites. The allelic capture of seeds reached 86% of the alleles from wild populations, including rare and locally common variants, which is an accurate representation of the wild source.

Based on a specific sampling strategy, more than 60 target species have already been collected in Great Britain. Seed collections were made from every Forestry Commission seed zone across all target taxa. The seed zones were created according to biogeographic zones and the acting environmental conditions which may influence dispersal and gene flow (Herbert *et al.*, 1999; Kallow and Trivedi, 2017).

An overall sampling from the whole area of distribution is however not a full guarantee of the conservation success, as it heavily depends on further *ex situ* management. *Pinus koraiensis* Siebold & Zucc. is a nationally endangered species of China preserved in clonal seed orchards (CSO) that include provenances from the whole distribution area. Tong *et al.* (2020) used nine SSR markers to study the genetic diversity and population structure of the provenances in the CSO, and compared the genetic variation of maternal populations and offsprings. High genetic diversity was detected in both the maternal and progeny populations, indicating that CSO can preserve an adequate diversity within the *ex situ* gene pool. In offsprings, the genetic diversity was even higher, which was likely due to the efficient mixing of the gene pools of the mother plants. However, the primary population differentiation due to regional adaptation was missing in the new *ex situ* generation, and the gene pool was homogenized, causing conservation concerns.

*The importance of optimal sample size*

To establish and maintain genetically and phenotypically diverse living collections, one of the most challenging tasks is to choose species-specific sample sizes for optimal *ex situ* conservation. If sampling is suboptimal and the number of sampled individuals is low, the resulting small populations will be exposed to genetic risks (Allendorf *et al.*, 2013). A first theoretical minimum sample size able to capture non-rare alleles was approximated by Marshall and Brown (1975) to between 30 - 60 individuals for an optimal allelic capture. Based on this recommendation, a minimum sample size of 50 individuals per population has been commonly used by conservationists for decades (Hoban and Strand, 2015). Recent evidence however clearly suggest that the appropriate minimum sampling size differs by species, it is not predictable by genus or population structure and simplistic sampling protocols applied equally to all taxa within a genus result in unsatisfactory outcomes, thus it is time to abandon this rather rigid approach (Hoban and Schlarbaum, 2014; Hoban and Strand, 2015; Hoban, 2019; Hoban *et al.*, 2020).

Most current-day *ex situ* collections have suboptimal sizes according to Hoban *et al.* (2020) and the root of the problem is often the ignorance, or lack of knowledge of effective population size, and consequently missampled wild populations. A molecular study based on microsatellite markers in *Ceroxylon quinduense* (H. Karst.) H. Wendl., an endemic species of the cloud forests of Colombia, showed that the material from *ex situ* collections originated from a single wild population and exhibited low genetic diversity and a low percentage of private alleles compared to the wild population. Several alleles of the *in situ* populations were completely missing and there was a higher rate of full-siblings in the *ex situ* collection. This outcome was likely the result of a poorly planned sampling design, i.e., seed collection from only a few parents (Chacón-Vargas *et al.*, 2019). Likewise, non-representative seed sampling and small-sized *ex situ* population (27 individuals compared to natural populations with more than 100 individuals), as well as differences in habitat ecology between the garden and natural populations caused low average gene diversity within populations and high genetic differentiation among populations of *Loropetalum subcordatum* (Benth.) Oliv., an endangered species endemic to China with high conservation priority (Li *et al.*, 2018).

*Cochlearia polonica* E. Fröhlich, a narrow endemic perennial plant of Poland had only one transplanted population left in the wild. As the source material contained only five individuals, the *ex situ* stand exhibited very low genetic variation i.e. three unique bands out of the 11 detected with ISSR markers in the wild populations (Rucińska and Puchalski, 2011). Further major differences in genetic composition reinforced that the garden population preserved only a very limited amount of the species' genetic variability.

Other attempts were more successful at calibrating the sampling protocols, securing the long-term survival of the *ex situ* collections. In a study based on ISSR markers of *Sinocalycanthus chinensis* W.C.Cheng & S.Y.Chang. the genetic diversity metrics (Na, Ne, h, and I) were not significantly different between *ex situ* and natural populations. This positive result was attributed to a sample size sufficient for this species, which included eight natural populations with 20 individuals from each population (Chen *et al.*, 2013). In another collection of *Leucothrinax morrisii* (H.Wendl.) C. Lewis and Zona established *ex situ* with just 58 plants, the allelic capture was greater than 94%. In this particular species, modeling exercises indicated that 15 individuals are sufficient to conserve a representative sample from species' genetic diversity (Namoff *et al.*, 2010).

Overall, species' biogeographical and demographic history such as the frequency of reproduction events are better predictors of an optimal sampling than the number of individuals alone, but more evidence is required before emerging any generalizations. As general guiding principles, it is advisable to tailor the sampling design to species' biology, possibilities of collection maintenance, and desired characteristics of the collection (e.g., number of allele copies and type of alleles targeted). Genetic data may be used to calculate the optimal specimen number, and sampling could be improved by collecting fewer seeds per plant but from several unique maternal plants, as well as by frequent sampling from the wild populations. Generally, a sufficient minimum collection size for a species seems to be ranging between 30-200 (300) plants (Hoban and Strand, 2015; Hoban *et al.*, 2020).

*Consequences of limited space and generation time in ex situ collections*

As mentioned earlier, exact numbers necessary for establishing an effective population size are particularly difficult to ascertain and depend on species-specific mating types and reproductive strategies (Ensslin, 2011; Basey *et al.*, 2015; Ensslin *et al.*, 2015). Other recommendations include periodic immigrations from the wild source population, increasing (tripling) the sample size in each generation which mitigates the effect of genetic drift by mimicking natural pollen flow patterns, and controlled pollination that may assist in preventing inbreeding depression (Havens *et al.*, 2004, 2006). Near-natural cultivation methods, which allow generation overlap and interspecific competition, help to reconstruct a species' original environment under *ex situ* conditions and better address the challenges of *ex situ* populations. A study by Lauterbach *et al.* (2012) based on AFLP markers compared three *ex situ* and their source *in situ* populations of the endangered *Silene otites* (L.) Wibel in Germany. Two *ex situ* populations were maintained in a single-species garden bed, with regular weed control and with biennial rejuvenation, and one population was cultivated under near-natural conditions. The genetic variation in the *ex situ* garden-bed populations was lower than in the wild source populations and the high level of  $F_{ST}$  value suggested a strong differentiation between the *in situ* and *ex situ* populations. The founder effect, genetic drift and inbreeding depression increased effect with the growing cultivation time in all *ex situ* populations. However, under near-natural conditions, the loss of genetic diversity was comparatively lower, and thus the population conservation was more successful.

The genetic risks to which small *ex situ* populations are exposed are exacerbated in species with short generation time especially in annuals, because a fast generation turnover results in very early manifestations of population genetic mechanisms (Schaal and Leverich, 2004). In a study of the short-lived *Cynoglossum officinale* L. based on nuclear microsatellite markers, four garden populations had almost zero genetic diversity that was correlated with cultivation time in the botanical garden. This was likely due to long-term genetic drift that may have increased fast with the number of generations. The mixed mating system with a high selfing rate (up to 70%) of the species could have additionally contributed to the strong genetic drift observed (Vrieling *et al.*, 1999; Ensslin *et al.*, 2011). Another study of five annual species of arable fields (*Anagallis arvensis* L., *Anagallis foemina* Mill., *Bupleurum rotundifolium* L., *Consolida regalis* L., *Nigella arvensis* L.) assessed the impact of *ex situ* cultivation on the genetic diversity and composition based on RAPD markers (Brütting *et al.*, 2012). In four out of five annual species, *ex situ* collections exhibited low levels of genetic diversity, an underrepresented allele structure and they even missed common alleles. Species that showed lower genetic diversity *ex situ* were mostly short-lived and frequently self-pollinating (Brütting *et al.*, 2012). These observations underscore the necessity to tailor not just the sampling design in the wild, but also the long-term *ex situ* maintenance plans to species' life form and breeding system (Schaal and Leverich, 2004).

*The importance of ex situ planting design*

Pollen dispersal is one of the main mechanisms of gene flow, which influences the long-term survival of populations. The often higher than the usual density of plants in collections inevitably alters the natural pollen dispersal distances, with important genetic consequences that need careful examination. While some *in situ* populations may experience biparental inbreeding due to small size and limited gene flow, the corresponding *ex situ* populations planted in high densities may overcome both challenges and have a lower rate of crosses between relatives. *Dipteryx alata* Vogel is an evergreen tree and an important biological and agricultural resource in the Brazilian Cerrado. The species is vulnerable due to intensive agricultural use and therefore its *ex situ* conservation has high priority (World Conservation Monitoring Centre, 1998; IUCN, 2020). The genetic diversity and the level of polymorphism of the germplasm collection had higher values than the *in situ* populations because the germplasm collection was established from different populations within a region (Corrêa *et al.*, 2000). Thus, the *ex situ* conditions preserved a larger number of alleles across generations. However, paternity correlation and pollen dispersal distance were lower in *ex situ* than under natural conditions, due to the higher density of individuals compared to the natural populations that reduced the pollinator traveling distance (Guimarães *et al.*, 2019).

High *ex situ* individual densities often cause inbreeding, and correct planting design and species-specific spatial structuring in transplants are needed to alleviate the risk of inbreeding. Aguiar *et al.* (2019) studied *Balfourodendron riedelianum* (Engl.) Engl., an endangered tree of the Atlantic Forest. *Ex situ* seedlings and adult trees were genotyped with microsatellite markers and indicated that the effective population size was adequate for short-term conservation. Analyses of pollen dispersal distance showed an isolation-by-distance (IBD) pattern, likely due to the high population density in the trial population and enhanced pollination due to shorter flying distances of the pollinators. Seedlings were predominantly outcrossed (93-96.2%), but selfing (3.8-7%) and mating between relatives (20-28.1%) resulted in biparental inbred seeds and seedlings with lower genetic diversity. This was most probably the consequence of the planting design, as provenances were grouped in plots within blocks (maternal families) and individuals of the same provenances were located in close proximity (Aguiar *et al.*, 2019).

Comparative genetic studies between *in situ* and *ex situ* conditions are thus useful tools for quantifying cross-pollination rates and pollen dispersal distance between populations, as well as for detecting potential shortages of animal pollinators under *ex situ* conditions (Allendorf, 2013; Ellstrand, 2014).

#### *The consequence of unwanted gene flow within ex situ collections*

Unwanted gene flow can undermine the genetic integrity of *ex situ* collections, causing hybridization through cross-pollination with different species and introgression between different populations of the same species (garden accessions or garden and wild crosses) (Maunder *et al.*, 2004; Ensslin and Godefroid, 2019).

Interspecific hybrids may exhibit superior qualities compared to their parents, which may further jeopardize the species' genetic integrity. *Sinojackia xylocarpa* Hu is a Chinese endemic tree extinct in the wild (Yao *et al.*, 2005), therefore its reintroduction in the wild would be critical. The plant is at great hybridization risk in the Wuhan Botanic Garden due to the presence of *Sinojackia rehderiana* Hu, a congener species. A study based on microsatellites revealed that 32.7% of the collected seeds were hybrids, and the paternity of 93 out of 249 seedlings was assigned to *S. rehderiana*. Hybrids had longer pollen dispersal distances than their parents. Avoiding extensive hybridization would require the establishment of a buffer zone, controlled pollination and testing the seeds before reintroduction (Zhang *et al.*, 2010).

Controlled inter-specific crosses can evaluate the chance of possible hybridization events and their negative outcomes. Lozada-Gobilard *et al.* (2020) performed controlled inter-specific crosses between *Minuartia smejkalii* M. Dvoráková (endemic to the Czech Republic) and its congener *Minuartia caespitosa* (Willd.) Degen. The results confirmed that the two species may hybridize and hybrid individuals had higher germination rates of the viable seeds, a higher number of flowers and also higher dispersal potential.

Hybrids can outcompete the original endangered species upon repatriation, therefore a greater caution is recommended before any restoration attempt in the wild. In studies with *Plantago lanceolata* L. and *Lotus corniculatus* L., Schröder and Prasse (2013) observed that hybrids from cultivated varieties outcompeted wild sources due to advantageous germination traits. But it is often difficult to disentangle the effects of cultivation on species' life history from the effects of unwanted gene flow. Schröder and Prasse (2013) also observed that cultivated individuals germinated faster and were more abundant than the wild sources, and it was not just the hybridization per se that had a great impact on species' life-history traits, but cultivation also.

Genetic diversity studies revealed important aspects related to *ex situ* gene stocks established in garden collections all over the world. Rich evidence has accumulated about the current state of the existing *ex situ* collections, as well as about the main forces that shape the genetic make-up of species maintained in *ex situ* collections. These experiences represent a strong handrail for future conservation efforts. In Table 1, we summarize a list of case studies performed to reveal differences in the genetic make-up of populations and fundamental successful conservation activities.



**Table 1.** List of case studies showing main genetic markers associated with comparative analyses of *ex situ* collections and *in situ* stands of plant species

Examined taxon	Markers used to characterize gene stock	Main conclusions	References
Case studies conducted on trees			
<i>Vatica guangxiensis</i> S.L. Mo	RAPD markers	Intermediate genetic variation, that showed to be representative at the species level.	Li <i>et al.</i> (2002)
<i>Leucothrinax morrisii</i> (H. Wendl.) C. Lewis & Zona	ISSR markers	Similar genetic diversity and low genetic distance between <i>ex situ</i> and <i>in situ</i> populations.	Namoff <i>et al.</i> (2010)
<i>Sinojackia xylocarpa</i> Hu and <i>Sinojackia rehderiana</i> Hu	Microsatellite markers	Extensive hybridization between the two species in garden conditions.	Zhang <i>et al.</i> (2010).
<i>Zelkova carpinifolia</i> (Pall.) K. Koch and <i>Zelkova abelicea</i> (Lam.) Boiss.	cpDNA spacer (trnH-psbA) and cpDNA intron (trnL)	New haplotypes and genetic representativeness in <i>ex situ</i> collection. Low number of haplotypes represented in <i>ex situ</i> garden collections.	Christe <i>et al.</i> (2014)
<i>Taxus yunnanensis</i> W. C. Cheng & L. K. Fu	Microsatellite markers	Lower genetic diversity in <i>ex situ</i> collections, but 23 unique alleles preserved.	Miao <i>et al.</i> (2015)
<i>Balfourodendron riedelianum</i> (Engl.) Engl.	Microsatellite markers	Lower pollen dispersal distance, biparental inbred seeds and seedlings in <i>ex situ</i> collection.	Aguiar <i>et al.</i> (2019)
<i>Ceroxylon quindiuense</i> (H. Karst.) H. Wendl.	Microsatellite markers	Lower genetic diversity, higher rate of full-siblings in <i>ex situ</i> .	Chacón-Vargas <i>et al.</i> (2019)
<i>Dipteryx alata</i> Vogel	Microsatellite markers	Higher genetic diversity in <i>ex situ</i> , but lower paternity correlation and decreased pollen dispersal distance.	Guimarães <i>et al.</i> (2019)
<i>Taxus baccata</i> L.	Microsatellite markers	Allelic capture, including rare and locally common alleles are representative in <i>ex situ</i> seed bank collection.	Gargiulo <i>et al.</i> (2019)
<i>Pinus koraiensis</i> Siebold & Zucc.	SSR markers	Higher genetic diversity in the progeny due to gene pool mixing.	Tong <i>et al.</i> (2020)
Case studies conducted on shrubs			
<i>Berberidopsis corallina</i> Hook.f.	RAPD markers	Representative genetic diversity and a high number of genotypes preserved in <i>ex situ</i> collection.	Etisham-Ul-Haq <i>et al.</i> (2001)
<i>Sinocalycanthus chinensis</i> W. C. Cheng & S. Y. Chang.	ISSR markers	Representative seed sampling resulted in similar genetic diversity between <i>in situ</i> and <i>ex situ</i> populations.	Chen <i>et al.</i> (2013)

<i>Loropetalum subcordatum</i> (Benth.) Oliv.	SRAP markers	Strong population differentiation and lower level of genetic diversity in <i>ex situ</i> population.	Li <i>et al.</i> (2018)
Case studies conducted on herbaceous species			
<i>Cochlearia polonica</i> E. Fröhlich	ISSR markers	Reduced genetic variability and high level of differentiation between <i>in situ</i> and <i>ex situ</i> populations.	Rucińska and Puchalski (2011)
<i>Cynoglossum officinale</i> L.	Microsatellite markers	Genetic diversity reduced with the cultivation time.	Ensslin <i>et al.</i> (2011)
<i>Silene otites</i> (L.) Wibel	AFLP markers	Reduced genetic variability and high level of differentiation in <i>ex situ</i> populations.	Lauterbach <i>et al.</i> (2012)
<i>Anagallis arvensis</i> L., <i>Anagallis foemina</i> Mill., <i>Bupleurum rotundifolium</i> L., <i>Consolida regalis</i> L., <i>Nigella arvensis</i> L.	RAPD markers	Lower genetic diversity and strong differentiation of <i>ex situ</i> collections.	Brütting <i>et al.</i> (2012)
<i>Plantago lanceolata</i> L. and <i>Lotus corniculatus</i> L.	-	Faster and more abundant germination in cultivated plants and hybrids.	Schröder and Prasse (2013)
<i>Zizania texana</i> Hitchc.	Microsatellite markers	Lower allelic diversity in <i>ex situ</i> collections.	Wilson <i>et al.</i> (2017)
<i>Minuartia smejkali</i> Dvoráková and <i>Minuartia caespitosa</i> (Willd.) Degen	-	Hybridization potential between the two species.	Lozada-Gobilard <i>et al.</i> (2020)

### Trait-based studies in *ex situ* populations

While interest in the genetic representation of *ex situ* collections has increased in the recent decades, the trait-based shifts such as fitness decline or changes caused by maladaptation have received less attention (Ensslin *et al.*, 2015). The genetic drift experienced by small and isolated plant populations has a visible impact on the phenotype and it may result in the decline of main fitness parameters in *ex situ* stands as well (Ellstrand and Elam, 1993). Important shifts in a life-history trait can occur when wild plants are introduced into novel environments such as botanic gardens or arboreta. In this case, plants are exposed to new selection pressures, which may result in evolutionary adaptation over time. In gardens, intensive horticultural care carries high risks of unconscious selection. Gardeners protect plants by daily watering, manuring or treatment against pathogens and with time, *ex situ* collection may lose the ability to tolerate less favourable environmental conditions. At the same time, selection pressures such as drought stress or lack of nutrients continue to shape populations in the wild causing many adaptive changes that remain uncaptured in *ex situ* stands. In addition, under *ex situ* conditions the weeding process eliminates the interspecific competition pressure, and individuals may lose their ability to face competition. If changes incurred by plants are maladaptive, they can cause the failure of the repatriation process (Ensslin and Godefroid, 2019). Therefore, tracking phenotypic changes over time in garden populations is of great importance for conservation (Husband and Campbell, 2004; Ensslin and Godefroid, 2019).

*Trait shifts in novel environments*

*Ex situ* cultivation may have a negative impact on fitness and other phenological traits. In a study of four native wild species (*Carlina vulgaris* L., *Corynephorus canescens* P. Beauv., *Jasione montana* L., *Melilotus officinalis* L.) *ex situ* plants clearly showed lower fitness performance than their wild source relatives (Ensslin *et al.*, 2015). In the case of *Carlina vulgaris* the different years of cultivation (3 to 31 years) affected negatively the number of flowering stems. The regeneration method (artificial *vs.* natural propagation) did not influence the fitness of *ex situ* plants, and thus the fitness decline was caused by genetic drift under garden conditions rather than by selection.

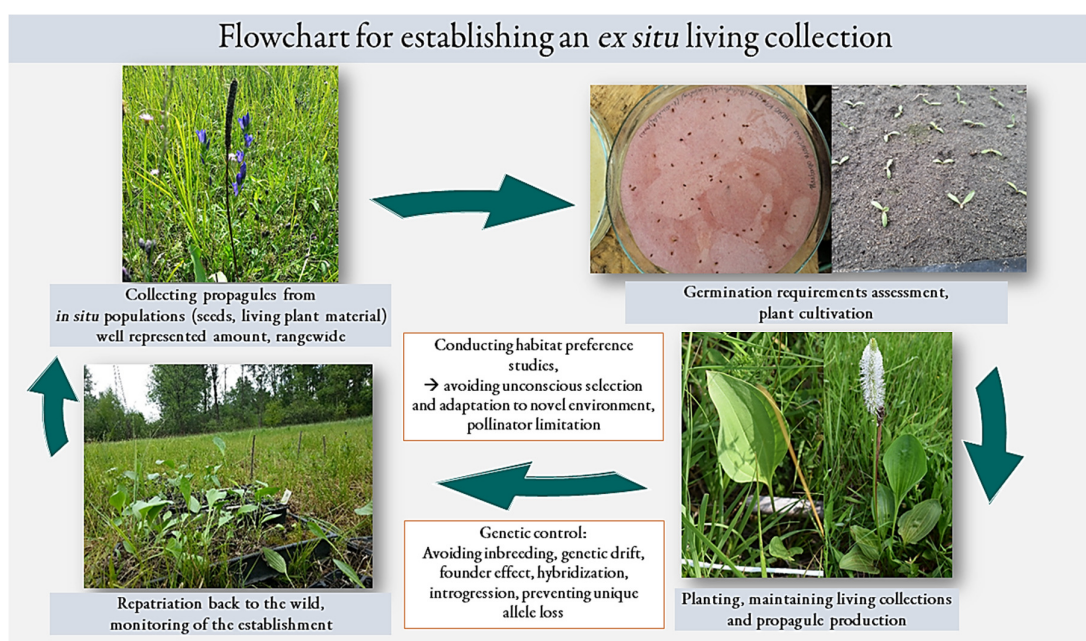
Life-history traits such as seed traits can experience important changes in germplasm collections. Seed germination rates can increase in germplasms as the result of unconscious or non-intentional selection by gardeners, who tend to plant out only the early germinating seedlings (Ensslin *et al.*, 2011; Ensslin, 2019). In a study of *Cynoglossum officinale* L. populations, both *in situ* and *ex situ* stands had similar seed production, but the *ex situ* stand, which had lower diversity, had lower mean seed mass and significantly higher germination percentage (Ensslin *et al.*, 2011). Ensslin *et al.* (2018) studied the germination characteristics of 72 species. Across all species, *ex situ* cultivated plants exhibited higher germination percentages and lower dormancy index compared to their wild sources. However, differences decreased with a longer seed storage time as a result of seed aging.

Life history traits connected with seed dormancy patterns can be used to detect selection pressure on garden populations. In a study by Ensslin *et al.* (2018), plant life span explained the differences in seed dormancy between wild and *ex situ* populations. In 22 short-lived plant species, seed dormancy loss and increased germination *ex situ* were remarkably stronger compared to perennial species that were more tolerant to dormancy loss. In short-lived species characterized by fast generation turnover, regeneration via seeds is a critical step in conservation. In the case of these species, keeping seeds in cold storage and regenerating them only for immediate use is the recommended practice (Ensslin *et al.*, 2018). Species' life history may further influence changes in germination characteristics, growth and phenology. Rauschkolb *et al.* (2019) investigated the influence of cultivation on trait differentiation in three threatened plant species (*Trifolium spadiceum* L., *Sisymbrium austriacum* Jacq., *Bromus grossus* Desf. ex DC.) and detected species-specific changes in life-history strategies associated with reduced performance of *ex situ* individuals. The differentiation happened rapidly, due to relaxed selection, genetic drift, inbreeding depression and fast adaptation to garden conditions.

Shifts in morphological traits under *ex situ* conditions can occur even in relatively short periods. Solberg *et al.* (2015) conducted an experiment on *Trifolium pratense* L. in *in situ* and *ex situ* populations, measuring 13 morphological and phenological traits. Four of the examined traits changed significantly within a single generation. Interestingly, the direction of changes shifted toward individuals that were similar to the commercial cultivars. The "cultivar type" was taller and larger than the wild type and had higher seed production. The increased frequency of genes associated with the morphology of the cultivars could have occurred due to the addition of genes from garden cultivars during regeneration or as a result of selection (Solberg *et al.*, 2015).

One of the environmental triggers of trait shifts is the increased light pollution in *ex situ* conditions, which can have a great impact on plant species' survival and can hamper conservation efforts in botanic gardens and arboreta. Peregrym *et al.* (2019) studied the effects of artificial light at night (ALAN) on living collections from Central, Southern and Eastern Europe. They reported a very high level of light pollution in this region and it is possible that plants conserved *ex situ* change their flowering phenology, growth form and resource allocation in response to increased light availability. Herbivores and pollinators also shifted their physiology, behaviour and ecology (for instance nocturnal pollinators visit plants less frequently), which can lead to decreased fruit production and seed productivity failure (Bennie *et al.*, 2016; Peregrym *et al.*, 2019). The breadth of adaptive changes triggered by artificial lightening is far from being well-investigated and more research is needed to understand their effects on *ex situ* collections.

Differences in habitat conditions can influence trait shifts in a variety of ways. For instance, differences in soil water regimes and maintenance level can have a fast, significant impact on morpho-phenological characteristics of the *ex situ* individuals. *Plantago maxima* Juss. ex Jacq. is a strictly protected species of Hungary, therefore the establishment and evaluation of the *ex situ* collection is extremely important to ensure the long-term preservation. *Ex situ* flowchart was designed to start a successful *ex situ* conservation of the species (Figure 1). Three stands were set up in a near-natural habitat (mesophilic fen meadow) in the Soroksár Botanical Garden, and two others were established in a perennial bed with regular weed control and frequent watering. Two years after planting, individuals from the fen meadow showed the best results: plants developed properly, peroxidase enzyme activity indicative of stress response was low and the survival rate was the highest. However only the individuals under horticultural maintenance flowered in the year of planting, and also the number of leaves significantly exceeded the ones in the meadow. This result can be due to horticultural care and lack of competition linked to weeding. Even slight differences between *ex situ* habitats can help evaluate the habitat preference of a species, and at the same time substantiate the success of repatriation by guiding the choice of the appropriate habitat type in the field (Kovács *et al.*, 2019).



**Figure 1.** Flowchart for the establishment of the living collection of *Plantago maxima* and the acting forces on which special attention is required

Habitat differences can affect the internal content of plants such as nutrient levels. Marler and Lindstrom (2020) compared the leaf nutrient content of *Cycas micronesica* K.D. Hill and *Cycas nongnoochiae* K.D. Hill. between *in situ* and *ex situ* locations and observed higher macronutrient levels under garden conditions. Differences between the soil nutrient content of the sites did not explain the discrepancy, in turn, it was likely influenced by the growing environment.

Individuals transferred from the wild into garden conditions may undergo severe stress. *Valeriana wallichii* DC. is an important medicinal plant and therefore possible biochemical composition shifts in *ex situ* collections are important to study. Asgher *et al.* (2020) revealed severe stress in the *ex situ* stand after three years of maintenance in Lead Botanical Garden of the Department of Botany, BGSBU Rajouri. Photosynthetic and growth attributes decreased, prolin, ornithine aminotransferase, pyrroline-5-carboxylate reductase increased, and higher reactive oxygen species (ROS) accumulation indicated oxidative stress. These changes concurred with better reproductive attributes (for instance better germination ability) *ex situ* due to better

pollinator availability. The study shows a clear adaptive mechanism to the novel environment, which can be due to different habitat conditions but also to the introduction of selected chemical phenotypes into *ex situ* collections.

Cultivation for a longer time can at the same time negatively influence stress response. Ensslin and Godefroid (2020) conducted a multi-species experiment, where they found reduced trait variability (germination rate, biomass and leaf length) and a slight reduction of response to drought stress. The change was greater in drought-tolerant than drought-intolerant species, which was due to greater environmental differences during cultivation relative to the source habitats. Near-natural habitat conditions are therefore recommended to prevent the reduction of species' evolutionary potential, which could jeopardize its ability to survive following reintroduction.

#### *Changes driven by pollination and pollinator limitation*

Pollination and seed dispersal are important indicatives of plant reproduction success. Lack of pollinators and seed dispersers under garden conditions can lead to the decline of fitness and diversity, and negatively influence the conservation success (Gong *et al.*, 1998, Biesmeijer, 2006). Tang *et al.* (2019) studied the pollination biology of four threatened species endemic to China (*Hibiscus aridicola* J. Anthony, *Amorphophallus albus* P.Y. Liu & J.F. Chen, *Stemona parviflora* C.H. Wright and *Stemona japonica* (Blume) Miq.). The study showed a reduced number of pollinators or no pollinators at all under garden conditions, which resulted in fruit set failure or a strong decrease in seed yield. *Amorphophallus albus* and the two *Stemona* species were self-incompatible, which increased the risk of extinction in garden conditions due to the absence of natural pollinators (Biesmeijer, 2006; Tang *et al.*, 2019).

Another study examined two *ex situ* populations of the partially self-compatible outcrosser *Iris ensata* Thunb. which has a disjunct distribution in East Asia (Zhao *et al.*, 2000). The two *ex situ* populations, located at relatively low elevations, initiated earlier flowering and fruit set compared to native populations. However, pollinator visits were less frequent, and no pollinator was observed in one of the *ex situ* populations. The consequence was inbreeding depression that limited seed set and germination, but it did not affect seedling survival (Xiao *et al.*, 2019).

In Table 2 there we present a list of case studies on trait changes in *ex situ* collections.

**Table 2.** List of case studies showing main traits analysed comparatively between *ex situ* collections and *in situ* stands

Examined taxon	Main conclusions	References
Case studies conducted on trees and shrubs		
<i>Cycas micronesica</i> K.D. Hill and <i>Cycas nongnoochiae</i> K.D. Hill.	Higher macronutrient content in <i>ex situ</i> .	Marler and Lindstrom (2020)
<i>Hibiscus aridicola</i> J. Anthony, <i>Stemona parviflora</i> C.H. Wright, <i>Stemona japonica</i> (Blume) Miq.	Reduced number of pollinators/no pollinators – fruit set decrease and failure.	Tang <i>et al.</i> (2019)
Case studies conducted on herbaceous species		
<i>Carlina vulgaris</i> L., <i>Corynephorus canescens</i> P. Beauv., <i>Jasione montana</i> L., <i>Melilotus officinalis</i> L.	Fitness decline in <i>ex situ</i> collection.	Ensslin <i>et al.</i> (2015)
<i>Trifolium pratense</i> L.	Rapid morphological trait shift in <i>ex situ</i> .	Solberg <i>et al.</i> (2015)
<i>Cynoglossum officinale</i> L.	Changed seed mass, increase in germination percentage in <i>ex situ</i> collection.	Ensslin <i>et al.</i> (2011)

72 short-lived or long-lived herbaceous species with orthodox seeds	Short-lived plant species were vulnerable to the seed dormancy loss and germination increase in <i>ex situ</i> .	Ensslin <i>et al.</i> (2018)
<i>Plantago maxima</i> Juss. ex Jacq.	Rapid morpho-phenological response to different habitat types and level of maintenance under <i>ex situ</i> condition.	Kovács <i>et al.</i> (2019)
<i>Trifolium spadicum</i> L., <i>Sisymbrium austriacum</i> Jacq. <i>Bromus grossus</i> Desf. ex DC. & A. Camus	Rapid changes of life-history trait in <i>ex situ</i> .	Rauschkolb <i>et al.</i> (2019)
<i>Amorphophallus albus</i> P.Y. Liu & J.F. Chen	Reduced number of pollinators/no pollinators – fruit set decrease and failure.	Tang <i>et al.</i> (2019)
Living collections	Artificial light at night (ALAN) produced shifts in flowering phenology, growth form and resource allocation.	Peregrym <i>et al.</i> (2019)
<i>Iris ensata</i> Thunb.	Changed phenology, pollinator limitations.	Xiao <i>et al.</i> (2019)
12 drought-tolerant or drought-intolerant herbaceous perennial species	Reduced trait variability and decreased response to drought stress in cultivated species. Increased resource acquisition strategy in drought-tolerant species.	Ensslin and Godefroid (2020)
<i>Valeriana wallichii</i> DC.	Increased stress related biochemical components and reproductive attributes. Decreased photosynthetic and growth attributes in <i>ex situ</i> .	Asgher <i>et al.</i> (2020)

### Attempts to regenerate and repatriate *ex situ* generations

One of the most important and crucial last steps in the *ex situ* conservation is the reintroduction of rare plant species to their natural habitats. The repatriation and reintroduction techniques are important conservation actions that help restoring the original population of endangered taxa and facilitates the reconstruction of the natural community structure (Haskins and Pence, 2012). Plant propagules (for example seedlings, cuttings or *in vitro* propagated plants) of *ex situ* origin are not fully adapted to conditions in their original natural habitat, but environmental differences can trigger the acclimatization needed for the successful reintroduction process (Haskins and Pence, 2012).

Perhaps the biggest challenge in the repatriation attempts is represented by individuals propagated *in vitro*. In a study of three *Dianthus* species (*D. callizonus* Schott & Kotschy, *D. glacialis* Haenke ssp. *gelidus* (Schott, Nyman & Kotschy) Tutin and *D. spiculifolius* Schur) Cristea *et al.* (2013) compared the acclimatization of wild plants and of plantlets propagated *in vitro* when replanted in *ex situ* conditions. *D. spiculifolius* had the best ability to acclimatize, likely due to the climatic and phytosociological conditions similar to the natural habitats secured for the species in the botanical garden. Plantlets propagated *in vitro* adapted and survived much better in outdoor conditions than the replanted wild individuals, but the

advantages of *in vitro* propagation unfold with longer-term monitoring of the somaclonal variability necessary for genetically certified gene stock conservation.

*Dianthus diutinus* Kit. is an endemic species of the Pannonian biogeographic region. Species' populations can only be found in the region between the Danube and Tisza rivers in Central Hungary. The Botanical Garden of the University of Szeged carried out a population genetic survey of the wild population to secure the success of the collection of the *ex situ* plant material. *Ex situ* plants were grown in conditions similar to the *in situ* environment. Following repatriation, soil seed bank and seed biology, population genetic study with RAPD markers, and habitat preference were examined to control the success of repatriation. The high survival rate and further *in situ* reproduction supported the effective conservation efforts (Németh and Makra, 2011).

In another case study in Finland, a natural population of *Rubus humulifolius* C.A.Mey. has gone through a severe decline and it was destroyed during construction work. A few individuals survived in a private cottage garden; therefore the *in vitro* propagation of the species was the single way for recovery. The *in vitro* propagated plants were introduced into several Finnish botanical gardens. The *ex situ* collections had low seed production, likely due to bottleneck or inbreeding depression, which anticipated possible recovery failure in the long run. Nonetheless, the reintroduced population was successful in the wild (Hyvärinen, 2020). Also, a successful cryopreservation protocol for micropropagated individuals were developed, to enable a long-term conservation of the germplasm. The regenerated plants were successfully transferred into common garden, which is an important step for future restoration (Edesi *et al.* 2020).

Successful repatriation attempts are most easily guided by analyses of different population traits. *Salix lapponum* L. is a relict species of eastern Poland. Pogorzalec *et al.* (2020) conducted habitat quality research and comparatively examined four plots representing possible suitable habitats for the reintroduction process. The study assessed the survival rate, the number of flowering plants, mean leaf length and width, and mean plant height of plants outplanted from the *ex situ* collection. Significant differences in functional traits were detected depending on the habitat conditions, and survival rates finally guided the successful reintroduction of the species.

## Conclusions

The number of *ex situ* collections has increased greatly in recent years intending to achieve GSPC targets. The acceleration of published case studies provided a high amount of data on factors that influence the long-term success of conservation and the effectiveness of *ex situ* cultivation in garden conditions. Knowledge harnessed from these case studies is extremely important for supporting the optimal maintenance and management of wild resources in botanical gardens.

Overall, the genetic representativeness of the living collections was generally low. Even though many new recommendations regarding *ex situ* conservation have been made, major knowledge gaps still remain. For instance, despite the importance of pollinators' presence or absence, only a few studies have focused on this topic. Little is known on the effects of hybridization, which represents a great risk within the garden *ex situ* populations and may end in the shift of genetic variation compared the to source populations.

Finally, changes in the environmental factors linked to human activity, for example, artificial light, is not thoroughly researched. Even though trait changes linked to human activity have a serious impact on *ex situ* garden collections, the direction of these changes is less known. A considerable number of studies are focusing on the genetic representativeness of the *ex situ* populations, but in-depth studies of morpho-phenological trait shifts, especially of threatened species, are still lacking.

## Authors' Contributions

ZSK designed the manuscript, wrote and authored most of the manuscript; MH authored part of the manuscript and reviewed drafts of the paper and made corrections; PCS and AMCS reviewed drafts of the paper and corrected the manuscript. All authors read and approved the final manuscript.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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