Ciencia y Tecnología, 39: 35-44, 2025
ISSN: 2215-5708

PRELIMINARY GENETIC DIVERSITY DATA OF TWO NEOTROPICAL TREES IN THE OLD-GROWTH FOREST OF COSTA RICA

Heidy M. Villalobos-Barrantes[1,2*], Federico J. Albertazzi[1,3], Gabriel Macaya[1,2].

[1] Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, 11501, San José, Costa Rica.
[2] Escuela de Química, Universidad de Costa Rica, 11501, San José, Costa Rica.
[3] Escuela de Biología, Universidad de Costa Rica, 11501, San José, Costa Rica.

Received October 2024; accepted August 2025

Abstract

Minquartia guianensis and *Hyeronima alchorneoides* are two valuable woody species in the Costa Rican tropical wet forest. Little is known about the genetic diversity of these species. Using 20 random amplified polymorphic DNA (RAPD) primers, we estimated the levels of diversity and variance within and among three distinct areas for each species within La Selva Biological Station. Genetic diversity was calculated using classical and Bayesian estimators, and their values were similar. The results indicated high levels of variance within patches (78% in *M. guianensis* and 87% in *H. alchorneoides*). We also estimated the genetic distance, and the dendrograms indicated a lack of differentiation between the patches for both species. Our data are consistent with studies of genetic variation in other tropical trees.

Key words: genetic diversity, RAPD, La Selva, dioecious tree, tropical tree, molecular markers.

Resumen

Minquartia guianensis y *Hyeronima alchorneoides* son dos especies de árboles maderables del bosque tropical húmedo de Costa Rica. Poco se sabe sobre la diversidad genética de estas especies. Utilizando 20 imprimadores de ADN polimórfico amplificado al azar (RAPD), estimamos los niveles de diversidad y varianza dentro y entre tres áreas de la Estación Biológica La Selva para cada especie. La diversidad genética se calculó mediante estimadores clásicos y bayesianos, y los resultados fueron similares. Estos indicaron una alta varianza dentro de los parches (78% para *M. guianensis* y 87% para *H. alchorneoides*). Además, estimamos la distancia genética, y los dendogramas indicaron una falta de diferenciación entre los parches para ambas especies. Nuestros resultados son consistentes con estudios sobre la diversidad genética en otras especies de árboles tropicales.

Palabras clave: diversidad genética, RAPD, La Selva, árbol dioico, árbol tropical, marcadores moleculares.

DOI: 10.15517/9txeeh12

I. INTRODUCTION

Hyeronima alchorneoides Allemao (Euphorbiaceae) is a dioecious, non-pioneer tree. Its distribution ranges from Mexico to Peru and Bolivia[1] and from sea level to 900 meters above sea level (masl) in the wet forest. *H. alchorneoides* has economic importance due to the high quality of its wood and is widely used in reforestation programs in the northeastern region of Costa Rica. The hermaphroditic Neotropical tree *Minquartia guianensis* Aublet (Olacaceae) has a broad geographic range, from southern Nicaragua to Peru and Brazil, and from sea level to 1000 masl[2,3]. Its wood is prized for rural construction in the humid lowlands because of its resistance to fungi and termites. Traditional medical applications have also been reported for this species[4,5].

Traditional reforestation efforts have focused on the maintenance of genetic diversity over geographical distances[6,7]. Genetic differentiation in populations within fragmented forests has also been analyzed in plants with different reproductive strategies, such as *Swietenia macrophylla*[8], *Enterolobium cyclocarpum*[9], and *Samanea saman*[10].

* Corresponding author: heidy.villalobosbarrantes@ucr.ac.cr

The number of studies on tropical tree genetic diversity has increased in recent years due to decreasing biodiversity and conservation policies[11–16]. One indicator related to the loss of biodiversity is genetic diversity, and recent arguments support a link between genetic diversity and population fitness[17,18].

Insights into the relative gene diversity among and within populations would be useful in developing strategies for the conservation of plant genetic resources. Molecular markers have been used to evaluate gene diversity in plants. In particular, RAPDs have been used to determine genetic variation in tropical trees[11,19–21] and dioecious plants[22,23]. Disadvantages of RAPDs include dominant markers and co-migrating bands[20]. However, scoring an appropriate number of RAPD fragments (usually >30) and using suitable analysis techniques[24,25] can reduce the impact of these limitations.

We provide preliminary information about genetic diversity and differentiation in two tropical trees, *Minquartia guianensis* (Olacaceae), known as "Manú," and *Hyeronima alchorneoides* Allemao (Euphorbiaceae), known as "Pilón," located in separate areas within a continuous old-growth wet forest at La Selva Station, using random amplified polymorphic DNA (RAPD)[26].

The tropical wet forest of La Selva is one of the most studied sites in Costa Rica[27–32]. A long-term project has studied Pilón, Manú, and four other canopy trees for more than 15 years[29,32]. Data on flowering patterns from 302 tree species have been collected[33]. Studies on population structure in the palm *Iriartea deltoides*[34] and in the monoecious tree *Symphonia globulifera*[35] have also been performed.

II. MATERIALS AND METHODS

Study Locality and Sample Procedure.— Leaf samples of *Minquartia guianensis* and *Hyeronima alchorneoides* were collected from trees along three different trails within the old-growth forest of La Selva Biological Station in Sarapiquí, Heredia (10° 26' N, 84° 00' W, elevation 35 to 150 m a.s.l.), in the Caribbean lowlands of Costa Rica (**Figure 1**). To define a patch, the distance within trails had to be at least 1 km. Samples were taken from trees in each trail rather than through strictly random sampling. These trees were already mapped in a database using Geographic Information System (GIS) at La Selva (**Figure 1**). We collected leaves from 41 *M. guianensis* individuals, distributed across three patches: 13 individuals from both the "Camino Experimental" (CE) and "Sendero Suroeste" (SSO) patches, and 15 from the "Camino Central" (CC) patch. For *H. alchorneoides*, leaves were collected from 41 individuals in three patches: 15 trees from the CE and CC patches, and 11 trees from the "Sendero Holdridge" (SHO) patch. Samples were folded in aluminum foil, kept on dry ice, transported to the Centro de Investigación en Biología Celular y Molecular (CIBCM) in San José, and stored at -70°C until DNA extraction.

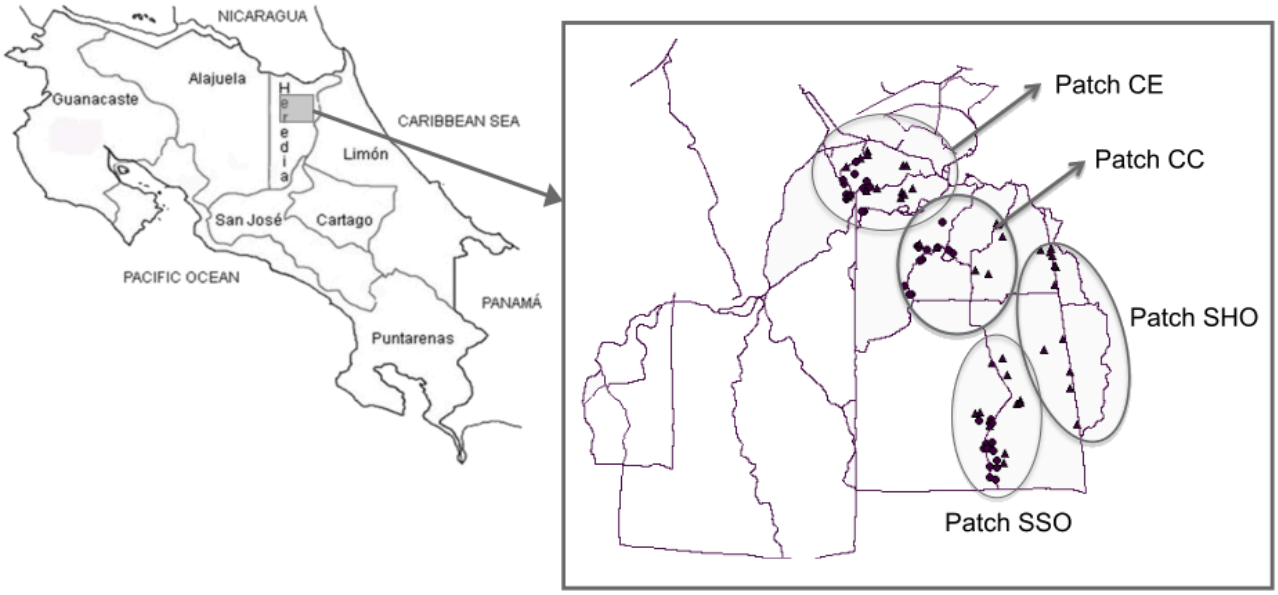

Figure 1: Map of La Selva Biological Station in Costa Rica. Circles represent Minquartia guianenesis individuals and triangles depict Hyeromina alchorneoides trees sampled in *this work. From Clark (1998).*

DNA EXTRACTION. Total DNA for PCR amplification was extracted from frozen leaves (approximately 1 g) following the CTAB protocol described by Lodhi et al.[36] and modified by Villalobos[37]. DNA quality was assessed by visualization on 0.8% agarose gels stained with ethidium bromide, and DNA concentration was measured using a Beckman UV-Vis 7500 spectrophotometer.

RAPD PROTOCOL. PCR amplification was carried out in a 15 µL reaction mix containing 25 ng DNA, 1× PCR Buffer, 2.0 mM $MgCl_2$, 0.1 mM dNTPs, 0.05 U/µL Taq Polymerase (Perkin Elmer, USA), and 0.25 µM primer (Serie OPC, Kit C, Operon Technologies Inc., USA). Controls containing all the above except template DNA were run in parallel for each set of reactions, using a Rapidcycler 1002 (Idaho Technologies, Inc., USA). Optimal amplification conditions for *M. guianensis* and *H. alchorneoides* RAPDs were one cycle of 2 minutes at 94°C (denaturation) followed by 40 cycles of 5 seconds at 92°C (denaturation), 7 seconds at 36°C (annealing), and 70 seconds at 72°C (extension), with a final cycle of 4 minutes at 72°C. Each PCR was repeated twice to test for PCR artifacts and ambiguous band readings.

GEL ELECTROPHORESIS. RAPDs products were visualized using a UV transilluminator after being separated on 1.8% agarose gels, stained with ethidium bromide, and photographed. The presence or absence of each fragment was recorded in a binary data matrix.

DATA ANALYSIS. POPGENE ver. 1.31[38] software was used to estimate the percentage of polymorphism, Nei's gene diversity[39], and Shannon's diversity index. HICKORY ver. 1.0 was used to estimate gene diversity (average panmictic heterozygosity) using a Bayesian method. We used all recommended default settings described by Holsinger et al.[25] and Holsinger and Wallace[40]. The Bayesian estimator of genetic diversity was calculated for each of the four models:

1) full model (includes priors for f, pI, and q)
2) $f = 0$ (assumes no inbreeding)
3) $q^B = 0$ (assumes no population structure)
4) f-free (allows for the incorporation of uncertainty about f into the analysis).

The four models were applied to the data and evaluated based on measures of deviance criterion (DIC and Dbar). An analysis of molecular variance (AMOVA) was calculated from the pairwise genetic distance (f_{ST} analogous to F_{ST}) measures using Winamova ver. 1.55[41], to estimate variance components and percent of variance: i) within patches and ii) between patches, for each species. We obtained estimators of F_{ST} under a random-effects model of population sampling, q^B, and G_{ST}-B, a Bayesian analogue for the three patches, using HICKORY as described by Holsinger and Wallace[40].

A Mantel test was used to estimate the association between the matrix of geographic distance (m) and the matrix of pairwise genetic distance[42] using GenAlEx version 6.0[43] with 999 permutations. The binary data matrix was used to determine genetic distance according to Nei and Li[44] between each individual and patch using TREECON ver. 1.3 for Windows[45]. Dendrograms for each species were inferred using the UPGMA clustering method.

III. RESULTS AND DISCUSSION

RAPD PATTERNS AND GENETIC DIVERSITY. Ten primers produced 57 polymorphic fragments in *M. guianensis*, with sizes ranging from 200 to 1600 bp. In *H. alchorneoides,* nine primers generated 46 polymorphic bands (Table 1). The percentage of polymorphic loci varied from approximately 79% to 98% in *M. guianensis* and from 74% to 100% in *H. alchorneoides* (Table 1). The diversity parameters (Nei's gene diversity, Shannon's diversity index, and the Bayesian estimator) were highest in the CC area for *M. guianensis*. For *H. alchorneoides*, Nei's gene diversity and Shannon's diversity index were highest in the CE area, while the Bayesian estimator had the highest value in the CC area (Table 1). Shannon's diversity index exhibited the greatest values compared with the other parameters in both species.

Table 1 **Genetic variation in three patches of Minquartia guianensis and Hyeronima alchorneoides based on RAPD data**

M. guianensis			No. of polymorphic amplification products (proportion)			
Primer	Sequence	Band size range (bp)	No. of loci	Area CE	Area CC	Area SSO
OPC-1	TTCGAGCCAG	500-1600	5	5(1.00)	5(1.00)	5(1.00)
OPC-5	GATGACCGCC	500-1000	5	4(0.80)	5(1.00)	4(0.80)
OPC-6	GGGGGTCTTT	500-1050	4	2(0.50)	4(1.00)	4(1.00)
OPC-8	TGGACCGGTG	500-2000	7	5(0.71)	7(1.00)	6(0.85)
OPC-11	AAAGCTGCGG	500-1600	8	7(0.87)	7(0.87)	8(1.00)
OPC-12	TGTCATCCCC	500-1500	8	8(1.00)	8(1.00)	8(1.00)
OPC-14	TGCGTGCTTG	400-1600	4	2(0.50)	4(1.00)	4(1.00)
OPC-15	GACGGATCAG	400-1600	6	4(0.67)	6(1.00)	5(0.83)
OPC-16	CACACTCCAG	200-1000	5	3(0.60)	5(1.00)	5(1.00)
OPC-20	ACTTCGCCAC	300-1000	5	5(1.00)	5(1.00)	5(1.00)
Total			57	45	56	54
% Polymorphism				78.9	98.2	94.7
Nei's gene diversity				0.3216	0.3506	0.3381
Shannon's diversity index				0.4717	0.5264	0.5088
Bayesian gene diversity				0.3672	0.3775	0.3728

H. alchorneoides						
Primer	Sequence	Band size range (bp)	No. of loci	CE	CC	SHO
OPC-2	GTGAGGCGTC	500-1000	5	5(1.00)	5(1.00)	5(1.00)
OPC-3	GGGGGTCTTT	500-1300	4	4(1.00)	4(1.00)	4(1.00)
OPC-4	CCGCATCTAC	500-1100	4	4(1.00)	4(1.00)	3(0.75)
OPC-5	GATGACCGCC	600-1300	5	5(1.00)	5(1.00)	5(1.00)
OPC-7	GTCCCGACGA	200-1600	7	7(1.00)	7(1.00)	2(0.28)
OPC-8	TGGACCGGTG	200-1400	6	6(1.00)	6(1.00)	5(0.83)
OPC-16	CACACTCCAG	300-1600	5	5(1.00)	4(0.80)	3(0.75)
OPC-19	GTTGCCAGCC	400-1000	5	5(1.00)	5(1.00)	3(0.60)
OPC-20	ACTTCGCCAC	300-1000	5	5(1.00)	4(0.80)	4(0.80)
Total			46	46	44	34
% Polymorphism				100	95.6	73.9
Nei's gene diversity				0.3757	0.3679	0.2587
Shannon's diversity index				0.5552	0.5410	0.3889
Bayesian gene diversity				0.3755	0.3764	0.3100

bp: base pair

RAPD data for both species were evaluated using a Bayesian approach under four models (Table 2). The full model, which displayed the lowest DIC, was preferred over the alternatives with $f = 0$ or $q^B = 0$.

Table 2: **Posterior mean, standard deviation (SD) and 95% confidence interval (CI) of f and parameters of the posterior distributions for RAPD data in M. guianensis and H. alchorneoides under four alternative Bayesian models.**

Posterior mean, standard deviation (SD) and 95% credible interval (CI) of *f* and q^B for RAPD data under four alternative models — Parameters of the posterior distributions under four alternatives

M. guianensis

	f			q^B			Bayesian models			
Model	**Mean**	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Dbar**	**Dhat**	**pD**	**DIC**
Full	0.6596	0.1747	0.3112-0.9686	0.1442	0.0221	0.1037-0.1882	618.9762	501.5201	117.4561	736.4324
f=0				0.1130	0.0179	0.0815-0.1509	616.3481	489.5646	126.7834	743.1315
q^B=0	0.7376	0.1378	0.4538-0.9725				906.9040	852.7565	54.1475	961.0515
f free	0.5212	0.2823	0.0398-0.9701	0.1414	0.0225	0.0997-0.1890	625.2660	501.3784	123.8876	749.1536

H. alchorneoides

	f			q^B						
	Mean	**SD**	**95% CI**	**Mean**	**SD**	**95% CI**	**Dbar**	**Dhat**	**pD**	**DIC**
Full	0.9181	0.0735	0.7241-0.9977	0.1601	0.0263	0.1135-0.2165	493.7494	398.9903	94.7591	588.5084
f=0				0.1182	0.0217	0.0809-0.1641	491.5657	393.9195	97.6462	589.2119
q^B=0	0.9288	0.0638	0.7602-0.9979				734.7579	691.1161	43.6418	778.3997
f free	0.5046	0.2922	0.0285-0.9756	0.1468	0.0268	0.0987-0.2015	495.6486	398.0642	97.5844	593.2330

The mean distance to the nearest individual was 47.9 m for *M. guianensis* and 90 m for *H. alchorneoides*. The Mantel test revealed that the matrix of pairwise genetic distances did not significantly correlate with the matrix of pairwise geographic distances in *M. guianensis* (r = 0.3678, P < 0.02) and *H. alchorneoides* (r =0.1058, P < 0.025P). These results do not support isolation by distance. Dendrograms generated by UPGMA clustering (Nei, 1973; Nei & Li, 1979) showed different association patterns in both species. The most distant groups were the SHO and CC areas for *M. guianensis* and *H. alchorneoides,* respectively (Figure 2a and 2b). Analysis of all individuals in each species showed a distribution along the same cluster with very low bootstrap values (data not shown).

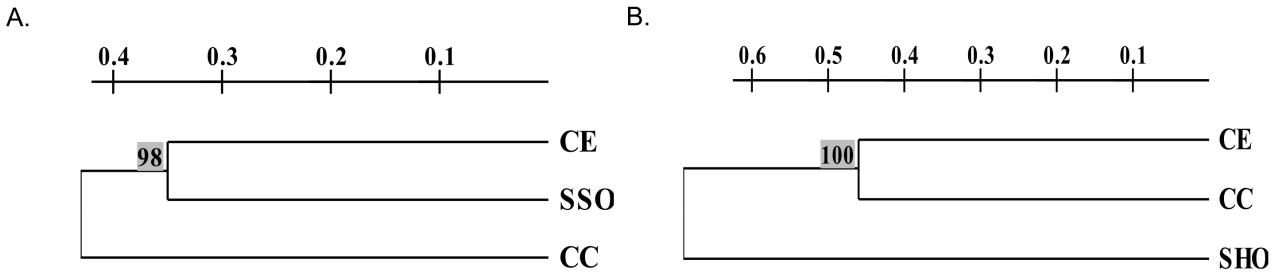

Figure 2: UPGMA dendrogram based on Nei and Li (1979) genetic distance of M. guianensis (A.) and H. alchorneoides (B.) at La Selva forest. The values above the branches indicate the support determined with 1000 bootstrap permutations.

GENETIC DIFFERENTIATION AND PARTITIONING OF MOLECULAR VARIANCE. Variance, estimated with AMOVA for the two species, showed higher values within patches than among them (Table 3). *H. alchorneoides* patches exhibited less structure (ϕ_{ST} = 0.131) compared to *M. guianensis* (ϕ_{ST} = 0.221). However, the opposite result was obtained using the Bayesian estimator of F_{ST} (q^B) and the Bayesian analogue of Nei's G_{ST} (G_{ST}-B) (Table 3). This incongruence may be due to an artifact in the Bayesian approach, potentially caused by a small sample size or the low number of polymorphic bands generated by RAPD analysis.

Table 3: **Analysis of molecular variance (AMOVA) and Bayesian Fst estimators for M. guianensis and H. alchorneoides RAPD data.**

	AMOVA			Bayesian estimators	
Source of variation	**df**	**%V**	**Øst**	**q^B (SD)**	**$G_{st}B$ (SD)**
Among patches					
M. guianensis	2	22.1	0.221*	0.1442 (0.0221)	0.1117 (0.0145)
H. alchorneoides	2	13.1	0.131*	0.1601 (0.0263)	0.1194 (0.0157)
Within patches					
M. guianensis	38	77.9			
H. alchorneoides	38	86.9			

The characterization of genetic diversity in two valuable native tropical trees, *Minquartia guianensis* and *Hyeronima alchorneoides* (two species with different mating systems in a continuous forest), provides useful information for understanding their population structure. The proportion of polymorphic RAPD bands ranged from 79% to 95% in *M. guianensis* and from 74% to 100% in *H. alchorneoides*. The lowest polymorphic values in both species were observed at different sites: the CE patch for *M. guianensis* and the SHO patch for *H. alchorneoides* (Table 1).

The genetic diversity estimates for *M. guianensis* and *H. alchorneoides* were within the range of values found in other studies of Neotropical trees using dominant DNA markers, such as *C. odorata*[12], *S. macrophylla*[7], *G. sepium*[6], *P. reticulata*[13], and *T. amazonica*[46]. These values ranged from 0.22 in *S. macrophylla* to 0.42 in *C. odorata.* The genetic diversity estimated using the Bayesian approach was more like Nei's diversity values than those obtained with the Shannon index. Similar patterns of gene diversity values for these three estimators were observed in the Neotropical fruit tree *Spondias purpurea*, using AFLP dominant markers (Miller and Schaal, 2006). Despite the different mating systems of these species, the genetic diversity values estimated by the three approaches were similar. On the other hand, the Shannon index estimates of phenotypic diversity for *Plathymenia reticulata*[13] were more like our estimates of Nei's and Bayesian diversity. Hall et al.[47] studied the genetic variation of *Pentaclethra macroloba* at La Selva using allozymes. They found low values of polymorphism and heterozygosity (F_{ST} = 0.038 from adults with three loci) within La Selva compared with samples outside the reserve, attributing these results to the limits of species distribution.

Bayesian analysis to detect inbreeding using dominant markers is a recent tool. The *f* and q^B values obtained with our RAPD data were higher for *H. alchorneoides* than for *M. guianensis*, indicating evidence of inbreeding within the patches ($f > 0$) and of genetic differentiation among populations ($q^B > 0$) for both species. However, the $f = 0$ model displayed the lowest Dbar and Dhat values, suggesting that the full model should be interpreted with extreme caution, as noted by Holsinger and Wallace[40].

Additionally, the mean value of the Bayesian estimator of F_{IS} (*f*) was higher than estimator q^B. A similar pattern was observed in *Spondias purpurea* using AFLP data[48]. The q^B estimator was used to gain more information about genetic diversity and to compare the different estimator values[40]. The preference of the full model over $f = 0$ or $q^B = 0$ in both trees aligns for *Platanthera leucophaea* (Orchideaceae) using RAPD data[40]. Moreover, in both cases using RAPD data, the Dbar value was smaller in the $f = 0$ model. The consistency of this result in both species suggests that RAPD data may not be a reliable source for inferring inbreeding. Future studies would benefit from a larger sample size and the use of other dominant markers, such as AFLPs.

GENETIC STRUCTURE. The variance within patches of the dioecious tree *H. alchorneoides* was higher compared to the hermaphroditic *M. guianenesis*. Therefore, genetic variability distribution was slightly different between the two species.

This pattern of genetic variance has been observed in other studies and may indicate effective gene flow among patches or sites[7,12,19,22]. This is a common phenomenon in outcrossing, perennial, woody plants, which typically exhibit greater genetic variation within populations than among populations. This can be attributed to their large gene pool size and, potentially, effective pollen and seed dispersal mechanisms[19].

According to Loveless and Hamrick[49], dioecious species typically exhibit higher genetic variation within populations than among populations. This pattern results from a combination of factors, including pollination mechanisms, breeding systems, population density, and modes of reproduction, all of which influence the genetic structure of these species. Our results align with this general model of genetic structure proposed for tropical species[50–55].

H. alchorneoides is dioecious while *M. guainensis* is hermaphroditic, and very little is known about the pollinators for these species. Bawa et al.[51] mentioned that the pollinators could be small insects, bees or bats that visit the different trees or different flowers (males and females) enabling an effective gene flow between them. Bawa[56] also mentioned that the flying range distance of small insects could be around 2 km. In the case of "Pilón", the maximal distance between individuals was 1,598 m and the aggregation index was 0.466 (data not shown). This value is within the range of distance reported for tropical species. No reports were found for "Manú".

The frequency of pollinator visits to trees depends on the resources they obtain, primarily food. Different plants produce various types of food, and some substances are more attractive to pollinators than others. In tropical trees, little is known about the specific substances that pollinators prefer. Quantifying and characterizing carbohydrate production in female and male flowers could provide key insights into the varying visit frequencies by pollinators in tropical trees. However, Bullock[57] found no sex-based differences in the total concentration of nonstructural carbohydrates in the dioecious trees *Jacaratia mexicana* (Caricaceae) or *Spondias purpurea* (Anacardaceae).

In conclusion, our data align with the prediction that outcrossing perennial, woody plants—whether dioecious or hermaphroditic—exhibit higher genetic diversity within populations than among populations. Effective long-distance pollen transfer minimizes differentiation among patches on a local scale, leading to high genetic diversity within these patches[49,56]. The results suggest that all trees belong to a single population with no significant differentiation between patches, despite the different mating systems of the species.

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ACKNOWLEDGEMENTS

This work was supported by AID grant No. 10-105 awarded to the Tropical Studies Organization. We would like to extend our special thanks to Deborah Clark, as well as Leonel Campos and William Brenes, for collecting the samples; Barbara Schaal and Gustavo Caetano-Anollés for sharing information and techniques; Mathew Clark for assisting with the GIS database; and Alfonso García and James Karkashian for their valuable comments on this manuscript.