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The Critically Endangered Haitian endemic palm *Attalea crassispatha* (Arecaceae) and its living collections in Fairchild Tropical Botanic Garden: Perspectives from conservation surveys and DNA microsatellite (SSR) data

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Abstract. With only 24 plants located in the wild in 2017, the Haitian endemic *Attalea crassispatha* (Mart.) Burret (Arecaceae) is one of the major priorities for palm conservation in the Caribbean Island Biodiversity Hotspot and is the only member of the genus (~ 30 species) that occurs in the region. A project of ex situ conservation and field surveys published in 1994 involved Fairchild Tropical Botanic Garden (FTBG) as a major distributor of wild collected germplasm with other botanic gardens/institutions from South Florida and the tropics in 1989 and 1991. Part of this material was also grown in FTBG. Over 25 years after this conservation initiative was established, new field surveys were made in 2017 where the species occurs in Peninsule de Tiburon, southern Haiti. The number of living plants recorded in this new inventory was 24 (vs 25 reported in 1994). DNA microsatellite data (SSRs) were used to compare levels of genetic variation in the FTBG ex situ conservation collections and the wild. We found that the FTBG genotypes did not capture most of the already limited genetic diversity found in the wild. Cluster analyses based on Bayesian statistics recognized three major genetic groups in the wild, and three of them were found in plants occurring mostly in the northern slopes of Peninsule de Tiburon; in contrast, only two of the genetic clusters were predominant in the southern portion of this peninsula mostly in the Cavaillon area. Our results concur with those recently published based on Single-nucleotide polymorphisms (SNPs) molecular markers for ex situ collections of this palm species conserved in five botanic gardens/research institutes.

Keywords: Conservation genetics, Greater Antilles, Tropical islands, Biodiversity Hotspots.

INTRODUCTION

Almost 30 years ago the first-known work focusing on an ex situ conservation program for a Critically Endangered plant species endemic in Haiti was published by Timyan and Reep (1994). The study concerned one of the most threatened palms of the Caribbean Islands, *Attalea crassispatha* (Mart.) Burret, and it provided an account on the status of this species in the wild as well as a germplasm collection initiative that had two aims: (1) introducing the species in private gardens of Haiti and (2) developing ex situ conservation collection of living plants in botanic gardens and horticulture stations.

The target species is restricted to southern Haiti (departments of Sud and Nippes at Peninsule de Tiburon) and it is the only Caribbean Island endemic in the Neotropical genus *Attalea* (~30 species; Henderson 2020). In 1994 only 25 plants (19 adults– including a senescent plant, and six juveniles) were found in eleven sites, but none of these localities had more than ten individuals (Figure 1A, Table 1). A more recent survey made by Timyan (2001) reported 18 seedlings, six juveniles, and 24 adults along the distribution range of the species.

Based on the age of the original description, this suggests that this palm was more abundant in the late 17th century (Martius 1844: 110–112, as *Maximiliana crassispatha* Mart.), and even Charles Plumier (1646–1697) drew and published a plate and a description of this species (Plumier 1703: 2, Plate 1; Figure 2). This pre-Linnean French botanist and monk traveled twice to Haiti between 1689 and 1693 (Mottram 2002).

Table 1. Localities of *Attalea crassispatha* reported by Timyan and Reep (1994: Table 1).

Population code	Locality	Coordinates	Number of individuals (adults/ juveniles)
A	Rivière Seche	73°14' 18°22'	2/0
B	Baron – Le Blanc	73°23' 18°22'; 73°15' 18°23'	2/0
C	Pemel	73°12' 18°22'	1/0
D	Grande Savanne	73°12' 18°23'; 73°13' 18°22'	2/0
E	Dipa	73°07' 18°22'	1/0
F	Perine	73°07' 18°17'	1/1
G	Rousseau	73°35' 18°22'	2/0
H	Bonne Fin	73°37' 18°23'	2/0
I	Christine	73°41' 18°17'	1/0
J	Dumay, Bois Nègre and Desvarine	73°42' 18°16'; 73°16' 18°16'	4/5
K	Boulier	73°43' 18°16'	1[a]/0
Total individuals in the wild			19/6

[a] Senescent individual.

Based on our own field observations and palm conservation work conducted in the Caribbean with other species, reasons for its decline are unsustainable harvesting of seeds for human consumption, introduced herbivores, and clearance of land for agriculture (Timyan and Reep 1994; Jestrow et al. 2018; Freitas et al. 2019).

As part of this ex situ conservation program implemented in the late 1980s and early 1990s, seeds were sent to 16 botanic gardens or agricultural research stations in South America, Central America, Germany, Australia, and Asia (Timyan and Reep 1994). According to Timyan and Reep (1994), two main shipments of seeds were distributed. The first happened in 1989, and was the most extensive one, having Fairchild Tropical Botanic Garden (FTBG), Miami, Florida as the hub for germplasm distribution. It involved sending seeds that were collected from five different mother plants from four places (localities A, D, G, and J; Figure 1A). Germplasm was sent to 13 botanic gardens and five other sites (Timyan and Reep 1994: Table 4). These seeds were also sent to Tropical Research and Education Center (TREC) and resulted in the plants currently cultivated in this research station of the University of Florida. The second consignment (harvested in 1991) was more limited in number of germplasm samples; and seeds were shipped only to botanic gardens in Australia, St. Vincent (Lesser Antilles), and Guyana (Timyan and Reep 1994), with samples received by FTBG as well. This second batch had the progeny of nine different mother plants from nine different places (localities A–H and J, Figure 1A); however, FTBG successfully cultivated plants from only A, B, and D (Fond-des-Nègres region, in the northeastern area of the species distribution range, Figure 1A). It is worth mentioning that Timyan and Reep (1994) found only six plants in these three localities. Regarding material distributed in Haiti, a total of 117 seedlings were sent to private gardens and non-government organizations between 1990 and 1991. Fairchild Tropical Botanic Garden received a third shipment of seeds in 2001 (also from Fond-des-Nègres region), as the ex situ conservation project continued.

The contribution here presented has two main objectives. The first one concerned revisiting the localities reported by Timyan and Reep (1994) to determine if there have been any changes regarding the *Attalea crassispatha* population demographics of these sites. This is particularly relevant in Haiti, a country that it is considered to have one of the highest deforestation rates of the Americas, and has experienced major socio-political turmoils in the last 50 years. In addition, this Carib-

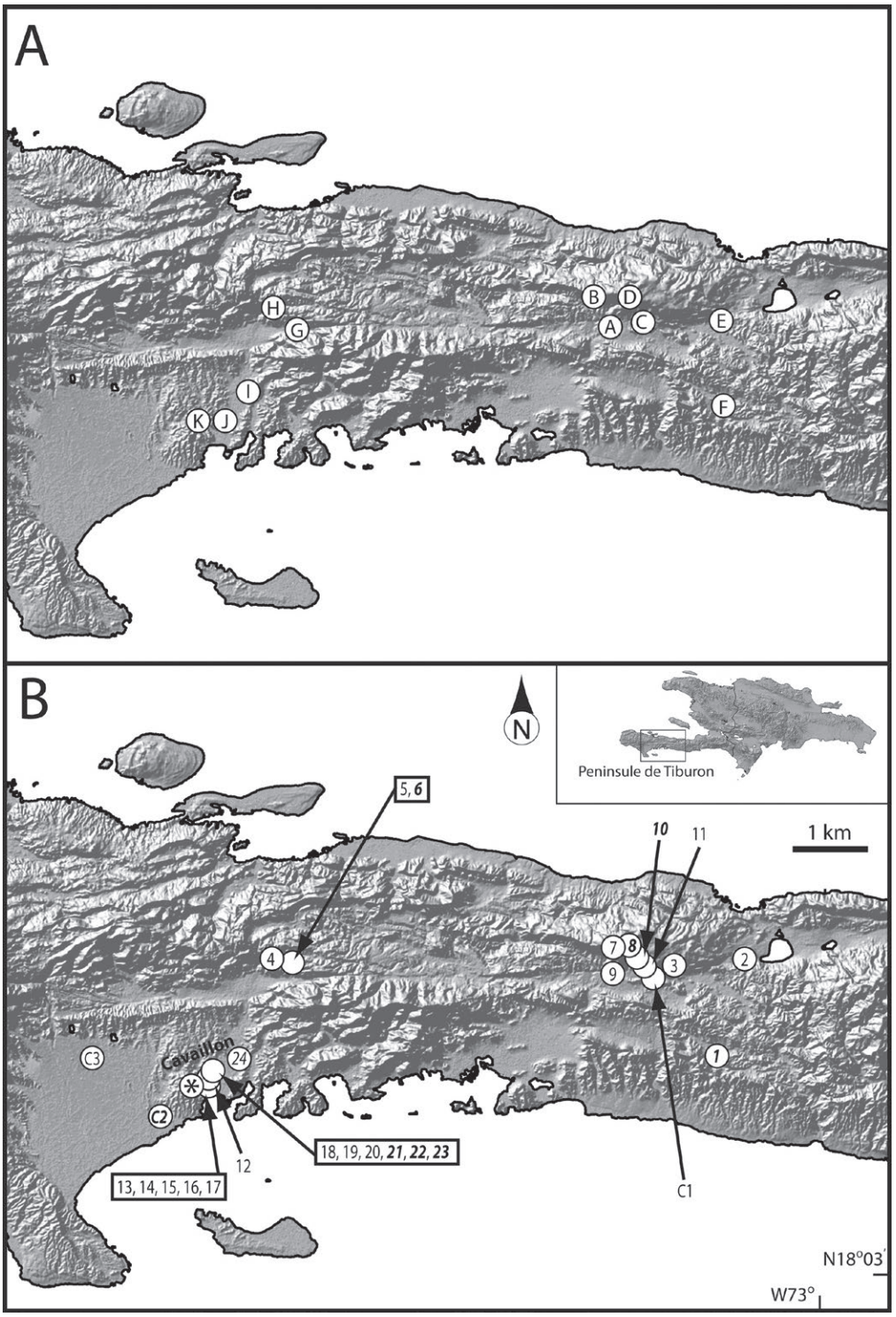

Figure 1. (A) The eleven localities where Timyan and Reep (1994) found wild plants of *Attalea crassispatha* in Peninsule de Tiburon. Locations plotted based on the geographical coordinates published by Timyan and Reep (1994). Number of individuals found in each of these eleven sites are reported in Table 2. Localities are coded as A: Rivière Sèche; B: Baron - Le Blanc; C: Pemel; D: Grande Savanne; E: Dipa; F: Perine; G: Rousseau; H: Bonne Fin; I: Christine; J: Dumay; Bois Nègre and Desvarine; and K: Boulier. (B) The geographical location of the individuals found during the 2017 field surveys conducted for this study in Peninsule de Tiburon. SSR profiles were obtained for all of the plants except for those indicated in bold-italic font (individuals 1, 6, 8, 10, 21–23, and C2). Individuals C1–C3 were found in cultivation. Locality where dead individual was located is coded with an asterisk (*). See Table 2 with geographical coordinates and names of the collecting sites

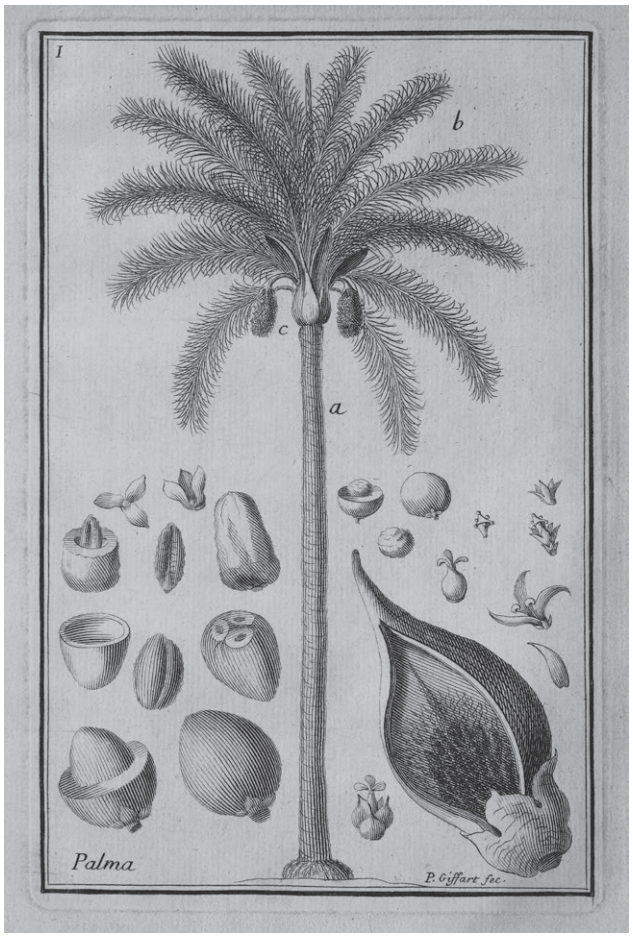

Figure 2. Pre-Linnean illustration of *Attalea crassispatha* as drawn and published by Plumier (1703) based on observations made during his botanical expeditions to Haiti between 1689 and 1693. Courtesy of the Linnean Society of London.

bean country has been affected by major environmental disasters such as the catastrophic 2010 and 2021 earthquakes and six devastating hurricanes (*Georges* in 1998, *Dennis* in 2005, *Gustav* in 2008, *Tomas* in 2010, *Matthew* in 2016, and *Laura* in 2020) that hit the area where this palm occurs. We wanted to assess the conservation status of one of the most threatened Caribbean palms since the last field conservation-oriented actions were taken in the 1990s by Timyan and Reep (1994) and Timyan (2001). The results of this first objective could provide some insights regarding long-term conservation goals performed in situations in which external detrimental conditions can evolve.

The second objective was to investigate the current ex situ genetic conservation status of the collected material at FTBG. Our results are complementary to those recently published in an exhaustive and comprehensive genetic conservation study of populations of this species in (1) ex situ living collections of Montgomery Botanical Center, FTBG, Singapore Botanic Garden, USDA Chapman Field Station at Miami, and the Tropical Research and Education Center (TREC) of the University of Florida at Homestead, Florida, and (2) eight wild sites of Haiti. This work was published by Diaz-Martin et al. (2023) based on 6093 single nucleotide polymorphisms (SNPs) and to identify optimal breeding pairs for future ex situ conservation actions.

In many instances ex situ conservation goals concern sample strategies in small populations in order to maximize the amount of genetic diversity that can be preserved in living individual germplasm banks. This is relevant in the tropics where the vast majority of species have recalcitrant seeds and cannot be massively stored in seed banks (Normah et al. 2019). Therefore, living plant germplasm banks with a very limited number of individuals is in many cases the only feasible alternative to ex situ conservation. The use of highly variable co-dominant molecular markers known as SSR microsatellites provide cost-effective quick tools to determine levels of genetic variation both in the natural populations and in those that are ex situ conserved (Griffith et al. 2017, 2020). These co-dominant markers were not so commonly available in 1994 when conservation activities targeting *Attalea crassispatha* were implemented. The strategy for ex-situ conservation established by Timyan and Reep (1994) was based on seeds collected from a limited number of mother plants across the distribution range of the species. Because in its natural environment this species has very few individuals it was assumed that this sampling strategy would not result in a major genetic bottleneck between the original populations and those established in the ex situ collections. Therefore, our genetic conservation goal at FTBG was to use SSRs to investigate the success of establishing genetic conservation in an ex situ living collection of a Critically Endangered species when the available germplasm comes from a very reduced number of individuals.

METHODS

Field surveys

Field work was conducted during January 2017 including authors BJ, WC, and AF. The geographic coordinates provided by Timyan and Reep (1994) were not recorded with GPS devices but were based on geographical maps; furthermore, they were reported using only degree and minute digits. The eleven populations that were identified by Timyan and Reep (1994) were

Table 2. Localities of *Attalea crassispatha* found during the 2017 surveys.

Palm number	Locality	Coordinates		Number of individuals (adults/ juveniles)
1	Perine	18.283183	-73.108383	1/0
2	Dipa	18.3626	-73.10645	1/0
3	Pemel	18.364517	-73.195	1/3[a]
4	Demoulin	18.396867	-73.6146	1/0
5	Carré-1	18.3905	-73.604633	0/1
6	Carré-2	18.390317	-73.60505	1/0
7	Baron	18.385333	-73.248917	1/0
8	Poitié	18.3853	-73.239917	1/0
9	Le Blanc	18.367683	-73.24855	1/0
10	Ca Michaud	18.373267	-73.23035	1/0
11	Fond-des-Nègres	18.368683	-73.224433	1/0
12	Kasosent-1	18.258083	-73.698033	1/0
13-17	Kasosent-2	18.256917	-73.697783	5/0
18-23	Desravine	18.260967	-73.6964	6/0
24	Nan Guildiv	18.291133	-73.671833	1/0
C1	Riviere Sèche	18.36415	-73.22	1[b]/0
C2	Charpentier	18.2123	-73.753633	1[b]/0
C3	Levy	18.303417	-73.854383	1[b]/0
Total individuals in the wild				23/1
Total individuals in cultivated sites				3/3[a]

[a] Seedlings in cultivation.
[b] Adult individuals found in cultivation.

labeled with a letter system from "A" to "K" as shown in Figure 1A. These localities represent two different watersheds involving the rivers Côtes-de-Fer (sites A–F) and Cavaillon (sites G–K). Therefore, one of the main challenges that we had during our field studies was to locate the actual places that were sampled by Timyan and Reep (1994); their geographic data were used as the initial framework for our own field surveys. In each of the sites we made demographic inventories and recorded the number of adults and juveniles.

Sampling

We obtained SSR genetic profiles of samples from 18 of the 23 wild plants collected in Haiti during our field studies (Figure 3). We were unable to obtain DNA data for seven of the wild collected individuals (plants 1, 6, 8, 10, 21, 22, 23; Table 2, Figure 3). Also, we obtained genetic data for the two of three individuals that were cultivated in this country (plants C1 and C3). Fairchild Tropical Botanic Garden currently has a total of 38 living individuals of *Attalea crassipatha*, and 30 of these were included in the molecular study (Figure 3). Two are from historical collections, the first one, plant F22 (recently died), confirmed as received from USDA in 1938 (accession number PI 129884), from germplasm originally collected by botanist and palm taxonomist Orator F. Cook (1867–1949) when he was working in Haiti in agriculture development projects (Todd 2009; Francisco-Ortega et al. 2018). The other early accession, represented by three living palms with F23 included, was received in 1940 of questionable source but most likely via the USDA as well, either with USDA accession number PI 129884, PI 138962, or PI 138963. Thirty-two of the 38 individuals found in FTBG belong to the material that reached FTBG in 1991 and 2001. As the living collections of FTBG developed, three young plants (one included, individual F31) were the progeny of palms already present in this garden, and considered part of the living collections.

Microsatellite protocols

DNA was isolated from fragments of leaflets that were fast-dried using Drierite (W. A. Hammond Drierite Co. Ltd). Liquid nitrogen was used to disrupt the leaf tissues, and this was followed by the actual DNA isolation using DNeasy Plant Mini Kit (Qiagen). Ten of the 14 microsatellite loci, originally developed for *Attalea phalerata* Spreng. (Choo et al. 2010), were used as molecular markers for our study (Table 2). First, the regions were amplified and sequenced to verify both the presence of microsatellite loci in the amplicon and the repeat motifs as described in Choo et al. (2010). We used PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences, Piscataway, NJ, USA) for the polymerase chain reaction (PCR) amplification reaction mix. For all regions, the PCR conditions were 94°C for 2 min, 30 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 30 s, and a final extension of 72°C for 10 min. We ran PCR products on a 2% agarose gel stained with SYBR safe (Invitrogen, Carlsbad, CA) and used UV light to visualize them and confirm amplicon lengths. Cycle sequencing was performed in both directions with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The primers used for PCR amplification were also utilized for the cycle sequencing reactions. Nucleotide sequences were visualized on an ABI PRISM 377 DNA Sequencer (Applied Biosystems) at the Florida International University (FIU) DNA Core Facility. All sequences were assembled and visually aligned, using Geneious 11.1.5 (https://www.geneious.com).

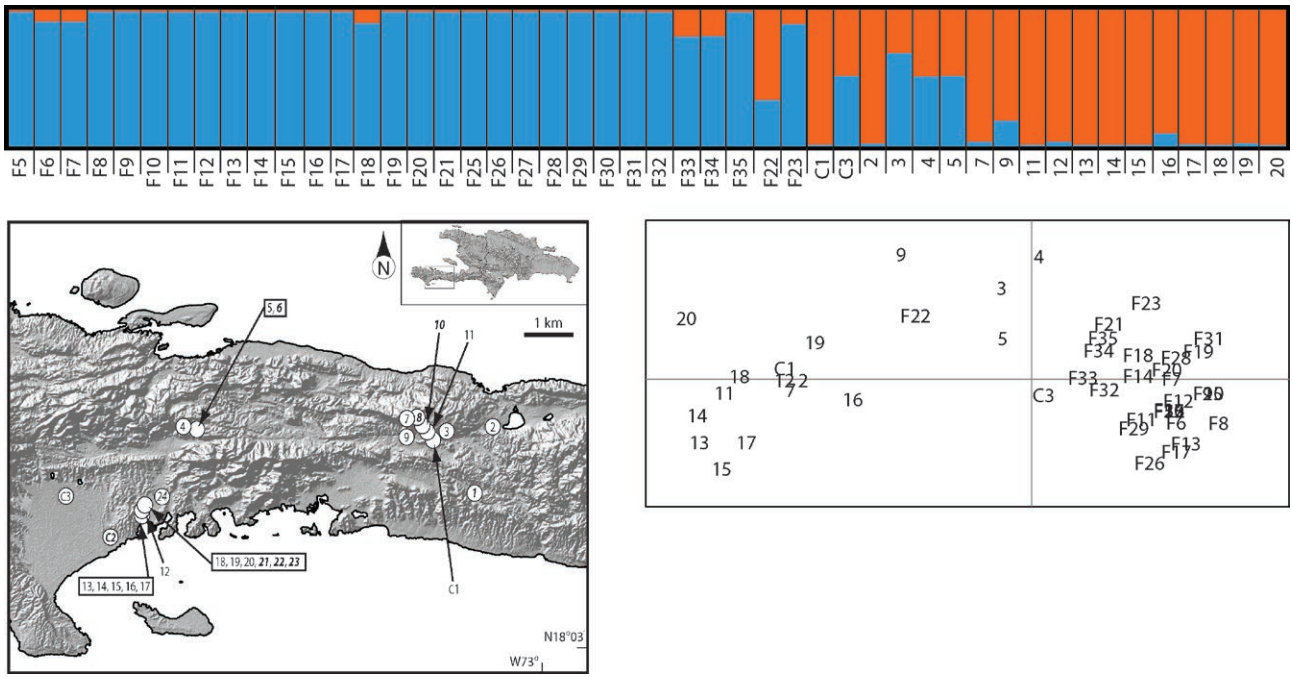

Figure 3. Genetic profiling results of the wild and cultivated populations of *Attalea crassispatha* included in this study. Top diagram shows results of the STRUCTURE analysis for $K = 2$. Bottom-right scatter diagram shows results of the Principal Coordinate Analyses. Geographic provenances of material collected in Haiti is shown in the map. SSR profiles were obtained for all of the plants except for those indicated in bold-italic font (individuals 1, 6, 8, 10, 21–23, and C2). Individuals C1-C3 were found in cultivated stands from Haiti. Plants from Fairchild Tropical Botanic Garden are coded with the letter "F" followed by individual number.

Once confirmed, samples for fragment analysis were prepared using the following PCR reactions of 10 µL volumes containing, 6.25 µL Apex Master Mix, 1.75 µL ddH20, 2.50 µL TBT-PAR [prepared as indicated by Samarakoon et al. (2013)], 0.75 µL of 10 µM fluorescent primer, 0.75 of 10 µM unlabeled primer, and 0.5 µL DNA extract. The PCR conditions were the same, but changed the annealing temperature to 56° C, and occasionally increased to 32 cycles to improve the signal. The samples were then sent to the DNA Core Facility at FIU for fragment analysis. The PCR fragments were separated using an ABI 3100 Genetic Analyzer and visualized with GeneMapper (Applied Biosystems).

Genetic analyses

Percentage of polymorphic loci (*P*), average number of alleles per locus (*A*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), number of loci that deviate significantly from HWE (*n*ds; $p < 0.05$), estimates of genetic differentiation (*F*st), and percentage of paired loci showing linkage disequilibrium (LDL; $p < 0.05$)] were calculated with ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer, 2010). Inbreeding coefficients (*F*is) were estimated using GDA (Weir, 1996). GenAlEx v. 6.5 (Peakall and Smouse 2006, 2012) was used to estimate the number of private alleles (*np*) and of identical genotype pairs per population. GenAlEx was also used to perform a principal coordinate analysis (PCA) among all the individuals included in the study. STRUCTURE v. 2.3.4 (Pritchard et al. 2000), a program based on Bayesian searches was utilized to reveal the genetic structure among populations. K values (1 to10) were estimated through 20 replicate runs of 1,000,000 iterations with burn-in of 100,000. The Dk method of Evanno et al. (2005) as implemented in CLUMPAK (Kopelman et al. 2015) was performed to determine the optimal value of K across samples. CUMPLAK was also used to obtain a consensus Q-matrix from the 20 runs of each k value and to visualize the final consensus cluster diagrams.

RESULTS AND DISCUSSION

Field surveys

The geographical data found in Timyan and Reep (1994) were used as the initial framework for our own field surveys. Furthermore, Ossin Jean helped relocate

these palms as he was with Timyan and Reep during their original search. We located 24 living plants in 15 wild sites (Table 2, Figure 1B). We also found cultivated mature individuals in three different places (Figure 1B). Interestingly the three plants grown in Pemel were small seedlings propagated in pots for future cultivation because of the ethnobotanical value of this palm, particularly for seed consumption.

One site (marked with an asterisk in Figure 1B) had a single dead individual, deceased in 2016 from Hurricane *Matthew*, likely the same individual listed as "Senescent" by Timyan (1991). The vast majority of the visited sites had a single individual except the Kasosent-2 and Desravine localities that had five and six adult plants, respectively. A comparison can be made between the 1994 and 2017 surveys, the number of adult individuals increased from 19 to 23; however, the number of juveniles decreased from six to one; this suggests that there has been a recruitment crisis (cf. Griffith et al. 2019). We hypothesize that the increase of adult plants is because individuals that were reported as juveniles in 1994 became adults prior to our 2017 field work. Plants were located in different environments but mostly in secondary forest or areas with severe deforestation (Figures 4–5).

Genetic analyses

Because of the scattered distribution of the few wild individuals and the very reduced number of plants encountered in each site, the population genetic statistics considered all the wild plants as belonging to a single population. Genetic diversity statistics show that the living collection of FTBG harbors much less diversity than that from Haiti. For instance, only 40% of loci (e.g., A106_2, B101_4, B102_8, B121_4) were polymorphic in FTBG (vs 100% in the wild). Other genetic diversity indicators also revealed that the FTBG individuals do not capture most of the genetic diversity found in the wild [e.g., number of private alleles (2 vs 20), average number of alleles per locus (2.7 vs 3.5), number of identical genotypes (7 vs 0), and percentage of loci showing linkage disequilibrium (15.56 vs 0)]. Concerning the FTBG ex situ collection, its values of expected heterozygosity almost double those of the observed one (0.464 vas 0.293). In contrast these two population genetic estimators were very similar in the wild population (He = 0.302 vs. Ho = 0.464). However, unexpectedly the wild population exhibited a much higher degree of inbreeding (Fis) than that from FTBG (0.310 vs 0.026)

Our genetic diversity data match those obtained by Diaz Martin et al. (2023) based on SNPs, as they found that plants grown in FTBG, Montgomery Botanical Center, Singapore Botanic Garden, USDA Chapman Field Station, and the TREC of the University of Florida do not capture most of the genetic diversity found in the wild. The broad study of germplasm genetic diversity conducted by Diaz Martin et al. (2023) has relevant conservation implications as they resulted in the identification of optimal breeding pairs in collections from different sites (including FTBG) and showed cases in which plants were likely mislabeled as accessions were moved between institutions.

Results yielded by the PCA and the Cluster Analyses based on Bayesian methods were concordant with those displayed by the genetic diversity statistic data (Figure 3). The PCA scatter diagram showed all genotypes from FTBG to have positive values along the first coordinate (38.2% of the multivariate variation vs 11.25% along the second coordinate), except for plant F22, which is the oldest plant found in this botanic garden (introduced in 1938). The narrow range of values along this coordinate shown by the FTBG individual provides evidence for the genetic bottleneck that happened when populations were sampled in the wild prior to their introduction in Miami. It suggests that few in situ individuals were the source of the living collections and matches the fact that only four loci were polymorphic.

These PCA findings were supported by the STRUCTURE analyses (optimal $K = 2$, the two obtained genetic groups are shown in orange and blue colors in Figure 3). Individuals from Fairchild that resulted from germplasm introduced in 1991 and 2001, showed virtually no admixture and were dominated by the Blue Genetic Cluster. There were only seven exceptions in which admixture was clearly detected for the ex situ collection. They concerned genetic profiles from five (F6, F7, F18, F33 and F34) of the 27 plants introduced 1991 and 2001 (17% of them); and from the two oldest trees planted in the Garden prior 1991 (F23– introduced in 1940, and the already mentioned F22). Regarding the two Haitian cultivated adult plants, one of them (C1) only grouped with the Orange Genetic Cluster; however, the second one (C2) showed admixture for the two genetic groups. Five of the 16 wild-collected (31%) plants (individuals 3–5, 9, and 16) also had admixture for the two genetic groups with the Blue Genetic Cluster mostly found in these plants. These individuals are mostly found in northern areas of the species range. In contrast, most of the plants growing on southern regions of this peninsula, particularly in the Cavaillon area, did not show admixture and were dominated by the Orange Genetic Cluster. The Blue Genetic Cluster was widespread among the 1991 and 2001 plants at FTBG, confirming that the germplasm

Figure 4. Examples of the isolated palms from the northern distribution. (A) A cultivated palm from the collection of Timyan growing at the tomb of a relative of Ossin Jean. (B) Ossin Jean holding an inflorescence of Palm 1. (C) Palm 7 used for storing corn, no longer flowers. (D) Palm 8 was among the tallest found.

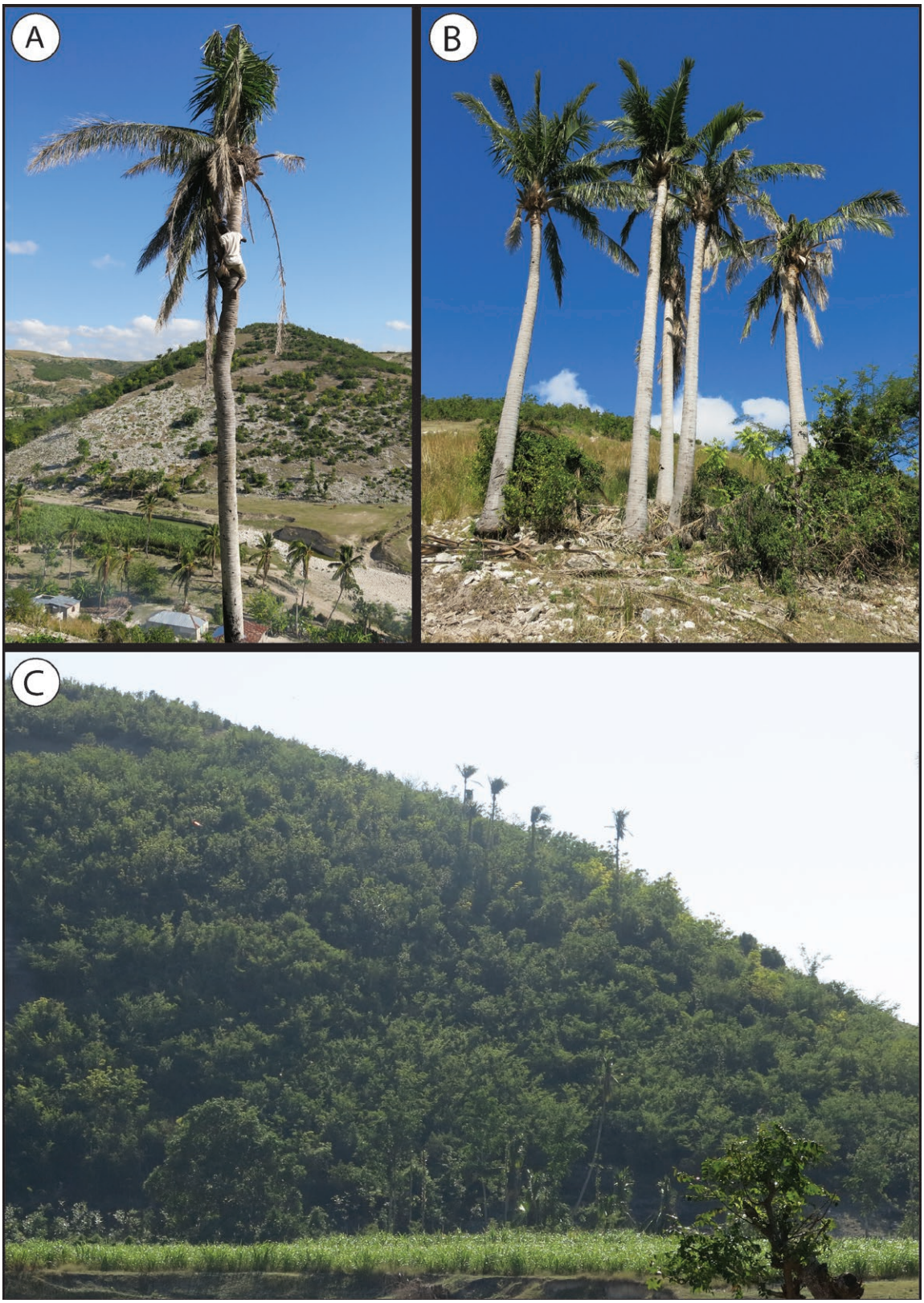

Figure 5. Palms from the Southwest populations of the Cavaillon area. (A) Collecting leaf material from palm 12. (B) Palm cluster of individuals 13–17. (C) Palm cluster of individuals 18–23.

Table 3. The ten SSR loci included in this study. Based on Choo et al. (2010).

Locus	Primer (5'-3') sequence	Repeat motif	Number of alleles
A103_3	F: CAATGCAAGAGACAAGCATAC R: GCACACTTGATGACATTTTATG	GT_9	2
A106_2	F: CATTTGGCATTCTTACACATTC R: CTTGGGGTGAACTGACTTTAC	CA_{11}	3
B101_4	F: CCTGGTCATCCGATTATTTCA R: TGTCGCCATTCTTTCGTTTAT	TC_{19}	5
B102_8	F: AGCACTAATGTGCATGTATGTG R: CCATTCCCTCTACAAGGATAAC	GA_{21}	4
B103_3	F: ATGCTGCTTGCGGTGTAG R: GAGGTATTGATGGGAGGAAGAC	TC_{17}	2
B121_4	F: CCTGGAGCATCAATGGAC R: TCCGAGAACCCTAAACCTG	TC_{12}	4
C5_4	F: AAGATGACCGTAGCATTAACAG R: TCCCATGTTTTCTTTAGTCTTC	GTT_9	2
C11_7	F: AGTCGTGAACTCTACCACTTTC R: TGTTGCCCTTCAGATATAGATC	CAA_9	2
D106_3	F: ACCACCCCATCACAAAAG R: GGACCATTTCAGCCAGAG	$AGAT_7$	5
D124_5	F: GGTGGTGATTGAACTGAACTC R: GCTGATGCTTGCTGACAG	$ATCT_{10}$	5

originated from the northern region of the peninsula, specifically the Fond-des-Nègres area.

In a subsequent STRUCTURE exploratory analysis, we noticed that for the wild population the optimal *K* value was 3 (shown as clusters in orange, red, and dark purple in Figure 6). The PCA scatter diagram (20.8% of variation along the first axis, and 16.07% along the second one) for the wild population is also presented in Figure 6. The results agreed with those yielded by STRUCTURE when the wild and cultivated populations were run together. Genotypes found in the northern area of Peninsule de Tiburon mostly displayed positive values along the first component, whereas those from the Cavaillon area exhibited negatives values along this axis. These multivariate ordination results agreed with the STRUCTURE outcome. Plants occurring in northern Peninsule de Tiburon were the only ones with admixture for Orange, Blue and Dark Purple Clusters. In contrast the Dark Purple Cluster was very uncommon in individuals found in the Cavaillon area (Figure 6).

Recommendations

Our study demonstrates that even when source populations have very few individuals, genetic bottlenecks can happen when ex situ conservation collections are established. We are aware that there is limited time for field exploration and collection of germplasm, and that in many instances not all individuals of the original population are fruiting or have mature seeds to be collected during field surveys. For example, no seed was collected in 1990 since the same mother palms that were harvested in 1989 either failed to produce fruits or fruited poorly (Timyan & Reep, 1994). In this case, collecting more seed from the south Cavaillon population is recommended as germplasm from this area did not reach FTBG. The use of molecular markers are opening new horizons on the management of ex situ collections of living plants, particularly of Critically Endangered species.

Table 4. Population genetic statistics for the wild collected plants and for those grown in Fairchild Tropical Botanic Garden (FTBG). P = percentage of polymorphic loci, np = number of private alleles, A = average number of alleles per locus, Ho = observed heterozygosity, He = expected heterozygosity, nds = number of loci that deviate significantly from HWE ($p < 0.05$), Fis=inbreeding coefficient, #id= number of identical genotype pairs, LDL = percentage of paired loci showing linkage disequilibrium ($p < 0.05$)

	P	np	A	Ho	He	nds	Fis	#id	LDL
Wild	100	20	3.5	0.295	0.302	4	0.310	0	0
FTBG	40	2	2.7	0.293	0.464	1	0.026	7[a]	15.56

[a] One of these seven genotypes was shared by six individuals and another one was shared by three individuals.

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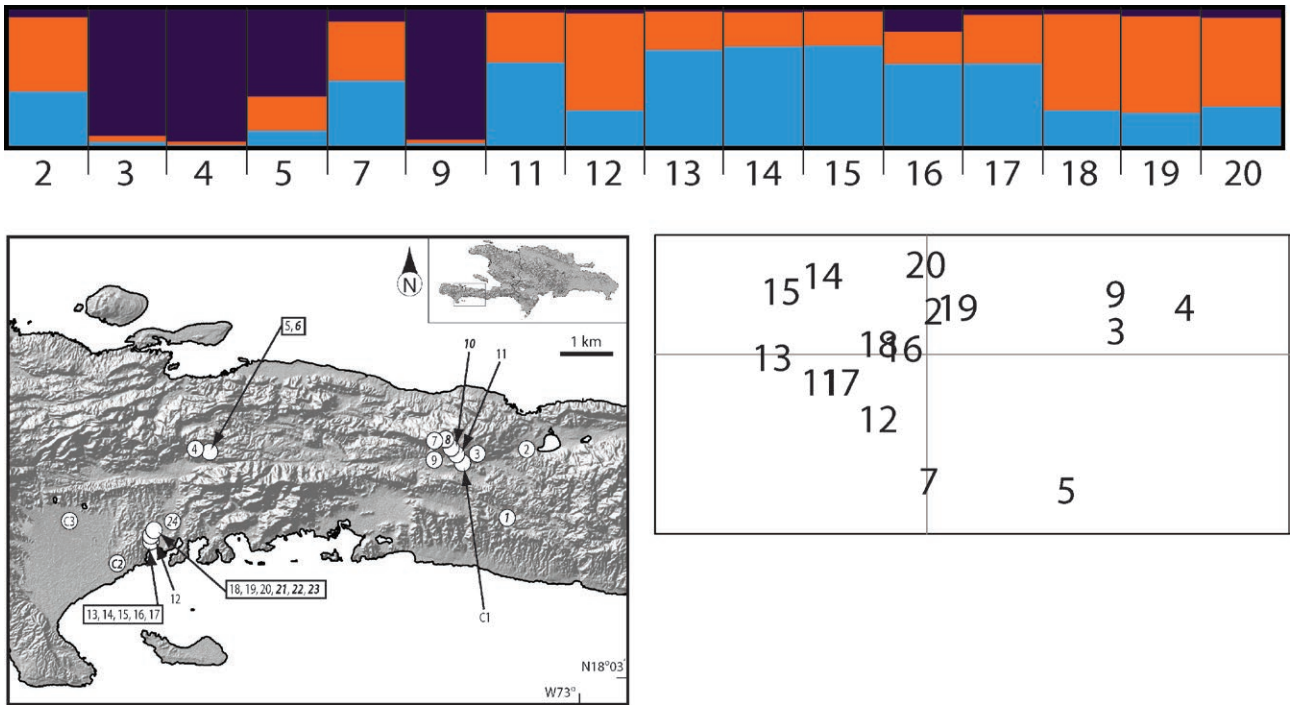

Figure 6. Genetic profiling results of the wild populations of *Attalea crassispatha* included in this study. Top diagram shows results of the STRUCTURE analysis for $K = 3$. Bottom-right scatter diagram shows results of the Principal Coordinate Analyses. Geographical provenances of material collected in Haiti is shown in the map. SSR profiles were obtained for all of the plants except for those indicated in bold-italic font (individuals 1, 6, 8, 10, 21–23, and C2). Individuals C1-C3 were found in cultivated stands and were not included in the analysis.

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