

# Sweet Drinks Are Made of This: Conservation Genetics of an Endemic Palm Species from the Dominican Republic

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## Abstract

*Pseudophoenix ekmanii* is a threatened palm species endemic to the Dominican Republic. Sap from trees is extracted to make a local drink; once they are tapped the individual usually dies. Plants are also illegally harvested for the nursery trade and destroyed by poachers hunting the endemic and threatened Hispaniolan parrot. We used 7 DNA microsatellite markers to assist land managers in developing conservation strategies for this palm. We sampled 4 populations along the known distribution range of this species (3 populations from the mainland and 1 from the small island of Isla Beata), for a total sample of  $n = 104$ . We found strong evidence for genetic drift, inbreeding, and moderate gene flow (i.e., all populations had at least 4 loci that were not in Hardy–Weinberg equilibrium, at least 9 loci pairs were in linkage disequilibrium, the pairwise  $F_{ST}$  values ranged from 0.069 to 0.266, and had positive  $F_{IS}$  values). Data supported an isolation-by-distance model, and cluster analyses based on genetic distances resolved 2 groups that match a north–south split. The population from Isla Beata had the lowest levels of genetic diversity and was the only one in which we found pairs of individuals with identical shared multilocus genotypes.

**Key words:** *Areaceae*, *Biodiversity Hotspots*, *Caribbean*, *ethnobotany*, *Hispaniola*, *in situ conservation*, *protected areas*, *SSRs*, *tropical islands*, *sugar palms*

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Palms, with more than 121 endemic species, represent one of the most important floristic elements of the Caribbean Island Biodiversity hotspot (Zona et al. 2007; Roncal et al. 2008). The approximately 1000 islands and islets of this region have a highly threatened flora due largely to massive habitat loss (reviewed by Maunder et al. 2008). The Caribbean palms provide examples of this biodiversity decline (Zona et al. 2007) and illustrate the conservation concerns for this group on islands (see Chapin et al. 2004, 2006). The 4 species of *Pseudophoenix* H. Wendl. ex Sargent (Ceroxyloideae: Cyclospatheae) are indicative of the challenges being faced by

conservation biologists in the Caribbean Islands. Three of these species are restricted to Hispaniola [*Pseudophoenix ekmanii* Burret, *P. lediniana* Read, and *P. vinifera* (Mart.) Becc.]. The fourth one (*P. sargentii* H. Wendl. ex Sargent) is widespread, ranging through the Bahamas, Cuba, Florida, Hispaniola, Dominica (Lesser Antilles), Mona Island (Puerto Rico), and the Yucatan Peninsula. Two of the Hispaniolan endemics are categorized as “Critically Endangered” sensu IUCN (IUCN 2009). *Pseudophoenix lediniana* is endemic to a single locality in southern Haiti, whereas *P. ekmanii* is restricted to Jaragua National Park in the southwestern

corner of the Dominican Republic at the Barahona Massif (Read 1968, 1969; Zona 2002).

The sap of *P. ekmanii* is a source of sugar that is locally tapped to produce a nonalcoholic beverage popularly known as “Mabí de Cacheo.” The tapping procedure involves removing a large portion of the crown meristem and often results in the death of the plant (Figure 1). It has been suggested that this traditional utilization is one of the main reasons for the decline of *P. ekmanii* in the wild (Hoppe 1998; Zona 2002). This species is facing 2 additional threats. Adults are illegally removed from the wild for gardens, particularly for the many tourist resorts in coastal regions (Jiménez F, unpublished data). The third threat is related to illegal poaching of the endemic Hispaniolan parrot [*Amazona ventralis* (Statius Muller)]. The trunks of *P. ekmanii* are a favorite nesting place for this bird (Jaragua G, unpublished data). Poachers destroy the palms



**Figure 1.** Individuals of *Pseudophoenix ekmanii* in wild populations at Jaragua National Park. (a) Healthy individual that has not had its sap harvested; (b) details of the procedure utilized to extract the sap from a healthy individual; (c) individual with fallen crown shortly after part of the leaf meristem has been removed to extract the sap; and (d) dead individual without crown. Photo credit: Scott Zona.

to gain access to the nests and remove chicks for the illegal bird trade.

The Caribbean Island Biodiversity Hotspot is a clear priority for plant biodiversity conservation (Smith et al. 2004; Maunder et al. 2008); however, few studies have used molecular techniques to address plant population genetic questions in this region (Francisco-Ortega et al. 2007, 2008). To our knowledge, microsatellite (simple sequence repeats [SSRs]) markers have only been used to investigate the genetic structure of the *Zamia pumila* L. complex (Meerow and Nakamura 2007; Meerow et al. 2007, forthcoming); a cycad restricted to the Greater Antilles, the Bahamas, Florida, and Georgia. Likewise, Derien and Duvall (2009), based on nucleotide sequence data from 2 chloroplast DNA markers, investigated the genetic diversity of the largest population of *Guaiaicum sanctum* L. (Zygophyllaceae) from the Florida Keys. In addition, Maskas and Cruzan (2000) used restriction site data of the chloroplast genome to infer phylogeographic patterns of the *Piriqueta caroliniana* Urb. (Passifloraceae) complex in southeastern North America and the Bahamas. Restriction site data were also used to document gene flow between *Borrchia frutescens* (L.) DC. (Asteraceae) and *B. arborescens* (L.) DC. in the Florida Keys (Cattell and Karl 2004) and provides a good example of the potential inherent in studies using molecular markers in conjunction with morphological data for understanding patterns of genetic diversity in the Caribbean. Other studies focusing on genetic diversity of Antillean plants have been based on allozyme data, and they included endemic gymnosperms (Walters and Decker-Walters 1991; Zheng and Ennos 1999, Pinares et al. 2009) or flowering plants (Glover and Barrett 1987; Wendel et al. 1992; Negrón-Ortiz and Hickey 1996; Ackerman and Ward 1998; Dunphy et al. 2004). Most of these isozyme studies were based on more than 10 polymorphic loci and have been useful to understand patterns of genetic variation of these groups, including the critically endangered cycad *Microcycas calocoma* (Miq.) A.DC., restricted to a few populations from Western Cuba (Pinares et al. 2009).

Conservation of critically endangered species often requires an understanding of the genetic structure of their populations (Hamrick et al. 1991; Hamrick and Godt 1996). Most of these species exhibit reduced numbers of both populations and individuals per population. They usually occur along a small geographical area with strict ecological requirements or within a highly fragmented landscape (IUCN 2009). Under such environmental conditions, these populations are severely affected by stochastic events: genetic drift coupled with inbreeding tends to decrease their genetic diversity. The immediate effects are 1) an increase in the number of homozygotes and the attendant risk of inbreeding depression and 2) loss of potential gene combinations with adaptive value that will enable the species to respond to habitat changes (Peterson and McCracken 2005). Therefore, population genetic studies can be crucial for providing information germane to the long-term survival of endangered species. Genetic diversity data can be extremely useful for developing management strategies for

both ex situ and in situ conservation (Holsinger and Gottlieb 1991).

In this study, we use 7 microsatellite loci to determine the patterns of genetic variation of *P. ekmanii* throughout its distribution range. Being a slow-growing, self-compatible, and critically endangered species, with an unsustainable ethnobotanical use (see below), we hypothesized that populations of this palm would depart from Hardy–Weinberg equilibrium (HWE), have an excess of homozygotes, and exhibit low levels of genetic diversity within populations.

## Materials and Methods

### Plant Species

Species of *Pseudophoenix* are hermaphroditic and are notoriously slow growers. The breeding system of this palm is unknown although isolated individuals in cultivation produce viable seeds, suggesting self-compatibility (Zona S, personal communication). However, based on our field observations, the flowers of *Pseudophoenix* are highly attractive to insect pollinators. During flowering season, they are extensively visited by honey and bumble bees, which suggests that outcrossing may play a major role in the reproductive biology of this palm.

The demographic dynamics of *P. ekmanii* are poorly understood; however, a recent ecological study of *P. sargentii* showed that reintroduced individuals mature faster than naturally recruiting individuals. Reintroduction of seedling and juveniles also balanced the age structure, which had been skewed by lack of natural recruitment and slow maturation, and ultimately improved the viability of the population (Maschinski and Duquesnel 2007). This study also found that wild population growth is positively correlated with seedling recruitment and removal of key conservation threats (e.g., elimination of harvesting and habitat preservation).

### Study Sites

*Pseudophoenix ekmanii* is mostly restricted to 3 areas in Jaragua National Park, 2 on the mainland (at Sabana del Algodón and Trudillé), and a third on Beata Island, offshore from the southernmost extreme of the Dominican Republic (Figure 2). The region where this species occurs is one of the least developed and economically poorest of the Dominican Republic (Bolay 1997). Charcoal extraction, near-shore fishing, hunting of feral pigs and goats, subsistence agriculture, and illegal trading of Mabí de Cacheo and poaching of the Hispaniolan parrot are the main economic activities of the region (Bolay 1997; Veloz A, personal communication). None of the populations of *P. ekmanii* are accessible by road, and our field work required several days of overnight camping and the hiring of local guides who led us to the study sites on mule rides. At least 5 populations are found in Trudillé, whereas 8 populations are known at Sabana del Algodón (Veloz A, personal communication). Only one population is known on Isla Beata (Figure 1). The

area where this palm occurs is mostly covered by dry thorny and semi-deciduous forests on limestone (Bolay 1997).

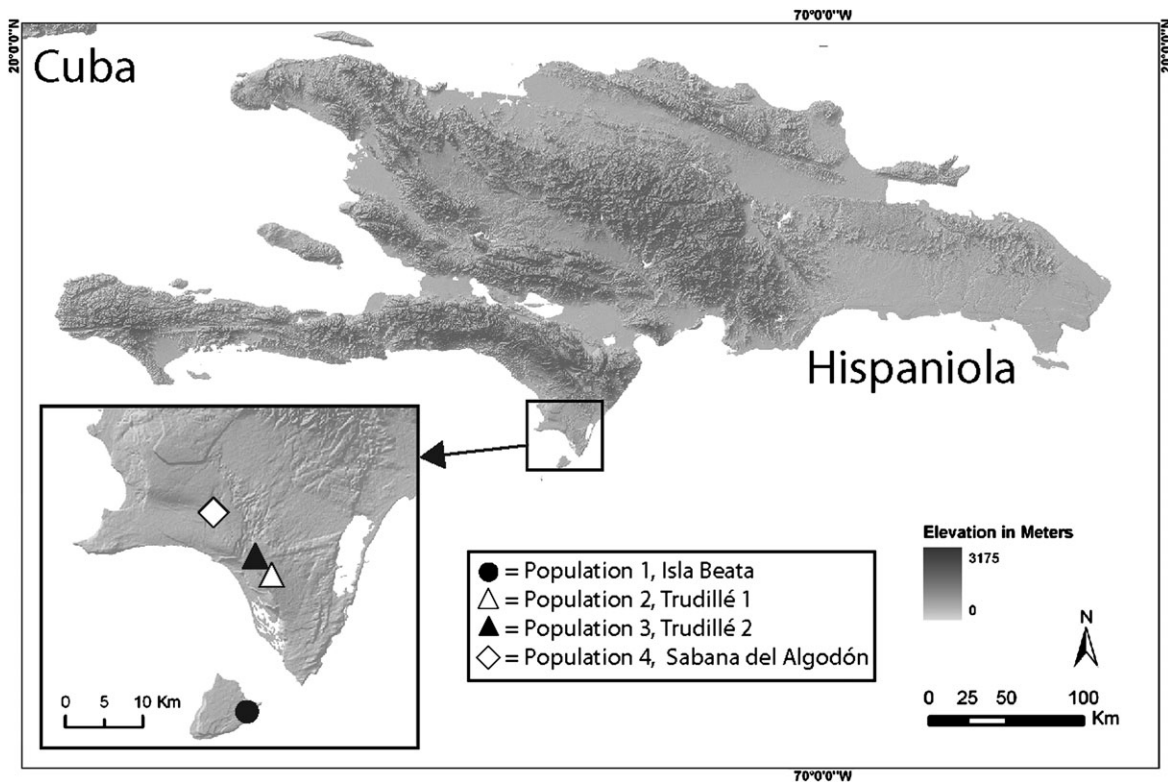
Our study was based on 4 populations, 1 from Isla Beata, 2 from Trudillé, and 1 from Sabana del Algodón (Figure 1). During our field studies, we were unable to sample additional populations, mostly because of the difficult terrain and inaccessibility where the species occurs. These 4 populations were selected on the basis of accessibility and size. Among the visited sites, they appeared to have the highest number of individuals and to cover the largest area. Within Trudillé, the average pairwise distance among sites is 2.5 km, and the 2 populations that we sampled were 4.5 km apart representing the 2 extremes of the distribution area of the species in this area (Supplementary Figure 1). Within Sabana del Algodón, the average pairwise distance among sites is much lower, approximately 0.45 km (Supplementary Figure 1).

### Demographic Structure of Populations

Within each site, individuals were evenly distributed but because of the difficult terrain and the thickness of the forest we could not conduct demographic inventories that included all the individuals of these populations. Therefore, demographic studies were performed on plots (~15 × 15 m<sup>2</sup> each) that covered approximately 10% of the surface area of each sampled population. These plots were randomly distributed over the whole distribution area of each collecting site. Data from these plots were subsequently extrapolated to the whole population area; therefore, they represent approximately estimates of the actual census population. The area covered by these populations ranged approximately between 2 and 6 ha for Isla Beata and Sabana del Algodón, respectively. The 2 populations of Trudillé had an intermediate area of approximately 4 ha. For each population, we recorded the number of individuals within 6 different plant classes, including 1) seedlings (plants with fewer than 3 leaves), 2) juveniles (plants smaller than 1.5 m in height), 3) adults (plants greater than 1.5 m in height), 4) total number of dead plants due to sap harvesting, 5) total number of individuals that were recently tapped prior to our population studies, and 6) total number of living plants that were tapped in previous years.

### Sampling, DNA Isolations, and Polymerase Chain Reaction Amplifications

Between 22 and 31 adult individuals were randomly collected from these 4 localities of *P. ekmanii* at Jaragua National Park (Figure 2). These individuals were sampled (intervals ≥ 15 m) along the whole distribution area of the species in each population. Leaf samples were fast dried in silica gel and subsequently used for DNA isolation using a DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Liquid nitrogen was used to aid in the disruption of the leaf tissue. Seven microsatellite loci originally developed for *P. sargentii* were used as molecular markers for our study (Table 1) (Namoff et al., 2010). The polymerase chain



**Figure 2.** Geographical area where *Pseudophoenix ekmanii* occurs. The 4 populations included in our study are also shown.

reaction (PCR) mixture (in 25  $\mu$ l volume) had the following concentrations: 0.8  $\mu$ M primer, 1 unit GoTaq (Promega), 1 $\times$  reaction buffer, 2.0 mM MgCl<sub>2</sub>, 0.2 mM each dNTP, and 1  $\mu$ l total DNA (concentration not determined). One primer in each pair was labeled with a fluorescent dye. Cycling conditions were 94  $^{\circ}$ C for 3 min followed by 36 rounds of 94  $^{\circ}$ C for 1 min, annealing for 1 min, 72  $^{\circ}$ C for 2 min, and then final extension at 72  $^{\circ}$ C for 10 min. Annealing temperatures were determined experimentally and are reported in Table 1. The PCR fragments were separated using an ABI 3100 Genetic Analyzer and visualized with GeneMapper (Applied Biosystems).

### Statistical Analysis

The program Arlequin v.3.11 (Schneider et al. 2000) was used to determine the percentage of polymorphic loci (P), the average number of alleles per locus (A), the observed heterozygosity ( $H_O$ ), and expected heterozygosity ( $H_E$ ) across the 4 populations included in our study. For each population, we also obtained the number of private alleles ( $n_p$ ) and number of identical shared multilocus genotypes (MLGs) with the program GenAlEx v.6 (Peakall and Smouse 2005). The probability of obtaining matching MLGs was also estimated with GenAlEx. Inbreeding coefficients ( $F_{IS}$ ) were calculated for each population using Genepop (Raymond and Rousset 1995; Rousset 2008).

The significance of deviations of  $F_{IS}$  from 0 was accessed by permutation tests (1000 permutations with 0.05 alpha level for Bonferroni correction). These calculations were performed with FSTAT (Goudet 1995). We used the exact test method of Guo and Thompson (1992), as implemented in Arlequin (i.e., Markov chain of 100 000 steps and 1000 dememorization steps), to assess deviations from HWE for each locus population. The program Genepop was used (1000 dememorization steps, 100 batches, and 1000 iterations per batch) to estimate global HWE deviations for each population. We also used Arlequin to perform a likelihood ratio test of linkage disequilibrium (LD) (10 000 permutations and 10 initial conditions) between loci pairs for each population following the method of Slatkin and Excoffier (1996).

Principal coordinate analysis (PCO) among all the individuals included in our study was computed with GenAlEx based on the algorithm developed by Orloci (1978) after conversion of the individual-by-individual genetic distance matrix, as defined by Smouse and Peakall (1999), to a covariance matrix and data standardization. This ordination technique was used to detect the genetic structure of populations projected in a continuous space (Abbott et al. 1985). The aim of this multivariate analysis was to produce a scatter diagram that summarized the original multidimensional data set and revealed the presence of groups.

The program Populations (Langella 1999) was used to calculate the chord distance of Cavalli-Sforza and Edwards (1967) among populations. A neighbor joining (NJ) tree

**Table 1** Microsatellite loci used in this study

Locus	Repeat <sup>a</sup>	Primer sequence (5'–3')	GenBank <sup>b</sup>	T <sub>a</sub> (°C) <sup>c</sup>	Allele size	No. of alleles
pse2.1	(CA) <sub>21</sub>	F: TCTTCAGTCTTCACCTTCTGCCATGC R: GAGTCTTACAGATAGCGAAGAACACAG	GU124750	54	264–284	8
pse3.33	(TA) <sub>6</sub> (GA) <sub>7</sub>	F: GATTCTTCAAAAATGCCAAGTTCAGCCTG R: ATCCATGCCATTCTCATCCTGGTCC	GU124752	55	264–274	5
pse3.6	(CA) <sub>4</sub> (GA) <sub>19</sub>	F: AGTTACGGTGGCTTGAATTGGAGTTAG R: TCTGCCATCATCCACAGCCATCAAC	GU124754	68	179–223	8
pse5.2	(AT) <sub>8</sub> (GT) <sub>21</sub>	F: AATGCAAGACTCGGTCAACCAAAAGT R: TGATTCTGGTGGAAAATGTTGCATCA	GU124755	55	438–551	9
pse5.5	(GT) <sub>12</sub> (GA) <sub>20</sub>	F: CTGACCCTGTAGCTTGATCCAACCTAG R: CCATCAAAAATTAGCCATCCTAGACCTAATC	GU124757	54	229–244	11
pse5.6	(GT) <sub>17</sub> (GA) <sub>21</sub>	F: CCAGCTCCAGTGTATTATGATCC R: TATCTCCAACACCACCTCTAGCCG	GU124758	65	364–372	4
pse7.26	(AAG) <sub>10</sub>	F: ACTGGAAGGTGATGACATAAGATG R: CTCAACGTGACATCCGACGGTAG	GU124759	64	306–319	3

<sup>a</sup> Repeat motif.

<sup>b</sup> GenBank accession number.

<sup>c</sup> Optimized annealing temperature.

based on this distance was obtained and branch support was assessed by 10 000 bootstrap replications (over loci). The final dendrogram was visualized with Tree View (Page 1996).

Analysis of molecular variance (AMOVA) among populations (based on Euclidean squared distance matrix and with *P* values obtained after 1023 replicates) and *F*<sub>ST</sub> pairwise comparisons between populations (with *P* values obtained after 110 random permutations) were computed with the algorithms implemented in Arlequin.

Relationships between geographical distance and *F*<sub>ST</sub>/(1 – *F*<sub>ST</sub>) values were determined in order to assess if the spatial genetic structure of the populations followed an isolation-by-distance model. This was performed with the program Isolation by Distance Web Service (Jensen et al. 2005) with 30 000 randomizations (significance was determined with 1000 jackknife permutation steps).

The program Micro-Checker (Van Oosterhout et al. 2004) was used to evaluate the presence of null alleles and allelic dropouts, employing 3000 randomizations.

## Results

### Demographic Structure of Populations

Palm tapping occurred in all sampled populations. The percentage of harvested palms ranged between 4% (Isla

Beata) to 16% (Trudillé 2) (Table 2) and very few of them survived this unsustainable harvesting (<4% average of survival within the tapped individuals, across populations). With an estimate of 329 individuals, Isla Beata had the smallest population size. In contrast, Sabana del Algodón had an estimate of 2475 individuals. In all mainland populations, we found that seedlings and juveniles represented the greatest proportion of individuals, ranging between approximately 81% and 67% for the Trudillé and Sabana del Algodón populations, respectively. In contrast, Isla Beata had the largest proportion of adults (64%).

### Genetic Variability

All loci were polymorphic in all populations, and the average number of alleles per locus varied between 2.8 (Isla Beata) to 5.4 (Trudillé 2) (Table 3). Isla Beata had the lowest number of private alleles (*n*<sub>p</sub> = 1), whereas the highest number (*n*<sub>p</sub> = 7) was found in Trudillé 2 (Table 3). The global analyses showed that all populations deviated significantly from HWE. Five loci were not in HWE for the populations of Isla Beata (loci pse2.1, pse3.33, pse5.5, pse5.6, and pse7.26), Trudillé 1 (loci pse3.33, pse3.6, pse5.2, pse5.5, and pse5.6), and Sabana del Algodón (loci pse3.6, pse5.2, pse5.5, pse5.6, and pse7.26). In contrast, 4 of them deviated from HWE in the Trudillé 2

**Table 2** *Pseudophoenix ekmanii* population demography data based on information recorded on sample plots

Population	Locality	Estimated number of living individuals				Sugar tapping effect		
		Total	Adults	Juveniles	Seedlings	Sugar tapped	<i>N</i> <sub>nonsurviving</sub> <sup>a</sup>	<i>N</i> <sub>surviving</sub> <sup>b</sup>
1	Isla Beata	329	211	59	59	14	14	0
2	Trudillé 1	550	110	264	176	71	69	2
3	Trudillé 2	496	95	253	148	80	77	3
4	Sabana del Algodón	2475	820	1,550	105	53	50	3

Plots covered approximately 10% of the surface area of each population.

<sup>a</sup> Estimated number of individuals that were harvested and did not survive.

<sup>b</sup> Estimated number of individuals that were harvested and survived.

population (loci pse2.1, pse5.2, pse5.5, and pse5.6; Table 3). Therefore, each locus departed from HWE in at least one population. Isla Beata was the only population where we found individuals (3) with identical MLGs. The average probability of obtaining a pair of individuals with the same MLG for this population was 0.0014. Nine loci pairs were in LD for the populations of Trudillé 1, Trudillé 2, and Sabana del Algodón; in contrast, Isla Beata had 19 loci pairs in LD. All populations were inbred, displaying positive values for  $F_{IS}$ , with Isla Beata having the highest (0.3) (Table 3).

The program Micro-Checker did not detect allelic dropout, but there was general excess of homozygotes for those loci–population combinations that were not in HWE (see above), suggesting that null alleles might be present for these loci. Although we cannot rule out that these loci had null alleles, the high number of homozygotes found in those loci might well be the outcome of genetic drift and inbreeding.

### Genetic Differentiation among Populations

Pairwise  $F_{ST}$  values among populations ranged between 0.266 (Isla Beata vs. Sabana del Algodón populations) and 0.069 (Sabana del Algodón vs. Trudillé 2) (Table 4), and they were significant ( $P < 0.001$ ) for all comparisons, with an overall mean of 0.16 across loci. AMOVAs showed that 83.96% of variance was within populations and 16.04% among populations.

The first and second axes of the PCO analysis accounted for approximately 50% of the genetic variation. Most of the individuals from the Isla Beata and Trudillé 1 populations have positive values along the first coordinate; in contrast those from Sabana del Algodón and Trudillé 2 displayed negative scores along this axis (Figure 3), suggesting that the 4 populations formed 2 groups.

Additional support for these 2 groups was obtained after NJ analyses of genetic distances among populations. These groups were supported by a 90% bootstrap value (Figure 4). The first group had the populations from Isla Beata and Trudillé 1 and the second one those from Sabana del Algodón and Trudillé 2. The isolation-by-distance analysis showed an increase of divergence among populations with

geographical distance (Figure 5). The  $F_{ST}/(1 - F_{ST})$  values among the 4 populations included in our study had a significant correlation with  $\log_{10}$  (Geographic Distance) for the SSR loci.

## Discussion

### Genetic Structure

There is a clear geographic signature in the patterns of microsatellite variation and this was supported by the clustering and ordination analyses. Two groups were revealed after the NJ and the PCO analyses. One of the groups had the southernmost populations of Isla Beata and Trudillé 1, whereas the second group had those from the north, at Sabana del Algodón and Trudillé 2. The isolation-by-distance analysis showed a highly significant correlation between genetic divergence and geographical distance values. Despite this outcome from the isolation by-distance analysis, the 2 most adjacent populations of Trudillé did not cluster in the same group. These 2 populations are only 4.5 km apart (Supplementary Figure 1), but they are at the extreme ends of the distribution area of the species in Trudillé; however, during our field studies, we did not identify any barrier for gene flow between these 2 populations. Indeed, the relatively low  $F_{ST}$  value between them (see below) suggests that there is gene flow. In addition, there are 3 additional populations of *P. ekmanii* between these 2 sites. We believe that unsustainable harvesting, illegal poaching of *P. ekmanii*, and historical changes to the habitat have increased stochastic evolutionary processes linked to genetic drift.

Within the mainland populations,  $F_{ST}$  values were lower than 0.167, a relatively low value supporting at least moderate amounts of gene flow among these populations (reviewed by Hamrick and Godt 1996). Further evidence for gene flow among these populations came from the ordination analysis in that there was overlapping among them in the PCO diagram.

The population Trudillé 2 displayed the highest number of unique alleles and average alleles per locus. In addition, it had the highest values for observed and expected

**Table 3** *Pseudophoenix ekmanii* population genetic statistics

Population <sup>a</sup>	Locality	P	$n_p$	A	$H_O$	$H_E$	$n_{ds}$	$F_{IS}$	$N_{ig}$	LDL
1 (25)	Isla Beata	100	1	2.8	0.2879	0.4507	5	0.300***	3	19
2 (31)	Trudillé 1	100	3	4.9	0.4423	0.6662	5	0.211***	0	9
3 (22)	Trudillé 2	100	7	5.4	0.5281	0.5912	4	0.255***	0	9
4 (26)	Sabana del Algodón	100	3	4	0.4591	0.5798	5	0.214***	0	9
Pooled values		100	14 <sup>b</sup>	6.9		0.636				

P, percentage of polymorphic loci;  $n_p$ , number of private alleles; A, average number of alleles per locus;  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity;  $n_{ds}$ , number of loci that deviate significantly from HWE ( $P < 0.05$ );  $F_{IS}$ , inbreeding coefficient;  $N_{ig}$ , number of identical genotype pairs; LDL, percentage of paired loci showing linkage disequilibrium.

<sup>a</sup> Number of sampled individuals are given inside the parenthesis.

<sup>b</sup> Total number of alleles across all loci = 48.

\*\*\*Values deviate significantly from 0 ( $P < 0.05$ ).

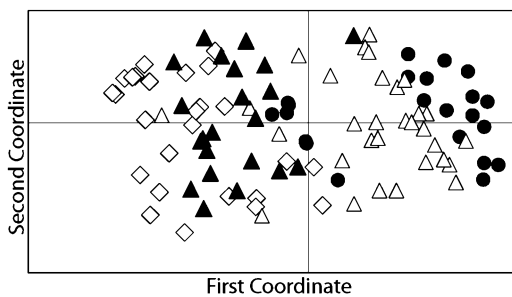
**Table 4** Geographical distances (km) (top) and  $F_{ST}$  values (bottom) among the 4 populations of *Pseudophoenix ekmanii* included in our study

	Isla Beata	Trudillé 1	Trudillé 2	Sabana del Algodón
Isla Beata	—	19.2	21.8	29.1
Trudillé 1	0.124	—	4.5	11.5
Trudillé 2	0.214	0.123	—	7.5
Sabana del Algodón	0.266	0.167	0.069	—

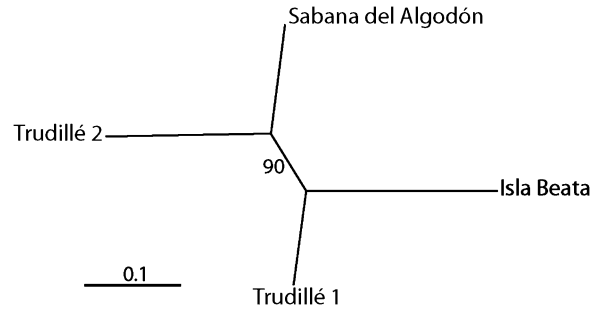
$F_{ST}$  values deviate significantly from 0 ( $P \leq 0.01$ ).

heterozygosity. The presence of a large number of unique alleles indicates that this population has probably been relatively large until recently. That it currently appears to have experienced the highest amount of sugar tapping may support this conclusion. In contrast, the population of Isla Beata had the fewest number of individuals and reduced levels of diversity relative to the other sites. This small island is separated from the mainland by a narrow 7 km sea-strait known as “Canal de la Beata” that acts as a barrier to gene flow with the other populations; interestingly, the highest  $F_{ST}$  values were recorded between the population of this island and those from the mainland supporting higher genetic differentiation than that occurring on the mainland.

Our results suggest that genetic drift and inbreeding have been important factors in shaping the genetic structure of *P. ekmanii*. Exact tests for heterozygote deficiency or excess (Rousset and Raymond 1995) indicated significant departure from HWE under the assumption of heterozygote deficiency, and a corresponding high degree of LD together with  $F_{IS}$  values greater than zero provided further evidence of inbreeding. We are aware that null alleles can result in HW disequilibrium and that gametic phase (unknown for our sample) is one of the possible explanations for LD. However, it is also well known that stochastic events linked to reduced population size also result in an increase of homozygosity and LD because of genetic drift and nonrandom mating (Allendorf and Likart 2006). Therefore, it seems that these 2 processes are relevant to the interpretation of our results because our species is extremely



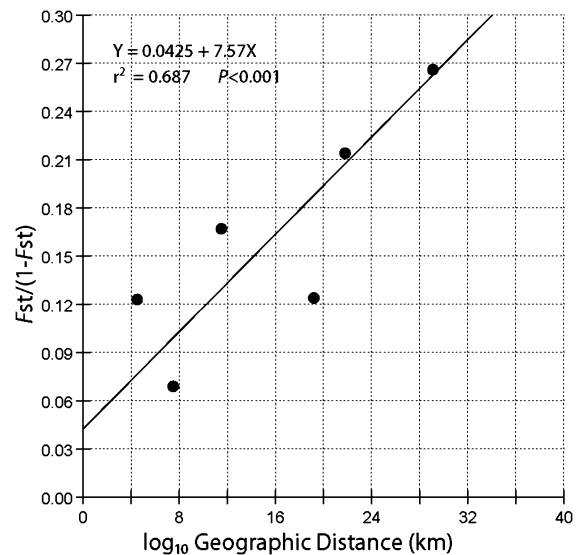
**Figure 3.** Principal coordinate analysis scatter diagram along the 2 first coordinates. Solid circles: Isla Beata; open triangles: Trudillé 1; solid triangles: Trudillé 2; open diamonds: Sabana del Algodón.



**Figure 4.** Neighbor joining dendrogram showing the genetic relationship among populations. Branch lengths are proportional to distances and bootstrap support for the 2 identified clusters is also shown.

rare and under threat from human activities. Isla Beata, with the highest inbreeding coefficient, also had the highest probability of a repeating MLG of the 4 populations, providing further evidence of inbreeding and overall lower genetic diversity.

Based on our field observations, we know that adult individuals of *P. ekmanii* are heavily tapped for the production of the “Mabí de Cacheo” drink. We have also observed that many plants are destroyed by horticultural and parrot poaching activities. These impacts have had detrimental effects on population size and contribute to genetic drift and inbreeding. In addition, it is well known that since the arrivals of Europeans to the region in the late 15th century, the habitats of the islands have been severely modified if not destroyed (Westermann 1952, 1953; Maunder et al. 2008). These historical patterns have had an impact on this palm and the area where it thrives.



**Figure 5.** Isolation-by-distance test showing relationship between  $F_{ST}/(1 - F_{ST})$  versus log-transformed geographic distance among the studied populations.

## Conservation Genetic Implications and Traditional Utilization of Palms

Despite being one of the most emblematic groups of the tropics (see Johnson 1996), relatively few studies have used microsatellite data to address population/conservation genetic and systematic questions with palms (i.e., Gaiotto et al. 2003; Henderson et al. 2006; Couvreur et al. 2007; Shapcott et al. 2007, 2009; Conte et al. 2008; Hernández Ugalde et al. 2008; Cibrián-Jaramillo et al. 2009).

One of the major challenges in conservation biology is how to preserve the evolutionary potential of populations within a viable environment (Mills 2007). *Pseudophoenix ekmanii* is subject to damaging human pressures including habitat destruction and continuous unsustainable use. We are aware that there are no studies concerning the breeding system of *P. ekmanii*; therefore to what extent casual observations in botanic gardens suggesting self-compatibility can also apply to natural populations is unknown. Our results detected inbreeding and reduced genetic diversity within populations, an expected outcome from an autogamous species. However, inbreeding is also reported for other palms that are harvested for sugar: the fishtail palm (*Chamaedora ernesti-augusti* H. Wendl.) and the palmito palm (*Euterpe edulis* Mart.) (Gaiotto et al. 2003; Cibrián-Jaramillo et al. 2009). For these allogamous species, it appears that unsustainable harvesting is one of the reasons for this genetic pattern as a reduction of adult population size can result in genetic drift and inbreeding (Frankham et al. 2002). Based on our field observations, clearly *P. ekmanii* is regularly exploited in the wild; however, a question that still needs to be answered is to what extent the breeding system of this Caribbean palm is also an important factor for the population genetic results obtained in our study. Our data show that genetic drift and inbreeding are key players in the evolutionary genetics of this species; however, it is uncertain to what extent this is causing inbreeding depression. Our demographic studies show that seedlings and juveniles represent the largest proportion of individuals in the mainland populations. In addition, a significant proportion of individuals at Isla Beata also belong to these categories. These results indicate that despite unsustainable harvesting and other threats, populations of *P. ekmanii* still have the ability to regenerate. If given proper protection, adult population sizes should gradually increase.

Palm-derived sap is a source of sugar that remains relatively common in the tropics, subtropics, and the Mediterranean. Unsustainable palm tapping has driven the Chilean wine palm [*Jubaea chilensis* (Molina) Baill.] to the verge of extinction (González et al. 2009) and similar procedures are driving the decline of the Coyol palm (*Acrocomia mexicana* Karw.) in Mexico and Central America (Balick 1990). In contrast, sustainable extraction of sap in the Canary Islands has been the key factor for the conservation of the endemic *Phoenix canariensis* Hort. ex Chabaud on the island of La Gomera (Quintero 1985). This island has the most numerous and largest natural populations of this palm. Farmers from the Middle East and the

Indian subcontinent also harvest the sweet sap of the date palm (*P. dactylifera* L.) and of the sugar palm [*P. sylvestris* (L.) Roxb.] (Barrevel 1993; Kamaluddin et al. 1998) without killing individual palms. Other palms that are sustainably harvested are the Palmyra palm (*Borassus flabellifer* L.), the Sago palm [*Arenga pinnata* (Wurmb) Merr.], and the Jaggery palm (*Caryota urens* L.) in Tropical Asia (Fox 1977; Moge et al. 1991; De Zoysa 1992). We believe that finding a nondestructive harvesting method for *P. ekmanii* can be a major factor for the future of this species as indicated by these examples.

## Supplementary Material

Supplementary Figures 1 and 2 can be found at <http://www.jhered.oxfordjournals.org/>.

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