Population structure and genetic diversity of *Magnolia cubensis* subsp. *acunae* (Magnoliaceae): effects of habitat fragmentation and implications for conservation

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Abstract Genetic data on threatened plant populations can facilitate the development of adequate conservation strategies to reduce extinction risk. Such data are particularly important for species affected by habitat fragmentation such as *Magnolia cubensis* subsp. *acunae*, a Critically Endangered magnolia subspecies endemic to Cuba. Using genetic data from 67 individuals, we aimed to evaluate the effect of habitat fragmentation on two subpopulations in the Guamuhaya mountain range, in Topes de Collantes Protected Natural Landscape and Lomas de Banao Ecological Reserve. We characterize the structure and genetic diversity of these subpopulations, with the objective of managing their conservation more effectively. We used Landsat satellite images to determine land-cover types at the two locations and calculated indices of habitat fragmentation. For genetic analyses, we extracted DNA from the leaf tissue of individuals from the two subpopulations and used 11 microsatellite markers to genotype them. We calculated heterozygosity, allelic richness and the *F*-statistics, to evaluate genetic variability. The montane rainforest in Topes de Collantes was most affected by habitat fragmentation, with smaller patches of more irregular shapes, compared to submontane forest at this location and both montane and submontane forests in

Lomas de Banao. Genetic diversity was higher in Topes de Collantes, but we found no genetic differentiation between subpopulations. Our findings suggest the two subpopulations can be considered a single evolutionary unit and conservation entity. We propose to use individuals from both subpopulations for reinforcement to increase the overall genetic diversity of the subspecies.

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Introduction

Genetic data on threatened plant populations can facilitate the development of adequate conservation strategies to reduce extinction risk (Hedrick, 2001). This is particularly important for species affected by habitat fragmentation, which reduces the number of individuals per population and leads to isolation between populations, raising the probability of local extinction (Heinken & Weber, 2013).

The genus *Magnolia* is represented in Cuba by seven endemic taxa (Palmarola et al., 2016). The Critically Endangered subspecies *Magnolia cubensis* subsp. *acunae* Imkhan. is endemic to the Guamuhaya mountain range (González-Torres et al., 2013, 2016) and is threatened by deforestation and land conversion for cattle farming and coffee production. Recent studies on the distribution and conservation status of the subspecies' populations (Palmarola et al., 2012; Granado, 2015) reported that the two main subpopulations have 416 and 70 individuals, respectively. However, there is no information on genetic diversity, the degree of habitat fragmentation or the interaction between these factors. Our study aimed to evaluate the effect of habitat fragmentation on the structure and genetic diversity of the *M. cubensis* subsp. *acunae* subpopulations in the Guamuhaya mountain range, to support effective conservation management of this subspecies.
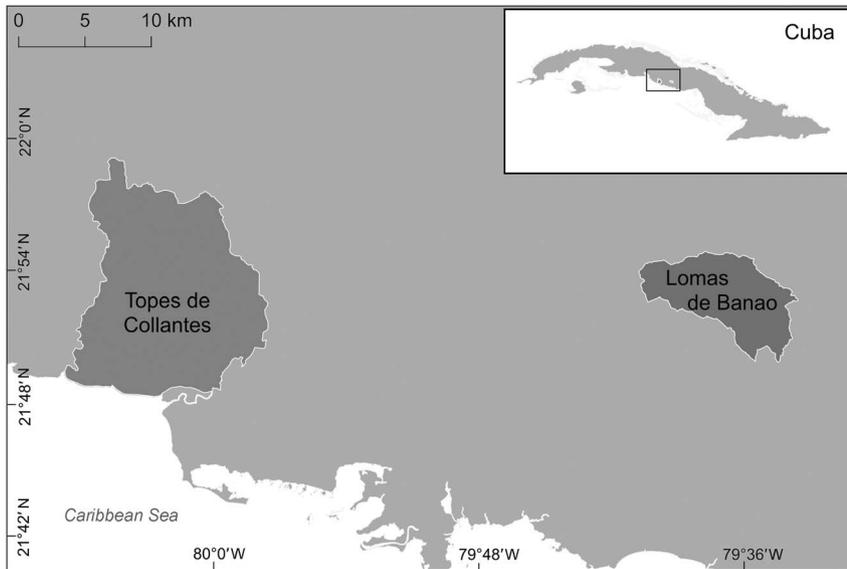
Study area

The study was carried out in the 201.35 km² Protected Natural Landscape Topes de Collantes and the 60.91 km² Ecological Reserve Lomas de Banao, in the Guamuhaya mountain range in central Cuba (Fig. 1; CNAP, 2014). Each of these protected areas contains one of the two main subpopulations of *M. cubensis* subsp. *acunae* (Granado, 2015).

The climate of Guamuhaya corresponds to the Western Caribbean subregion and is classified as humid tropical, although at high elevations it could be considered mild warm (Dominguez & Acosta, 2012). The variety of ecosystems in the region contain high levels of biodiversity, which, particularly with respect to the flora, makes the Guamuhaya one of the most biodiverse and endemics-rich localities in Cuba (Ruiz et al., 2011).

Methods

Fragmentation analyses

We used a Landsat 8 satellite image of Guamuhaya mountain range, taken in February 2014, with 30 m spatial resolution and 11 spectral bands (Roy et al., 2014), and cropped the areas of interest using a map of protected areas (CNAP, 2014). We extracted georeferenced points of the habitat types in which *M. cubensis* subsp. *acunae* occurs from the vegetation map of Estrada et al. (2012). The points were used for supervised categorization of satellite images using the maximum likelihood method. In this process, pixels with a known land-cover type, located within the training areas, are used to categorize pixels of unknown land-cover type. We used four land-cover categories: submontane rainforest, montane rainforest, water and matrix, the latter

defined as non-forested areas, including other vegetation, and agricultural and urban areas.

We converted the raster land-cover map to vector format and calculated seven fragmentation indices (Table 1). The variety of habitat patch shapes was classified according to Henao (1988) for the mean shape index and Hargis et al. (1998) for the mean patch fractal dimension index. We used *IDRISI Selva 12.1* (Eastman, 2012) for the supervised categorization and the *Patch Analyst* extension of *ArcGis 9.3* (Esri, Redlands, USA) for the fragmentation analyses.

Population genetic analyses

Sampling

We collected samples from 67 *M. cubensis* subsp. *acunae* individuals and stored them in self-sealed bags with silica gel. Of these, 58 were from Topes de Collantes (39 leaf samples from adult plants, 19 leaf samples from juveniles obtained from seeds) and 9 from Lomas de Banao (all leaf samples from adult plants), representing 10% and 13% of all individuals in these subpopulations, respectively. We collected seeds from fruits randomly selected from the Topes de Collantes subpopulation, using only one fruit per tree and one seed per fruit. The seeds were planted in nurseries in December 2014 and after two months, when the seedlings had six leaves, one leaf was collected for genetic analysis. Here we define population as the total number of individuals of the subspecies and subpopulation as the different population nuclei within the distribution area, according to IUCN (2001).

DNA extraction and genotypification

We extracted DNA from dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle & Doyle, 1990), with MagAttract

TABLE 1 Fragmentation indices used for the characterization of submontane and montane rainforest in the Protected Natural Landscape Topes de Collantes and the Ecological Reserve Lomas de Banao, two protected areas in the Guamuhaya mountain range, Cuba (Fig. 1).

Index	Description
Number of patches	Number of patches of a particular land-cover type
Mean patch size (km^2)	Average size of patches of a particular land-cover type
Patch size standard deviation (km^2)	Variability in relation to average size of patches in a particular class
Mean shape index	Average shape of patches of a particular land-cover type; the index varies from 1 to infinity, whereby 1 represents a compact patch & infinity an irregular patch
Mean perimeter-area ratio	Ratio of patch perimeter length to patch area
Mean patch fractal dimension	Mean shape of the patch, considering the ratio of patch perimeter to patch area
Edge density	Ratio of actual patch edge in relation to total length of edge possible

Suspension G solution mediated cleaning (Xin & Chen, 2012). We genotyped individuals with 11 microsatellite markers or simple sequence repeats (Table 3) developed on four Neotropical *Magnolia* species: *M. lacandonica* (MA39), *M. mayae* (MA40), *M. dealbata* (MA41) and *M. cubensis* subsp. *acunae* (MA42) (Veltjen et al., 2018), using the three-primer PCR multiplex method. PCR conditions and primer labelling followed Veltjen et al. (2018). Fragment analyses were executed by Macrogen Inc. (Seoul, South Korea) and we analysed the results in *Geneious 8.0.5* (Kearse et al., 2012), using the microsatellite plugin.

Simple sequence repeats marker testing

We calculated the deviation from Hardy–Weinberg equilibrium, linkage disequilibrium and the inbreeding coefficient per locus (F_{IS}) cf. Weir & Cockerham (1984) for each locus and for each subpopulation, using 10,000 dememorization steps, 100 batches and 5,000 iterations per batch in *Genepop 4.3* (Rousset, 2008). We calculated deviations of both the uncorrected (Waples, 2015) and (sequential Bonferroni) corrected p-values to the nominal level of $\alpha = 0.05$ for both analyses.

Population structure and genetic diversity

We applied the classification method according to Manel et al. (2005) and performed runs in *Structure 2.3* (Pritchard et al., 2000) under the following conditions: 10,000 Markov chain Monte Carlo replicates after an initial burn-in of 10,000, using correlated allelic frequencies and assuming the admixture model. To obtain probability values of allocation of individuals to each genomic group, we used five repetitions for each set K (number of groups), with K set to run from 1 to 8. We determined the most probable number of groups from the value of ΔK obtained according to the method of Evanno et al. (2005). The results were visualized in *Structure Harvester Web 0.6.94* (Earl & von Holdt, 2012). An individual was considered to be a member of a genetic group when its probability of belonging to that group was > 0.5.

We quantified genetic diversity within each subpopulation and within maturity classes (i.e. adult or juvenile individuals) using the number of alleles per locus (A), number of private alleles (A_p), allelic richness (A_R), observed heterozygosity (H_o), expected heterozygosity (H_e), percentage of polymorphic loci (P) and inbreeding coefficient (F_{IS}). We estimated genetic differentiation between subpopulations and across generations through pairwise comparisons of F_{ST} values (Weir & Cockerham, 1984) and interpreted these following the criteria of Hartl & Clark (1997). Measures of genetic diversity were calculated with *GenAlEx 6.1* (Peakall & Smouse, 2012). We calculated F_{ST} values, allelic richness and significant deviations from zero for F_{IS} values with *FSTAT 2.9.3.2* (Goudet, 2001).

We estimated genetic distances between individuals and subpopulations and carried out a principal coordinates analysis from the matrix obtained. To identify possible patterns of isolation by distance in the genetic differentiation of the studied subpopulations, we performed Mantel correlation tests with 10,000 permutations between genetic distances and geographical distances for all pairs of adult individuals of both subpopulations. We used *GenAlEx* for both analyses.

To investigate the occurrence of any bottlenecks in the sampled subpopulations, we characterized allele frequency distribution by locus, and evaluated deficit or excess of heterozygotes for the infinite alleles model, the two phase model and the stepwise mutation model, using the Wilcoxon test in *Bottleneck 1.2.02* (Cornuet & Luikart, 1996).

Results

Fragmentation analyses

The supervised categorization projected on the distribution map of the habitat of *M. cubensis* subsp. *acunae* in Topes de Collantes and Lomas de Banao showed submontane rainforest covered a larger area than montane rainforest (Fig. 2). Lomas de Banao had greater landscape homogeneity and a smaller area categorized as matrix (other vegetation, and agricultural and urban areas). In Topes de

TABLE 2 Fragmentation indices of submontane and montane rainforest in the Protected Natural Landscape Topes de Collantes and the Ecological Reserve Lomas de Banao in the Guamuhaya mountain range, Cuba.

Location & land-cover type	No. of patches	Mean ± SD patch size (km²)	Mean shape index	Mean perimeter–area ratio	Mean path fractal dimension	Edge density	Total area (km²) of all patches
Topes de Collantes							
Submontane rainforest	114	0.0224 ± 0.15	1.87	2405.17	1.52	94.29	64.59
Montane rainforest	115	0.0029 ± 0.01	2.36	2289.99	1.54	53.93	39.20
Lomas de Banao							
Submontane rainforest	169	0.1671 ± 1.12	1.98	1003.87	1.40	78.88	28.24
Montane rainforest	107	0.2054 ± 1.89	2.06	1775.94	1.40	52.39	21.91

TABLE 3 Locus names, repeat motifs and measures of genetic diversity of the 11 simple sequence repeat markers designed for the genetic characterization of *Magnolia cubensis* subsp. *acunae* in the Topes de Collantes and Lomas de Banao subpopulations.

		Topes de Collantes[1]						Lomas de Banao[1]					
Locus name	Repeat motifs (5′–3′)	A	A_R	H_o	H_e	P	$F_{IS\ (W\&C)}$	A	A_R	H_o	H_e	P	F_{IS}
MA39_333	AGG(5)	3	2.358	0.351	0.333	1.000	−0.042	2	2.000	0.222	0.346	0.341	0.407
MA41_264	AG(15)	9	7.020	0.718	0.858	0.032	0.156	3	2.993	0.333	0.438	0.106	0.294
MA41_076	AG(26)	2	1.205	0.026	0.025	NI		1	1.000	NI			
MA42_255	AG(26)	6	4.228	0.513	0.695	0.012	0.174	3	3.000	0.625	0.617	0.229	0.054
MA42_274	AG(18)	6	3.783	0.564	0.512	0.029	−0.088	2	2.000	0.000	0.219	0.067	1.000
MA42_083	AAG(17)	9	4.807	0.711	0.618	0.507	−0.137	6	5.765	0.778	0.722	0.414	−0.018
MA40_045	AG(13)	9	5.985	0.684	0.758	0.240	0.111	4	3.882	0.444	0.599	0.109	0.312
MA42_166	AG(13)	4	3.048	0.541	0.449	0.806	−0.191	2	1.993	0.222	0.198	1.000	−0.067
MA42_063	AG(14)	11	7.710	0.895	0.853	0.567	−0.035	5	4.765	0.667	0.525	1.000	−0.215
MA42_279	AG(14)	5	3.104	0.385	0.575	0.002*	0.342*	3	2.889	0.444	0.512	0.638	0.189
MA42_265	AAG(12)	2	1.889	0.179	0.204	0.405	0.134	2	2.000	0.222	0.346	0.341	0.407

[1]A, average number of alleles per locus; A_R, allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity; P, exact probability of Hardy–Weinberg equilibrium test; $F_{IS\ (W\&C)}$, F_{IS} values of Weir & Cockerham (1984) per locus; *$p < 0.005$ Bonferroni corrected probability were considered statistically significant; NI, non-informative comparison because it is a monomorphic locus or presents low values of H_o and H_e.

Collantes the matrix land-cover type was primarily in the south and to a lesser extent at higher altitude, in the central part of the protected area. In Lomas de Banao, matrix areas were mainly in the submontane rainforest regions, whereas the montane rainforest was less fragmented.

The number of patches in the four Subpopulation × Class categories was 107–169 (Table 2); in Topes de Collantes patches of both forest types were smaller and less variable in their size compared to Lomas de Banao.

The mean shape index indicated that for the submontane rainforest, the most common patch form was rectangular-oblong, whereas for the montane rainforest, most patches could be considered amorphous. The mean path fractal dimension for both forest types showed complex forms analogous to fractal objects (Table 2).

Population genetic analyses

Simple sequence repeats marker

We found no significant deviations from Hardy–Weinberg equilibrium for 10 out of the 11 tested loci. Only MA42_279 in Topes de Collantes significantly deviated from Hardy–Weinberg proportions. The linkage analysis showed that the loci were not in allelic association, with the exception of MA40_045 and MA42_279; MA42_279 was discarded from all subsequent analyses.

Of the 11 microsatellite loci, 10 were polymorphic for both subpopulations; MA41_076 was monomorphic in Lomas de Banao. We found 68 alleles among all loci (1–11 per locus), with a mean of 6.18 alleles per locus. Of the 24 rare alleles found, 22 (91.67%) were private alleles of Topes de Collantes. Detailed information on the genetic diversity estimates per locus is given in Supplementary Table 1.

Population structure and genetic diversity

The optimal value for K was 2 (Fig. 3). Of the adult trees, 75% were assigned to a genetic group that aligned with their sampling location. All individuals of Lomas de Banao clustered in genetic group 2, together with 30.77% of the adult individuals and 100% of the juveniles of Topes de Collantes. This means 12 of 39 adult individuals from Topes de Collantes had a probability of > 50% to belong to genetic group 2,
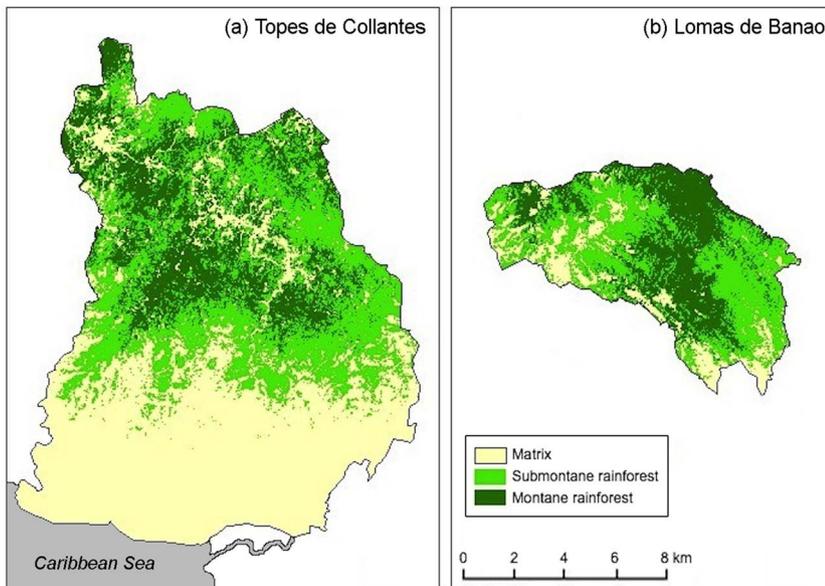
FIG. 2 Land-cover types in (a) Protected Natural Landscape Topes de Collantes, (b) Ecological Reserve Lomas de Banao, Cuba, resulting from satellite Landsat image classification (Roy et al., 2014). Matrix refers to other vegetation, and agricultural and urban areas.
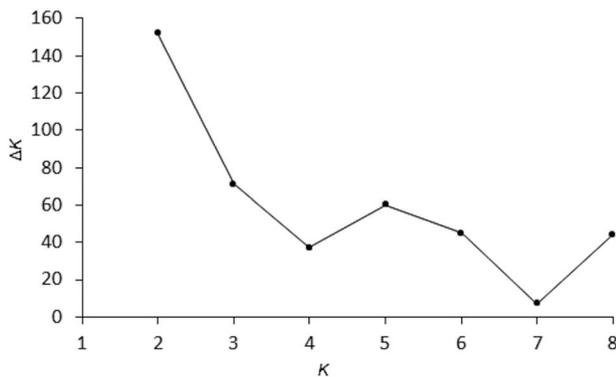
FIG. 3 ΔK values obtained with K (number of groups) from $K = 2$ to $K = 8$, and five simulations per analysis, visualized with *Structure Harvester Web 0.6.94* (Earl & von Holdt, 2012), to determine the most probable number of groups of *Magnolia cubensis* subsp. *acunae* individuals from the Topes de Collantes and Lomas de Banao subpopulations, according to the method of Evanno et al. (2005).

which aligns with the individuals of Lomas de Banao. This analysis did also not distinguish between juveniles from Topes de Collantes and adults from Lomas de Banao within the second genetic group (Fig. 4).

Values for all diversity measures were lower in Lomas de Banao than Topes de Collantes. Similarly, juveniles of Topes de Collantes were less diverse than the adult population of this locality. The inbreeding coefficient (F_{IS}) was higher, and statistically significant, in Lomas de Banao compared to Topes de Collantes (Table 4).

No genetic differentiation was apparent between the two subpopulations using the F_{ST} fixation index (Table 5). The principal coordinates analysis showed no differential clustering for individuals from different subpopulations or maturity classes (Fig. 5). The Mantel correlation test had a low correlation coefficient ($r = 0.034$), considered statistically non-significant ($p = 0.32$), indicating no effect of distance on genetic variation.

The Wilcoxon test for Topes de Collantes showed differences between the estimated heterozygosity value at equilibrium and that obtained by simulating diversity under the infinite alleles model. For Lomas de Banao, the analysis also revealed differences for the two stage mutation model and stepwise mutation model (Table 6). The distribution of allelic frequencies displays an L-shaped distribution (with a high proportion of low frequency alleles and a few frequency alleles near 1) for Topes de Collantes, which is not the case for Lomas de Banao (Fig. 6).

Discussion

Fragmentation analyses

Habitat fragmentation was most severe in the montane rainforest of Topes de Collantes, with smaller, irregularly shaped patches and greater patch perimeter to area ratio. Edge effects and the quality of the surrounding area also influence the characteristics of remnant habitat patches (Heinken, & Weber, 2013). A matrix with a similar structure to a remnant forest fragment will have less influence than one with a different structure (Fischer & Lindenmayer, 2007). In the montane rainforest, where many fragments are surrounded by submontane rainforest, the matrix effect is thus smaller, whereas the submontane rainforest is mainly surrounded by matrix areas.

The high degree of forest fragmentation was expected, given the history of land-use changes in the area. Coffee has been produced in Topes de Collantes since the 19th century, resulting in selective logging across large areas and the replacement of natural vegetation by a monoculture.
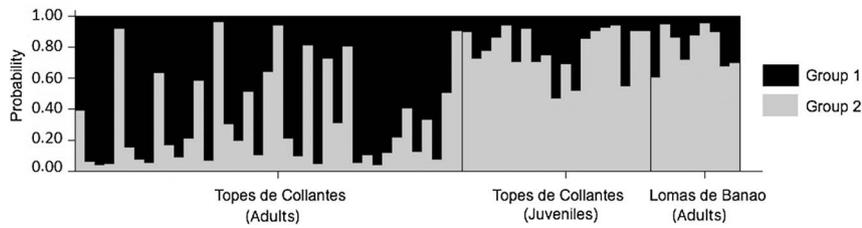
Fig. 4 Probability of genetic group allocation of *M. cubensis* subsp. *acunae* individuals of Topes de Collantes and Lomas de Banao subpopulations, inferred at *K* = 2 based on allelic frequencies on simple sequence repeats data.

Table 4 Measures of genetic diversity in adult and juvenile individuals of *M. cubensis* subsp. *acunae* in the Topes de Collantes and Lomas de Banao subpopulations.

Subpopulation	No. of individuals	Genetic diversity measures[1]						
		A (range)	A_R	H_o	H_e	HW	F_{IS}	P (%)
Topes de Collantes (adults)	39	6.000 (2–11)	4.103	0.506	0.535	2	0.037	100
Topes de Collantes (juveniles)	19	3.545 (2–8)	3.100	0.435	0.461	2	0.067	100
Lomas de Banao (adults)	9	3.000 (1–6)	2.935	0.360	0.411	0	0.183*	90.91

[1]A, mean number of alleles; A_R, allelic richness; H_o, mean observed heterozygosity; H_e, mean expected heterozygosity; HW, number of loci deviated from the proportions of Hardy–Weinberg; F_{IS}, population inbreeding coefficient, significant deviations from zero are indicated with * (p = 0.05); P, percentage of polymorphic loci.

Table 5 Fixation index F_{ST} (above diagonal) and F_{IT} (below diagonal) values, determined from 10 microsatellite loci, for all paired comparisons, of sampled subpopulations of *M. cubensis* subsp. *acunae*.

Subpopulation/ generation	Topes de Collantes (adults)	Topes de Collantes (juveniles)	Lomas de Banao (adults)
Topes de Collantes (adults)		0.037	0.043
Topes de Collantes (juveniles)	0.182		0.024
Lomas de Banao (adults)	0.164	0.262	

Furthermore, the wood of native tree species such as *M. cubensis* subsp. *acunae* is also used to build coffee plantation infrastructure (Domínguez et al., 2012).

Population genetic analyses

Simple sequence repeats markers

Deviations from the Hardy–Weinberg equilibrium found in the MA42_279 locus in both adults and juveniles of Topes de Collantes can be explained by the presence of inbreeding. F_{IS} was significantly larger than zero, which indicates a deficit of heterozygotes. Although we cannot discard this phenomenon, the presence of the so-called Wahlund effect (i.e. a reduction of heterozygosity in a population caused by subpopulation structure) should affect the loci equally (Waples, 2015). It therefore seems most plausible that apparent inbreeding at one of 11 loci is a chance effect, rather than a result of actual inbreeding, which should be evident across most loci. Another possible interpretation is the presence

of null alleles or errors in genotyping (Pompanon et al., 2005). However, in our study the markers that presented such errors were eliminated from the final selection (Veltjen et al., 2018).

Population structure and diversity

The analysis of population structure showed two genetic groups that partly corresponded with the two adult demographic groups defined prior to the analysis, and diversity analyses showed absence of differentiation between subpopulations. The subpopulation in Topes de Collantes was more diverse, whereas that in Lomas de Banao showed lower values for all diversity indices. This could be a result of the different subpopulation sample sizes. This is supported by the fact that 50% of the loci used in our study showed higher values for genetic diversity indices with samples from 39 adult plants from Topes de Collantes, compared to the analyses carried out by Veltjen et al. (2018) on 20 individuals. The Topes de Collantes subpopulation has the highest number of individuals, with 75% of the global population (Granado, 2015), whereas Lomas de Banao includes 15% of the global population. The sample size was thus larger for Topes de Collantes compared to Lomas de Banao, but both sample sizes are considered comprehensive and cover a similar proportion of the known individuals per subpopulation. However, allelic richness by locus for a fixed sample size (Allendorf et al., 2013), was also higher for Topes de Collantes in most loci evaluated.

The diminution of the diversity across generations in Topes de Collantes may reflect a decrease of pollen and seed dispersal. Pollen dispersal distances for trees visited by small insects such as beetles in closed-canopy forests
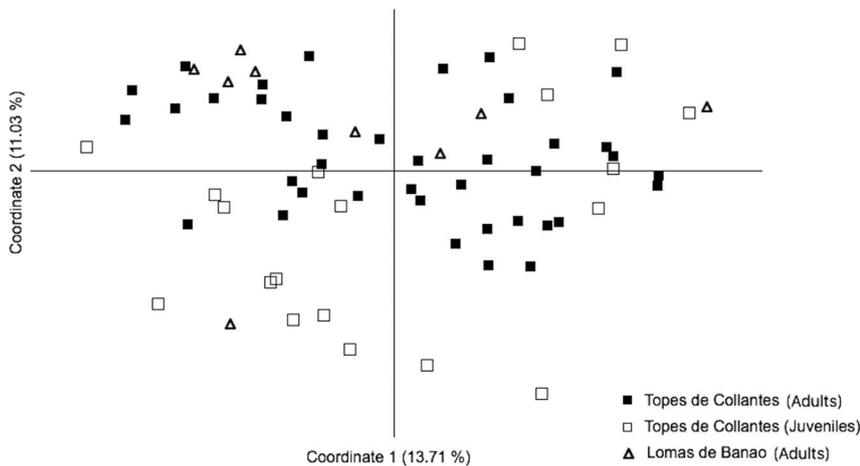
Fig. 5 Principal coordinates analysis of genetic distances matrix with 10 microsatellite markers in *M. cubensis* subsp. *acunae* in Guamuhaya, Cuba.

Table 6 Probability of deficiency and excess heterozygosity (H_e) observed at equilibrium, compared to that estimated under the infinite alleles model, two phase model and stepwise mutation model for the subpopulations of *M. cubensis* subsp. *acunae*.

| | Topes de Collantes | | Lomas de Banao | |
Model	Deficiency H_e	Excess H_e	Deficiency H_e	Excess H_e
Infinite alleles model	0.995	0.007*	1.000	0.001*
Two phase model	0.984	0.042	1.000	0.001*
Stepwise mutation model	0.903	0.116	1.000	0.001*

*p < 0.01 was considered statistically significant.

often do not exceed 300 m (Dick et al., 2008), and it is possible that the loss of one genetic group in the juveniles of Topes de Collantes compared to the adult trees of this subpopulation is a result of reduced pollen movement. However, given the mix of c. two-thirds genetic group 1 and one-third genetic group 2 in the adult generation, a similar representation was expected in the juveniles. There are three potential explanations for this:

(1) Gene flow between subpopulations: the geographical proximity of the subpopulations increases the gene flow mediated by fruit dispersal and tends to homogenize the present genetic variation. In a fragmented habitat, the dispersion is affected by both the isolation of patches and the movement of the dispersers (Laaka-Lindberg et al., 2003). The potential dispersers of this subspecies, the Cuban trogon *Priotelus temnurus*, fieldfare *Turdus pilaris* and western spindalis *Spindalis zena*, are permanent or seasonal residents in Cuba (Garrido & Kirkconnell, 2011) and could move between the two subpopulations, which are 33 km apart. However, detailed studies of the ecology, behaviour and foraging strategies of potential seed dispersers in this mountainous region are required to elucidate the flow of diaspores between the subpopulations. It is unlikely that pollen moves between the subpopulation because there are only few reports of pollen travelling distances > 10 km for insect-pollinated species (Petit & Hampe, 2006).
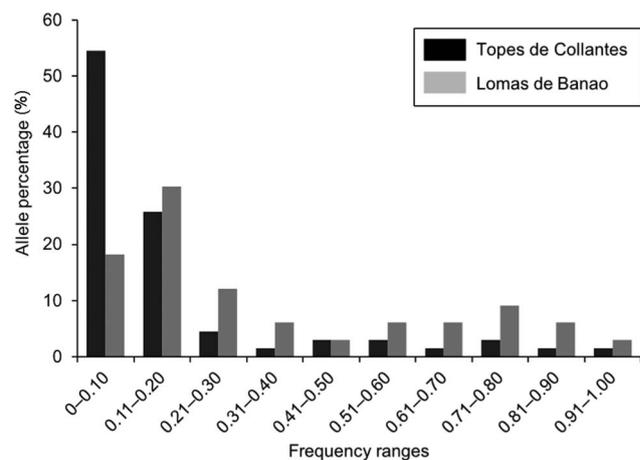
Fig. 6 Allele percentage distribution by frequency ranges for 48 adult individuals of subpopulations of *M. cubensis* subsp. *acunae* in Guamuhaya, Cuba.

(2) Evolutionary history: the simplest interpretation of small genetic distances between subpopulations is that they share a recent common ancestor. The homogeneity in Lomas de Banao could be an indication of the founder effect (Slatkin, 2004); i.e. a few, genetically similar individuals from Topes de Collantes could have founded this subpopulation. However, it is more likely that both subpopulations once belonged to a single, larger population.

(3) The species' crossing system: cross-pollination is one of the mating systems of *Magnolia* (Thien, 1974), guaranteeing that a few migrants per generation are sufficient to counter genetic differentiation (Holsinger & Weir, 2009). These species are thus generally characterized by low genetic differentiation between populations. This could explain the genetic similarity between the two subpopulations of *M. cubensis* subsp. *acunae*.

Topes de Collantes tested positive for a past bottleneck, assuming the infinite alleles model. However, for microsatellite markers, the stepwise mutation model is considered more appropriate (Putman & Carbone, 2014), which would indicate no trace of a past bottleneck for this subpopulation. Furthermore, the distribution of allelic frequencies in Topes de Collantes is typical for a population that has not suffered a recent bottleneck (Allendorf et al., 2013). In Lomas de Banao the existence of a bottleneck is possible considering the low number of individuals, the low genetic diversity and the high values of inbreeding. However, the results should be interpreted with caution because the Wilcoxon test requires at least 10 individuals (Cornuet & Luikart, 1996) and could be compromised by the small sample size from Lomas de Banao (9 individuals).

Implications for conservation

The principal consequences of ongoing habitat fragmentation are progressive reduction of subpopulation sizes and increased distance between habitat fragments, which can affect a population's genetic variation, heterozygosity, inbreeding, gene flow and genetic divergence between subpopulations (Heinken & Weber, 2013). These potential genetic consequences of fragmented habitats are reflected in our results, with high levels of inbreeding and low genetic diversity in Lomas de Banao, reduction of the genetic diversity across generations in Topes de Collantes and low differentiation between subpopulations. Our findings merit a new approach to the conservation and management of *M. cubensis* subsp. *acunae*.

The Lomas de Banao subpopulation should be considered a priority for the population reinforcement actions currently being carried out in the Guamuhaya region. The results of the structure analyses, principal coordinates analysis plots and F_{ST} values predict there is no marked genetic structure in the subpopulations. Our findings suggest the two subpopulations can be considered a single evolutionary unit and conservation entity (Moritz, 1994). Granado (2015) proposed nine areas for *Magnolia* population reinforcement in Guamuhaya, taking into account the species' modelled (potential) distribution, the area's protection status and landscape use. From these analyses, and considering the similarity between the subpopulations, we suggest using individuals from both subpopulations for such reinforcement actions.

Despite past habitat fragmentation and loss of natural vegetation cover in these protected areas, the improvement of the legal framework on biological diversity in Cuba in recent years, which prohibit agricultural, forestry and fruit production in protected areas and set goals to reduce the impact of agroforestry, will support the recovery of the studied subpopulations and the ecosystems to which they belong.

Historically, there was little interest in conserving *M. cubensis* subsp. *acunae* in Topes de Collantes, but after 12 years of conservation projects focussing on this subspecies, awareness has increased. People are now interested in protecting this subspecies and its habitat because it represents a symbol for the community and its cultural identity. Many farmers cultivate coffee in the shade of *M. cubensis* subsp. *acunae* and claim that coffee quality is superior when shade is provided by native species such as *Magnolia*. Restoration of the montane and submontane rainforest, population reinforcement and support for these matters amongst local communities are essential for the long-term improvement of the conservation status of this endemic Cuban subspecies.

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