Review

Meta-analysis of genetic representativeness of plant populations under ex situ conservation in contrast to wild source populations

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Abstract: Ex situ conservation is widely used to protect wild plant species from extinction. However, it remains unclear how genetic variation of ex situ plant collections reflects diversity of wild source populations. We conducted a global meta-analysis of the genetic representativeness of ex situ populations by comparing genetic diversity (i.e., AR, allelic richness; H_e, expected heterozygosity; PPB, percent polymorphic bands; and SWI, Shannon–Winner index), inbreeding coefficient (F_{IS}), and genetic differentiation between ex situ plant collections and their wild source populations. Genetic diversity (i.e., H_e, PPB, and SWI) was significantly lower in ex situ populations than their wild source populations, whereas genetic differentiation between ex situ and wild populations (ex-situ-wild F_{ST}), but not that among ex situ populations, was significantly higher than among wild populations. Outcrossing species, but not those with mixed mating system, had significantly lower genetic diversity in ex situ populations and significantly higher ex-situ-wild F_{ST}. When the collection size for ex situ conservation was ≥30 or 50, PPB, H_e, and ex-situ-wild F_{ST} were not significantly different between ex situ and wild populations, indicating a relatively high genetic representativeness. Collecting from the entire natural distribution range and mixing collections from different sources could significantly increase the genetic representativeness of ex situ populations. Type of ex situ conservation (i.e., planting or seed bank) had no effect on genetic representativeness. The effect size of H_e decreased and the effect size of ex-situ-wild F_{ST} increased as the duration of ex situ conservation increased. Our results suggest that current ex situ plant collections do not effectively capture the genetic variation of wild populations. Low genetic representativeness of ex situ populations was caused by both initial incomplete sampling from wild populations and genetic erosion during ex situ conservation. We emphasize that it is necessary to employ more thorough sampling strategies in future collecting efforts and to add new individuals where needed.

Keywords: biodiversity conservation, genetic differentiation, genetic erosion, living ex situ collection, sampling strategy, seed bank, small population size

Resumen: La conservación ex situ se usa ampliamente para proteger de la extinción a las especies silvestres de plantas. Sin embargo, todavía no está claro cómo la variación genética de las colecciones ex situ de plantas reflejan la diversidad de las poblaciones de origen silvestre. Realizamos un metaanálisis mundial de la representatividad genética de las poblaciones ex situ al comparar la diversidad genética (es decir, RA: riqueza alélica, H_e: heterocigosidad esperada, BPP: bandas polimórficas porcentuales, e ISW: índice de Shannon-Weiner), el coeficiente de endogamia (F_{IS}) y la diferenciación genética entre las colecciones ex situ de plantas y sus poblaciones de origen silvestre. La diversidad genética (i.e., H_e, BPP e ISW) fue significativamente más baja en las poblaciones ex situ que en sus poblaciones con origen silvestre, mientras que la diferenciación genética entre las poblaciones ex situ y las silvestres (ex situ-silvestre F_{ST}), pero no aquella entre las poblaciones ex situ, fue significativamente más alta que entre las poblaciones silvestres. Las especies con fertilización cruzada, pero no aquellas con un sistema de

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***Article impact statement**: Low genetic representativeness of living plant collections is a worldwide problem in ex situ conservation.*
Paper submitted July 2, 2020; revised manuscript accepted August 18, 2020.

DOI: 10.1111/cobi.13617

apareamiento mixto, tuvieron una diversidad genética significativamente más baja en las poblaciones ex situ y significativamente más alta ex situ-silvestre F_{ST}. Cuando el tamaño de la colección para la conservación ex situ fue ≥30 o 50, las BPP, H_e, y el F_{ST} ex situ-silvestre no fueron significativamente diferentes entre las poblaciones silvestres y las ex situ, lo que indica una representatividad genética relativamente alta. La recolección a partir de puntos en toda la extensión de la distribución natural y la mezcla de colecciones a partir de diferentes orígenes podría incrementar significativamente la representatividad genética de las poblaciones ex situ. El tipo de conservación ex situ (es decir, plantación o banco de semillas) no tuvo efecto sobre la representatividad genética. El tamaño del efecto de H_e disminuyó y el tamaño del efecto de la F_{ST} ex situ-silvestre incrementó conforme incrementó la duración de la conservación ex situ. Nuestros resultados sugieren que las colecciones actuales de plantas ex situ no capturan de manera efectiva la variación genética de las poblaciones silvestres. La baja representatividad genética de las poblaciones ex situ fue causada tanto por el muestreo inicial incompleto en las poblaciones silvestres y la erosión genética durante la conservación ex situ. Recalcamos que es necesario emplear estrategias de muestreo más minuciosas en los siguientes esfuerzos de colecta y que se deben añadir nuevos individuos en donde sea necesario.
Metaanálisis de la Representatividad Genética de Poblaciones de Plantas bajo Conservación Ex Situ en Contraste con Poblaciones de Origen Silvestre

Palabras Clave: banco de semillas, colección viviente ex situ, conservación de la biodiversidad, diferenciación genética, erosión genética, estrategia de muestreo, tamaño poblacional pequeño, 生物多样性保护, 遗传分化, 遗传侵蚀, 活体迁地种群, 取样策略, 种子库, 小种群

摘要: 迁地保护是应对植物灭绝风险的重要手段。当前迁地保护种群是否涵盖了其野生来源种群的遗传变异尚不明确。我们用集合分析研究了全球范围内迁地保护种群的遗传涵盖度, 比较了遗传多样性 (等位基因丰富度、预期杂合度、多态性位点百分比和香农维纳指数) 、近交系数和遗传分化。迁地保护种群的遗传多样性显著低于野生种群。迁地保护种群与野生种群间的遗传分化, 而非迁地保护种群间的遗传分化, 显著高于野生种群间的遗传分化。与具有混合交配系统的物种相比, 异交物种迁地保护种群的遗传多样性显著低于其野生种群, 而其迁地种群与野生种群间的遗传分化则显著高于野生种群间的遗传分化。当迁地保护的样本数不小于30或者50时, 迁地保护种群与野生种群的遗传多样性无显著差异, 且迁地种群与野生种群间的遗传分化与野生种群间的遗传分化也无显著差异, 这表明迁地保护种群具有较高的遗传涵盖度。此外, 从物种的整个自然分布区而非局部收集迁地保护样本, 以及从多个野生种群收集样本, 均可以有效提高迁地保护种群的遗传涵盖度。迁地保护种群的遗传涵盖度在不同迁地保护类型 (活体种植和人为种子库) 间无显著差异。遗传多样性 (预期杂合度) 的效应值随迁地保护时间的增加而降低, 而迁地保护种群与野生种群间遗传分化的效应值则随迁地保护时间的增加而升高。我们的研究结果表明, 现有的迁地保护种群并未有效涵盖其野生种群的遗传变异。迁地保护种群的低遗传涵盖度可以归因于不完善的取样策略和迁地保护过程中的遗传侵蚀。因此, 我们强调在未来的迁地保护实践中全面采样策略的必要性, 也强调在必要时在已有的迁地保护种群中补充新的个体。

Introduction

Anthropogenic activities are causing rapid climate warming, habitat fragmentation, and habitat loss. These changes have led to an increased risk of extinction for wild plant populations (Pimm et al. 2014). Indeed, local extirpation of wild plant populations and losses of local biodiversity are occurring across the world (Wiens 2016; González et al. 2020). Because of these impacts, ex situ conservation strategies have become an essential approach to protecting wild plants and preventing total extinction (Havens et al. 2006; Mounce et al. 2017; Abeli et al. 2020). Living plant collections and seed banks are the 2 main types of ex situ conservation for wild plants (Schoen & Brown 2001; Oldfield 2009; Mounce et al. 2017).

Preserving genetic variation is one of the primary goals of ex situ plant conservation (Maunder & Byers 2005). Genetic representativeness, which indicates how much of the genetic variation in wild populations has been captured in ex situ populations, is a crucial parameter for quantifying the success of ex situ conservation strategies (Cibrian-Jaramillo et al. 2013). Several theoretical reviews point to the critical role of genetic diversity in individual plant fitness and population persistence (Booy et al. 2000; Reed & Frankham 2003; Jump et al. 2009). Empirical studies also show that genetic diversity is of importance for the survival, growth, and reproduction of transplanted individuals at ex situ conservation sites (Williams 2001; Enßlin et al. 2011; Evans et al. 2018).

Genetic representativeness of ex situ populations can be influenced by species characteristics, sampling strategies for ex situ conservation, and types of ex situ conservation. These can exert impacts during the following processes. First, whether ex situ plant populations capture enough genetic variation from the source populations can be determined at the beginning of the ex situ conservation action (Haven et al. 2006; Oldfield 2009; Kashimshetty et al. 2017). This process involves several key sampling strategies, including collection size

(i.e., number of samples collected for ex situ conservation), collection range, and mixing collections for ex situ conservation (Koskela et al. 2013; Guerrant et al. 2014; Hoban & Strand 2015; McGlaughlin et al. 2015; Bucharova et al. 2019; Hoban 2019; Hoban et al. 2020). Collection range, which refers to whether collections for ex situ conservation were from part of or the entirety of the target species' natural distribution range (Christe et al. 2014), is related to spatial sampling design (Neel & Cummings 2003; Hoban & Schlarbaum 2014; Hoban & Strand 2015; Kashimshetty et al. 2017; Hoban et al. 2018). For example, small collection size and biased collection range could reduce genetic variation through founder effects or genetic bottlenecks (Christe et al. 2014). In contrast, mixing of collections from different sources in a given ex situ population can increase its genetic representativeness (Yuan et al. 2010; Bucharova et al. 2019).

Second, ex situ plant populations may undergo genetic erosion over time (Enßlin et al. 2011; Lauterbach et al. 2012). Ex situ populations have many of the same negative genetic consequences as small populations in the wild (Havens et al. 2006). Theoretical and empirical studies show that founder effects or genetic drift, as well as inbreeding effects, can result in a decline in genetic diversity in small and isolated populations (Ellstrand & Elam 1993; Aguilar et al. 2008). Plant species with different intrinsic characteristics (e.g., life form and mating system) exhibit distinct responses in the face of decreased population size and increased spatial isolation (Aguilar et al. 2008, 2019; Müller et al. 2017). Because genetic erosion is expected to be stronger after several generations, negative genetic consequences are more likely to occur in herbs, which have short life cycles, than in woody plants (Young et al. 1996; Aguilar et al. 2008). In small populations, outcrossing and self-incompatible plants are more prone to lose genetic variation than self-compatible plants with selfing or mixed mating systems (Aguilar et al. 2008, 2019). Furthermore, extrinsic characteristics of plants, such as economic value and threat status, can also influence ex situ conservation efforts and thus genetic representativeness. Plants with economic value have typically been cultivated and domesticated (Yuan et al. 2010). Artificial selection for desired traits may result in decreases in genetic diversity beyond those from the bottleneck effect alone (Doebley et al. 2006). Due to the heavy ex situ load but limited resources, conservation organizations have to devote more resources to the ex situ conservation of threatened plants (Havens et al. 2006), which are expected to have a higher genetic representative than nonthreatened plants.

Third, the type of ex situ conservation (i.e., planting or seed bank) can also influence the genetic representativeness of ex situ populations. Seed banks can store seeds at very high density, reducing space requirement, and are thus expected to harbor larger population sizes and to facilitate higher genetic representativeness than is practical in living plant collections (Wyse et al. 2018; Dalrymple & Abeli 2019). For species with long-lived seeds, seed banks may also have the advantage that collections suffer less genetic erosion caused by genetic drift or artificial selection during the process of ex situ conservation (Dalrymple & Abeli 2019). For some species, however, seed collections lose genetic variation during the periodical regeneration cycles needed to maintain seed viability (Schoen & Brown 2001).

Due to the different species characteristics and conservation efforts, the results of empirical research on the genetic representativeness of ex situ plant populations remain mixed. Results of multiple studies show that genetic diversity of ex situ populations is lower than in natural populations (Li et al. 2005; Lauterbach et al. 2012; Yokogawa et al. 2013; Wilson et al. 2017; Chacón-Vargas et al. 2020), whereas others indicate similar (Enßlin et al. 2011) or even higher (LaBonte et al. 2017) levels of genetic diversity in ex situ populations. Inbreeding coefficient also exhibits mixed results among ex situ conservation genetic studies (Aavik et al. 2012; Yokogawa et al. 2013). Furthermore, the levels of genetic differentiation among ex situ populations (among-ex-situ F_{ST}) or between ex situ and wild populations (ex-situ-wild F_{ST}) are similar or higher compared with those among wild populations (among-wild F_{ST}) (Li et al. 2005; Lauterbach et al. 2012; Yokogawa et al. 2013; Miao et al. 2015).

We conducted a global meta-analysis of genetic representativeness of ex situ plant collections. Specifically, we asked the following questions. Do ex situ plant populations capture the genetic diversity found in wild populations? Is among-ex-situ F_{ST} or ex-situ-wild F_{ST} higher than among-wild F_{ST}? Does genetic representativeness of ex situ populations vary between species with different characteristics (i.e., life form, mating system, threat status, and economic value), sampling strategies for ex situ conservation (i.e., collection size, collection range, and mixing of collections), and ex situ conservation types (i.e., planting and seed bank)?

Methods

Literature Search and Data Extraction

We used the ISI Web of Science and the online databases of 3 major publishers (Elsevier, Springer, and Wiley) to search for peer-reviewed articles published from 1900 to July 2017. We used the following 3 keyword combinations: ("*ex situ conservation*" OR *plantation* OR *planted* OR *cultivation* OR *cultivated*) AND (*plant*) AND (*genetic*∗), ("*ex situ conservation*" OR *plantation* OR *planted* OR *cultivation* OR *cultivated*) AND (*plant*) AND (*genotypic*∗), and ("*ex situ conservation*") AND ("*seed bank*") AND (*genetic*∗). We also obtained 42

articles through cross-references, within which there were some papers published after July 2017. In total, we obtained 3599 relevant articles. We selected these papers according to their title, abstract, or full text. We selected articles that met the following 5 criteria: living plants or seed banks were used for ex situ conservation; genetic variations between ex situ and wild populations were compared; population sample size was ≥5; there were ≥3 populations for both ex situ and wild populations; data on at least 1 measure of genetic diversity, inbreeding coefficient (F_{IS}), or genetic differentiation were used. Here, measures of genetic diversity included allelic richness (AR), expected heterozygosity (H_e), percentage of polymorphic bands (PPB), and Shannon–Wiener index (SWI). Measures of genetic differentiation included F_{ST}, D_{EST}, R_{ST}, G_{ST}, and Φ_{ST}. Because there were few data points for each of the last 4 measures, we combined all 5 measures of genetic differentiation in further analyses. In the end, we obtained 84 publications (Appendix S1). A detailed flowchart depicting the process and outcome of the publication search and selection is provided in Appendix S2.

We compiled the following data: the value of every genetic parameter, number of populations, and sample size of each population. We obtained more than one set of data for a given parameter from some studies that focused on more than one species or used more than one kind of molecular marker. Because the measures of genetic diversity and genetic differentiation and the number of data points varied among studies, the total number of cases for each genetic parameter varied. As a result, we had 207 data points among the 4 measures of genetic diversity, 18 data points for F_{IS}, and 58 data points for genetic differentiation measures from the 84 studies with 98 cases (Appendix S3).

We also recorded the following information: article publication year, molecular marker, country where the study was conducted, study species, family of study species, duration of ex situ conservation, species characteristics (i.e., life form, mating system, threat status, and economic value), sampling strategies for ex situ conservation (i.e., collection size, collection range, and mixing of collections), and ex situ conservation type (i.e., planting or seed bank). If not stated, we used the sample size of the ex situ population, which is no more than the ex situ population size, as collection size because the initial collection size for ex situ conservation is not always available in case studies. We considered a plant had economic value if it was used for an economic purpose, including agriculture, medicines, ornamentals, or timber. Information was extracted from the source literatures, not from other data sets, except for species threat status, which was categorized following the IUCN Red List of Threatened Species (IUCN 2020).

Statistical Analyses

We calculated mean and standard error for each genetic parameter. The differences in genetic parameters between ex situ and wild populations were tested with paired *t* tests. We tested the influence of sample size on each genetic parameter with regression analysis. We correlated effect size of each genetic parameter based on the duration of ex situ conservation. If the data did not satisfy the assumptions of normality and homogeneity of variance after transformations, we used nonparametric tests or Spearman's rank correlation coefficient. We used SPSS 16.0 (SPSS, Chicago, Illinois, U.S.A.) to perform all statistical analyses.

We used Hedge's *d* as measure of effect size and estimated it as the unbiased standardized mean difference between the mean values of the genetic parameter for ex situ and wild populations:

$$d = \frac{\underline{x}_e - \underline{x}_i}{S} J, \tag{1}$$

where $\underline{x}_e$ and $\underline{x}_i$ are the mean values of a given genetic parameter for ex situ and wild populations, respectively, *S* is the pooled standard deviation across all populations, and *J* is a correction factor for the effect of small sample size (Gurevitch & Hedges 2001). For genetic differentiation, $\underline{x}_e$ is the mean value of among-ex-situ F_{ST} or ex-situ-wild F_{ST}, and $\underline{x}_i$ is among-wild F_{ST}. Following Reed and Frankham (2003), we weighted each study according to $[(K\text{-}2)N]^{1/2}$, where *K* is the number of populations studied and *N* is the mean number of samples per population. This weighting allowed accounting for not only the number of populations, but also the number of samples genotyped (Honnay & Jacquemyn 2007). A negative *d* value implies lower genetic diversity or F_{IS} in ex situ populations than in wild populations. For genetic differentiation, a positive value of effect size implies that among-ex-situ F_{ST} or among-ex-situ F_{ST} is higher than among-wild F_{ST}.

We used standard meta-analysis to estimate the overall effect size of each genetic parameter. We conducted subgroup meta-analysis to test whether genetic representativeness varied among different species characteristics, sampling strategies for ex situ conservation, and ex situ conservation types. Categories with <3 data points were excluded because of low reliability (González et al. 2020).

Closely related species are probably more similar in morphological or physiological characteristics than distantly related species. Therefore, shared phylogenetic history for closely related species can result in similar estimates of effect sizes (Koricheva et al. 2013). To avoid the potential effect of this phylogenetic signal, we used a Brownian-motion model to perform a phylogenetic

meta-analysis. Species that were not found in the Phylomatic database were removed prior to analysis. With the remaining species, we made a phylogenetic tree in Phylomatic version 3 (http://phylodiversity.net/phylomatic/) and estimated branch lengths with Phylocom version 4.2 (Webb et al. 2008).

For standard meta-analysis, conventional random-effect models were used to combine effect sizes across studies. For subgroup meta-analysis, although studies within each subgroup share a fixed effect, there was also other random variation among studies. Therefore, we also used traditional random-effect models to combine effect sizes across studies for each subgroup. For phylogenetic meta-analysis, we used phylogenetic random-effect models to estimate the grand mean effect size (Wallace et al. 2017). In contrast to fixed-effect models, random-effect models are often used in ecology because, besides random sampling variation, there is true variation in study-specific effects caused by different experimental conditions or natural environments (Koricheva et al. 2013). We conducted the analysis with restricted maximum likelihood estimators to calculate the parameters. If the 95% confidence interval (CI) of d did not overlap with 0, the effect size was considered significant, indicating that the genetic parameter was significantly different between different groups.

Studies with significant results have a greater possibility of being published than those with nonsignificant results, and this could result in publication bias, which means studies included in ecological meta-analysis may have more cases with significant results. Therefore, we tested publication bias in our sample with 2 commonly used methods. First, we generated a funnel plot to show the effect size plotted against standard error (Egger et al. 1997). A symmetrical funnel shape of the scatterplot around the mean effect size indicates the results of the meta-analysis are robust to publication bias. Next, we calculated the fail-safe number (FSN) for significant effect with the Rosenthal approach (Rosenthal 1979). Here, if FSN is $> 5k + 10$ (where k is the number of data points in the analysis), the results of the meta-analysis are expected to be robust to publication bias. We used OpenMEE software to complete all meta-analyses and publication bias tests (Wallace et al. 2017).

Results

Summary of Data Set

The 98 studies from the 84 publications were carried out in 49 countries or regions, including China (30 cases), Germany (12), United States (10), Brazil (6), Mexico (6), Spain (5), Turkey (5), and others with <5 cases (Appendix S4). Although approximately 30% of the 98 cases were from China, there was no effect of country on the significance of effect size of any parameter, except SWI (Appendix S5).

After the 2000, the number of studies increased rapidly, although there was significant interannual variability (Appendix S6). Eight kinds of molecular markers were recorded (Appendix S6). The most commonly used markers were nuclear simple sequence repeats (nSSR; 35.7%), followed by random amplification of polymorphic DNA (RAPD) (18.4%), intersimple sequence repeats (ISSR) (15.3%), amplified fragment length polymorphisms (AFLP) (12.2%), and allozymes (12.2%). The use of different molecular markers (i.e., ISSR+RAPD vs. others) had no influence on the significance of effect size of any parameter, except PPB (Appendix S7).

Paired t *Test and Regression Analyses*

Ex situ populations had significantly lower genetic diversity than wild populations for 3 of the 4 measures (H_e: $Z = -3.041$, $p = 0.002$; PPB: $t = -3.403$, $p = 0.001$; SWI: $Z = -3.743$, $p < 0.001$; but not AR: $t = -0.328$, $p = 0.746$) (Appendix S8). The F_{IS} was not significantly different between ex situ and wild populations (F_{IS}: $Z = -0.283$, $p = 0.777$) (Appendix S8). None of the genetic diversity measures or F_{IS} was significantly related to sample sizes of either ex situ or wild populations, except that F_{IS} was negatively correlated with sample size of wild populations (Appendix S9).

Among-ex-situ F_{ST} was not significantly different from among-wild F_{ST} ($t = 0.937$, $p = 0.356$), but ex-situ-wild F_{ST} was significantly higher than among-wild F_{ST} ($t = -3.565$, $p = 0.001$) (Appendix S8).

Meta-analyses

Both standard and phylogenetic meta-analyses revealed that the overall effect sizes of H_e, PPB, and SWI, but not AR and F_{IS}, were significantly negative, whereas the effect size of ex-situ-wild F_{ST}, but not that of among-ex-situ F_{ST}, was significantly positive (Fig. 1).

Subgroup meta-analyses revealed that the effect sizes of AR and F_{IS} were not significant under any kind of species characteristics (Appendix S10). We observed significant lower genetic representativeness for both H_e and SWI regardless of life form, threat status, or economic value of the study species (Fig. 2). The PPB showed similar results, with the exception that the effect size of PPB was not significant for plants with economic value but was significantly negative for plants with no information on economic value (Fig. 2). For H_e, PPB, and SWI, the effect sizes of outcrossing plant species were significantly negative, whereas the effect sizes of plants with a mixed or selfing mating system were nonsignificant (Fig. 2).

The effect size of among-ex-situ F_{ST} was not significant for any species characteristic (Appendix S10). However, the effect size of ex-situ-wild F_{ST} was

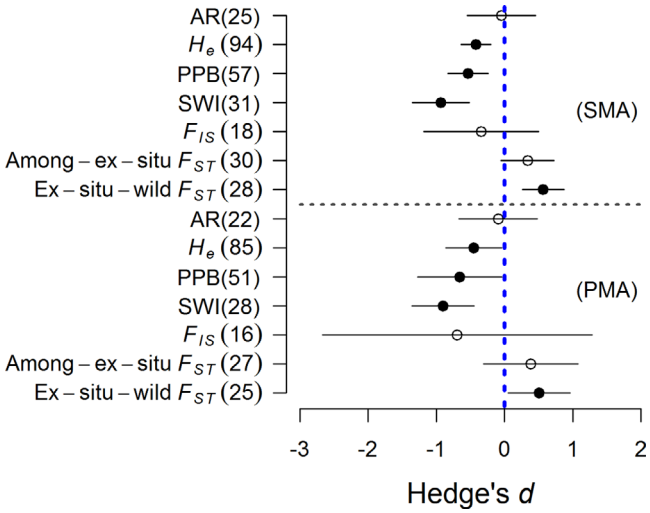

Figure 1. Effect sizes (Hedge's d*) and 95% CIs of genetic parameters based on both standard meta-analysis (SMA) and phylogenetic meta-analysis (PMA) (parentheses, number of data points for each measure; vertical dashed lines, Hedge's* d = *0; filled dots, significant effect size; AR, allelic richness;* F_{IS}*, inbreeding coefficient;* H_e*, expected heterozygosity; PPB, percentage of polymorphic bands; SWI, Shannon–Wiener index; among-ex-situ* F_{ST}*, genetic differentiation among ex situ populations; and ex-situ-wild* F_{ST}*, genetic differentiation between ex situ and wild populations). A mean effect size is significantly different from 0 when its 95% CI does not include 0. A negative mean effect size indicates ex situ populations had lower genetic diversity or* F_{IS} *than wild populations. A positive mean effect size suggests that among-ex-situ* F_{ST} *or ex-situ-wild* F_{ST} *was higher than among-wild* F_{ST}*.*

significantly positive regardless of economic value, but not significant for both woody plants and herbs (Fig. 2). The effect size of ex-situ-wild F_{ST} for outcrossing plants was significantly positive, but not significant for plants with mixed mating systems (Fig. 2). The effect size of ex-situ-wild F_{ST} for nonthreatened plants was significantly positive, but not significant for threatened plants (Fig. 2).

Subgroup meta-analyses revealed that the effect sizes of AR and F_{IS} were not significant under any sampling strategy or type of ex situ conservation (Appendix S11). The effect sizes of H_e and SWI were significantly negative whether the collection size for ex situ conservation was ≥30 or <30 (Fig. 3). The effect size of PPB was not significant when the collection size was ≥30, but it was significantly negative when the collection size was <30 (Fig. 3). The effect sizes of H_e and PPB were not significant when the collection size was ≥50, but the effect sizes of H_e, PPB, and SWI were all significantly negative when the collection size was <50 (Fig. 3). The effect sizes of H_e, PPB, and SWI were all significantly negative for ex situ collections obtained from only part of target species' natural distribution ranges, whereas none were significant when ex situ collections covered the entirety of their ranges (Fig. 3). The effect sizes of H_e, PPB, and SWI were all significantly negative if a mixed collection strategy was not adopted, whereas none were significant when collections from different sources were mixed for ex situ conservation (Fig. 3). The effect size of H_e for both planting and seed bank was significantly negative (Fig. 3).

The effect size of among-ex-situ F_{ST} was not significant under any sampling strategy or type of ex situ conservation, although it was significantly positive when the collection size was ≥30 for ex situ conservation (Appendix S11). The effect size of ex-situ-wild F_{ST} was significantly positive when the collection size was <30 or 50, whereas it was not significant when the collection size was ≥30 (Fig. 3). The effect size of ex-situ-wild F_{ST} was significantly positive when the ex situ collections obtained from only part of target species' natural distribution ranges, whereas it was not significant when collections covered the entire ranges (Fig. 3). The effect size of ex-situ-wild F_{ST} was significantly positive if a mixture strategy was not adopted, whereas it was not significant when collections from different sources were mixed for ex situ conservation (Fig. 3).

The effect sizes of H_e and ex-situ-wild F_{ST} were negatively ($r = -0.375$, $p = 0.006$) and positively ($r = 0.660$, $p = 0.007$) correlated with duration of ex situ conservation, respectively (Fig. 4).

For all 3 kinds of meta-analysis, examination of the funnel plots for each genetic parameter revealed a generally symmetric funnel shape (Appendix S12; Appendix S13). Likewise, FSN suggested that the results for H_e, PPB, SWI, and ex-situ-wild F_{ST} were all robust to publication bias. However, the results of both standard and subgroup meta-analyses for ex-situ-wild F_{ST} (FSN < $5k$ +10) should be interpreted with caution (Appendix S3).

Discussion

It is widely recognized that ex situ conservation plays an important role in protecting wild plant species and species diversity (Mounce et al. 2017). However, our findings demonstrate that genetic diversity, the other level of biodiversity, in ex situ plant populations is significantly lower than in their wild source populations, and genetic divergence between ex situ and wild populations was higher than that among wild populations (Fig. 1).

Lower Genetic Diversity in Ex Situ Populations

Our results showed that H_e, PPB, and SWI, but not AR, were significantly lower in ex situ populations compared

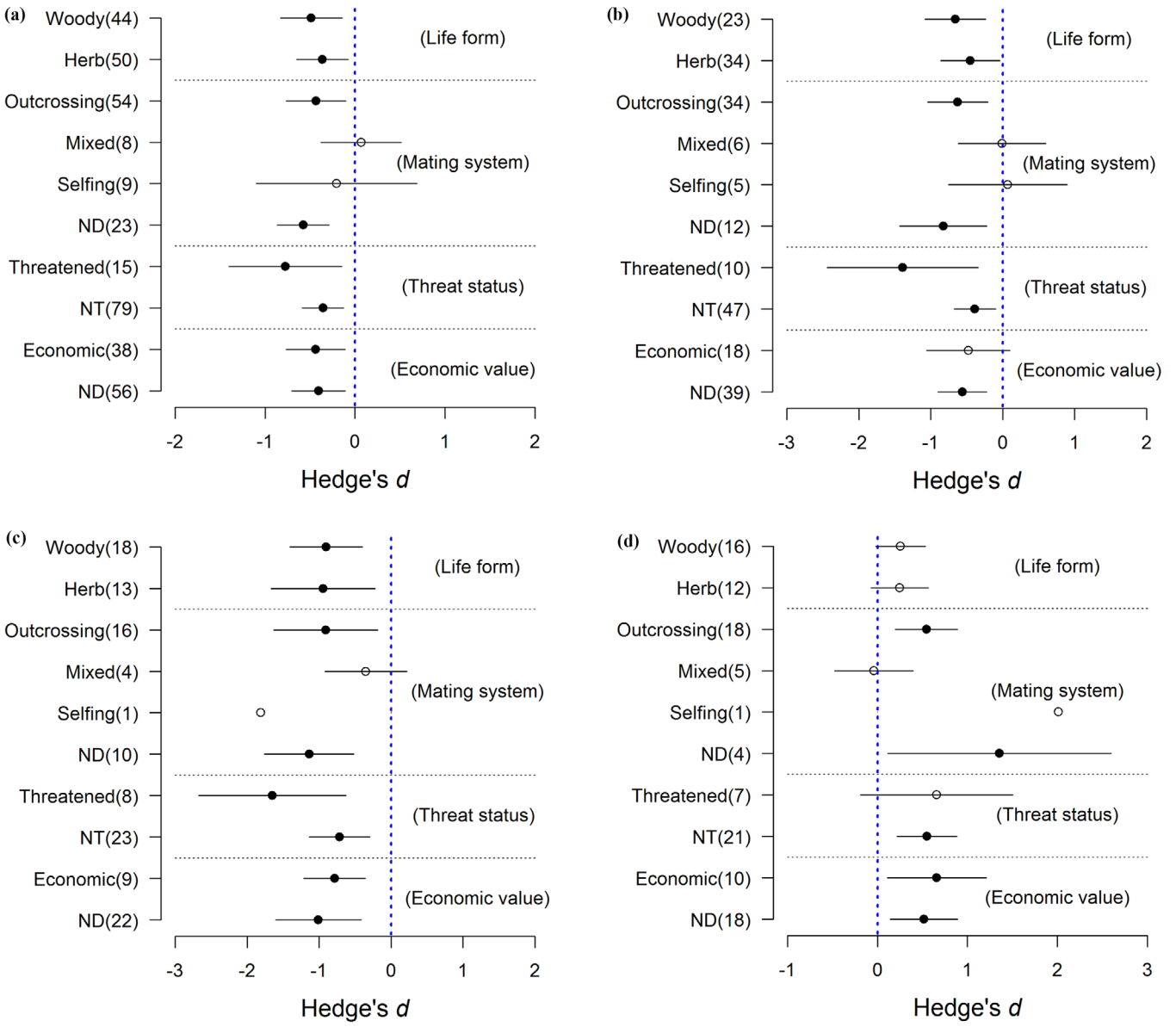

Figure 2. Effects of species characteristics on effect sizes (Hedge's d*) of genetic parameters ((a) He, (b), PPB, (c) SWI, (d) ex-situ-wild FST) based on subgroup meta-analyses (parentheses, number of data points for each measure; vertical dashed lines, Hedge's* d *= 0; filled dots, significant effect size;* H_e*, expected heterozygosity; PPB, percentage of polymorphic bands; SWI, Shannon–Wiener index; ex-situ-wild* F_{ST}*, genetic differentiation between ex situ and wild populations; ND, no data; and NT, not threatened). A mean effect size is significantly different from 0 when the 95% CI does not include 0. A negative mean effect size indicates that ex situ populations have lower genetic diversity than wild populations. A positive mean effect size suggests that ex-situ-wild* F_{ST} *is higher than among-wild* F_{ST}*.*

with wild populations, regardless of a species' economic value, threat status, or life form. This reflects the prevalence of low genetic representativeness in ex situ plant conservation practices. However, in contrast to our expectation, AR, which is more likely to change in small and isolated populations than H_e (Nei et al. 1975), was not significantly different between ex situ and wild populations. This may be because AR was always rarefied to the smallest sample size used in each individual study, and the similar and relatively small sample sizes used to estimate AR for both ex situ and wild populations resulted in comparable values.

We found evidence for 2 primary explanations of the lower genetic diversity in ex situ plant populations. First,

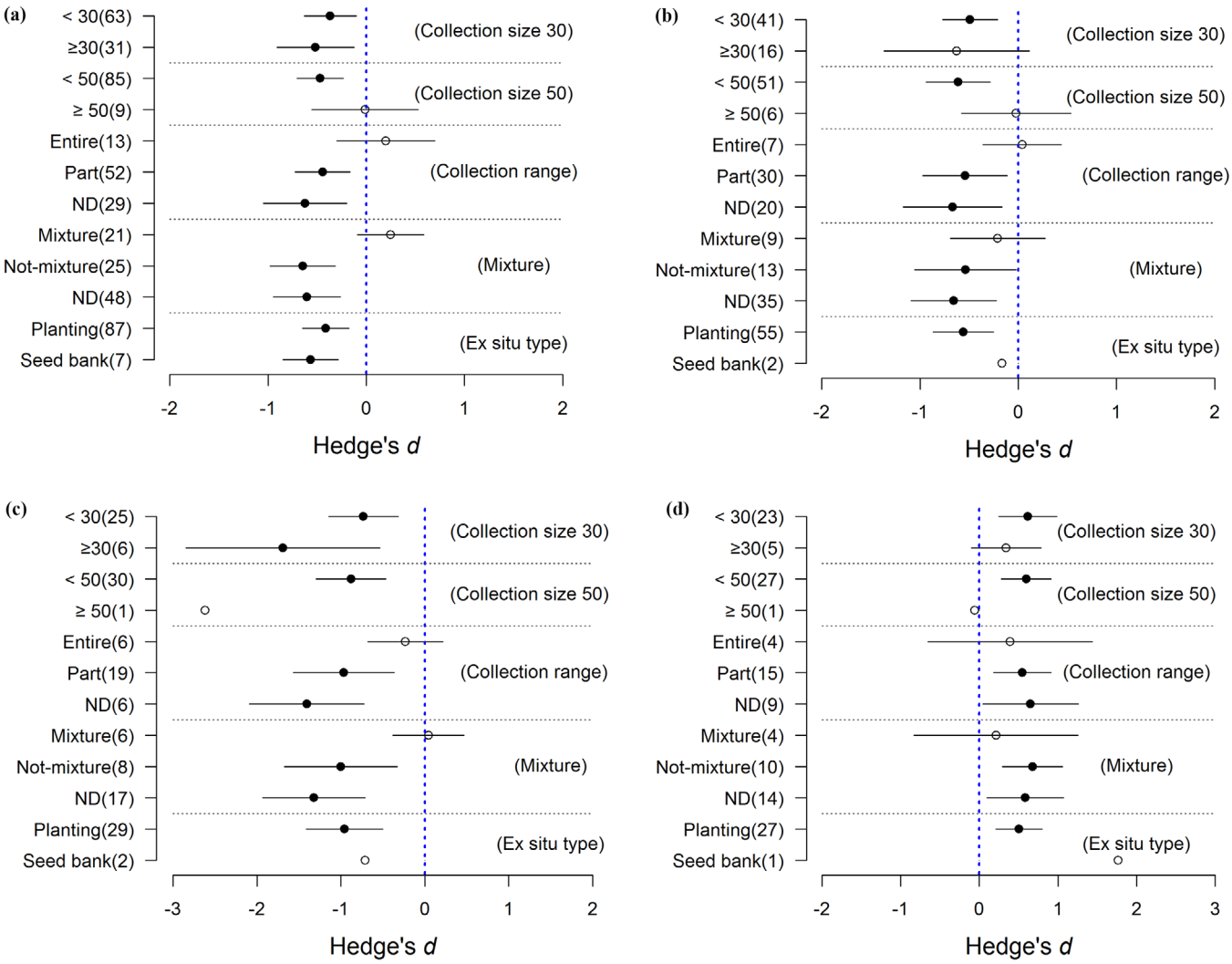

Figure 3. Effects of sampling strategies and types of ex situ conservation on effect sizes (Hedge's d*) of genetic parameters ((a) He, (b), PPB, (c) SWI, (d) ex-situ-wild FST) based on subgroup meta-analyses (parentheses, number of data points for each measure; vertical dashed lines, Hedge's* d *= 0; filled dots, significant effect size;* H_e*, expected heterozygosity; PPB, percentage of polymorphic bands; SWI, Shannon–Wiener index; ex-situ-wild* F_{ST}*, genetic differentiation between ex situ and wild populations; ND, no data; and NT, not threatened). A mean effect size is significantly different from 0 when the 95% CI does not include 0. A negative mean effect size indicates that ex situ populations have lower genetic diversity than wild populations. A positive mean effect size suggests that ex-situ-wild* F_{ST} *is higher than among-wild* F_{ST}*.*

ex situ plant populations did not capture enough genetic variation from the wild populations at the beginning of the ex situ conservation practice due to poor sampling strategies. We found evidence that ex situ populations had low genetic representativeness for some genetic parameters when the collection size was <30 or 50, but high genetic representativeness when the collection size was ≥30 or 50 (Fig. 3). As compared with collecting only from part of the natural distribution ranges of target species, collections from the entire distribution ranges can also increase the genetic representativeness (Fig. 3). In contrast to not mixing collections from different sources, mixtures of collections showed similar genetic diversity in ex situ and wild populations (Fig. 3). For example, mixing collections from multiple wild populations for ex situ conservation can make genetic diversity of the ex situ populations comparable to or even higher than wild populations (Li et al. 2005; Yuan et al. 2010; Christe et al. 2014; Labonte et al. 2017). Our findings reflect that there are effective approaches (i.e., with collection size ≥30 or 50, collecting from the entire natural distribution ranges, and mixing collections from different sources) that can increase genetic representativeness, but they have not yet been widely adopted. We

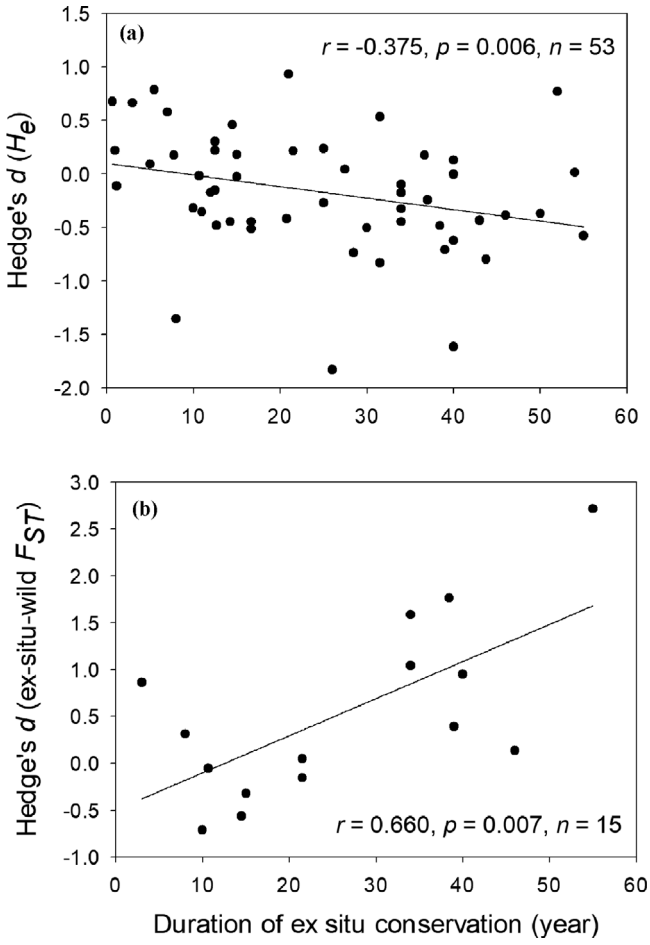

Figure 4. Correlations between the duration of ex situ conservation and the effect sizes of (a) H_e *and (b) ex situ-wild* F_{ST} *(ex-situ-wild* F_{ST}*, genetic differentiation between ex situ and wild populations;* H_e*, expected heterozygosity).*

emphasize the importance of thorough sampling strategies for high levels of genetic representativeness in future studies.

Second, ex situ plant populations lost genetic diversity during ex situ conservation. The direct evidence for this is that the effect size of H_e decreased significantly as the duration of ex situ conservation increased (Fig. 4a). Empirical studies show that genetic diversity of rare or endangered plant species decreases as cultivation time in botanic gardens increases (Enßlin et al. 2011; Lauterbach et al. 2012). The negative effect of artificial selection on genetic diversity in ex situ populations, especially for economic plants, is also prone to increase as cultivation time increases (Doebley et al. 2006). Furthermore, we found that outcrossing species, but not plants with mixed or selfing mating system, had lower genetic diversity in ex situ populations than in wild populations. Given that our findings echo the results of studies of wild isolated and small populations under intensifying human disturbance (Aguilar et al. 2008; 2019), we stress that both wild and ex situ populations with small population sizes and increased spatial isolation are threatened by negative genetic consequences, such as genetic erosion, which is challenging the maintenance of genetic variation in wild plant species.

Higher Genetic Differentiation between Ex Situ and Wild Populations

We found that among-ex-situ F_{ST} was not significantly different from among-wild F_{ST}. This supports the assumption that it is better if among-ex-situ F_{ST} is comparable to among-wild F_{ST} because biased genetic composition of ex situ populations is thought to jeopardize the effectiveness of ex situ conservation (Li et al. 2005). However, this is only meaningful if the numbers and geographic distributions of ex situ populations are similar to these of the wild populations. That is because both genetic composition and geographic connectivity, 2 important factors determining genetic differentiation between populations, are mainly determined by sampling strategies for ex situ populations but are naturally formed for wild populations (Li et al. 2005). However, we cannot verify whether the ex situ and wild populations of the studies we obtained the 30 data points of among-ex-situ F_{ST} had similar distributions.

We also found that ex-situ-wild F_{ST} was significantly higher than among-wild F_{ST}. This means that gene flow between ex situ and wild population was greatly reduced. Similar to genetic diversity, the higher ex-situ-wild F_{ST} could also be caused at the beginning of ex situ conservation by incomplete sampling and during the cultivation of the transplanted individuals. Incomplete sampling strategies, such as with collection size <30 or 50, collecting only from part of the natural distribution ranges, and no mixture of collections from different sources, strengthened ex-situ-wild F_{ST} and vice-versa (Fig. 3). Because of the small population sizes, increased spatial isolation, and artificial selection caused by incomplete sampling strategies, high levels of ex-situ-wild F_{ST} have been reported in several empirical studies, even between ex situ populations and the wild populations where they were collected (Lauterbach et al. 2012; Miao et al. 2015; Müller et al. 2017). Furthermore, the effect size of ex-situ-wild F_{ST} significantly increased as the duration of ex situ conservation increased (Fig. 4b), indicating that cultivation time is also a critical factor to determine the level of ex-situ-wild F_{ST}. Additionally, high level of ex-situ-wild F_{ST} can also be ascribed to the lack of effective pollinators in ex situ collections (Xiao et al. 2019).

Comparable Inbreeding Coefficient in Ex Situ and Wild Populations

Our meta-analysis results showed that F_{IS} was not significantly different between ex situ and wild populations

for any of the species characteristic, sampling strategy, or type of ex situ conservation. Two potential scenarios are likely to account for this finding. First, if a species has only a few wild populations with a small number of individuals, one may expect to see similar levels of inbreeding in both ex situ and wild populations. Second, if the cultivation time of ex situ conservation for a long-lived species is shorter than its life history, one can only detect F_{IS} of the transplanted individuals because an increase in inbreeding coefficient can only be detected after at least one generation. Theoretically, the time elapsed since the beginning of transplantation is an important factor in determining the expression and magnitude of inbreeding depression in ex situ populations (Aguilar et al. 2008; Fernández & González-Martínez 2009). In small populations, as is the norm for most ex situ populations, F_{IS} could increase immediately in the next generation of the transplanted individuals (Aguilar et al. 2008). For long-lived species, however, most studies were conducted on transplanted individuals with a relatively short period at ex situ sites.

Caveats

We recorded 8 kinds of molecular markers, including ISSR and RAPD, which have been criticized as unreliable and irreproducible. However, the use of different molecular markers did not affect the significance of effect sizes, except that the effect size of PPB was significantly negative for studies in which ISSR or RAPD were used, but not for those in which other molecular markers were used (Appendix S7). This is probably because, for PPB, the percentage of studies with a collection size $\geq$30 within the studies in which ISSR or RAPD was used was lower than that within the studies that used other kinds of molecular markers (ISSR or RAPD: 5 of 32; others: 11 of 25).

There was bias in the geographic distribution of the case studies we used (Appendix S4). More than 30% (30/98) of the cases were done partly or completely in China. However, this bias did not significantly influence the significance of effect sizes except that the effect size of SWI was significantly negative for studies from China but not those from all the other countries and regions (Appendix S5). This is probably because, for SWI, the percentage of studies for which samples were collected from only parts of the target species' natural distribution ranges was higher in China than that in the rest of the world (China: 14 of 20; not China: 5 of 11).

In most cases, there was a period between collecting materials (seeds or seedlings) for ex situ conservation and sampling for comparison of genetic variation between wild and ex situ populations. During this period, the wild populations may be changed through ecological and evolutionary processes in a shifting environment (Aguilar et al. 2008; Moritz & Agudo 2013). However, up to now, almost all the cases studies neglected this factor. For wild plant species with extremely small populations, transplanting individuals from wild populations to ex situ populations can reduce the population size and genetic diversity of wild populations (Furlan et al. 2020). This can lead to an overestimate of the genetic representativeness of ex situ populations.

There was a large difference in the number of studies focusing on the genetic representativeness of planting collections and seed banks (Fig. 3). We had only enough data points ($\geq$3) to analyze H_e when we used planting (87) and seed bank (7) as categories, and both types of ex situ conservation did not capture enough genetic variation from the wild populations (Fig. 3). We emphasize the need to increase the genetic representativeness of both types of ex situ plant conservation. The limited number of studies available for seed banks highlights the need for more investigations of the genetic representativeness of this important approach to ex situ plant conservation in the future (Schoen & Brown 2001; Gargiulo et al. 2019).

Recommendations

Because studies comparing genetic variation between ex situ and wild populations have steadily increased over the past 2 decades, genetic representativeness of ex situ populations can now be assessed for a wide range of plant species worldwide. In general, genetic diversity of ex situ populations is lower than that of wild source populations and ex-situ-wild F_{ST} is higher than among-wild F_{ST}. To prevent low genetic representativeness, we recommend the following. First, a thorough sampling design based on integrating information about spatial distribution range, genetic diversity, and genetic structure, as well as life-history traits of target plant species, should be employed. Ideally, collections should occur across the entire distribution range of the target species (Hoban & Schlarbaum 2014; Hoban & Strand 2015; Kashimshetty et al. 2017; LaBonte et al. 2017; Hoban et al. 2018; Hoban 2019). Our results also showed that the collection size should be $\geq$30 or 50, and this is largely consistent with previous studies (Hoban & Schlarbaum 2014; Hoban et al. 2018, 2020). At each ex situ site, mixing collections from different sources within the nearby region is also an effective approach to increase genetic variation. Given the limited resources of most botanical gardens and arboreta, it is challenging to achieve all of these, but as many as possible need to be done to increase genetic representativeness.

Second, it is necessary to assess levels of genetic diversity and genetic composition of the ex situ populations that already exist but have not yet been assessed and to add new individuals to the collections when necessary (Kashimshetty et al. 2017; Wilson et al. 2017). Third, genetic variation, inbreeding, and functional trait

composition of different cohorts should be simultaneously compared between ex situ and wild populations to reveal the potential genetic erosion and fitness decline in both the transplanted individuals and their progenies. That is because, in contrast to the huge number of studies focusing on genetic representativeness, only a few studies have assessed changes in performance of ex situ populations (i.e., plant size, leaf traits, flower traits, fertility, seed mass, seed germination, etc.), which may be caused by genetic erosion, artificial habitats, or both (Williams 2001; Enßlin et al. 2011; Li et al. 2012; Ensslin et al. 2015, 2018; Rauschkolb et al. 2019). To do so, conservation genomic tools (such as genomic sequencing and genotype-trait association) should be used in future studies (Benestan et al. 2016); these methods more powerfully associate selective genetic variation with fitness than the neutral molecular markers that are commonly used (Appendix S6).

Acknowledgments

We thank S. Wang, L. Chen, M. Liu, Y. Xu, Z. Yang, and T. Yang for assistance with data collection and compilation. The comments and suggestions of 3 anonymous reviewers have contributed greatly to the improvement of our manuscript. We thank R.T. Corlett and M. Stephen for their assistance with English language and grammatical editing of the manuscript. This work was supported by the National Key Research and Development Program of China (2016YFC0503105) and the National Natural Science Foundation of China (31870510 and 31770572).

Supporting Information

Additional information is available online in the Supporting Information section at the end of the online article. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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