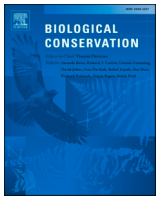
Biological Conservation 280 (2023) 109955

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon



Current management practices do not adequately safeguard endangered plant species in conservation collections

Zoe Diaz-Martin [a,*], Jeremie Fant [a], Kayri Havens [a], William Cinea [b], Joanna M. Tucker Lima [c], M. Patrick Griffith [c]

[a] Chicago Botanic Garden, Glencoe, IL, USA
[b] Jardin Botanique des Cayes, Haiti
[c] Montgomery Botanical Center, Coral Gables, FL, USA

ARTICLE INFO

Keywords:
Attalea crassispatha
Haiti
Conservation breeding
Parentage analysis
Relatedness
Exceptional species
Conservation
Conservation genetics

ABSTRACT

Imperiled plant species can benefit from ex situ cultivation to safeguard against loss of genetic diversity and possible extinction in the wild. Few studies use genetic monitoring in endangered plant species to evaluate how well current management practices maintain genetic diversity and limit inbreeding and relatedness after plants are brought into cultivation. We examine this question using *Attalea crassispatha*, a palm species with fewer than 100 palms surviving worldwide, and only 25 remaining in their native habitat. We sampled all accessible palms of this species (both in situ and ex situ) to (1) investigate how well garden collections capture in situ genetic diversity, (2) evaluate how well genetic diversity is carried forward into subsequent generations ex situ, (3) determine the number of wild and founding individuals contributing to ex situ breeding efforts, and (4) identify optimal breeding pairs that would maximize diversity and limit inbreeding. We found higher genetic diversity in situ and that current propagation practices lead to self-fertilization in the ex situ population and therefore fail to adequately steward genetic diversity in the conservation collection. Using relatedness analyses, we identified optimal breeding pairs in collections at different locations, highlighting the need for coordinated breeding efforts to maximize diversity ex situ. We also identified putative *A. crassispatha* that are genetically unrelated to the rest of the study cohort and are likely mislabeled. This study highlights the utility of genetic monitoring and the importance of careful coordination and record keeping within and among collections to ensure genetic diversity is maintained for future conservation efforts.

1. Introduction

Ex situ collections remain a vital component of plant conservation (Maunder et al., 2004), and botanic gardens are ideally suited to promote such efforts (Westwood et al., 2021). Retaining high levels of genetic diversity and low levels of inbreeding in ex situ collections of threatened plant species is important to bolster the evolutionary potential and fitness of the collection, which maximizes the success of any future re-introduction efforts (Frankham et al., 2010; Fant et al., 2016). However, genetic diversity of a closed population will inevitably decrease over generations and given the limited resources of most botanic gardens, ex situ populations at these institutions are often small, which further increases their risk of decreasing genetic diversity and increasing inbreeding over time (Basey et al., 2015; Willoughby et al., 2015). Genetic tools are used to evaluate the amount of genetic diversity brought into ex situ collections from the in situ population (Griffith et al., 2015; Griffith et al., 2017; Hoban et al., 2020) and lend insight into curation practices (van der Merwe et al., 2021; Clugston et al., 2022; Zumwalde et al., 2022). An important finding has been that pooling garden holdings into 'meta-collections' across botanic gardens enhances the stewardship of vital genetic resources (Christe et al., 2014; Griffith et al., 2019, 2020). Practical lessons on how to cooperatively manage meta-collections among institutions are readily available from the zoological community (Conde et al., 2013), and these methods are now being adapted for plant species (Fant et al., 2016; Wood et al., 2020). Zoos often use a combination of a pedigree approach and genetic monitoring across institutions to understand the genetic makeup of the captive metapopulation and ensure that the loss of genetic diversity is minimized over time by coordinating breeding efforts. If we are to safeguard the genetic diversity of highly endangered plant species in

* Corresponding author at: Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60022, USA.
E-mail address: zdiaz-martin@chicagobotanic.org (Z. Diaz-Martin).

https://doi.org/10.1016/j.biocon.2023.109955
Received 1 September 2022; Received in revised form 2 February 2023; Accepted 6 February 2023
Available online 16 February 2023

conservation collections, then genetic monitoring is critical to ensure that we are using the best practices available to us to maintain genetic diversity and guarantee future reintroduction success (Attard et al., 2016). However, genetic monitoring is rarely employed at botanic gardens and little is known about how well current management practices are safeguarding the genetic resources of rare and endangered plant species.

As the loss of genetic diversity reduces the conservation value of collections (Cibrian-Jaramillo et al., 2013; Basey et al., 2020), genetic monitoring of ex situ meta-collections can lend important insight into two important aspects regarding the genetic diversity of a meta-collection. One question asks the extent to which remnant wild individuals are genetically represented across a meta-collection. The makeup of the 'founders' (i.e., individuals collected from the wild and held at gardens) is critical, as they represent the full extent of genetic diversity that is brought into collections (Lacy, 2013; Willoughby et al., 2015). A genetically robust meta-collection would be one where the founders represent the full range of genetic diversity found in the wild (Hoban et al., 2021). If genetic diversity is higher in situ, then important genetic variation may not have been captured in the conservation collection, and this genetic variation could be lost over time in situ due to decreasing population sizes. As a result, the use of genetic monitoring can help identify collection strategies to further develop the conservation collection (van der Merwe et al., 2021; Clugston et al., 2022; Zumwalde et al., 2022) and build an effective metacollection that retains the maximum amount of genetic diversity that is conserved ex situ (Griffith et al., 2015; Griffith et al., 2017; Hoban et al., 2020).

The other important aspect of genetic monitoring is to evaluate how well the founding genetic diversity is maintained under cultivation (Basey et al., 2015). This is critical given that the random loss of genetic diversity, or genetic drift, within a collection is inevitable over time, especially if there is no replacement from the wild to add new founders (Lacy, 2013; Willoughby et al., 2015). Genetic monitoring can measure how well plants 'born' (to borrow the zoo term) at botanic gardens carry genetic diversity forward and identify the risk of inbreeding depression. Loss in genetic diversity from founder plants to F1 or later generations reduces the conservation value of garden holdings (Cibrian-Jaramillo et al., 2013), and inbreeding depression can reduce fitness (Walsh et al., 2019). Both a pedigree approach and genetic monitoring are used in managing zoo populations to promote active conservation breeding, which identifies optimal (i.e., highly unrelated) breeding pairs to maintain representation of the founding genetic diversity and minimizes inbreeding in the in situ or captive population (Wood et al., 2020). Management of conservation collections at botanic gardens does not always consider the genetic makeup of individuals when generating plants and new accessions are often propagated passively, with individuals allowed to set seed without controlled pollination, or pollinated without consideration of genetic diversity. The lack of attention given to the genetic consequences of passive breeding may result in increased rates of inbreeding, which can lead to a loss of fitness (Walsh, 2015; Foster et al., 2022) and increase the rate of diversity lost through drift. Zoos also employ a pedigree-based management approach that tracks relatedness between individuals and can identify the parentage of captive born individuals in relation to founders. Building pedigrees can also identify shared parentage between founders and ensure that all founders are contributing to future generations of the ex situ population. This is especially critical when managing species across multiple institutions where optimal breeding pairs are likely found at separate institutions, and therefore cooperative plans need to be implemented to optimize breeding (Wood et al., 2020).

To demonstrate the value of genetic monitoring in safeguarding genetic diversity in a critically endangered plant, we examined current management practices in a meta-collection of the highly imperiled exceptional species (sensu Pence et al., 2022) *Attalea crassispatha* (Mart.), or the Carossier Palm. A recent survey found only 25 Carossier Palm individuals in the wild, which places this taxon in the IUCN Redlist category of Critically Endangered (Jestrow and Franck, 2017; Timyan and Cinea, 2018) and extant individuals mainly grow near homesteads (Burney and Burney, 2009). The greatest threats to the species are tropical storms (given their single meristem habit; Griffith et al., 2008), diminishing habitat, and lower annual precipitation due to climate change (Timyan and Cinea, 2018). Most remaining wild palms are mature, nearing or at senescence, and seedlings have been continuously depleted by grazing livestock in recent decades, hindering demographic recruitment (cf. De Freitas et al., 2019; Klimova et al., 2021). In addition, seed germination rates are strikingly low (6 % per records at Montgomery Botanical Center), the cause of which remains unknown. We collected genetic samples from all accessible living individuals and genotyped them using ddRAD sequencing. We first evaluated genetic structure between wild, founder, and captive born individuals to ensure individuals pertain to the same population and to assess the genetic similarity between founder and wild individuals. We then compared genetic diversity, inbreeding, and relatedness between the in situ population and two generations (founder and captive born) of ex situ plants found in botanic garden collections. We used the genetic data to identify the parentage for founder and captive born individuals and to suggest optimal future breeding pairs to retain genetic diversity and minimize inbreeding in the meta-collection. Finally, we offer recommendations to avoid inadvertent degradation of ex situ genetic resources via current breeding and curation practices. This work provides important insight into developing effective strategies for the management of exceptional species in botanic garden collections.

2. Materials and methods

2.1. Study species and sampling

Attalea crassispatha is a large, single-trunked palm endemic to Haiti (Fig. 1A) (Timyan and Reep, 1994). The species is thought to be primarily outcrossing as it produces either predominantly staminate or co-sexual inflorescences that hold pistillate flowers proximally and staminate flowers distally (Henderson and Balick, 1991). Observations of ex situ plants indicate that either all or the majority of inflorescences produced by a single palm in a given year are entirely staminate (J.M. Tucker Lima *personal observation*). *Attalea crassispatha* is a narrow endemic, native to the southern peninsula of Haiti (Timyan and Reep, 1994) (Fig. 2). The species occurs 50–504 m above sea level in subtropical moist forest (Timyan and Reep, 1994), although many sites are now cleared with the palms growing in the open. Timyan and Reep (1994) provided *Attalea crassispatha* seed to 22 botanic gardens and nurseries in Australia, Costa Rica, Cuba, Dominican Republic, Ecuador, Germany, Guyana, the Philippines, St. Vincent, Thailand, UK, and USA.

We collected leaf samples of all known, accessible individuals of *A. crassispatha* (Table 1). The Haitian (i.e., in situ or wild) population sampling includes 14 individuals (out of the 25 known palms) that were accessible via non-destructive sampling. The ex situ or captive population consists of 60 individuals collected as seed in Haiti and living in botanic gardens (i.e., the founder generation), and 15 individuals resulting from reproduction events between founders (i.e., the captive born generation). Most founders were collected in 1989 and 1991 (Timyan and Reep, 1994; Noblick and Tucker Lima, 2021). Information associated with the collection for each individual varied (Table S1). Leaf material was dried on silica gel and stored at room temperature.

2.2. DNA extraction and genomic sequencing

We extracted genomic DNA using a DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands). We used a modified double-digestion restriction site-associated sequencing, or ddRAD-seq, protocol (Peterson et al., 2012) using *Eco*RI and *Msp*I restriction enzymes for digestion (New England Biolabs, Ipswich, MA, USA) (See Supplementary Materials Appendix A). We used STACKS v 2.2 (Catchen et al., 2011, 2013) to call

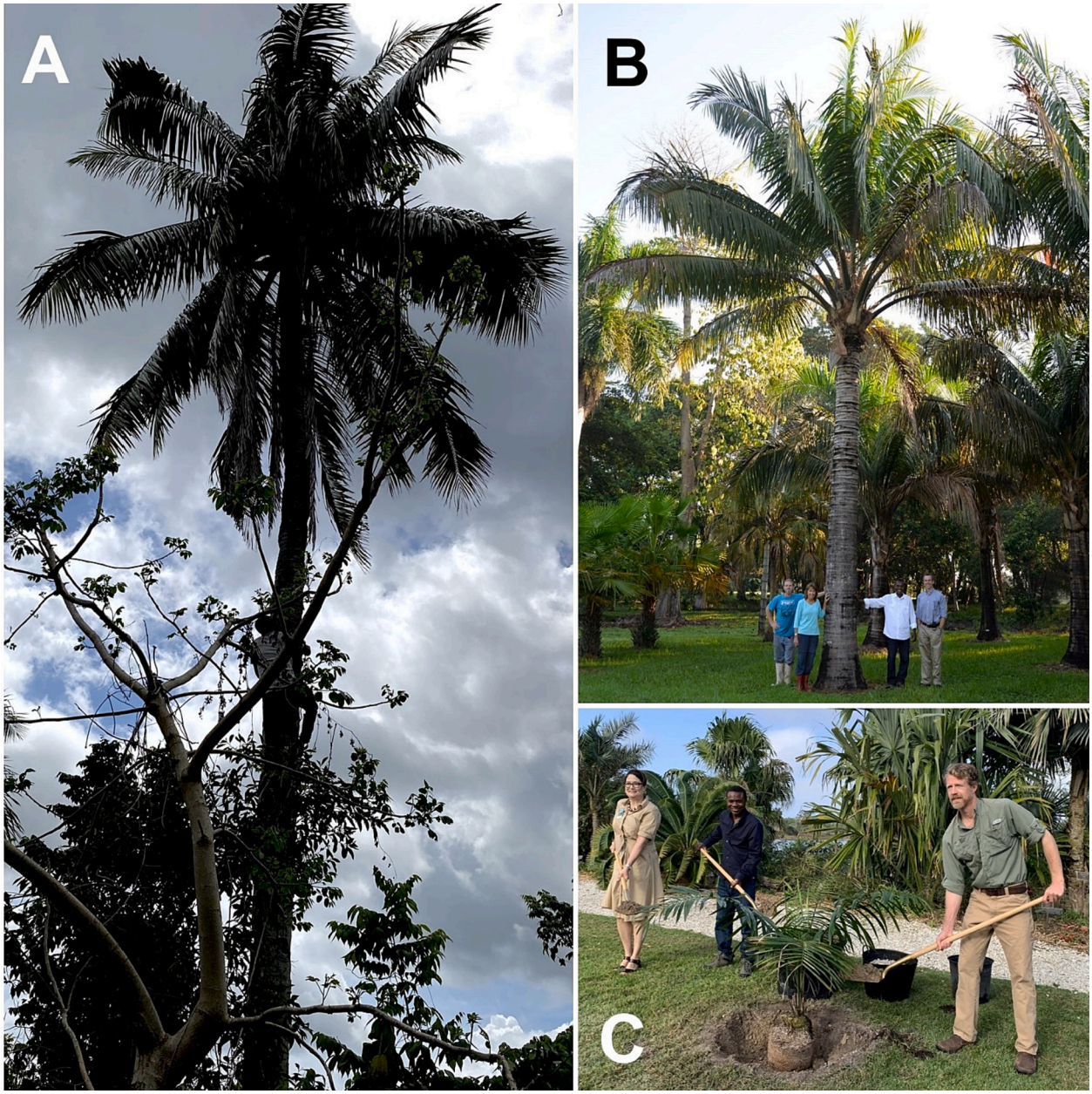


Fig. 1. *Attalea crassispatha*, a critically endangered palm from southern Haiti. (A) Mature palm in habitat at Bonne Fin, Haiti (i.e., "wild" cohort; note botanist climbing trunk; 2020 photo, WC). (B) Mature palm ("founder" cohort) in an ex situ collection (plant 91327*H at Montgomery Botanical Center), grown from seed collected in 1991 (2015 photo, MPG). (C) Offspring from plant B (i.e. "captive born" cohort) being planted at Naples Botanical Garden (2021 photo, Chad Washburn).

single nucleotide polymorphisms (SNPs) de novo for each species to generate eight datasets (See Supplementary Materials Appendix B). Each dataset was filtered for high quality SNPs using VCFTools v. 0.1.12 (Danecek et al., 2011). The first dataset included all quality filtered SNPs called for all individuals together (Table S2). Our analyses revealed that a subset of captive-born individuals at the United States Department of Agriculture (USDA) are genetically distinct from all other *A. crassispatha*, are likely not the same species (see results below), and were introducing a large number of loci that were only variable in those individuals but monomorphic in all other *A. crassispatha* in our dataset. As a result, we also generated a dataset with SNPs excluding those individuals at the USDA. This dataset was further divided in subsets to generate three additional datasets that were used to estimate relatedness and included either, only SNPs from wild individuals, only SNPs from founders, or only SNPs from captive born individuals, that were in Hardy-Weinberg Equilibrium (Table S2). The final two datasets were also used for parentage analysis and included quality filtered SNPs in Hardy-Weinberg Equilibrium and in linkage equilibrium with and without the USDA samples (Table S2). We evaluated SNP quality per locus and individual (Table S2).

2.3. Genetic structure, genetic diversity, and inbreeding

The biology of *A. crassispatha* (i.e., outcrossing and long-lived) coupled with its narrow distribution on the southern peninsula of Haiti suggests that the species occurs in a single population (Fig. 1; Timyan and Reep, 1994). To be certain, we used the complete dataset with all quality filtered SNPs and all individuals to generate a scaled and centered principal components analysis (PCA) generated using the program adegenet() v. 2.1.5 (Jombart, 2008) in R v. 4.0.2 (R Core Team, 2020) (Table S3). We also generated a scaled and centered PCA using all quality filtered SNPs and all individuals, except those captive-born

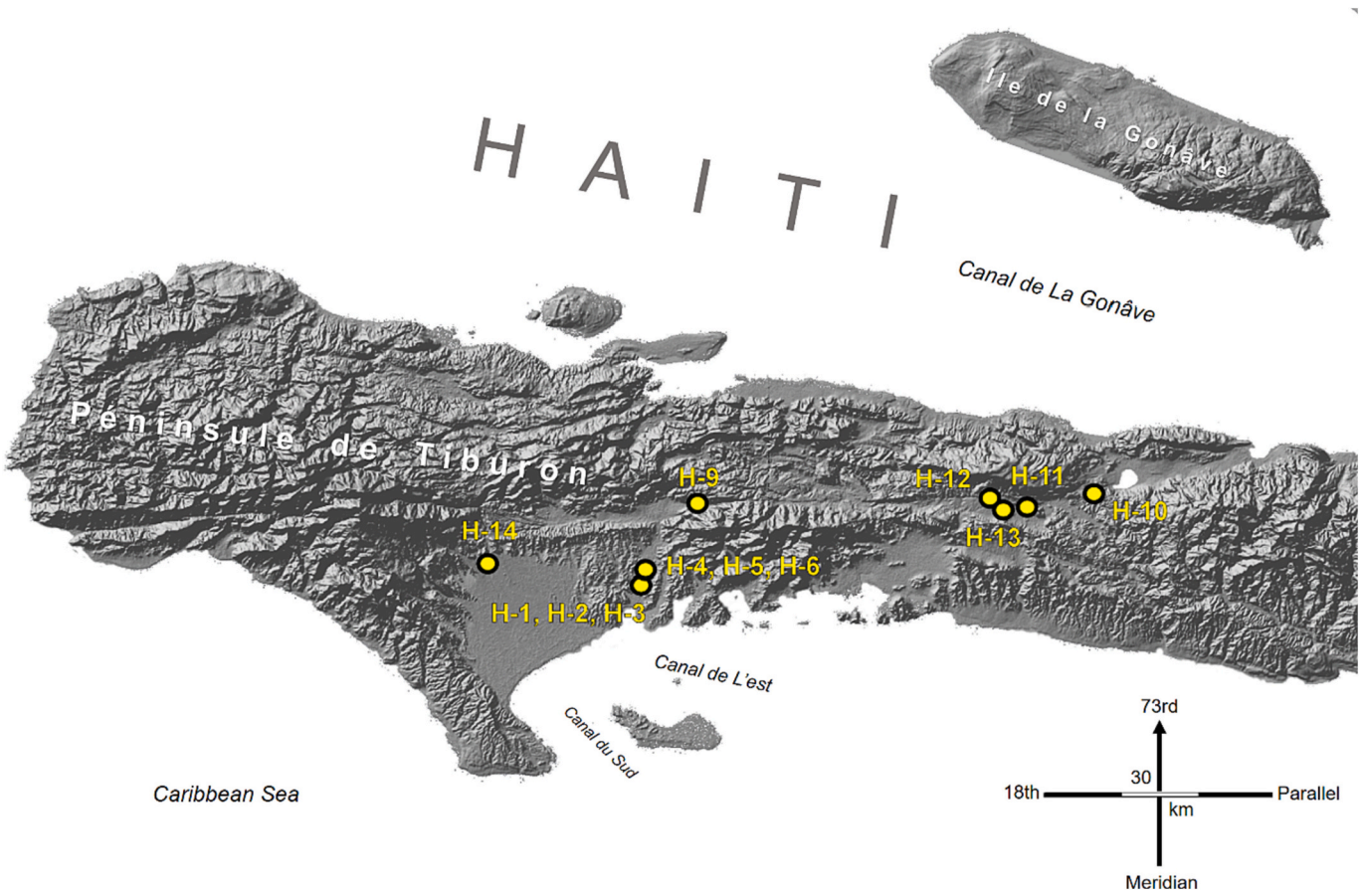


Fig. 2. Location of the wild or in situ population of *Attalea crassispatha* genotyped using single nucleotide polymorphisms (SNPs). Shown is the location of each individual, the elevation (gray scale), and major geographic features.

Table 1
Sampling structure for all *Attalea crassispatha* included in analyses. Shown is the generation (in situ/wild, ex situ founder, and ex situ captive born), source (current location), and number of individuals.

Generation and source	N
In situ and inter situ ("Wild")	
Wild plants in Haiti (H)	14
First Generation ex situ ("Founders")	
Fairchild Tropical Botanic Garden (FTG)	31
Montgomery Botanical Center (MBC)	10
UF Tropical Research and Education Center (TREC)	15
Singapore Botanic Garden	2
Second Generation ex situ ("Captive Born")	
Fairchild Tropical Botanic Garden	2
Montgomery Botanical Center	5[a]
USDA Chapman Field (USDA)	7
Total	86

[a] One of these plants is now kept at Naples Botanical Garden, Collier County, Florida, USA.

individuals at the USDA.

We measured genetic diversity and inbreeding at the locus and individual level across the wild, founder, and captive-born groups. As we are interested in *A. crassispatha* individuals that are part of the species' conservation collection, we used the SNP datasets that excluded the genetically distinct individuals at the USDA facility, unless noted otherwise. We first used the basic.stats() function in the package hierfstat v.0.5–11 (Goudet and Jombart, 2022) to determine per locus measures of inbreeding (F_{IS}) and gene diversity (H_S) for the wild, founder, and captive-born individuals. For each of the three groups, we calculate the proportion of SNPs that showed fixed homozygosity (i.e., $H_S = 0$). In addition, we compared gene diversity between groups and tallied the number of loci with higher gene diversity between groups (e.g., captive-born vs. wild, founder vs. captive-born). For each group, we also used the allelic.richness() function in hierfstat v.0.5-11 (Goudet and Jombart, 2022) to estimate per locus allelic richness with rarefaction to seven individuals, which is the number of captive-born individuals excluding those from the USDA. We used the populations' summary output from STACKS to tally the number of private alleles for each group. Next, we evaluated genetic diversity and inbreeding at the individual level. To do so, we used the genhet() function in R v. 4.0.2 (R Core Team, 2020), which calculates the individual level proportion of heterozygous loci (PHt), or the number of heterozygous loci over number of genotyped loci (Coulon, 2010; Cristescu et al., 2022; Silver et al., 2022). We estimated the inbreeding coefficient (F) for each individual using the –het flag in PLINK 2 (Purcell et al., 2007). We tested for differences in PHt and F between the wild population, the founder generation, and the captive born generation using a non-parametric pairwise Wilcoxon rank sum test with a Bonferroni correction in the FSA() package v.0.9.3 (Ogle et al., 2022).

2.4. Relatedness and breeding pairs

We estimated pairwise relatedness for wild, founder, and captive born individuals using the SNPs called for each group separately, via the program CoAncestry v. 1.0.1.9 (Wang, 2011). For each group, we simulated relatedness values for all seven estimators provided in the program. In simulations, we used per locus empirical allele frequencies and percent missing data to estimate relatedness for the following relationship categories: unrelated, parent-offspring, full siblings, half

siblings, first cousins, second cousins, and double first cousins. The simulation was carried out using 100 reference individuals with a 1 % genotyping error rate since the sequencing error rate for the NovaSeq Illumina platform is as is ~0.1 % (Stoler and Nekrutenko, 2021). The best estimator had the smallest variance for most of the relationship categories and the highest correlation coefficient (i.e., Pearson's r) with the true value of relatedness (Hogg et al., 2019) (Table S4). For the wild, founder, and captive born groups, the DyadML and TrioML had similarly low levels of variance for relationship categories and comparable correlation coefficients. In addition, the two measures were highly correlated with one another ($r^2 = 0.997$, $p < 0.0001$). We chose to use the DyadML as the correlation coefficient for this measure was higher with the true value of relatedness for wild, founder, and captive born individuals compared to the TrioML measure. After estimating relatedness between all pairs of individuals in the wild population, the founder generation, and the captive born generation, we tested for differences in pairwise relatedness estimates between the groups using a non-parametric pairwise Wilcoxon rank sum test with a Bonferroni correction in the FSA() package v.0.9.3 (Ogle et al., 2022). Finally, we identified optimal breeding pairs as those founders with the most conservative pairwise relatedness estimates indicating unrelated individuals (i.e., 0).

2.5. Parentage analysis and genetic representation

We performed parentage analyses with the final dataset to evaluate (1) the proportion of wild individuals with no offspring represented in the founders and (2) the proportion of founders that were not represented in the subsequent generation born in captivity. To do this we used CERVUS v. 3.0.3 and critical Δ values to assign parentage (Marshall et al., 1998) for the subset of filtered SNPs. For the founders, we simulated parent pairs with known sexes including parameters with 10,000 offspring genotyped, 14 candidate mothers and fathers, assuming 50 % of parents have been sampled, a 3 % genotyping error rate, a minimum of 100 loci. After conducting the parentage analysis, we tallied the number of living wild individuals that were not assigned as parents to a founder. For the captive born generation, the simulation parameters for parent pairs with known sexes included 10,000 offspring genotyped, 58 candidate mothers and fathers, assuming 95 % of parents have been sampled, a 3 % genotyping error rate, a minimum of 100 loci. After parentage assignments were complete, we tallied the number of founders that do not have offspring in the captive born generation. We also ran a parentage assignment using the dataset that includes the captive-born individuals at the USDA site and the same simulation parameters.

3. Results

Using publicly available data on collections holdings (Botanic Gardens Conservation International (BGCI), 2020) and contacting botanic gardens listed in Timyan and Reep (1994), we determined that *Attalea crassispatha* survives in only 6 living collections (Table S1), and the majority of founders are not yet reproductively active. Four of these collections are located in Miami-Dade County, Florida, USA, one is in Collier County, Florida, and one is in Singapore (Table 1).

We genotyped 14 wild individuals, 58 founders, and 14 captive born individuals (Table S2). After de-multiplexing, we retained an average of 6,535,471 raw reads per individual (range = 2,202,915–16,880,778). After quality filtering, the dataset with all single nucleotide polymorphisms (SNPs) contained 6093 SNPs with an average of 5.64 % (±9.68 %) missing data per individual and an average read depth coverage of 47.53× (±19.02×) (Table S2). The quality filtered dataset with all SNPs that excluded the United States Department of Agriculture (USDA) individuals contained 5910 SNPs with an average of 4.45 % (±8.16 %) missing data per individual and an average read depth coverage of 45.73× (±18.97×) (Table S2). The additional datasets used in the analyses have fewer SNPs, slightly higher rates of missing data, and slightly lower read depth coverage (Table S2). The founders included in this study are currently held at 4 sites and the captive born individuals are held at 3 sites (Table S1).

3.1. Genetic structure, genetic diversity, and inbreeding

The first two axes of the principal components analysis (PCA) using all SNPs and all individuals explained 47.18 and 9.76 % of the variation in the data, respectively. Most of the variation in this PCA was between the subset of captive-born USDA samples that varied along the first principal component, while all other individuals varied along the second axis (Fig. 3a). The first two axes of the principal components analysis using all SNPs and all individuals except for the USDA individuals explained 20.80 and 11.25 % of the variation in the data, respectively (Fig. 3b). The majority of wild individuals were distributed within the first quadrant of this PCA (85.7 %) while eight (13.8 %) of founders and one (14.3 %) of captive born individuals were in the same quadrant (Fig. 3b; Table S3).

Locus-level measures of rarefied allelic diversity (A_r) and the number of private alleles were lowest for the captive-born individuals (excluding USDA), intermediate for founders, and highest for wild individuals (Table 2). Locus-level estimates of inbreeding (F_{IS}) was highest for captive-born individuals (excluding USDA), intermediate for founders, and lowest for wild individuals (Table 2). Loci in captive-born individuals had the highest proportion of fixed homozygosity but intermediate levels of gene diversity, while loci in wild individuals had intermediate levels of fixed homozygosity and the highest levels of gene diversity, and loci in founders have the lowest levels of gene diversity and lowest proportion of fixed homozygosity. (Table 2). We found more loci with higher H_S in captive-born and wild individuals compared to founders (Fig. 4a,c) and comparable number of loci with higher gene diversity between captive-born and wild individuals (Fig. 4b).

The individual proportion of heterozygous loci (PHt) was significantly lower in the ex situ captive born generation than the wild individuals ($z = -3.29$, $p = 0.003$) (Fig. 5a; Table 2). While not statistically significant, PHt tended to be lower in the ex situ captive born group compared to the ex situ founders ($z = -2.28$, $p = 0.068$) and tended to be lower in the founders compared to the wild individuals ($z = -2.06$, $p = 0.12$) (Fig. 5a; Table 2). Similarly, individual level inbreeding (F) was significantly higher in the captive-born group compared to the wild born ($z = 3.25$, $p = 0.003$) (Fig. 5a; Table 2). Again, inbreeding tended to be higher in the captive born individuals than the founders ($z = 2.21$, $p = 0.082$) and higher in the founders compared to the wild individuals ($z = 2.09$, $p = 0.11$) (Fig. 5b; Table 2).

3.2. Pairwise relatedness and breeding pairs

Estimates of pairwise relatedness varied considerably within groups (Fig. 5c). We found no difference in relatedness between the captive born and founder generation ($z = -1.23$, $p = 0.65$) or captive born and wild individuals ($z = 0.17$, $p = 1.00$) (Fig. 5c). Pairwise relatedness was significantly higher in the ex situ founder generation ($z = 2.89$, $p = 0.012$) compared to the wild population (Fig. 5c; Table S1). For the founder generation, 911 (55 %) of possible individual pairings had a relatedness value of zero and were identified as optimal breeding pairs to minimize inbreeding (Table S5). Of the possible pairings, 734 (81 %) involve individuals which are currently located at different institutions.

3.3. Parentage analysis and genetic representation

For the parentage analysis of founders, the simulation for parent pairs with known sexes was able to assign pairs in 15 % of cases using strict confidence and 28 % of cases using relaxed confidence. In the parentage assignment analysis, we identified parent pairs (i.e., both maternal and paternal sources) for two of the 58 founders (3.45 %) with high confidence (Table S1, S6). For these two founders, the maternal and

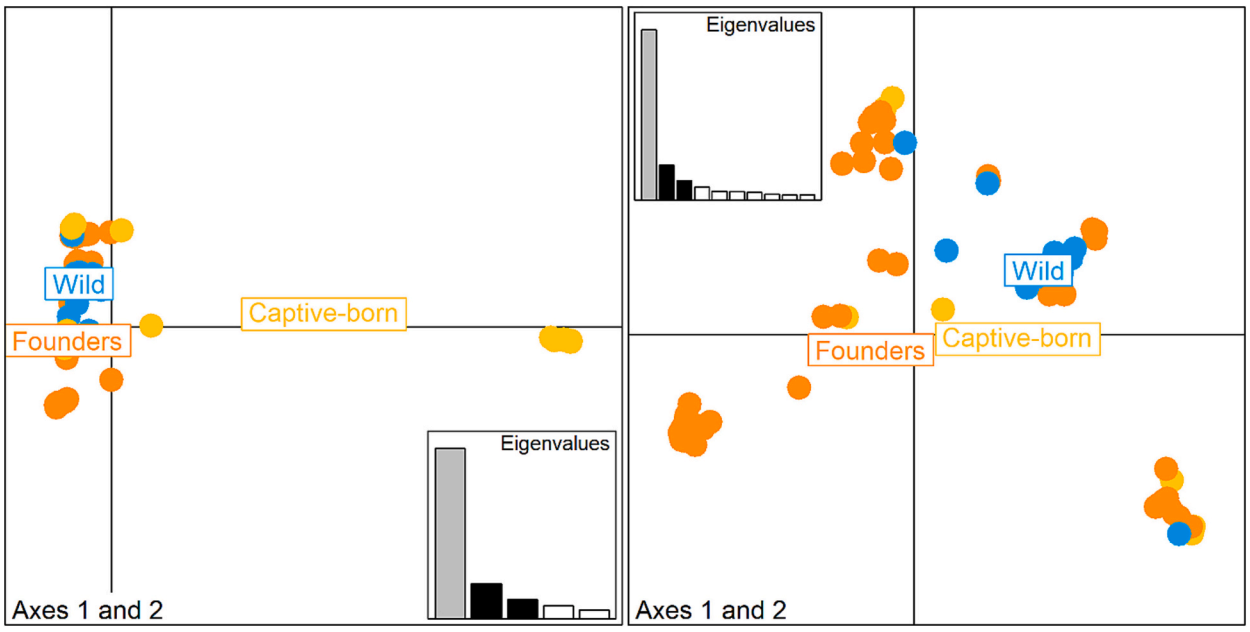


Fig. 3. The first two axes of principal components analyses where each point is an individual. Color corresponds to either the wild individuals (blue), the ex situ founder individuals (orange), or the ex situ captive born individuals (yellow). Eigenvalues indicate the proportional amount of variance represented by each principal component. Panel A includes all individuals and panel B excludes the captive-born individuals at the United States Department of Agriculture site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Measures of genetic diversity and inbreeding for captive-born, founder, and wild *Attalea crassispatha*. Shown are locus-level measures of average gene diversity (H_S), the percentage of loci with fixed homozygosity (i.e., $H_S = 0$), allelic richness rarefied to seven individuals, the inbreeding coefficient (F_{IS}), and the number of private alleles. Also shown are individual level measures of the average proportion of heterozygous loci (PHt) and inbreeding (F). ±1 SD is shown in parentheses.

Status	Locus measures					Individual measures	
	H_S	% fixed homozygosity in Hs	A_r	F_{IS}	Private alleles	PHt	F
Captive-born	0.336 (±0.233)	27.4	1.679 (±0.426)	0.603 (±0.489)	1	0.0966 (±0.0432)	0.686 (±0.0993)
Founders	0.315 (±0.148)	1.35	1.726 (±0.256)	0.502 (±0.343)	37	0.157 (±0.0575)	0.519 (±0.181)
Wild	0.377 (±0.144)	2.5	1.822 (±0.234)	0.375 (±0.362)	79	0.219 (±0.0949)	0.332 (±0.277)

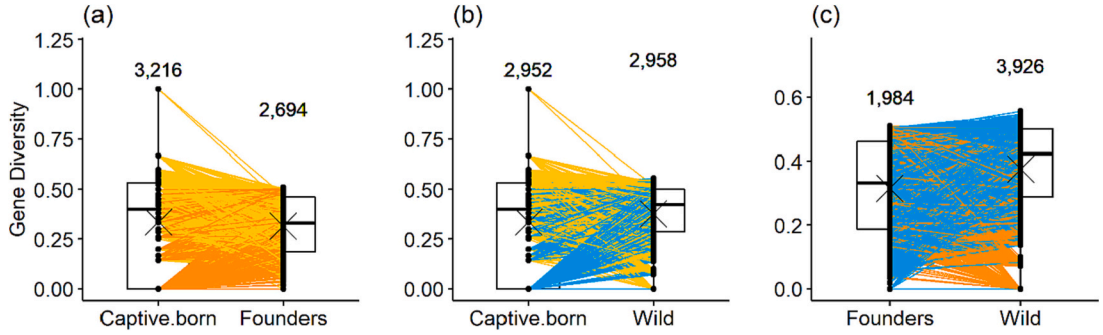


Fig. 4. Boxplots showing paired, per locus gene diversity (H_S) between (a) captive-born and founder, (b) captive-born and wild, and (c) founder and wild *Attalea crassispatha*. Gene diversity was measured using 5910 shared single nucleotide polymorphisms (SNPs) across groups but excluding individuals from the United States Department of Agriculture site. X denotes group mean. Number above boxplot indicates number of loci with higher H_S in that group. A yellow line indicates a locus with higher gene diversity in captive-born individuals, an orange line indicates higher gene diversity in founder individuals, and a blue line indicates higher H_S in wild individuals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

paternal source was the same individual. We also identified four founders whose maternal and paternal assignment pair assignments exhibited high confidence, but low trio confidence – for these founders the same individual (H-14) was the assigned mother and father (Table S6). We identified two wild individuals (H-12, H-14) as a parental source with high confidence for seven founder individuals (Table S6). In total, three of fourteen wild individuals (21.4 %) were assigned as parents to the 58 founders. In all cases, founders clustered near their assigned parental sources in the PCA (Table S3).

The parent pair simulation for captive born individuals was able to assign parents pairs in 77 % of cases using strict confidence and 100 % of cases using relaxed confidence. For the captive born individuals, parentage analysis assigned the same individual as the mother and father to all non-USDA individuals – in each case the assigned mother

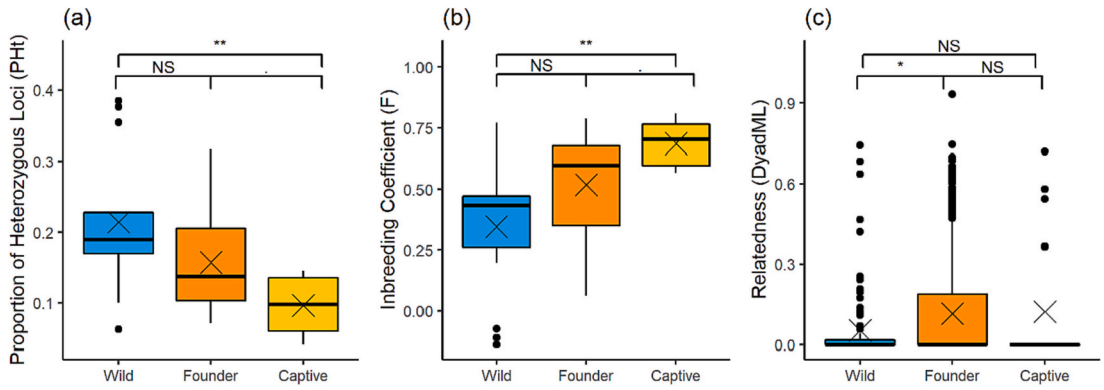


Fig. 5. Genetic diversity, inbreeding, and relatedness for two generations, captive born and founders, of the ex situ population as well as the wild population of *Attalea crassispatha* individuals. Box plots show the (a) individual level proportion of heterozygous loci (PHt), the (b) inbreeding coefficient (F), and (c) pairwise relatedness (DyadML) for each group with means represented by X. • $p \sim 0.05$ * $p < 0.05$ ** $p < 0.01$.

aligned with accession data of recorded mothers. As three founders were assigned as parents, 95 % of founders are not represented in the captive born generation. Nearly all captive born *A. crassispatha* individuals clustered near their assigned parent on the PCA, with the exception of FTG-03 (Table S3). Of the seven captive born individuals that form their own distinct genetic cluster (i.e., those at the USDA site), we were unable to identify a maternal or paternal source with high confidence.

4. Discussion

This work highlights the utility of genetic monitoring and identifies challenges to the effective management of exceptional species across a meta-collection. Similar to other studies, we found that many wild individuals are not genetically represented in the conservation collection (i.e., are not parental sources) and that genetic diversity tends to be higher in the wild population compared to the founder generation ex situ (Hoban et al., 2020). However, just as importantly, we demonstrated that the 'passive' breeding management of the metacollection has not adequately carried the diversity from the wild population into the captive born generation, resulting in a significant loss of genetic diversity and increase in inbreeding. We also show that many of the optimal breeding pairs identified for effective genetic management of this species are held at different botanic gardens, emphasizing the need for a cooperative breeding program among botanic garden collections. One surprising result was that an entire cohort of captive born collections held at one site appears to be genetically distinct from all other *A. crassispatha* individuals, including samples from the wild, suggesting either distinct genetic origin or a potential curatorial error. Taken together, this work provides insight into improving management practices for endangered species held across botanic garden collections.

4.1. Genetic monitoring of the ex situ founder generation

Genetic monitoring of the in situ and ex situ founder population of *A. crassispatha* indicate how well the in situ or wild population was sampled in establishing our ex situ population that exists in botanic gardens. We verified that the individuals analyzed in this study comprise one population and that genetic structure within that population is heavily influenced by relatedness between individuals. Indeed, many individuals with similar scores on the principal components analysis (PCA) were identified as related either through the accession records, the parentage analysis, or both (Table S1, Table S3). However, we highlight that the majority of wild individuals cluster near few ex situ individuals on the PCA and that very few wild individuals were assigned as parents to founders. Importantly, these results reveal that many wild individuals that were the parents of the founders no longer exist in situ. Given that relatedness is significantly lower and that gene diversity, rarefied allelic diversity, and the number of private alleles tends to be higher in wild individuals (Table 2), our work suggests that the majority of in situ individuals are not genetically represented in the ex situ meta-collection. As a result, some amount of genetic diversity (i.e., alleles) exists in situ that is not safeguarded for conservation ex situ. As is the case in many founding populations, sampling error or drift has created a genetic bottleneck whereby genetic diversity is lower in our ex situ founder population (Nei et al., 1975). Maximizing the amount of genetic diversity that is represented in the ex situ founder generation is a vital step in creating a conservation collection with high adaptive potential (Noël et al., 2017) that is able to support restoration and reintroduction efforts (Frankham, 2010).

Our collections records verify that limited sampling and limited survival of seedlings are a likely explanation for the lower rates of genetic diversity ex situ – of 60 known ex situ founder plants, 15 (25 %) are from a single collection event in 1989, and 39 (65 %) are from a single collection event in 1991 (Table S1). Timyan and Reep (1994) state that seeds were originally collected from 5 trees in 1989, and from 9 trees in 1991. Limited germination and limited seedling survival are noted by Timyan and Reep (1994), and our inquiries at the 6 gardens holding the species confirm this low germination and survival rate. Low germination rates could be due to inbreeding depression in the seeds comprising the founding stock, as the founders are derived from the limited number of wild plants and we have documented high rates of selfing in captivity. In addition, low germination rates could reflect impatience in the nursery as *Attalea* seed are known to take up to 5 years to germinate (Riffle et al., 2012). Documentation differs among recipient gardens, but records indicate that surviving founder plants at Montgomery Botanical Center (MBC) comprise four maternal lines (Table S1). Recent work on other Hispaniolan palms, such as *Pseudophoenix ekmanii* (Burret) (Arecaceae), shows the challenges of field collections in representing the full range of in situ diversity, given that only some plants are reproductive each year (Griffith et al., 2020). Collections over multiple years can help capture additional maternal lines (Griffith et al., 2015). In this way, the two founder plants at Singapore Botanic Gardens are an important addition to the meta-collection, as they were collected in 2000. Despite the challenges, we suggest additional seed collections from all wild individuals that are not genetically represented in the ex situ population.

Both locus- and individual-based measures of inbreeding tended to be higher in the founder generation compared to the wild population. In both cases, inbreeding was relatively high, especially for a long-lived palm that is likely insect pollinated. While selfing is phenologically possible, acropetal inflorescence maturation is common to *Attalea* species (Henderson, 2002). The position of pistillate flowers at the base of *A. crassispatha* rachillae (Fig. 6) minimizes the opportunity for pollination within a single inflorescence; however, individual palms can open with multiple inflorescences simultaneously (Joanna Tucker Lima,

Fig. 6. Open inflorescences of *Attalea crassispatha*. (A) shows an androgynous inflorescence with pistillate flowers proximal and staminate flowers distal (2017 photo, JTL), and (B) shows an entirely staminate inflorescence (2020 photo, JTL). This alternation between staminate and androgynous inflorescences can present challenges in making optimal crosses for genetic diversity.

personal observation), which would enable self-pollination. As for many palms, sap beetles and small weevils are important pollinators for *Attalea* species (Barfod et al., 2011), and these pollinator types can mediate outcrossing and long-distance pollen flow, minimizing inbreeding (Browne et al., 2018; Diaz-Martin and Karubian, 2021). While mating among relatives becomes inevitable in small, closed populations (Frankham et al., 2010), we suggest that a decrease in or extirpation of pollinators in Haiti increased rates of inbreeding among wild born individuals (Eckert et al., 2010). Such a decrease in pollinators is likely coupled with increasing fragmentation of the *A. crassispatha* population and the greater distance between reproductive individuals has also contributed to increased inbreeding in the wild (Ghazoul 2005). Indeed, we observed that two founders had the same individual assigned as both parental sources, indicating self-fertilization in the wild population. High rates of inbreeding are often linked to inbreeding depression and the expression of deleterious alleles (Carr and Dudash, 2003), which we suggest is negatively affecting wild born *A. crassispatha*. An important consequence of inbreeding depression is a decrease in reproductive fitness, often due to post-zygotic mortality (Michalski and Durka, 2007; Neaves et al., 2015). Anecdotally, seeds collected from the wild and distributed to botanic gardens have very low germination rates, which in part explains why so few institutions hold this species in their collection and may help explain why recruitment in the wild is so low. We suggest that more research is needed to investigate *A. crassispatha* pollination ecology and identify the pollinators of this highly endangered palm to better explain the causes of inbreeding.

We found that the majority of optimal breeding pairs of founders reside at different institutions within the meta-collection, highlighting the need for a cooperative breeding program. In the zoological community, this kind of geographic barrier has been overcome using sperm banks where samples can be held long term and shipped to institutions without loss of viability, thereby facilitating mating with the female in the optimal breeding pair regardless of phenology or geography. A similar approach should be more widely adopted by botanic gardens to facilitate the exchange of pollen between garden sites, as shown in Calonje et al. (2011) and Staples and Singeo (2014). The pairwise relatedness analysis provides straightforward direction on exactly which breeding pairs would limit inbreeding and relatedness in the captive born generation. Alternatively, an equally effective conservation breeding approach can be achieved through the development of a pedigree through the sharing of information (such as the origin of founders and breeding events) across institutions. Botanic Gardens Conservation International is currently working to develop a pedigree module in the PlantSearch database, which will be similar to the Zoological Information Management System (ZIMS: Species360) used by Zoos for deciding mating pairs. This online system would centralize pedigree information globally allowing for informed decision making without need for expensive molecular techniques (Smith, 2016; Fant et al., 2016).

Through genetic monitoring we identified potential inaccuracies in the accession records of founder individuals, which are nearly complete for the meta-collection. For example, the maternal accession 91327

includes seven founder individuals that reside at Fairchild Tropical Garden (FTG-05 to FTG-11). Parentage analysis was able to identify a wild individual (H-14) as the maternal source (i.e., 91327) for four out of the seven FTG founders, but no parent was assigned to FTG-07. The pairwise relatedness values between FTG-07 and all other founders of the 91327 accession suggest that FTG-07 is not a half sibling and likely does not belong in this accession. Similarly, parentage analysis and relatedness estimates suggest that FTG-25 is likely not part of the accession 91411, as the records indicate, which includes seven other individuals. Genetic monitoring can be a costly and time-consuming method not yet accessible to many botanic gardens, making institutions reliant on accession records when evaluating relatedness and parentage in conservation collections. The inconsistencies revealed here highlight the need for rigorous documentation and labeling of individuals brought in from the wild as seed to ensure that future breeding decisions are made using accurate information.

4.2. Genetic monitoring of the captive born ex situ population

Our work suggests that the current passive breeding management (i.e., open pollination) of *A. crassispatha* does not effectively safeguard genetic resources ex situ. We found a significant reduction in genetic diversity between the wild population and the captive born plants as well as a significant increase in inbreeding. Furthermore, all captive born individuals with assigned parentage are a result of self-fertilization. Available records are consistent with a self-fertilization event in that the two captive-born *A. crassispatha* plants from MBC (MBCB-01 and -02) are derived from the maternal plant 91327*H (MBCA-02) (Fig. 1B), which was the only reproductively active individual recorded the year that the seed of captive born individuals were produced. Our data suggest that the high rates of self-fertilization and inbreeding observed in the captive born individuals is causing inbreeding depression given the low rates of germination ex situ. In addition, we found that captive born individuals have strikingly high rates of fixed homozygosity at a large portion of loci (~30 %). The remaining loci exhibit high rates of rates of heterozygosity, which we posit are elevated due to the preferential survival of the small subset of offspring who inherit the largest portion of heterozygous genotypes minimizing the effects of deleterious alleles (Moehring, 2011).

While the size of the captive born generation is quite low (see below), we caution that the stark lack of outcrossing seen here and a continuing shift towards self-fertilization may severely impact this species by increasing the chances of inbreeding depression (Charlesworth and Charlesworth, 1987; Barrett, 2002) and lowered reproductive fitness as documented in many inbred, captive populations of animals (Woodworth et al., 2002) and plants (Walsh et al., 2019; Foster et al., 2022). The observed decrease in genetic diversity in the captive born generation may diminish the species' ability to respond to changing environments, or its evolutionary potential (Snyder et al., 1996; Ren et al., 2014) and serve as a barrier to successful reintroduction efforts (Araki et al., 2008). For example, the successful reintroduction of *Pseudophoenix sargentii* (H. Wendl. ex Sarg) in the Florida Keys is attributed to the decrease in the levels of inbreeding and increase in genetic variation in situ (Fotinos et al., 2015). However, the genetic composition of restoration material is often overlooked and can be a possible explanation for the decreased success of many reintroduction efforts (Mijangos et al., 2015). Previous studies have shown that the survival rates of reintroduced plants increases when genetic diversity is considered in the project design (Godefroid et al., 2011), providing developmental stability and increasing population fitness (Booy et al., 2000). The consideration of genetic diversity is especially important for species such as *A. crassispatha* that are threatened by decreases in annual precipitation (Timyan and Cinea, 2018) and are likely to continue experiencing novel conditions (Mijangos et al., 2015).

Given the high rates of selfing, few founder individuals contribute to the captive born generation. One reason for the low number of founder parents in the captive born generation relates to the demographic history and reproductive phenology of the species. The species is characterized as long-lived and slow to mature (Fig. 6). While reproductive knowledge of *A. crassispatha* is limited, flowering was recorded between February and May at MBC, with at least a few individuals producing multiple inflorescences each year. *Attalea crassispatha* palms begin to produce staminate inflorescences within 20 years but may take 30–40 years before producing pistillate flowers in androgynous inflorescences (Timyan and Cinea, 2018). At MBC, a single maternal plant (91327*H) has produced all of the seed collected thus far on site, and was the first and most reproductive individual among the founder cohort at MBC, flowering consistently since 2009. In 2013, the year the captive cohort was produced, 91327*H was the only Carossier Palm observed in flower. On the other hand, in 2021, all but one of MBC's ten founder *A. crassispatha* flowered, but none of them developed any fruit. Then in 2022, only two of ten palms produced inflorescences (91327*H and 91441*B). Of these, 91327*H began to develop fruit but the seeds aborted before reaching maturity. Documenting life stages and phenology of exceptional species will be critical should breeding action (e.g., hand pollination, pollen storage) need to occur to safeguard genetic material from all individuals in the meta-collection.

Surprisingly, we found that the captive born cohort of 7 plants held at the USDA was genetically distinct from all other *Attalea crassispatha*, either ex situ or in situ. The diagnostic morphology of *Attalea* relies on flowering and fruiting structures, and these 2013 plants are years away from flowering. Ongoing, broader-scale genotyping of the genus (A. Grinage, in prep.) may identify how this cohort compares to other species in the group and its placement within *Attalea* and *A. crassispatha* specifically. Records at MBC indicate that this cohort was derived from 28 seeds that MBC provided to the USDA in August 2013, resulting from the open pollinated offspring of 91327*H. One hypothesis is that these seeds were hybrids from pollination by another *Attalea* species. Several other *Attalea* spp. are grown at MBC, many of which flower annually, and plant conservation scientists have long been concerned that hybridization within collections could decrease the conservation value of endangered species (Basey et al., 2015). However, additional samples collected from that same seed lot (MBCB-01 and MBCB-02) are not genetically distinct (Fig. 2A), and their assigned parentage matches to the MBC founders (Table S6). Therefore, the results are more consistent with a labeling error at some point before, during or after movement from MBC to USDA. The complete lack of genetic similarity or parental match between the USDA cohort and all other samples supports this hypothesis. This result provides a critical lesson for improving curation practices: a prompt and rigorous chain of custody for seeds, seedlings, and plantings is required for stewarding living conservation collections, especially when taxonomy is cryptic in young plants. Issues with mislabeling have been discovered in herbarium specimens with an estimated 50 % of tropical plants in collections having incorrect names (Goodwin et al., 2015). This work highlights the benefit of genetic monitoring for providing an important verification of plants kept for conservation and reintroduction purposes.

4.3. Recommendations for collection development and management

Botanic gardens are positioned to play an important role in the conservation of highly endangered plant species through careful and active coordination in managing conservation collections that enables restoration efforts (Fant et al., 2016; Griffith et al., 2019; Westwood et al. 2021). Retaining genetic diversity serves to promote the reintroduction of plant species (Abeli et al., 2019) and prevents a decline in fitness after ex situ cultivation (Enßlin et al., 2015). Our work uses genetic monitoring in the meta-collection of *Attalea crassispatha* to refine collection management practices used in botanic gardens to facilitate successful reintroductions. Together, we find that (1) some wild individuals are not genetically represented ex situ and that (2) garden propagated palms do not adequately safeguard the genetic resources for

this exceptional species as they are all the result of self-fertilization. One limitation is that the reproductive phenology of the species constrains seed collection opportunities and limits cross pollination, which restricts the genetic diversity that can be collected from the wild. Opportunities for cross pollination in garden collections thus far are few and far between, with few seed collections (1989 or 1991) having reached reproductive maturity. Moreover, the early years of *A. crassispatha*'s reproductive phase appears to be primarily invested in staminate inflorescences, delaying fruit and seed production even further. Given the findings above, we offer three recommendations to improve stewardship for *Attalea crassispatha*:

1. *Targeted collections development.* The current founder cohort is a solid start for capturing in situ genetic diversity, but additional collections from in situ plants would augment these efforts. We suggest parentage analysis that includes all living geo-referenced wild individuals to inform future collections, emphasizing wild plants that are distinct from contributors to the current founder pool. Since 2019, widespread challenges to safety in Haiti present a formidable barrier to this need (William Cinea, *personal observation*), highlighting the critical importance of resource and civic security for species conservation (Matthew et al., 2002).
2. *Deliberate coordination of breeding throughout the meta-collection.* Open pollinated palms do not carry forward the diversity of the wild population and instead encourage self-fertilization and inbreeding. Delayed reproductive maturity throughout the founder cohort and geographic distribution across the meta-collections restricts breeding efforts. We recommend the development of a coordinated system for sharing individual level information across botanic gardens as well as a pollen storage protocol that allows botanic gardens to bank pollen to send other institutions with the aim of facilitating mating between optimal breeding pairs.
3. *Rigorous chain of custody.* Some error in information management was discovered through our genetic monitoring. These types of errors can be minimized through rigorous, prompt, and regular attention to accessioning, labeling, and mapping of living plants. We suggest the development of a standardized record keeping system accessible to all sites.

Each of these recommendations is applicable to conservation meta-collections of any species. Coordinated, informed plant breeding represents an important improvement for botanic garden conservation going forward (Griffith et al., 2019; Pearce et al., 2020). This study provides firm evidence that moving towards these goals will yield tangible successes in preventing the extinction of endangered plant species.

CRediT authorship contribution statement

Zoe Diaz-Martin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jeremie Fant:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Validation, Supervision, Writing – review & editing. **Kayri Havens:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **William Cinea:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **Joanna M. Tucker Lima:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. **M. Patrick Griffith:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data have been published to FigShare and can be accessed at https://doi.org/10.6084/m9.figshare.22041026.v1

Acknowledgements

This work was funded by the Institute of Museum and Library Services (MG-60-19-0064-19) as part of the TREES (Tools and Resources for Endangered and Exceptional Species) Project, with additional funding from the Plant Exploration Fund of Montgomery Botanical Center. The authors thank Nura Abdul Karim, Chris Dunn, Romina Gazis, Brett Jestrow, Vickie Murphy, Larry Noblick, and Andrew Street, and Fairchild Tropical Botanic Garden, Montgomery Botanical Center, Singapore Botanic Garden, USDA Chapman Field, and University of Florida Extension Tropical Research and Education Center for keeping and providing living collections for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2023.109955.

References

Abeli, T., Dalrymple, S., Godefroid, S., Mondoni, A., Muller, J.V., Rossi, G., Orsenigo, S., 2019. Ex situ collections and their potential for the restoration of extinct plants. Conserv.Biol. 34 (2), 303–313.
Araki, H., Berejikian, B.A., Ford, M.J., Blouin, M.S., 2008. Fitness of hatchery-reared salmonids in the wild. Evol. Appl. 1 (2), 342–355.
Attard, C.R.M., Möller, L.M., Sasaki, M., Hammer, M.P., Bice, C.M., Brauer, C.J., Carvalho, D.C., Harris, J.O., Beheregaray, L.B., 2016. A novel holistic framework for genetic-based captive-breeding and reintroduction programs. Conserv. Biol. 30 (5), 1060–1069.
Barfod, A.S., Hagen, M., Borchsenius, F., 2011. Twenty-five years of progress in understanding pollination mechanisms in palms (Arecaceae). Ann. Bot. 108, 1503–1516.
Barrett, S.C.H., 2002. The evolution of plant sexual diversity. Nat.Rev. 3, 274–284.
Basey, A.C., Fant, J.B., Kramer, A.T., 2015. Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. Nat.Plants J. 16, 37–53.
Basey, A.C., Dunwiddie, P.W., Fant, J.B., Kaye, T.N., Kramer, A.T., 2020. Mixing source populations increases genetic diversity of restored rare plant populations. Restor. Ecol. 28 (3), 583–593.
Booy, G., Hendriks, R.J.J., Smulders, M.J.M., Van Groenendael, J.M., Vosman, B., 2000. Genetic diversity and the survival of populations. Plant Biol. 2, 379–395.
Botanic Gardens Conservation International (BGCI), 2020. PlantSearch Online Database. Botanic Gardens Conservation International, Richmond, U.K. https://tools.bgci.org/plant_search.php. [Accessed June 18, 2020].
Browne, L., Ottewell, K., Sork, V.L., Karubian, J., 2018. The relative contributions of seed and pollen dispersal to gene flow and genetic diversity in seedlings of a tropical palm. Mol. Ecol. 27, 3159–3173.
Burney, D.A., Burney, L.P., 2009. Inter situ conservation: opening a" third front" in the battle to save rare Hawaiian plants. Bgjournal 6 (1), 16–19.
Calonje, M., Kay, J., Griffith, M.P., 2011. Propagation of cycad collections from seed: applied reproductive biology for conservation. Sibbaldia 9, 77–96.
Carr, D.E., Dudash, M.R., 2003. Recent approaches into the genetic basis of inbreeding depression in plants. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 358 (1434), 1071–1084.
Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22 (11), 3124–3140.
Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J.H., 2011. Stacks: building and genotyping loci de novo from short-read sequences. G3: Genes Genomes Genet. 1 (3), 171–182.
Charlesworth, D., Charlesworth, B., 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Evol. Syst. 18, 237–268.
Christe, C., Kozlowski, G., Frey, D., Fazan, L., Bétrisey, S., Pirintsos, S., Gratzfeld, J., Naciri, Y., 2014. Do living 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 (12), 2945–2959.

Cibrian-Jaramillo, A., Hird, A., Oleas, N., Ma, H., Meerow, A.W., Francisco-Ortega, J., Griffith, M.P., 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 (4), 559–577.
Clugston, J.A., Ruhsam, M., Kenicer, G.J., Henwood, M., Milne, R., Nagalingum, N.S., 2022. Conservation genomics of an Australian cycad Cycas alcicole, and the Absence of Key Genotypes in Botanic Gardens. Conserv. Genet. 23, 449–465.
Conde, D.A., Colchero, F., Gusset, M., Pearce-Kelly, P., Byers, O., Flesness, N., Browne, R. K., Jones, O.R., 2013. Zoos through the lens of the IUCN Red List: a global metapopulation approach to support conservation breeding programs. PloS One 8 (12), e80311.
Coulon, A., 2010. GENHET: an easy-to-use R function to estimate individual heterozygosity. Mol. Ecol. Resour. 10 (1), 167–169.
Cristescu, R.H., Strickland, K., Schultz, A.J., Kruuk, L.E.B., de Villiers, D., Frère, C.H., 2022. Susceptibility to a sexually transmitted disease in a wild koala population shows heritable genetic variance but no inbreeding depression. Mol. Ecol. 31, 5455–5467.
Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., 2011. The variant call format and VCFtools. Bioinformatics 27 (15), 2156–2158.
De Freitas, J., Camilleri, J., van Eijk, S., Posno, V., Valdes, I., Coolen, Q., van Blerk, J., Griffith, M.P., 2019. Sabalpalm (Sabal antillensis) recovery over 40 years: lessons for successful palm conservation. Palms 63 (2), 57–68.
Diaz-Martin, Z., Karubian, J., 2021. Forest cover at landscape scales increases male and female gametic diversity of palm seedlings. Mol. Ecol. 30, 4353–4367.
Eckert, C.G., Kalisz, S., Geber, M.A., Sargent, R., Elle, E., Cheptou, P.O., Goodwillie, C., Johnston, M.O., Kelly, J.K., Moeller, D.A., Porcher, E., Ree, R.H., Vallejo-Marín, M., Winn, A.A., 2010. Plant mating systems in a changing world. Trends Ecol. Evol. 25 (1), 35–43.
Enßlin, A., Tschöpe, O., Burkart, M., Joshi, J., 2015. Fitness decline and adaptation to novel environments in ex situ plant collections: current knowledge and future perspectives. Biol. Conserv. 192, 394–401.
Fant, J.B., Havens, K., Kramer, A.T., Walsh, S.K., Callicrate, T., Lacy, R.C., Maunder, M., Meyer, A.H., Smith, P.P., 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.
Foster, J.A., Walsh, S.K., Havens, K., Kramer, A.T., Fant, J.B., 2022. Supporting long-term sustainability of ex situ collections using a pedigree-based population management approach. Appl. Plant Sci. 10, e11491.
Fotinos, T.D., Namoff, S., Lewis, C., Maschinski, J., Griffith, M.P., von Wettberg, E.J.B., 2015. Genetic evaluation of a reintroduction of Sargent's Cherry Palm, Pseudophoenix sargentii. J.Torrey Bot.Soc. 142 (1), 51–62.
Frankham, R., 2010. Challenges and opportunities of genetic approaches to biological conservation. Biol. Conserv. 143, 1919–1927.
Frankham, R., Ballou, J.D., Briscoe, D.A., 2010. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.
Ghazoul, J., 2005. Pollen and seed dispersal among dispersed plants. Biol. Rev. 80, 413–443.
Godefroid, S., Piazza, C., Rossi, G., Buord, S., Stevens, A., Aguraiuja, R., Cowell, C., Vanderborght, T., 2011. How successful are plant species reintroductions? Biol. Conserv. 144, 672–682.
Goodwin, Z.A., Harris, D.J., Filer, D., Wood, J.R.I., Scotland, R.W., 2015. Widespread mistaken identity in tropical plant collections. Curr. Biol. 25 (22), R1066–R1067.
Goudet, J., Jombart, T., 2022. Hierfstat: estimation and tests of hierarchical F-statistics. R package version 0.5-11. https://CRAN.R-project.org/package=hierfstat.
Griffith, M.P., Noblick, L.R., Dowe, J.L., Husby, C.E., Calonje, M.A., 2008. Cyclone tolerance in New World Arecaceae: biogeographic variation and abiotic natural selection. Ann. Bot. 102 (4), 591–598.
Griffith, M.P., Calonje, M., Meerow, A.W., Tut, F., Kramer, A.T., Hird, A., Magellan, T.M., Husby, C.E., 2015. Can a botanic garden cycad collection capture the genetic diversity in a wild population? Int. J. Plant Sci. 176, 1–10.
Griffith, M.P., Calonje, M., Meerow, A.W., Francisco-Ortega, J., Knowles, L., Aguilar, R., Tut, F., Sánchez, V., Meyer, A., Noblick, L.R., Magellan, T.M., 2017. Will the same ex situ protocols give similar results for closely related species? Biodivers. Conserv. 26, 2951–2966.
Griffith, M.P., Beckman, R., Callicrate, R., Clark, J., Clase, T., Deans, S., Dosmann, M., Fant, J., Gratacos, X., Havens, K., Hoban, S., Lobdell, M., Jiménez-Rodriguez, F., Kramer, A., Lacy, R., Magellan, T., Maschinski, J., Meerow, A.W., Meyer, A., Sanchez, V., Spence, E., Toribio, P., Walsh, S., Westwood, M., Wood, J., 2019. Toward the MetaCollection: Safeguarding Plant Diversity And Coordinating Conservation Collections. Botanic Gardens Conservation International US, San Marino, USA.
Griffith, M.P., Clase, T., Toribio, P., Encarnación Piñeyro, Y., Jimenez, F., Gratacos, X., Sanchez, V., Meerow, A., Meyer, A., Kramer, A., Fant, J., Havens, K., Magellan, T.M., Dosmann, M., Hoban, S., 2020. Can a botanic garden metacollection better conserve wild plant diversity? A case study comparing pooled collections to an ideal sampling model. Int. J. Plant Sci. 181, 485–496.
Henderson, A., 2002. In: Evolution And Ecology of Palms. The New York Botanical Garden Press, Bronx, New York, pp. 113–116.
Henderson, A., Balick, M., 1991. Attalea crassispatha, a rare and endemic Haitian palm. Brittonia 43 (3), 189–194.
Hoban, S., Callicrate, T., Clark, J., Deans, S., Dosmann, M., Fant, J., Gailing, O., Havens, K., Hipp, A.L., Kadav, P., Kramer, A.T., Lobdell, A., Magellan, T., Meerow, A. W., Meyer, A., Pooler, M., Sanchez, V., Snyder, E., Derrickson, N.F., Beissinger, S.R., Wiley, J.W., Smith, T.B., Spence, P., Thompson, R., Toppila, Walsh, S., Westwood, M., Wood, J., Griffith, M.P., 2020. Taxonomic similarity does not predict necessary sample size for ex situ conservation: a comparison among five genera. Proc. R. Soc. B 287 (1926), 20200102.
Hoban, S., Bruford, M.W., Funk, W.C., Galbusera, P., Griffith, M.P., Grueber, C.E., Heuertz, M., Hunter, M.E., Hvilsom, C., Stroil, B.K., Kershaw, F., Khoury, C.K., Laikre, L., Lopes-Fernandes, M., MacDonald, A.J., Mergeay, J., Meek, M., Mittan, C., Mukassabi, T.A., O'Brien, D., Ogden, R., Palma-Silva, C., Ramakrishnan, U., Segelbacher, G., Shaw, R.E., Sjögren-Gulve, P., Veličković, N., Vernesi, C., 2021. Global commitments to conserving and monitoring genetic diversity are now necessary and feasible. Bioscience 71 (9), 964–976.
Hogg, C.J., Wright, B., Morris, K.M., Lee, A.V., Grueber, C.E., Belov, K., 2019. Founder relationships and conservation management: empirical kinships reveal the effect on breeding programmes when founders are assumed to be unrelated. Anim. Conserv. 22, 348–1361.
Jestrow, B., Franck, A., 2017. In search of the Haitian oil palm Attalea crassispatha: revisiting it after 25 years. Trop.Gard. 72 (3), 17–20.
Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24 (11), 1403–1405.
Klimova, A., Ortega-Rubio, A., Gutiérrez-Rivera, N., Garcillán, P.P., 2021. Genomic insights into the historical population dynamics and spatial differentiation of an Endangered Island endemic palm, Brahea edulis. Int. J. Plant Sci. 182 (8), 695–711.
Lacy, R.C., 2013. Achieving true sustainability of zoo populations. Zoo Biol. 32 (1), 19–26.
Marshall, T.C., Slate, J., Kruuk, L.E.B., Pemberton, J.M., 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. 7, 639–655.
Matthew, R.A., Halle, M., Switzer, J. (Eds.), 2002. Conserving the Peace: Resources, Livelihoods And Security. International Institute for Sustainable Development, Winnipeg.
Maunder, M., Havens, K., Guerrant, E.O., Falk, D.A., 2004. Ex situ methods: a vital but underused set of conservation resources. In: Guerrant, E.O., Havens, K., Maunder, M. (Eds.), Ex Situ Plant Conservation: Supporting Species Survival in the Wild. Island Press, Washington, pp. 3–20.
Michalski, S.J., Durka, W., 2007. High selfing and high inbreeding depression in peripheral populations of Juncus atratus. Mol. Ecol. 16, 4715–4727.
Mijangos, J.L., Pacioni, C., Spencer, P.B.S., Craig, M.D., 2015. Contribution of genetics to ecological restoration. Mol. Ecol. 24, 22–37. https://doi.org/10.1111/mec.12995.
Moehring, A.J., 2011. Heterozygosity and its unexpected correlations with hybrid sterility. Evolution 65 (9), 2621–2630.
Neaves, L.E., Eales, J., Whitlock, R., et al., 2015. The fitness consequences of inbreeding in natural populations and their implications for species conservation – a systematic map. Environ.Evid. 4 (5) https://doi.org/10.1186/s13750-015-0031-x.
Nei, M., Maruyama, T., Chakraborty, R., 1975. The bottleneck effect and genetic variability in populations. Evolution 29, 1–10.
Noblick, L.R., Tucker Lima, J.M., 2021. The lone Attalea of the Caribbean, Attalea crassispatha, and the efforts to preserve it. Palm J. 221, 4–8.
Noël, E., Jarne, P., Glémin, S., MacKenzie, A., Segard, A., Sarda, V., David, P., 2017. Experimental evidence for the negative effects of self-fertilization on the adaptive potential of populations. Curr. Biol. 27 (2), 237–242.
Ogle, D.H., Doll, J.C., Wheeler, P., Dinno, A., 2022. FSA: Fisheries Stock Analysis. R package version 0.9.3. https://github.com/fishR-Core-Team/FSA.
Pearce, T.R., Antonelli, A., Brearley, F.Q., Couch, C., Campostrini Forzza, R., Gonçalves, S.C., Magassouba, S., Morim, M.P., Mueller, G.M., Nic Lughadha, E., Obreza, M., 2020. International collaboration between collections-based institutes for halting biodiversity loss and unlocking the useful properties of plants and fungi. PlantsPeoplePlanet 2 (5), 515–534.
Pence, V.C., Meyer, A., Linsky, J., Gratzfeld, J., Pritchard, H.W., Westwood, M., Bruns, E. B., 2022. Defining exceptional species—a conceptual framework to expand and advance ex situ conservation of plant diversity beyond conventional seed banking. Biol. Conserv. 266, 109440.
Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PloS ONE 7, e37135.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575.
R Core Team, 2020. R: A Language And Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.
Ren, H., Jian, S.G., Liu, H.X., Zhang, Q.M., Lu, H.F., 2014. Advances in the reintroduction of rare and endangered wild plant species. Sci. China Life Sci. 57, 603–609. https://doi.org/10.1007/s11427-014-4658-6.
Riffle, R.L., Craft, P., Zona, S., 2012. The Encyclopedia of Cultivated Palms, No. Ed. 2. Timber Press.
Silver, L.W., Cheng, Y., Quigley, B.L., Robbins, A., Timms, P., Hogg, C.J., Belov, K., 2022. A targeted approach to investigating immune genes of an iconic Australian marsupial. Mol. Ecol. 31, 3286–3303.
Smith, P., 2016. Building a global system for the conservation of all plant diversity: a vision for botanic gardens and for botanic gardens conservation international. Sibbaldia 14, 5–13.
Snyder, N.F., Derrickson, S.R., Beissinger, S.R., Wiley, J.W., Smith, T.B., Toone, W.D., Miller, B., 1996. Limitations of captive breeding in endangered species recovery. Conserv. Biol. 10 (2), 338–348.
Staples, G., Singeo, W., 2014. Desiccating palm pollen-a technique for pollinating rare palm species over long distance. Palms 58 (1), 45–51.
Stoler, N., Nekrutenko, A., 2021. Sequencing error profiles of Illumina sequencing instruments. NAR Genom.Bioinform. 3 (1), lqab019.

Timyan, J., Cinea, W., 2018. Attalea crassispatha. In: The IUCN Red List of Threatened Species: e.T38198A2868173.
Timyan, J.C., Reep, S.F., 1994. Conservation status of Attalea crassispatha (Mart.) Burret, the rare and endemic oil palm of Haiti. Biol. Conserv. 68, 11–18.
van der Merwe, M.M., Yap, J.Y.S., Wilson, P.D., Murphy, H.T., Ford, A., 2021. All populations matter: conservation genomics of Australia's iconic purple wattle,Acacia purpureopetala. Diversity 13 (4), 139.
Walsh, S., 2015. Floral Biology, Breeding System, Pollination Ecology, And Ex Situ Genetic Diversity of the Endangered Hawaiian Species, Brighamia insignis A. gray (Campanulaceae). University of Hawaii at Manoa, Honolulu.
Walsh, S., Pender, R.J., Junker, R.R., Daehler, C.C., Morden, C.W., Lorence, D.H., 2019. Pollination biology reveals challenges to restoring populations of Brighamia insignis (Campanulaceae), a critically endangered plant species from Hawai'i. Flora 259, 151448.
Wang, J., 2011. Coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol. Ecol. Resour. 11, 141–145.
Westwood, M., Cavender, N., Meyer, A., Smith, P., 2021. Botanic garden solutions to the plant extinction crisis. PlantsPeoplePlanet 3 (1), 22–32.
Willoughby, J.R., Fernandez, N.B., Lamb, M.C., Ivy, J.A., Lacy, R.C., DeWoody, J.A., 2015. The impacts of inbreeding, drift and selection on genetic diversity in captive breeding populations. Mol. Ecol. 24 (1), 98–110.
Wood, J., Ballou, J.D., Callicrate, T., Fant, J.B., Griffith, M.P., Kramer, A.T., Lacy, R.C., Meyer, A., Sullivan, S., Traylor-Holzer, K., Walsh, S.K., Havens, K., 2020. Applying the zoo model to conservation of threatened exceptional plant species. Conserv. Biol. 34 (6), 1416–1425.
Woodworth, L.M., Montgomery, M.E., Briscoe, D.A., Frankham, R., 2002. Rapid genetic deterioration in captive populations: causes and conservation implications. Conserv. Genet. 3 (3), 277–288.
Zumwalde, B.A., Fredlock, B., Beckman, E., Duckett, D., McCauley, R.A., Spence, E.S., Hoban, S., 2022. Assessing ex situ genetic and ecogeographic conservation in a threatened but widespread oak after range-wide collecting effort. Evol. Applic. 15 (6), 1002–1017.