EX SITU CONSERVATION OF LARGE AND SMALL PLANT POPULATIONS ILLUSTRATES LIMITATIONS OF COMMON CONSERVATION METRICS

M. Patrick Griffith,†,* Falon Cartwright,† Michael Dosmann,‡ Jeremie Fant,§ Ethan Freid,† Kayri Havens,§ Brett Jestrow,∥ Andrea T. Kramer,§ Tracy M. Magellan,*, Alan W. Meerow,* # Abby Meyer,** Vanessa Sanchez,* Eugenio Santiago-Valentín,‡† Emma Spence,‡‡ Jose A. Sustasche-Sustache,§§ Javier Francisco-Ortega,|| ||| and Sean Hoban‡‡

*Montgomery Botanical Center, Coral Gables, Florida, USA; †Bahamas National Trust, Nassau, Bahamas; ‡Arnold Arboretum of Harvard University, Boston, Massachusetts, USA; §Chicago Botanic Garden, Glencoe, Illinois, USA; ||Fairchild Tropical Botanic Garden, Coral Gables, Florida, USA; §§School of Life Sciences, Arizona State University, Tempe, Arizona, USA; **Botanic Gardens Conservation International US, San Marino, California, USA; ††Universidad de Puerto Rico–Río Piedras, San Juan, Puerto Rico, USA; ‡‡Morton Arboretum, Lisle, Illinois, USA; §§§Departamento de Recursos Naturales y Ambientales, San Juan, Puerto Rico, USA; and ||||Florida International University, Miami, Florida, USA

Editor: Stefanie Ickert-Bond

Premise of research. Ex situ plant conservation can be improved through genetic analysis. One area of interest is the relative value of conserving smaller or larger populations and how sampling strategies for these might differ. Current practice emphasizes collecting large sample sizes from some populations and limiting sampling from others and aims for the capture of allele diversity exceeding predetermined thresholds at the species level. Evaluating how well botanic garden collections can capture the genetic diversity of populations of different sizes can help refine guidance on conservation efforts.

Methodology. A model species, Pseudophoenix sargentii (Arecaceae), was chosen for its disjunct and insular range, variation in population size, and presence in collections. We compared 123 in situ plants from three discrete island populations with 94 ex situ conservation specimens via 10 microsatellite markers. Comparison of allelic diversity among the wild populations and collections allowed for the evaluation of genetic capture.

Pivotal results. Genetic distance analysis, fixation indexes, and Bayesian clustering analysis show discrete in situ geographic structure and close affinity between ex situ collections and in situ source populations. Yet collections from just the largest population met the Global Strategy for Plant Conservation Target 9 threshold for conservation success for that source population, for other smaller populations, and for all populations together.

Conclusions. Percent of genetic capture thresholds may need revision, as such thresholds overlook important diversity. Efficient genetic capture is maximized by emphasizing unique maternal lineages and limiting half-siblings in a collection, but this selectivity must be balanced against the need for redundancy in living collections. Large and small populations each contribute to meeting genetic diversity goals. We recommend that botanic gardens and their networks develop conservation priorities based on genetic diversity and resources, carefully consider existing thresholds for conservation success, define metrics for ex situ conservation goals, and integrate analysis into ex situ conservation efforts.

Keywords: Arecaceae, botanic garden, Caribbean, conservation genetics, Global Strategy for Plant Conservation Target 9, Pseudophoenix sargentii.

Introduction

Plant conservation often requires protective cultivation. As a critical complement to habitat protection and land management, growing imperiled plants as living conservation collections is increasingly the first step to long-term species survival—and in some cases, the only viable way to sustain remaining diversity (Dhar 1996; Owens and Rix 2007), particularly for “exceptional species” sensu Pence (2013). The vital importance of safeguarding such living treasures is codified in the Global Strategy for Plant Conservation (GSPC; Sharrock 2012). That guiding document establishes goals to ensure that conservation collections complement other actions to ensure species survival: GSPC Target 8 calls on botanic gardens to maintain imperiled plants for recovery programs set within a framework of improved understanding,
sustainable use, education and training, and in situ and ex situ conservation. As a worldwide endeavor to halt the loss of plant diversity, the GSPC puts forward a vision of a sustainable future for people and plants.

The botanic garden community values the conservation of habitats and landscapes (Miller et al. 2016) and understands the importance of holding diverse living collections. “Diverse” here can refer to taxonomic diversity (Mounce et al. 2017) but also geographic breadth (Migicovsky et al. 2019) or the number of maternal lines in a collection (Wood et al. 2020). Genetic diversity can be measured by alleles per locus, heterozygosity, effective population size, and other metrics. The metric most often used to assay the genetic diversity captured in living conservation collections is allele diversity (Gibrian-Jaramillo et al. 2013). Drawing on methods from forestry (Yang and Yeh 1992; Brown and Hardner 2000) and agricultural sciences (Volk et al. 2005; Richards et al. 2007; FAO 2014), botanic gardens have increasingly assayed the effectiveness of ex situ collections via allele diversity (Namoff et al. 2010b; Toppila 2012; Christe et al. 2014).

These advances in “collection genetics” underpin a benchmark for the 2011–2020 GSPC: Target 9 seeks the protection of genetic diversity in certain species. The full text of Target 9 reads, “70 per cent of the genetic diversity of crops including their wild relatives and other socio-economically valuable plant species conserved, while respecting, preserving and maintaining associated indigenous and local knowledge” (Convention on Biological Diversity 2020). Thus, this genetic capture objective applies to crops, expands to crop wild relatives, and, finally, includes “socio-economically valuable” species. Target 9 thus applies to any utilized species and could be effectively applied to any plant (Hoban et al. 2020b). Therefore, for this study, we consider genetic diversity per Target 9 of the GSPC, along with the concomitant North American Botanic Garden Strategy for Plant Conservation (BGCI 2016), which calls for species to have “good genetic representation” in botanic gardens, without a specific threshold. Note that higher allele capture thresholds, including those as high as 95% (Marshall and Brown 1975; Brown and Marshall 1995), have been proposed.

Achieving percentage-based targets prompts a focus on deliberate sampling. Consideration of sampling size and method has been largely modeled from in situ population genetic data (Hoban and Schlarbaum 2014; McGlaughlin et al. 2015; Kashimshetty et al. 2017). Recently, such study has seen a shift in emphasis to models that take into account logistic and biological factors involved with establishing ex situ living collections (Ensslin et al. 2015; Hoban and Strand 2015; Ensslin and Godefroid 2019; Hoban 2019) and make empirical assessments of such established collections, that is, direct comparison of the ex situ and in situ genetic diversity (Li et al. 2002; Hou et al. 2012; Choi et al. 2013; Bragg et al. 2020). Structured resampling of the genetic data from ex situ collections provides insight into how these biological factors may influence how well collections capture in situ genetic diversity (Namoff et al. 2010b; Griffith et al. 2015, 2017b, 2020; Hoban et al. 2020a).

A major factor differentiating populations is geographic distance (Hutchison and Templeton 1999), which can create a barrier to gene flow. Thus, modern guidelines for obtaining germplasm for ex situ collections emphasize covering the full geographic range of a species or population to help ensure that more alleles are obtained (Guerrant et al. 2014; Griffith et al. 2015; Hoban and Strand 2015), which affects better “genetic capture” of in situ diversity. For example, collections development for rare oaks emphasizes sampling from populations that are at least 50 km from other known collecting sites (Beckman et al. 2019), on the basis of likely maximal gene flow distance in this genus.

An area that deserves further attention, however, is the influence of population size on genetic capture. It is well established that smaller plant populations have unique concerns with inbreeding, genetic drift, and gene flow (Ellstrand and Elam 1993; Wood et al. 2016) and that populations in marginal habitats may be small but have unique adaptations (Lesica and Allendorf 1995; Hampe and Petit 2005; Sexton et al. 2014), making these populations of specific interest for ex situ conservation. Current best practices consider population size, most often emphasizing a size threshold of the total plants in a population from which to collect from every maternal line (e.g., 100 plants in Pence et al. 2019). Understanding the best sampling for the optimal genetic capture of small populations is vitally important given the deleterious impact of oversampling seeds from such sources (Menges et al. 2004). Unfortunately, previous simulation work has almost always considered simple situations in which populations are of equal size (e.g., Hoban and Schlarbaum 2014; Hoban 2019; though see Hoban et al. 2018), convenient for a model but not true for most plant species.

While such concerns apply to any plant population with low numbers, exceptional species in the palm family (Arecaceae) illustrate the importance of living conservation collections for very small imperiled stands. For example, Copernicia fallaensis is limited to 84 extant mature palms and is overexploited for thatching (Verdecia 2005), but an ex situ collection of 50 individuals was established to ensure its survival (Verdecia 2005; Hodel et al. 2016). Attalea crassispatha survives as fewer than 30 in situ individuals, and established ex situ collections augment these numbers (Johnson 1998). Only 25 mature Sabal lough-eedana survive in an area overgrazed by introduced herbivores (Griffith et al. 2019b), and ex situ cultivation is advised to help safeguard this population (De Freitas et al. 2019). Most alarmingly, only two wild Pritchardia aylmer-robinsonii survive, and these in situ trees have not been observed reproducing naturally— but palms in at least 30 botanic gardens have set seed (Chapin et al. 2004; Chapin 2005). The above examples do not even include those palm species surviving solely in gardens (e.g., Hyphorbe amaricaulis; Jackson et al. 1990). Every example above is located in an island habitat (Cuba, Hispaniola, Bonaire, Niihau, and Mauritius), highlighting the urgent and specific conservation challenges of these long-isolated island florae (Francisco-Ortega et al. 2000; Maunder et al. 2008).

For such island plant populations or for any fragmented species, ex situ collections can be improved through greater understanding of genetic capture. This improved understanding can be framed within some specific questions, and we structure our study around five questions as presented below. For example, many species are found in disjunct locations with variable population sizes. There are often limits to resources or access for collections development work, prompting questions of where to collect. When faced with such a choice, we first ask, (1) Can ex situ collections from a large population capture sufficient diversity to adequately conserve the diversity found in smaller populations? Or (2) is there additional conservation value gained from sampling smaller populations? Recent work also advances
that pooled ex situ collections (‘metacollections’; Griffith et al. 2019a, 2020) capture genetic diversity more efficiently. We wish to test this idea further, so we ask: (3) Do such pooled collections always improve genetic capture? Understanding the relative contributions of such pooled collections to species conservation can guide sampling efforts. Therefore, we also ask: (4) How should ex situ collections be developed when populations in the wild differ considerably in size? The questions above can help address a more central question: (5) Are percent genetic capture thresholds, such as the explicit 70% allele capture threshold listed in GSPC Target 9, always appropriate for evaluating ex situ conservation success? This study explores these questions via a case study of the palm species Pseudophoenix sargentii and illustrates ways that conservation benchmarks may be improved.

Material and Methods

Case Study

Pseudophoenix sargentii H. Wendl (buccaneer palm, palma pirata; fig. 1) was selected for this study for the following five reasons. (1) While this species is widespread in the Caribbean Basin (from Belize to Dominica) and therefore not considered globally threatened (Zona et al. 2007), many populations are highly imperiled. For example, P. sargentii is reduced to a single wild mature individual each on both Navassa Island (Zona 2002) and Elliott Key (Gonzalez et al. 2019) and has been completely extirpated from other islands (e.g., Wax Cay; Russell 1961; Jordan 1989). (2) These populations are of variable size and threat status and are separated by significant geographic distance (detailed below). (3) Adequate ex situ collections from three populations of very different sizes are established and available for study, and these collections were developed in parallel with population genetic sampling of in situ populations. (4) Genetic assay techniques are well established for the species (Rodríguez-Peña et al. 2014a, 2014b). Finally, (5) beyond the intrinsic value (O’Neil 1997; Batavia and Nelson 2017) of P. sargentii, the species is of significant economic value as an ornamental crop (Kumla et al. 2016), as evidenced by local extirpation for landscape use (Lippincott 1992; Maschinski and Duquesnel 2006). Mature palms have even been felled for animal fodder (Read 1988). Such economic uses place this species well within the scope of GSPC Target 9.

Evaluation of Genetic Conservation

We evaluate genetic conservation success on the basis of GSPC Target 9 for 2020, which seeks a 70% threshold of alleles conserved, but we recognize that a higher threshold may be warranted for threatened taxa. Hereafter, we also define conservation narrowly to mean ex situ conservation, that is, maintenance of living collections that hold the genetic diversity being measured.

Sampling

We assayed three in situ populations and the collections derived from those populations (table 1; fig. 1). The population at Leon Levy Native Plant Preserve, Eleuthera, Bahamas (fig. 1A), is the largest known population of P. sargentii (estimated at several thousand mature plants; M. P. Griffith, personal observation), was abundantly reproductive during collections made in 2013 and 2017, and is considered secure. Two other populations were collected on Mona Island in 2012. The Carabinero population (fig. 1B) occurs in a coastal forest on southern Mona, and of 22 mature plants, only one plant was reproductive that year. The Carabinero plants appeared to be in suboptimal health, and the population showed signs of decline (Santiago-Valentin et al. 2012). The Antenna population (fig. 1C) grows on an upland limestone plateau and consisted of five mature palms, all abundantly reproductive, and seven juvenile palms. These three populations were included in the study because they met criteria detailed in the ‘Case Study’ description above. Other small populations of P. sargentii are known (Rodríguez-Peña et al. 2014b) but were not included because of a lack of established ex situ collections from these sites. For purposes of evaluating conservation success relative to GSPC Target 9, these three sampled populations represent the breadth of the species (see ‘Discussion’) to allow for the exploration of ex situ collection success among large and small populations.

Leaflets were sampled from a total of 123 in situ plants (table 1) in wide transects throughout the populations. A total of 800 seeds were obtained (481 from Eleuthera and 319 from Mona) from a total of 25 mother plants. Seeds were kept separated by maternal line (i.e., accession) and were tracked separately. After cleaning, transportation, germination, and establishment, these 800 seeds yielded 94 ex situ plants from 19 maternal lines, all of which provided leaflets examined in the study.

DNA Extraction, Amplification, and Visualization

DNA isolation, PCR amplification, and visualization of simple sequence repeat fragments follow the methods detailed by Meerow and Nakamura (2007). We extracted total DNA from dried leaflet samples via FastDNA Kits (MP Biomedicals). We employed 10 microsatellite primers (Namoff et al. 2010) for the analyses, as follows: pse2.1, pse3.11, pse3.33b, pse3.34b, pse3.6, pse5.2, pse5.4, pse5.5, pse5.6, and pse7.26b. This primer set is well established as an appropriate assay for the genus Pseudophoenix (Rodríguez-Peña et al. 2014a, 2014b; Griffith et al. 2020).

PCR mix included 1 × buffer (15 mM MgCl2), 200 µM dNTPs, 250 µM each of forward and reverse primers, 0.25 U Taq DNA polymerase (New England Biolabs), 10 ng genomic DNA template, and nuclease-free distilled water to bring the total volume up to 10 µL. We employed the following PCR program on an ABI 9700 thermal cycler (Applied Biosystems): 2 min at 94°C; 38 cycles of 30 s at 94°C, 1 min at 54°–68°C depending on primer, per Namoff et al. (2010a), and 1 min at 72°C; 10 min at 72°C; and storage at 4°C.

Allele size was measured on an ABI 3730 Genetic Analyzer (Applied Biosystems) via capillary gel electrophoresis with a GeneScan 500 ROX size standard (Applied Biosystems). Analysis of the raw microsatellite data was performed using GeneMapper 4.0 (Applied Biosystems).

Population Genetic Assay and Estimation of Genetic Capture

Comparative estimates of alleles per locus, genetic distance (Nei 1978), fixation indexes (Wright’s $F_{ST}$), and multivariate
Fig. 1  Examples and provenance of *Pseudophoenix sargentii*, a palm species suited for ex situ conservation studies.  

A, Plant from a healthy, robust native population at the Leon Levy Native Plant Preserve, Eleuthera, Bahamas (2017), the largest known population of the species.  

B, One plant of 22 total at the Carabinero population, Mona Island (2012).  

C, Two plants of 12 (five mature and seven juvenile) total at the Antenna population, Mona Island (2012). Note the differences in robustness and apparent health between B and C, two populations with a fixation index ($F_{ST}$) of 0.467 (fig. 2; table 2).  

D, Group of ex situ plants at Montgomery Botanical Center, Coral Gables, Florida (2020). These plants were grown from seed collected in Eleuthera (A) in 2013. These in situ sites span the core of the species’ range, which extends to a few more disjunct sites in insular Florida, Dominica, and the Yucatán (Zona 2002).
Randomization was implemented in GenAlEx, and sampling to obtain model collections (hereafter referred to as resamples) were structured to include as few as one and as many as six plants (i.e., limited to the size of the smallest ex situ collection, from Carabinero). Limiting the size of each resample to six plants controls for the effect of larger numbers of plants (i.e., 79 plants from the pooled Eleuthera collection) on allele capture.

We also compared four different resampling regimes on the Eleuthera ex situ collection. These were (1) sampling only from the 2013 collection, (2) sampling only from the 2017 collection, (3) sampling at random without replacement from the pooled collection (2013 and 2017), and (4) sampling no more than one plant from each accession (i.e., excluding half-siblings and maximizing the number of maternal lineages). These ex situ resamples were structured to include as few as zero individual plants and as many as 16 plants (i.e., limited to the maximum number of plants obtained when limited to one per accession of 16 accessions).

We measured genetic capture for these random resamples by comparing each resample with the total in situ population via GenAlEx and comparing the proportion of private alleles with total alleles in the population and resample. We modeled allelic capture as a function of the number of plants in the collection via a logarithmic regression fit to the allele capture percentage using GenAlEx, yielding a curve of expected genetic capture as a function of collection size (Griffith et al. 2017b). Logarithmic regression offers the best fit to data on increasing genetic capture as a function of increased number of individuals in the collection (Namoff et al. 2010b).

Results

The average number of alleles per locus (table 2) ranged between 1.4 (populations and collections from Mona Island) and 11.2 (the total in situ data set). Genetic distances (table 2) ranged between 0.04 (between Eleuthera and the 2013 Eleuthera collection) and 0.58 (between the Eleuthera 2013 collection and the Carabinero population).

Multivariate analysis of the genetic distance data shows a consistent match of each ex situ collection to its source population (fig. 2). Among in situ populations, \( F_{ST} \) was highest

### Table 1

**Sampling of Pseudophoenix sargentii Plants Used in This Study**

| Group, description | No. plants |
|--------------------|------------|
| Mona Island:       |            |
| Antenna population:|            |
| In situ            | 10         |
| Ex situ            | 9          |
| Carabinero population: |        |
| In situ            | 10         |
| Ex situ            | 6          |
| Eleuthera:         |            |
| Leon Levy Preserve population: |       |
| In situ            | 103        |
| Ex situ, 2013      | 21         |
| Ex situ, 2017      | 58         |

*Note.* Numbers in parentheses represent the average number of alleles per locus. When pooled, average alleles per locus are 11.2 for all in situ populations, 9.9 for all ex situ collections, 2.2 for both Mona Island collections, and 9.2 for both Eleuthera collections.
between the two small populations (Antenna and Carabinero) on Mona (0.467), around double that between each of these Mona populations and Eleuthera (0.230, 0.275; table 2; fig. 2).

The Evanno method of determining genetic structure identified \( K = 2 \) as the optimal number of groups across all populations and collections in the study. The STRUCTURE analysis clearly divides the plants by the island groups Mona and Eleuthera (\( \Delta K = 996.9 \)), with very little admixture between the islands (fig. 3) and with ex situ collections clearly pairing with their in situ source islands. By contrast, lnPr\((K)\) identified \( K = 6 \) as optimal (not shown). The four MMK estimators were split evenly between \( K = 4 \) and 5. Increasing the assumed populations to four or more resolves the two Mona populations as distinct, with minimal admixture between them or with the Eleuthera plants (fig. 3). All that these higher estimates of \( K \) accomplished was to highlight underlying substructure in the Eleuthera populations; the Mona populations remained homogeneous (fig. 3). While the lnPr\((K)\) estimate of \( K = 6 \) seemed an overestimate of genetic structure, \( K = 4 \) was more informative than \( K = 2 \).

Genetic capture of in situ diversity by collections ranged according to population size and collection size (table 3). The lowest genetic capture of any ex situ population was seen for the Eleuthera population, with a maximum capture of 78.7% with all ex situ collections pooled, while the in situ diversity on Mona (either separated by population or pooled) was captured at 100% by the pooled collection. Each of the Mona collections could only capture less than 13% of the diversity on Eleuthera, while the Eleuthera collections captured at least 71% of the diversity on Mona.

Bootstrapped resamples of the collections showed a rank order of efficient genetic capture (figs. 4, 5). The smallest collection (Carabinero) captures the alleles of its source population most...
efficiently, with the Antenna collection nearly equivalent. The Eleuthera collections are not nearly as efficient at low collection sizes (fig. 3). Considering just the Eleuthera population, the 2013 and 2017 cohorts differ in efficiency, and a pooled collection of both of these is intermediate in genetic capture. Genetic capture is maximized by deliberately excluding half-siblings and maximizing the number of maternal lineages (fig. 5). In this case, simply pooling the 2013 and 2017 collections did not increase the efficiency of genetic capture unless half-siblings were explicitly excluded.

Discussion

The above results clearly demonstrate a close match between ex situ collections and their source populations (e.g., figs. 2, 3). This further confirms the well-established guidance to collect directly from specific source populations, especially those populations with high genetic structure (Hoban and Schlarbaum 2014; as seen in fig. 3), when building conservation collections (Griffith et al. 2015). The results are also consistent with the recommendation of curating population-based living collections separately (Cibrian-Jaramillo et al. 2010; Krishnan et al. 2013), even within a single species. The variation observed in genetic capture by collection or a pooled combination thereof (table 3; figs. 4, 5) also suggests that *Pseudophoenix sargentii* provides an apt model system for exploring the relative efficacy of conserving larger and smaller populations of threatened plants. Large populations do seem to hold more diversity than smaller ones: consistent with its status as the largest *P. sargentii* population thus far reported (see above; cf. Rodriguez-Pena et al. 2014b), Eleuthera’s alleles per locus (10.8; table 2) exceed all

| Ex situ collections | In situ populations |
|---------------------|---------------------|
|                     | Antenna population  | Carabinero population | Mona* | Eleuthera | Mona and Eleuthera |
| Antenna collection  | 92.85               | 50                    | 73.27 | 11.11     | 12.50               |
| Carabinero collection | 57                 | 92.85                | 73.27 | 10.18     | 11.60               |
| Pooled Mona collections | 100            | 92.85                | 95    | 14.81     | 17.80               |
| Eleuthera, 2013 collection | 57.14         | 78.57                | 60    | 62.04     | 60.70               |
| Eleuthera, 2017 collection | 71.42        | 78.57                | 70    | 68.51     | 66.00               |
| Pooled Eleuthera collections | 71.42      | 85.71                | 75.00 | 77.80     | 75                  |
| Total metacollection* | 100              | 100                  | 100   | 78.70     | 79.40               |

Note. All values are percentages.

* Pooled in situ populations, Antenna and Carabinero.
*b All pooled ex situ plants in the study (see table 1).
such values previously reported for any population in the genus *Pseudophoenix* (Rodriguez-Pena et al. 2014b). With the above results in place, we now consider the five questions posed in the introduction of this article.

**Can Collections from a Large Population Capture the Diversity of Smaller Populations?**

Table 3 shows genetic capture for each population for different subsets of the ex situ collection. The Eleuthera collection alone captures above the 70% threshold of each small population from Mona. In cases where resource limitations force a choice between accessing a larger or a smaller population of a given species for conservation purposes, these results indicate that collections from the larger population may concurrently and effectively capture much of the neutral genetic diversity found in smaller populations. In this case, ex situ plants from the Eleuthera population alone capture more than 70% of the genetic diversity found on Mona Island, thus meeting the GSPC Target 9 threshold despite 1600 km of physical distance, pronounced genetic structure (figs. 2, 3), and large $F_{ST}$ values.

Guidance for conservation collections development typically encourages collecting from multiple or even all populations (Schoen and Brown 1991; Guerrant et al. 2014; Hoban and Schlarbaum 2014; Griffith et al. 2015). Certainly, collecting from multiple populations will increase the chance of accessing more diversity, as also seen in this study (table 3), but on the basis of *alleles conserved* (see “Material and Methods”), collections from just one of three populations meet the GSPC thresholds for the plants in this study. If geographic diversity is being used as a proxy for genetic diversity (a common heuristic at botanic gardens; e.g., Kozlowski et al. 2012) or geographic diversity is an explicit goal in itself (see Beckman et al. 2019; Khoury et al. 2019), then collecting from multiple populations is certainly worthy (also see below). But strictly according to the criteria of GSPC Target 9, collections from one large population can meet conservation targets and conserve overall species genetic diversity despite uncultivated smaller populations. At the minimum, this suggests that the 70% threshold may be insufficient to ensure that all populations are represented; higher percentage thresholds (e.g., 95%; Marshall and Brown 1975) may be warranted.

**Is Conservation Value Gained from Sampling Smaller Populations?**

A clear result here is that, indeed, collections from an outside population can help meet the goal of capturing the genetic diversity of the target population. This was clearly demonstrated with the Antenna population, where the nine ex situ plants from Antenna conserved 93% of the in situ diversity from that population, but by adding the six ex situ plants from Carabinero, the full complement of alleles (100%) observed at the Antenna population was captured. The combined collection (Eleuthera and Mona) from all three sites can capture every allele observed on Mona Island (table 3). So, while the GSPC thresholds for the
entire study can be met merely by collecting from the largest population, collecting from smaller populations can efficiently capture additional diversity, and this value of sampling smaller populations is easily observable in the genetic structure in figure 3, which shows clear differentiation between the two islands.

Beyond considerations of genetic diversity evaluated here, phenotype, ecology, geography, and other botanical aspects are also worth representing in collections. Comparison of figure 1B and 1C shows this clearly: while the Eleuthera collections adequately capture the genetic diversity observed at Antenna and Carabínero, the differences in morphotype and fecundity present on Mona would go undocumented and unknown if these sites were not observed—and such documentation also contributes to the conservation value of an ex situ collection (Aplin 2008). The environmental conditions on Mona may have fostered adaptive genetic variation that differs substantially from that of the Eleuthera population, and these differences are often not resolved by microsatellite DNA, which is usually considered “neutral,” neither advantageous nor disadvantageous for fitness (Leinonen et al. 2008). Thus, we declare that every population is worth visiting and collecting—even on those islands with only a single surviving buccaneer palm.

We find that GSPC Target 9 is limited by evaluating only allele diversity as the main criterion, as in this study and, to our knowledge, in most past genetic evaluations of ex situ collections (but see, e.g., Ensslin et al. 2011). Criteria for effective genetic conservation should also recognize the importance of alleles with local adaptive value (Silva et al. 2020). Such alleles are not identified through standard molecular genetics approaches such as microsatellites, and future efforts should analyze DNA sequences at genes of known biological function. We also note that some alleles can be quickly lost through drift in small populations (Silva et al. 2020). Because of their small sizes, two small geographically close populations on a single island can be as genetically different as if they were separated by more than a thousand kilometers of open water (fig. 2).

Finally, we note that the GSPC threshold of 70% does not appear to have a scientific basis; there is as yet no consensus among scientists as to how much genetic diversity is needed to support resilient natural systems. Agricultural geneticists have suggested that 95% should be collected to capture some but not all local adaptations (Marshall and Brown 1975); others have argued for 99% (Lawrence et al. 1995).

Can Pooled Collections Improve Genetic Capture?

A recent study of a congeneric palm demonstrated that bringing together collections from multiple sites and years into a single metacollection improves genetic capture (Griffith et al. 2020). The current study provides further evidence that such pooling of resources serves the common good by providing a path forward to meet GSPC targets. Table 3 demonstrates that the total pooled collection of all ex situ plants captures the most genetic diversity from each population or combination of populations. Thus, meeting conservation targets will be easier when gardens steward collections collaboratively. Figure 5 also shows a path forward for increasing the efficiency of genetic capture. The most efficient genetic capture increases come from excluding half-siblings, that is, maximizing the number of maternal lineages included in a collection. Thus, when seeking efficient genetic capture, for both metacollections and single-source collections, maintaining many maternal lines (i.e., accessions) is preferable over maintaining large half-sibling groups from a single mother plant (Fant et al. 2016; Hoban 2019). A word of caution, however: it must be noted that attrition of collections happens much faster than perceived (Griffith et al. 2017a), and loss of plants at every life stage should be expected. Table 4 summarizes the results (thus far) of collections development efforts relevant to this study. While each seed was carefully sown, only around 12% of seeds currently survive as plants (a percentage perhaps more favorable than in natural systems). More alarming is the loss of entire maternal lines: only 75% of the accessions are currently extant. These losses of maternal lines appear to be more severe for Mona collections than for Eleuthera (50% loss vs. 16% loss, respectively), perhaps reflecting inbreeding depression as a result of small population sizes in the Antenna and Carabínero populations. Whether these attrition rates are in line with those of other exceptional palm species is currently under study. Thus, while maximizing maternal lines is essential, great redundancy within half-sibling groups is also critical; with the expectation of such losses, more than one copy of each allele must be maintained if genetic diversity is to be kept long term (Hoban 2019). Botanic gardens must project the number of plants needed when such losses are expected; Hoban (2019) suggests that to provide for such backup, a roughly linear increase in sample size is needed. For example, to capture five copies of an allele takes almost five times as many samples. Efficiency must be balanced with resiliency.

Table 4

| Attirion of Conservation Collections Used in This Study | Seeds collected | Plants held as of 2019 | Accessions (i.e., maternal lines) collected | Accessions held as of 2019 |
|--------------------------------------------------------|-----------------|-----------------------|--------------------------------------------|--------------------------|
| Antenna, 2012                                          | 215             | 9 (4)                 | 5                                          | 2 (40)                   |
| Carabínero, 2012                                       | 104             | 6 (6)                 | 1                                          | 1 (100)                  |
| Eleuthera, 2013                                        | 276             | 58 (21)               | 12                                         | 11 (92)                  |
| Eleuthera, 2017                                        | 205             | 21 (10)               | 7                                          | 5 (71)                   |

Note. Between collection and genetic analysis, seeds were sterilized, transported, sowed, and established in a greenhouse environment. Numbers in parentheses are percentages. Percent establishment numbers here are within the typical range for palm conservation collections (cf. Murphy et al. 2016).
Can the Relative Size of Populations Guide Collections Development?

One of the greatest differences observed in this study is seen in figure 3, which shows very different genetic capture curves for large and small populations. Both of the small populations (Antenna and Carabinero) quickly accumulate high percentages of genetic capture with collection sizes of fewer than six plants. Considered in the context of GSPC Target 9, these collections are very successful with just six or nine plants, respectively, each achieving above 90% genetic capture of their population (tables 1, 3), in some cases with seeds from a single plant. On the other hand, the large population on Eleuthera requires a much larger ex situ collection to approach the GSPC threshold for successful genetic capture: the 2013 and 2017 collections (n = 21 and 58, respectively) each fall short of that 70% target, but when pooled, these 79 plants capture 78% of Eleuthera’s diversity. These results are consistent with other studies of palms such as Leucothrinax morrisii (Namoff et al. 2010b), Chamaedorea ernesti-augustii (Cibrian-Jaramillo et al. 2013), and Pseudophoenix ekmani (Griffith et al. 2020), which showed that larger collection sizes are needed to reach adequate capture of larger populations. Thus, small populations may not require large collections to capture sufficient diversity. In this case, this is probably due to fewer alleles per locus observed on Mona Island (table 2), which may be the result of drift, selection, or founder effects in these tiny populations. A similar case is observed for the ancient relic Wollemi pine (Wollemia nobilis), a species with fewer than 100 extant wild trees. Until the recent discovery of three distinct chlorotypes (Greenfield et al. 2017), no genetic diversity was detected in any extant W. nobilis plants (Peakall et al. 2003). A single cutting could thereby represent 100% genetic capture of a wild population, and multiple cuttings could represent all known genetic diversity for the species. The emergent guidance here appears to be a simple maxim: small collections for small populations, large collections for large populations. Of note is that this finding contradicts some existing guidance that posits a maximum number of propagules to collect from larger populations (CPC 2019, pp. 2–9).

This work adds to a growing body of clear recommendations for how to allocate sampling across a species’ range. For example, Hoban et al. (2018) showed that in a widespread, common species (Fraxinus excelsior), the majority of the sampling effort could focus on the core of a species’ range, where the species is most abundant and where allelic diversity is highest. Nonetheless, some sampling was still needed at the margins, which sometimes have unique alleles not present in the core (as predicted by theory; Lesica and Allendorf 1995). Considering the geographic and genetic distribution of a species across its range can help optimize collection plans (Gapare et al. 2008; Hoban and Schlarbaum 2014; McGlaughlin et al. 2015). Our results regarding large and small populations of a species are analogous.

Are Percent Capture Thresholds Appropriate for Evaluating Ex Situ Conservation Success?

We confirm the value of percent capture thresholds for advancing plant conservation but propose that these thresholds are better applied at the population level. This study shows how simple measures of the allele capture of a species by ex situ collections may neglect important diversity. While table 3 confirms that collections from a single population (Eleuthera) can meet a common 70% threshold both for the species (per GSPC Target 9; Sharrock 2012) and for peripheral populations not directly visited, visualizing population structure via genetic distance data (fig. 2) or Bayesian clustering (fig. 3) suggests that important site-specific diversity may be missed by such a single-site collection.

The GSPC remains a vital worldwide guidepost for ensuring the survival of both plants and society, as boldly stated in its introduction: without plants there is no life. As we have reached the end year of the 2011–2020 GSPC Targets, this is an appropriate time to reconsider the use of a 70% allele capture threshold to evaluate successful species conservation (cf. Laikre et al. 2020). Given the broad set of challenges to plant conservation, a 70% target was an understandable benchmark designed to offer an achievable threshold that makes positive conservation progress. Since the past decade has also seen good advances in the understanding of how ex situ collections can contribute to safeguarding plant diversity (Abeli et al. 2020; Chacón-Vargas et al. 2020; Santo and Hamilton, forthcoming), perhaps it is time to revise Target 9. We propose that a simple shift in emphasis from “species” to “population” will preserve the benefits of this well-thought-out and carefully negotiated strategic target, allow for another achievable, widely applicable benchmark for the next decade, and greatly improve both population and species conservation. Recent focus on metacollection management by botanic gardens suggests that a network of gardens, perhaps each safeguarding a local population, can contribute significantly to overall species conservation (Walsh 2015; Fant et al. 2016; Griffith et al. 2019a, 2020; Wood et al. 2020). If a percent threshold is applied at the population level rather than at the species level, important local variation (e.g., that seen in fig. 1B, 1C) would be more adequately conserved.

Recommendations for Future Conservation Efforts

These results offer some insight into the dynamics of the ex situ conservation of large and small plant populations. This study has value as an empirical case study illustrating current guidance, models, and best practices and providing an evaluation of a current conservation collection. Nevertheless, there are some limitations to the study and how it can be applied. These limitations include the model species’ life history, reproductive biology, and range. As a long-lived, slow-to-flower palm with recalcitrant seeds and a widespread and heavily disjunct island distribution, P. sargentii can offer some specific guidance for other such tree species in similar habitats and perhaps inform collecting efforts for other perennials with large disjunctions. Expanded sampling of other smaller populations of Pseudophoenix throughout its range will also help fully illuminate this case (cf. Griffith et al. 2015, 2017b); our study used all currently available ex situ collections and their corresponding source populations, but inclusion of further ex situ collections and in situ populations can provide a more robust illustration of these ideas. With those caveats, the current study leads to these recommendations:

Recommendation 1: develop conservation priorities based on genetic diversity and resources. As shown above, concentrating
a collecting effort on a larger population may yield higher genetic capture of a species’ genetic diversity than collecting from a smaller population. But smaller populations can contribute important diversity to the total genetic landscape. Simply put, when no other information is available, collect fewer seeds from small populations and more seeds from large populations.

Recommendation 2: define metrics for ex situ conservation goals. Here, we define genetic diversity as the primary measure of success for ex situ efforts, in accordance with GSPC Target 9. However, we also discuss important reasons for developing collections that do not add significantly to the overall numbers of alleles maintained. For example, representing geographic breadth may be its own important goal, and such geographic breadth (e.g., Beckman et al. 2019) may capture important local adaptations that are overlooked when the simple allelic diversity thresholds are applied at the species level. Botanic gardens and their networks should clearly define how they will measure success for ex situ conservation.

Recommendation 3: carefully consider current thresholds for conservation success. While the GSPC provides vital and pragmatic guidance for conserving plants, conservation workers should not overly rely on a 70% species allele capture benchmark for conserving plants, conservation workers should clearly define how they will measure success for ex situ conservation.

Recommendation 4: recognize that ex situ conservation is a continuous process. Developing collections, networking these into metacollections, evaluating the conservation effectiveness of these collections, and using them to advance in situ conservation require constant effort. Living collections are easily diminished and lost over time—a garden is never finished. Collect, evaluate, plan, conserve, and begin again.

Acknowledgments

This project was supported by the IMLS (National Leadership Grant MG-30-16-0085-16 and Museums for America MA-30-18-0273-18). In addition to major support from IMLS, we are grateful to the following funders of this project’s fieldwork, conservation, and outreach: the Plant Exploration Fund, City of Coral Gables, International Palm Society, and the participating institutions. Ex situ collections in this study are also supported by awards from the US National Science Foundation (DBI 1203242, 1561346, and 1762781). In addition to the authors, the following people also contributed to this work: Taylor Callcrate, Claudia Calonje, Michael Calonje, John Clark, Laurie Danielson, Eliza Gonzalez, Tonya Meister-Griffith, Bob Lacy, Vickie Murphy, Larry Noblick, Kaylee Rosenberger, Andrew Street, Joanna Tucker Lima, Murphy Westwood, and Jordan Wood.

Literature Cited

Abeli T, S Dalrymple, S Godefroid, A Mondoni, JV Müller, G Rossi, S Orsenigo 2020 Ex situ collections and their potential for the restoration of extinct plants. Conserv Biol 34:303–313.

Aplin D 2008 How useful are botanic gardens for conservation? RHS Plantsman 7:190–193.

Batavia C, MP Nelson 2017 For goodness sake! what is intrinsic value and why should we care? Biol Conserv 209:366–376.

Beckman E, A Meyer, A Denvir, D Gill, G Man, D Pivorunas, K Shaw, M Westwood 2019 Conservation gap analysis of native U.S. oaks. Morton Arboretum, Lisle, IL.

BGCI (Botanic Gardens Conservation International) 2016 North American botanic garden strategy for plant conservation, 2016–2020. Botanic Gardens Conservation International, Glencoe, IL.

Bragg JG, P Caneo, A Sheriell, MR Rossetto 2020 Optimizing the genetic composition of a translocation population: incorporating constraints and conflicting objectives. Mol Ecol Resour 20:54–65.

Brown AHD, CM Hardner 2000 Sampling the gene pools of forest trees for ex situ conservation. Pages 185–196 in A Young, D Boshier, T Boyle, eds. Forest conservation genetics: principles and practice. Oxford University Press, Oxford.

Brown AHD, DR Marshall 1995 A basic sampling strategy: theory and practice. Pages 75–91 in L Gaurino, V Ramanatha Rao, R Reid, eds. Collecting plant genetic diversity: technical guidelines. CAB International, Wallingford, UK.

Chacón-Vargas K, VH García-Merchán, MJ Sanín 2020 From keystone species to conservation: conservation genetics of wax palm Cerroxyylon quindiuense in the largest wild populations of Colombia and selected neighboring ex situ plant collections. Biodivers Conserv 29:283–302.

Chapin MH 2005 Prritchardia aylmer-robinsonii St. John. International Union for Conservation of Nature Species Survival Commission, Gland, Switzerland. http://www.iucn.org/sites/dev/files/import/downloads/psg_pritchardia_aylmer_robinsonii.pdf.

Chapin MH, KR Wood, SP Perlman, M Maunder 2004 A review of the conservation status of the endemic Prrotchardia palms of Hawaii. Oryx 38:273–281.

Choi HJ, SK Kaneko, YS Song, DS Kim, SH Kang, Y Suyama, Y Isagi 2013 Population and genetic status of a critically endangered species in Korea, Eschrichtia japonica (Leguminosae), and their implications for conservation. J Plant Biol 56:251–257.

Christe G, K Kozlowski, D Frey, J Fazan, S Bétrisey, S Pirintos, J Gratzfeld, Y Naciri 2014 Do existing ex situ collections capture the genetic variation of wild populations? A molecular analysis of two relict tree species, Zelkova abelica and Zelkova carpinifolia. Biodivers Conserv 23:2945–2959.

Cibrian-Jaramillo A, R DeSalle, E Brenner, D Daly, T Marler 2010 When north and south don't mix: genetic connectivity of a recently endangered oceanic cycad, Cycas micronesica, in Guam using EST-microsatellites. Mol Ecol 19:2364–2379.

Cibrian-Jaramillo A, A Hird, N Oleas, H Ma, AW Meerow, J Francisco-Ortega, MP Griffith 2013 What is the conservation value of a plant in a botanic garden? using indicators to improve management of ex situ collections. Bot Rev 79:559–577.

Convention on Biological Diversity 2020 Targets. Convention on Biological Diversity, Montreal. http://www.cbd.int/gspc/targets.shtml.

CPC (Center for Plant Conservation) 2019 CPC best plant conservation practices to support species survival in the wild. CPC, Escondido, CA.

Dhar S 1996 Corypha taliera: endangered palm extinct in the wild. Palm J 130:10–11.

Ellstrand NC, DR Elam 1993 Population genetic consequences of small population size: implications for plant conservation. Annu Rev Ecol Syst 24:217–242.
Ensslin A, S Godefroid 2019 How the cultivation of wild plants in botanic gardens can change their genetic and phenotypic status and what this means for their conservation value. Sibbaldia 17:51–70.

Ensslin A, TM Sandner, D Matthies 2011 Consequences of ex situ cultivation of plants: genetic diversity, fitness and adaptation of the monocarpic Cynoglossum officinale L. in botanic gardens. Biol Conserv 144:272–278.

Ensslin A, O Tschöpe, M Burkart, J Joshi 2015 Fitness decline and adaptation to novel environments in ex situ plant collections: current knowledge and future perspectives. Biol Conserv 192:394–401.

Evanno G, S Regnaut, J Goudet 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620.

Farr J, K Havens, AT Kramer, SK Walsh, T Callicrate, RC Lacy, M Maunier, AH Meyer, PP Smith 2016 What to do when we can’t bank on seeds: what botanic gardens can learn from the zoo community about conserving plants in living collections. Am J Bot 103:1541–1543.

FAO (Food and Agriculture Organization of the United Nations) 2014 Genebank standards for plant genetic resources for food and agriculture. FAO, Rome. http://www.fao.org/3/a-i3704e.pdf.

Francisco-Ortega J, A Santos-Guerra, SC Kim, DJ Crawford 2000 Pseudophoenix sargentii (Arecaceae), a critically endangered, endemic palm species from the rear edge matters. Ecol Lett 8:461.

Francisco-Ortega J, A Santos-Guerra, SC Kim, DJ Crawford 2000 Pseudophoenix sargentii (Arecaceae), a critically endangered, endemic palm species from the rear edge matters. Ecol Lett 8:461.

Gonzalez E, C Chavez, D Noblick 2019 What this means for their conservation value. Sibbaldia 17:23–26.

Hoban S 2019 New guidance for ex situ gene conservation: sampling realistic population systems and accounting for collection attrition. Biol Conserv 235:199–208.

Hoban S, T Callricate, J Clark, S Deans, M Dorsom, J Fant, O Gailing, et al 2020 Taxonomic similarity does not predict necessary sample size for ex situ conservation: a comparison among five genera. Proc R Soc B 287:20200102.

Hoban S, N Cavender, MP Griffith 2020b Conservation through collaboration: ensuring genetic diversity in garden collections. BGIJournal 17:23–26.

Hoban S, S Kallow, C Trivedi 2018 Implementing a new approach to effective conservation of genetic diversity, with ash (Fraxinus excelsior) in the UK as a case study. Biol Conserv 225:10–21.

Hoban S, S Scharlbaum 2014 Optimal sampling of plant populations for ex situ conservation of genetic biodiversity, considering realistic population structure. Biol Conserv 177:90–99.

Hoban S, A Strand 2015 Ex situ conservation seed collections should consider spatial design and species’ reproductive biology. Bio Conserv 187:182–191.

Hodel DR, R Verdecia Pérez, D Suárez Oropesa, M Rodríguez Lima, LA Mera 2016 Copernicia fallalaenis: the greatest fan palm of all them. PalmArbor 2016:4–11.

Hou B, M Tian, J Luo, Y Ji, Q Xue, X Ding 2012 Genetic diversity assessment and ex situ conservation strategy of the endangered Den drubium officinale (Orchidaceae) using new trinucleotide microsatellite markers. Plant Syst Evol 298:1483–1491.

Huff DR, R Peakall, PE Smouse 1993 RAPD variation within and among natural populations of outnumbering buffalograss Buchloe dactyloides (Nutt.) Engelm. Theor Appl Genet 86:927–934.

Hutchison DW, AR Templeton 1999 Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914.

Jackson PW, QCB Cronk, JAN Parnell 1999 Notes on a critically endangered palm from Mauritius, Hyophorbe amaricandia Mart. Bot Gard Conserv News 1:24–26.

Janes JK, JM Miller, JR Dupuis, RM Malenfant, JC Gorrell, CI Cullingham, RL Andrew 2017 The K = 2 conundrum. Mol Ecol 26:3594–3600.

Johnson D 1998 The IUCN red list of threatened species: Attalea crassispatha. International Union for Conservation of Nature, Gland, Switzerland. https://doi.org/10.2305/IUCN.UK.1998.RLTS.T381987A101005.en.

Jordan KC 1989 An ecology of the Bahamian hutia (Geocromys ingrahami). PhD diss. University of Florida, Gainesville.

Kashimshetty Y, S Pelikan, SH Rogstad 2017 Effective seed harvesting strategies for the ex situ genetic diversity conservation of rare tropical tree populations. Biodivers Conserv 26:1311–1311.

Khoury CK, SL Greene, S Krishnan, AJ Miller, T Moreau 2019 A road map for conservation, use, and public engagement around North America’s crop wild relatives and wild utilized plants. Crop Sci 59:2302–2307.

Kozlowski G, D Gibbs, F Huan, D Frey, J Gratzerfeld 2012 Conservation of threatened relict trees through living ex situ collections: lessons from the global survey of the genus Zelkova (Ulmaceae). Biodivers Conserv 21:671–685.

Krishnan S, TA Ranker, AP Davis, JJ Rakotomalala 2013 The study of genetic diversity patterns of Coffea comorosana, an endangered coffee species from Madagascar: a model for conservation of other littoral forest species. Tree Genomes 9:179–187.

Kumla J, N Suzannarach, S Lumnong 2016 First report of Phoma leaf spot disease on cherry palm caused by Phoma berbarum in Thailand. Can J Plant Pathol 38:103–106.

Laikre L, S Hoban, MW Bruford, G Segelbacher, FW Allendorf, G Ga jardo, AG Rodriguez, et al 2020 Post-2020 goals overlook genetic diversity. Science 367:1083–1085.
Lawrence MJ, DF Marshall, P Davies 1995 Genetics of genetic conservation. I. Sample size when collecting germplasm. Euphytica 84:89–99.

Leinonen T, RB O’Hara, JM Cano, J Merila 2008 Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. J Evol Biol 21:1–17.

Lesica P, FW Allendorf 1995 When are peripheral populations valuable for conservation? Conserv Biol 9:753–760.

Li Q, Z Xu, T He 2002 Ex situ genetic conservation of endangered Vatica guangxiensis (Dipterocarpaceae) in China. Biol Conserv 106:151–156.

Li YL, JX Liu 2018 StructureSelector: a web-based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour 18:176–177.

Lippincott C 1992 Restoring Sargent’s cherry palm on the Florida Keys. Fairchild Trop Gard Bull 47:12–21.

Marshall DR, AHD Brown 1975 Optimum sampling strategies in genetic conservation. Pages 53–80 in OH Frankel, JG Hawkes, eds. Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge.

Maschinski J, J Duquesnel 2006 Successful reintroductions of the endangered long-lived Sargent’s cherry palm, Pseudophoenix sargentii, in the Florida Keys. Biol Conserv 134:122–129.

Maunier M, A Leiva, E Santiago-Valentin, DW Stevenson, P Acevedo-Rodríguez, AW Meewor, M Mejia, C Clubbe, J Francisco-Ortega 2008 Plant conservation in the Caribbean island biodiversity hotspot. Bot Rev 74:197–207.

McClughlin ME, L Riley, M Brandsrud, E Archibal, MK Helenurm, K Helenurm. 2015 How much is enough? minimum sampling intensity required to capture extant genetic diversity in ex situ seed collections: examples from the endangered plant Siberia filifolia (Brassicaceae). Conserv Genet 16:253–266.

Migócsy Z, E Warszchelsky, LL Klein, AJ Miller 2019 Using living germplasm collections to characterize, improve, and conserve woody perennials. Crop Sci 59:2365–2380.

Miller JS, P Popowry, J Aronson, S Blackmore, K Havens, J Maschinski 2016 Conserving biodiversity through ecological restoration: the potential contributions of botanical gardens and arboreta. Candollea 71:91–98.

Meewor AW, K Nakamura 2007 Ten microsatellite loci from Zania integrifolia (Zamiaaceae). Mol Ecol Notes 7:824–826.

Menges ES, EO Guer rant Jr, S Hamze 2004 Effects of seed collection on the extinction risk of perennial plants. Pages 305–324 in EO Guer rant Jr, K Havens, M Maunier, eds. Ex situ plant conservation: supporting species survival in the wild. Island, Washington, DC.

Mounce R, P Smith, S Brockington 2017 Ex situ conservation of plant diversity in the world’s botanical gardens. Nat Plants 3:795–802.

Murphy V, T Clase, RA Rodríguez-Peña, F Jiménez-Rodríguez, B Jestrow, CE Hushy, MP Griffith 2016 Conservation horticulture for yarey palm and buccaneer palm: substrate and sowing depth affect germination and early seedling growth.HORTTechnology 26:811–815.

Namoff S, J Francisco-Ortega, S Zona, CE Lewis 2010a Microsatellite markers developed for the Caribbean palm Pseudophoenix sargentii: two PCR-based methods. Conserv Genet Resour 2:85–87.

Namoff S, CE Hushy, J Francisco-Ortega, LR Noblick, CE Lewis, MP Griffith 2010b How well does a botanical garden collection of a rare palm capture the genetic variation in a wild population? BIol Consrerv 143:1110–1117.

Nei M 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

O’Neil R 1997 Intrinsic value, moral standing, and species. Environ Ethics 19:45–52.

Orloco I. 1978 Multivariate analysis in vegetation research. 2nd ed. Junk, The Hague.

Owens SJ, M Rix 2007 Franklinia alatamaha, Theaceae. Curtis’s Bot Mag 24:186–189.

Peckall R, D Ebert, LJ Scott, PF Meagher, CA Oftord 2003 Comparative genetic study confirms exceptionally low genetic variation in the ancient and endangered relictual conifer, Wollemia nobilis (Araucariaeae). Mol Ecol 12:2331–2343.

Peckall R, PE Smouse 2006 GENALEX 6: genetic analysis in Excel: population genetic software for teaching and research. Mol Ecol Notes 6:288–295.

——— 2012 GenALEX 6.5: genetic analysis in Excel: population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.

Pence VC 2013 In vitro methods and the challenge of exceptional species for Target 8 of the Global Strategy for Plant Conservation. Ann Mo Bot Gard 99:214–220.

Pence VC, M Westwood, J Maschinski, C Powell, N Sugii, D Fish, J McGuinness, et al 2019 Collecting and maintaining exceptional species in tissue culture and cryopreservation. Pages 4–21 in CPC best plant conservation practices to support species survival in the wild. Center for Plant Conservation, Escondido, CA.

Pritchard JK, M Stephens, P Donnelly 2000 Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Puechmaille SJ 2016 The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour 16:608–627.

Read RW 1988 Utilization of indigenous palms in the Caribbean (in relation to their abundance). Adv Econ Bot 6:137–143.

Richards CM, MF Antolin, A Reillely, J Poole, C Walters 2007 Capturing genetic diversity of wild populations for ex situ conservation: Texas wild rice (Zizama texana) as a model. Genet Resour Crop Evol 54:837–848.

Rodríguez-Peña RA, B Jestrow, W Cinea, A Veloz, F Jiménez-Rodríguez, R García, AW Meewor, MP Griffith, M Maunier, J Francisco-Ortega 2014 Conservation and genetics of two critically endangered Hispaniolan palms: genetic erosion of Pseudophoenix lediniana in contrast to P. ekmanii. Plant Syst Evol 300:2019–2027.

Rodríguez-Peña RA, B Jestrow, AW Meewor, T Clase, MP Griffith, E Santiago-Valentin, JA Sustache-Sustache, J Francisco-Ortega 2014b Genetic diversity and differentiation of Pseudophoenix (Arecaceae) in Hispaniola. Bot J Linn Soc 176:469–485.

Russell OS 1961 Preliminary report on the flora of the Exuma Cays. Pages 16–21 in C Ray, ed. Report of the Exuma Cays Park Project (revised edition). Bahamas National Trust, Nassau.

Santiago-Valentin E, JA Sustache-Sustache, J Francisco-Ortega, C Figueroa-Hernández, J Fumero-Cabán, P Griffith 2012 Pseudophoenix sargentii on Mona Island: conservation survey and a new discovery. Palms 56:78–90.

Santo LND, JA Hamilton Forthcoming Using environmental and geographic data to optimize ex situ collections and preserve evolutionary potential. Conserv Biol. https://doi.org/10.1111/cobi.13568.

Schoen DJ, AHD Brown 1991 Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants. Proc Natl Acad Sci USA 88:4494–4497.

Sexton JP, SB Hangartner, A Hoffmann 2014 Genetic isolation by environment or distance: which pattern of genetic structure in the wild? Evolut 68:1–15.

Sharrock S 2012 GSACP global strategy for plant conservation: a guide to the GSAP: all the targets, objectives and facts. Botanic Gardens Conservation International, Richmond, UK.

Silva AR, LC Resende-Moreira, CS Carvalho, FC Lanes, MP Ortíz-Vera, PL Viaña, R Jaffé 2020 Range-wide neutral and adaptive genetic structure of an endemic herb from Amazonian savannas. AoB Plants 12:p. plaa003.

Toppila R 2012 Ex situ conservation of oak (Quercus L.) in botanic gardens: a North American perspective. MS Thesis. University of Delaware, Newark.
Verdecia R 2005 *Copernicia fallaensis* León. International Union for Conservation of Nature Species Survival Commission, Gland, Switzerland. https://www.iucn.org/sites/dev/files/import/downloads/psg_copernicia_fallaensis.pdf.

Volk GM, CM Richards, AA Reilley, AD Henk, PL Forstline, HS Aldwinckle 2005 Ex situ conservation of vegetatively propagated species: development of a seed-based core collection for *Malus sieversii*. J Am Soc Hortic Sci 130:203–210.

Walsh S 2015 Floral biology, breeding system, pollination ecology, and ex situ genetic diversity of the endangered Hawaiian species, *Brighamia insignis* A. Gray (Campanulaceae). PhD diss. University of Hawaii, Manoa.

Wood J, JD Ballou, T Callicrate, JB Fant, MP Griffith, AT Kramer, RC Lacy, et al 2020 Applying the zoo model to conservation of threatened exceptional plant species. Conserv Biol 43:1416–1425.

Wood JLA, MC Yates, DJ Fraser 2016 Are heritability and selection related to population size in nature? meta-analysis and conservation implications. Evol Appl 9:640–657.

Yang RC, FC Yeh 1992 Genetic consequences of in situ and ex situ conservation of forest trees. For Chron 68:720–729.

Zona S 2002 A revision of *Pseudophoenix*. Palms 46:19–38.

Zona S, C Lewis, A Leiva-Sanchez, R Verdecia-Perez 2007 Conservation status of West Indian palms. Oryx 41:300–305.