



Genetic and ecological relationships of tree species of sub-humid tropical forest from southern Mexico

Relaciones genéticas y ecológicas de especies arbóreas del bosque tropical subhúmedo del sur de México

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ABSTRACT

Tree communities are shaped by eco-evolutionary processes, which in turn determine functional and ecological traits, as well as genetic diversity. However, the relationship between levels of genetic diversity and ecological traits remains understudied. This study examined the relationships between genetic diversity, estimated through the Internal Transcribed Spacer region of nuclear ribosomal DNA, and the dispersal syndrome, successional affinity, and leaf area of 14 tree species inhabiting a sub-humid tropical forest at the Selva El Ocote Biosphere Reserve. Differences in genetic diversity between groups of tree species clustered based on plant traits were assessed using a Kruskal-Wallis test. We explored the relationships between genetic parameters and the three plant traits through a Principal Components Analysis. Leaf area and successional affinity were not associated with any genetic diversity parameter, while it was detected that nucleotide diversity (π) and segregating sites (s) are related to the dispersal syndrome ($P < 0.05$). Dispersal by zoochory and zoochory/barochory varied for both genetic parameters ($P = 0.009$ for s ; $P = 0.01$ for π). The species dispersed only by zoochory showed the lowest genetic diversity, while the species dispersed by both zoochory and barochory were the most genetically diverse. This study improves our understanding of the relationship between the ecological and genetic aspects of tropical trees, which is particularly important for highly threatened ecosystems.

KEYWORDS: barochory, dispersal, ITS, nucleotide diversity, zoochory.

RESUMEN

Las comunidades arbóreas son formadas por procesos coevolutivos, que a su vez determinan los atributos funcionales y ecológicos, así como la diversidad genética. Sin embargo, la relación entre los niveles de diversidad genética y los atributos ecológicos han sido poco estudiados. Esta investigación examinó las relaciones entre la diversidad genética – estimada con la región del Espaciador Transcrito Interno del ADN ribosómico nuclear – y el síndrome de dispersión, afinidad sucesional, y área de la hoja de 14 especies arbóreas del bosque tropical subhúmedo de la Reserva de la Biosfera Selva El Ocote. Las diferencias en la diversidad genética entre grupos de especies arbóreas agrupadas en función de los rasgos de las plantas se evaluaron mediante una prueba de Kruskal-Wallis. Se exploraron las relaciones entre los parámetros genéticos y los tres atributos de las plantas a través de un análisis de componentes principales. El área de la hoja y la afinidad sucesional no se asociaron con ningún parámetro genético, mientras que sí se detectó que la diversidad nucleotídica (π) y los sitios segregativos (s) están relacionados con el síndrome de dispersión ($P < 0.05$). La dispersión por zoocoria y zoocoria/barocoria varió para ambos parámetros genéticos ($P = 0.009$ para s y $P = 0.01$ para π). Las especies dispersadas solo por zoocoria mostraron la menor diversidad genética, mientras que las especies dispersadas por zoocoria y barocoria fueron las más diversas genéticamente. Este estudio mejora el entendimiento de la relación entre aspectos ecológicos y genéticos de árboles tropicales, lo que es particularmente importante para ecosistemas altamente amenazados.

PALABRAS CLAVE: barocoria, dispersión, ITS, diversidad nucleotídica, zoocoria.

INTRODUCTION

The functional characteristics of tree species are morpho-physiological, biochemical, structural, or phenological traits of organisms that impact survival, growth, and reproduction (Dudley, 1996; Nock et al., 2016; Violle et al., 2007), with consequences in demographic dynamics (Worthy & Swenson, 2019). Plant species can be grouped according to functional and ecological traits, resulting in a community formed by several groups of species sharing biological traits and playing similar roles in ecosystem processes, irrespective of the taxonomic nature of the species within a group (Saldaña-Acosta et al., 2008).

The functional and ecological traits of tree species result from selective and neutral evolutionary processes operating throughout generations (Hedrick, 2000; Frankham et al., 2002; Junker et al., 2015). Trait similarity between species may emerge, at least partially and indirectly, from analogous responses to the changing environment, which are highly dependent on genetic diversity and the evolutionary processes that work upon it. Tree communities are assembled by eco-evolutionary processes such as drift, dispersal, speciation, and selection acting simultaneously (Overcast et al., 2021), impacting on different scales of biological organization (from genes to meta-communities, see Overcast et al., 2021), that include phenotypical characteristics, such as functional and ecological traits. Therefore, a relationship between genetic diversity levels and ecological traits can be expected. Knowledge of the genetic diversity associated with functional or ecological groups may contribute to understanding the evolutionary aspects involved in community and ecosystem processes (Kelly, 2006).

Successional affinity (inferred from the structure, diversity and disturbance history of the vegetation where it has occurred) and the dispersal syndrome are ecological traits strongly related with morpho-functional traits, such as mass seed/fruit type and leaf area (Tavera-Carreño et al., 2019; Delgado, 2012; Groenendijk et al., 2005; Kooyman & Rossetto, 2008; Makana & Tomas, 2006). Successional affinity is essential for the local coexistence of plant species

over the changing environmental conditions of forest succession (Quintana-Ascencio & González-Espinosa, 1993; Quintana-Ascencio et al., 2004; Ramírez-Marcial, 2003). The dispersal mechanism of propagules, seeds, fruits, or spores has consequences related to the distance of movement and migration (Pérez-Harguindeguy et al., 2013). Separately, the leaf area of a species is crucial for water transport and light uptake, which affect the photosynthetic rate (Saldaña-Acosta et al., 2008; Violle et al., 2007).

Genetic diversity information could contribute to formulating more robust predictions about the responses of ecological groups to external environmental conditions. For instance, Bâ and Rivera-Ocasio (2015) evaluated the association between the genetic diversity and functional attributes of the tree *Pterocarpus officinalis* Jacq and symbiotic microbial communities in Caribbean wetlands. These authors found a relationship between genetic diversity and particular strains of microorganisms that influenced plant growth and absorption of nutrients such as nitrogen. Honorio-Coronado et al. (2019) reported a higher genetic diversity in shade-tolerant tree species compared with pioneer species in western Amazon forests. Junker et al. (2015) explored the influence of functional and genetic diversity of plant communities on the richness and diversity of animal species. Their study reported that the functional diversity of plant communities supports a larger number of animal species.

The Selva El Ocote Biosphere Reserve (REBISO, for its acronym in Spanish) protects an extensive area of humid and sub-humid tropical forests in southeastern Mexico highly relevant worldwide for its high biodiversity and number of endemic species (Ramírez-Marcial et al., 2017; Ruiz-Montoya et al., 2017). However, the REBISO has been under strong pressures associated with forest fragmentation and land-use changes driven by the advance of the agricultural frontier (Manzanilla-Quiñones & Aguirre-Calderón, 2017), which threatens the structure and functioning of this tropical ecosystem (Ramírez-Marcial et al., 2017).



The REBISO vegetation has been studied little. Recently, Ramírez-Marcial et al. (2017) reported high levels of tree species diversity and turnover between localities at the REBISO core zone. In a separate study, tree species richness was marginally related to genetic diversity within the community (Cruz-Salazar et al., 2021), and Ruiz-Montoya et al. (2021) described the genetic diversity of four tree species that thrive at REBISO.

We selected the Internal Transcribed Spacer (ITS) as an approximation to the genetic diversity level of tree species because it is a universal marker for plants that is not subject to selection and allows examining genetic variation in several plant species simultaneously (Kirk & Freeland, 2011; Liu et al., 2018; Tan et al., 2017). Another advantage of ITS is that it accumulates nucleotide substitutions at a relatively high rate and is easily replicable due to its multiple copies in the genome (Nagy et al., 2012). Thus, it is a useful tool for species identification (Liu et al., 2018; Tan et al., 2017) and can reveal nucleotide variants for analyzing the intraspecific genetic variation of different species regardless of their phylogenetic relation (*e.g.*, Honorio-Coronado et al., 2019; Ornelas et al., 2016). They are nucleotide variants mainly neutral to natural selection and thus show random evolutionary and demographic process. When species with similar functional traits experience similar migration and demographic changes, these would lead to genetic diversity similarities (Wang et al., 2013). Therefore, our hypothesis was to detect similar genetic diversity levels in species displaying similar ecological traits. We used three plant ecological/functional traits to test this expectation: successional affinity, dispersal syndrome, and leaf area. Successional affinity (ecological trait) denotes the forest successional stage most frequently colonized by the species; two categories were recognized in this study: pioneer and late-successional species. Pioneer species (also known as early secondary species) are the first to colonize newly opened areas in mature vegetation and are characterized by rapid growth and shade intolerance. Late-successional (also known as mature) species settle in forests with a well-developed canopy, are shade-tolerant, and grow slowly. The dispersal syndrome (ecological trait) refers to the

mechanisms or agents involved in fruit and seed dispersal. Fruits and seeds can be dispersed by animals (zoochory), wind (anemochory), water (hydrochory), or through free fall to the ground (barochory).

Leaf area (functional trait) denotes the capacity or requirement of a species for capturing resources for germination, establishment, growth, and survival (Tavera-Carreño et al., 2019). Late-successional species from inner forest areas usually develop a larger leaf area than canopy species (Groenendijk et al., 2005; Kohyama et al., 2003; Poorter & Bongers, 2006), which increases their capacity to capture solar radiation. We followed the leaf-area categories of Webb (1959), based on Raunkiaer (1934): leptophyll ($<0.25 \text{ cm}^2$), nanophyll ($0.25 \text{ cm}^2 - 2.25 \text{ cm}^2$), microphyll ($2.25 \text{ cm}^2 - 20.25 \text{ cm}^2$), notophyll ($20.25 \text{ cm}^2 - 45 \text{ cm}^2$), mesophyll ($45 \text{ cm}^2 - 182.25 \text{ cm}^2$), macrophyll ($182.25 \text{ cm}^2 - 1640.25 \text{ cm}^2$), and megaphyll ($>1640.25 \text{ cm}^2$).

According to the classification by Pérez-Harguindeguy et al. (2013), leaf area is a functional individual trait, while the dispersal syndrome and fruit type are regenerative functional traits, and successional affinity is a proxy of age and longevity of individual trees throughout the successional process. Although these traits could be considered redundant because leaf area and the dispersal syndrome are related to the ecological strategy of the species (successional affinity) (Leishman et al., 1995), this relationship can be weak and unsteady (*e.g.*, Saldaña-Acosta et al., 2008). Therefore, this study considered the three traits separately.

Specifically, we expected anemochorous and late-successional species to show a higher genetic diversity than zoochorous pioneer species, because in the latter gene flow is higher and is not adversely affected by disturbance (Ruiz-Montoya et al., 2021), which generally reduces the genetic variation of populations and species (Honorio-Coronado et al., 2019; Freitas et al., 2008). The results of this investigation would advance our understanding of the interrelationships of functional and ecological traits and genetic processes governing the diversity patterns and functioning of seriously threatened tropical forests.

OBJECTIVES

The objective of this study was to examine the genetic diversity of trees in REBISO sub-humid tropical forests and its relationship with dispersal syndrome, successional affinity, and leaf area of selected tree species, as a possible converging effect of evolutionary processes.

MATERIALS AND METHODS

Study area

The Selva El Ocote Biosphere Reserve (REBISO) protects an area of 101.3 km² with one of the last remnants of sub-humid tropical vegetation in Mexico; it is located between the coordinates 16°45'42"–17°09'00" N, 93°54'19"–93°21'20" W in the State of Chiapas, southeast Mexico. The high environmental heterogeneity of REBISO favors diverse types of vegetation, including humid tropical forests (Flamenco-Sandoval et al., 2007). REBISO is biologically important for its high biodiversity, number of endemic species, and for being part of the Mesoamerican Biological Corridor (Comisión Nacional de Áreas Naturales Protegidas [Conanp] & Secretaría de Medio Ambiente y Recursos Naturales [Semarnat], 2001). Evergreen and sub-evergreen tropical forests (*sensu* Rzedowski, 1978) are the dominant vegetation types; the mean annual temperature exceeds 22 °C and the mean annual precipitation ranges between 1500 mm and 2500 mm (García, 1973; Orantes-García et al., 2013). Specifically, REBISO has altitudinal variations ranging from 300 m a.s.l. to 1450 m a.s.l., with predominantly warm-humid climates, abundant rainfall from May to September (1200 mm - 2500 mm) and an average monthly temperature of over 18 °C (Conanp/Semarnat, 2001).

Tree sampling and assignment of ecological traits

We collected young undamaged leaves from 14 tree species at five REBISO locations defined according to the access, security and previous experience working in multidisciplinary research: Nuevo San Juan Chamula, Veinte Casas,

El Encajonado, San Joaquín, and Emilio Rabasa (Table 1; Fig. 1). These species were selected according to their presence, and their relative abundance was estimated from forest inventories carried out in five sampling units (0.1 ha circular plots) at each locality to record the diversity of trees (Cruz-Salazar et al., 2021). For the present study, we selected those species with ≥ 3 individuals in the total sample by location (Cruz-Salazar et al., 2021) (Table 1). After collecting, all samples were transported in 1.5 ml vials with TRIS-EDTA buffer solution, pH 8.0 (Sigma-Aldrich), and stored at -70 °C until genetic analysis.

The species subjected to genetic testing were classified based on published information on successional affinity, dispersal syndrome, and leaf area (Table 1). Of the 14 species, 10 were classified as late-successional ($N = 80$ individuals) and four as pioneers ($N = 37$ individuals). According to the dispersal mechanism, two species are anemochorous ($N = 23$ individuals), seven are zoochorous ($N = 63$ individuals), and five have two dispersal mechanisms, i.e., barochorous and zoochorous ($N = 31$ individuals). Regarding leaf area, two of the 14 species have notophyll leaves ($N = 11$ individuals); eight, mesophyll leaves ($N = 68$ individuals); and four, megaphyll leaves ($N = 38$ individuals) (Table 1). The number of species was low, and the sequences analyzed by functional/ecological trait were variable, being small for some species such as *Allophylus psilospermus* Radlk, *Dendropanax arboreus* (L.) Decne. & Planch., and *Garcia nutans* Vahl. Nonetheless, we consider that the sample size allows exploring the relationship between genetic diversity and selected traits of tropical trees.

Genetic Data Recording

Genomic DNA was extracted from 115 leaf samples of 14 tree species from the REBISO sub-humid tropical forest (Table 1), using the CTAB method (Doyle & Doyle, 1990). DNA viability was determined by visualization on 1% agarose gel. The genetic diversity of each species was assessed by PCR amplification of the ITS-1 region of nuclear ribosomal DNA (TCCGTAGGTGAACCTGCGG) (GCTGCGTTCATCGATGC) (White et al., 1990).



The PCR was performed in a final volume of 50 µl with a master mix solution medium (PROMEGA) in a C1000 Touch™ (BIORAD) thermal cycler. The PCR reactions started with denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s each for denaturation, 57 °C during 30 s for alignment, and 72 °C for 2 min for extension; the final extension was carried out at 72 °C for

10 min (White et al., 1990). The reaction product was visualized by electrophoresis on 2% agarose gels with a 100 base-pair (bp) control marker (Ladder, PROMEGA). Reactions were sequenced at Macrogen Inc, Seoul, Korea, following the sequencing protocol developed by Applied Biosystems.

TABLE 1. Functional and ecological traits and genetic diversity of 14 tree species inhabiting the humid tropical forest of Selva El Ocote Biosphere Reserve, southeast Mexico.

Species	Family	N	Functional/ecological traits			Genetic diversity estimators			
			Suc	Dis	Area	s	π	h	Hd
<i>Allophylus psilospermus</i> Radlk. ^{4, 6, 8, 14, 15, 21}	Sapindaceae	3	L	Z	Mesophyll	2	0.005	2	0.67
<i>Dendropanax arboreus</i> (L.) Decne. & Planch. ^{4, 6, 13, 14, 15, 21}	Araliaceae	3	L	Z	Mesophyll	2	0.006	3	0.10
<i>Manilkara zapota</i> (L.) P. Royen ^{6, 14, 15, 20, 21}	Sapotaceae	17	L	Z	Mesophyll	3	0.002	4	0.42
<i>Psychotria chiapensis</i> Standley ^{6, 11, 14, 20, 21}	Rubiaceae	14	L	Z	Mesophyll	10	0.011	4	0.71
<i>Garcia nutans</i> Vahl ^{5, 6, 14, 21}	Euphorbiaceae	3	L	Z, B	Mesophyll	10	0.024	3	1.00
<i>Zanthoxylum caribaeum</i> Lam. ^{4, 6, 13, 14, 17, 21}	Rutaceae	10	L	Z, B	Mesophyll	$\frac{3}{2}$	0.070	3	0.64
<i>Brosimum alicastrum</i> Sw. var. <i>alicastrum</i> ^{4, 6, 14, 15, 16, 21}	Moraceae	6	L	Z	Notophyll	3	0.004	2	0.33
<i>Pseudolmedia spuria</i> (Sw.) Griseb. ^{6, 10, 14, 21}	Moraceae	5	L	Z	Notophyll	3	0.007	2	0.60
<i>Bursera simaruba</i> (L.) Sarg. ^{5, 6, 14, 15, 16, 18, 21}	Burseraceae	9	P	Z, B	Megaphyll	12	0.011	4	0.58
<i>Cordia alliodora</i> (R. & P.) Oken ^{3, 6, 14, 17, 21}	Boraginaceae	13	P	A	Mesophyll	5	0.004	4	0.53
<i>Guarea glabra</i> Vahl ^{6, 7, 9, 14, 17, 19, 21}	Meliaceae	4	L	Z, B	Megaphyll	$\frac{4}{6}$	0.085	4	1.00
<i>Heliocarpus appendiculatus</i> Turcz. ^{4, 6, 12, 14, 17, 21}	Malvaceae	10	P	A	Megaphyll	5	0.006	5	0.80
<i>Mariosousa centralis</i> (Britton & Rose) Seigler & Ebinger ^{1, 2, 6, 14, 21}	Fabaceae	5	P	Z, B	Mesophyll	8	0.012	2	0.40
<i>Trichilia moschata</i> Sw. subsp. <i>matudae</i> (Lundell) Penn ^{6, 9, 11, 14, 18, 21}	Meliaceae	15	L	Z	Megaphyll	1	0.001	2	0.13

N = number of sequences analyzed, Suc = successional affinity (L = late-successional or mature, P = early pioneer or secondary), Dis = dispersal syndrome (Z = zoochory, A = anemochory, B = barochory), Area = Leaf area (Notophyll = 20 cm² – 45 cm², Mesophyll = 45 cm² – 180 cm², Megaphyll = >180 cm²). s = segregating sites, π = nucleotide diversity, h = number of haplotypes, Hd = haplotype diversity.

¹Bustamante et al. (2006), ²Cruz-Salazar et al. (2021), ³Edward & Munishi (2009), ⁴Escobar-Ocampo & Ochoa-Gaona (2007), ⁵Galván-Guevara et al. (2009), ⁶González-Espinosa et al. (2005),

⁷Hamrick et al. (1993), ⁸Holl et al. (2017), ⁹Howe & De Steven (1979), ¹⁰Mancina & Castro-Arellano (2013), ¹¹Martín-Rodríguez et al. (2013), ¹²McDonald et al. (1995), ¹³Quintana-Ascencio & González-Espinosa (1993), ¹⁴Raunkiaer (1934), ¹⁵Rocha-Loredo et al. (2010), ¹⁶Rodríguez et al. (2009), ¹⁷Salmerón et al. (2016), ¹⁸Sánchez-Gallen et al. (2010), ¹⁹Thompson et al. (2015),

²⁰Tzab (2014), ²¹Webb (1959).

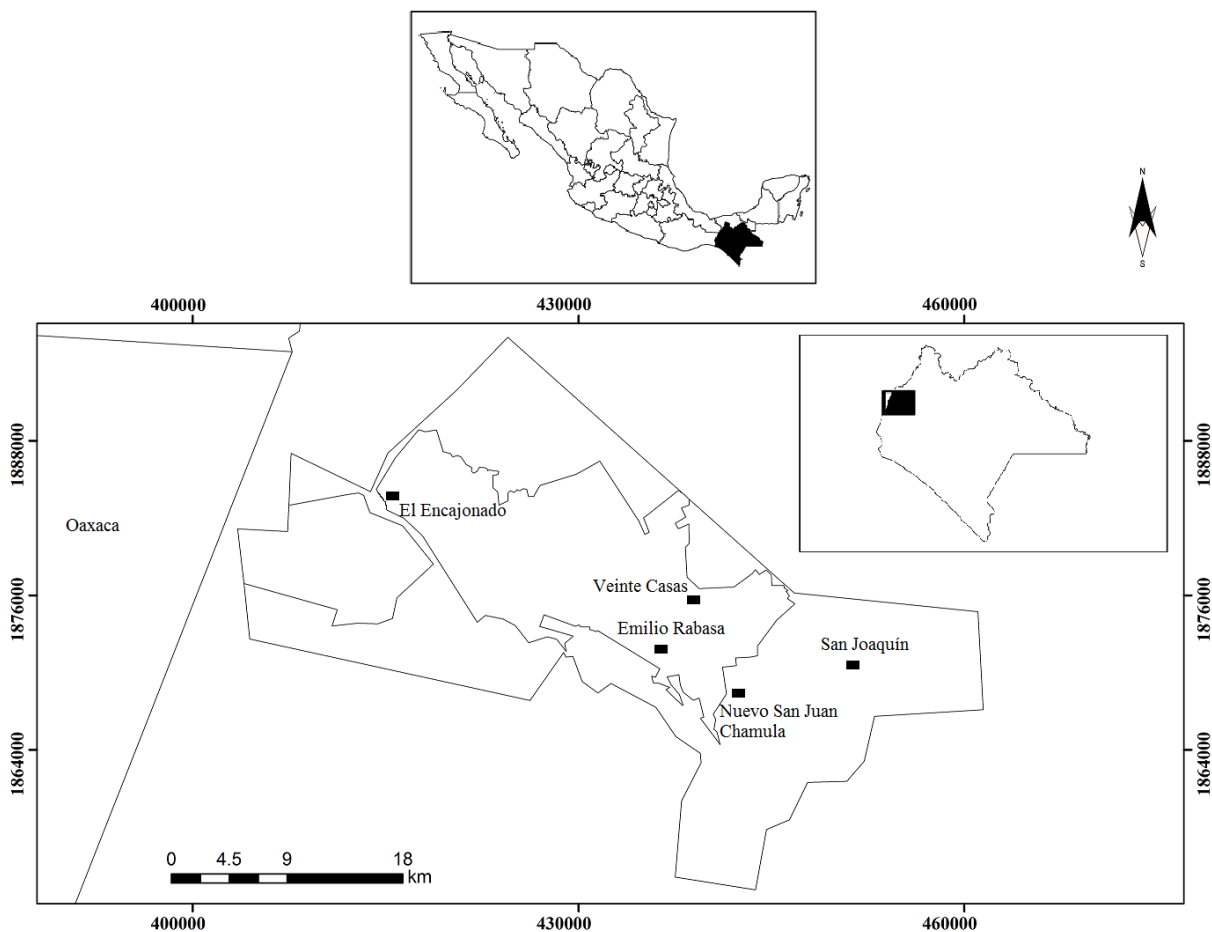


FIGURE 1. Location of the study area and collection sites of 14 tree species inhabiting the humid tropical forest of Selva El Ocote Biosphere Reserve, southeast Mexico.

The sequences obtained from the ITS-1 were edited with the software Chromas v. 1.5 (McCarthy, 1996), applying the following three criteria: (1) each nucleotide should show a unique and well-defined (> 90%) signal in the electropherogram, (2) the emitted signal should correspond to the nucleotide in the sequence, and (3) the taxonomic identity of each sequence, as evaluated with BLAST, should match its field identity. The edited sequences were then aligned using the software Clustal X v. 2.1 (Thompson et al., 1997). Four genetic diversity measures were calculated for each species using the software DnaSP v. 5 (Librado & Rozas, 2009): segregating sites (s), nucleotide diversity (π), number of haplotypes (h), and haplotype diversity (Hd).

Relationship between Genetic Diversity and Ecological/Functional Traits

We performed the Kruskal-Wallis test to determine whether the genetic diversity parameters differ significantly among trait groups. To this end, the genetic diversity parameters of the species within a given group were clustered for the Kruskal-Wallis test. Subsequently, we specifically identified the categories that were different within the groups yielding significant differences through a paired Nemenyi test. Finally, the relationship between genetic structure and traits was assessed through a Principal Components Analysis (PCA). These analyses were carried out using the R statistical package (PMCMR, FactoMineR, factoextra and MASS packages) (R Core Team, 2013;



Husson et al., 2020; Kassambara & Mundt, 2020; Ripley et al., 2020; Thorsten, 2021).

RESULTS

The ITS-1 region was made up of 290 base pairs (bp) in all species. All the sequences obtained were entered into GenBank under accession numbers MN077267 through MT071992 (Appendix 1).

Tree species that showed a high genetic diversity were *Guarea glabra* Vahl ($s = 46$, $\pi = 0.085$) and *Zanthoxylum caribaeum* Lam. ($s = 32$, $\pi = 0.070$). The lowest genetic diversity was recorded in other late-successional zoochorous species such as *Trichilia moschata* Sw. subsp. *matudae* (Lundell) Penn ($s = 1$, $\pi = 0.001$, $h = 2$, $Hd = 0.13$), and *Manilkara zapota* (L.) P. Royen ($s = 3$, $\pi = 0.002$) (Table 1).

In relation to the dispersal syndrome, nucleotide diversity was highest in species dispersed by zoochory/barochory ($\pi = 0.001$ – 0.085) and lowest ($\pi = 0.001$ – 0.011) in zoochore species; the species dispersed by anemochory showed intermediate values ($\pi = 0.004$ – 0.006) (Table 1). This same pattern was found regarding the number of polymorphic sites (s) and haplotype diversity (Hd), i.e., the lowest values ($s = 1$ – 10 , $Hd = 0.10$ – 0.71) were observed in zoochore species, intermediate values ($s = 5$; $Hd = 0.53$ – 0.80) in anemochore species, and the highest values ($s = 10$ – 46 , $Hd = 0.58$ – 1.00) in species dispersed by zoochory/barochory (Table 1). The number of haplotypes (h) showed a different pattern: fewer haplotypes ($h = 2$ – 4) were observed in species dispersed by zoochory or zoochory/barochory and more haplotypes ($h = 4$ – 5) in anemochore species (Table 1). No clear patterns could be discerned between genetic diversity and successional affinity or leaf area (Table 1).

The box plot shows variation in genetic diversity among species for almost all groups formed according to successional affinity, dispersal syndrome, and leaf area (Fig. 2 A-I). No variation was recorded for anemochory (A); zoochory showed low genetic diversity in segregating sites and nucleotide diversity (Fig. 2D, E); and notophyll leaves

(NT) also showed low diversity in s , π , and h (Fig. 2G-I). Segregating sites (s) and nucleotide diversity (π) were variable for species that displayed the zoochory-barochory (Z/B) dispersal syndrome (Fig. 2D; 2E) and megaphyll leaves (MG) (Fig. 2G; 2H). Segregating sites and nucleotide diversity were less variable in relation to successional affinity (Fig. 2A; Fig. 2B; respectively). The Kruskal-Wallis test showed that only nucleotide diversity (π) and the number of segregating sites (s) were significantly different among dispersal syndromes ($P = 0.011$ for π ; $P = 0.012$ for s) (Table 2). The Nemenyi test to recognized that zoochory and zoochory/barochory vary for s ($P = 0.009$) and π ($P = 0.01$).

The first and second principal components from the PCA accounted for 69% and 30% of the total genetic variation, respectively. The number of segregating sites (s) was the variable that most contributed (47%) to the variation on the first component, whereas the number of haplotypes (h) did so (91%) on the second component. The PCA showed the spatial distribution of genetic diversity related to zoochory, which did not occur with other dispersal mechanisms or in relation to successional affinity or leaf area (Fig. 3).

DISCUSSION

The genetic diversity detected in this study indicates that the tree community of the REBISO has a huge genetic pool distributed among species. Ruiz-Montoya et al. (2021) reported π values of a similar order of magnitude for populations of *Brosimum alicastrum* Sw. var. *alicastrum*, *Sapium macrocarpum* Müll. Arg., *Cecropia peltata* L., and *Heliocarpus appendiculatus* Turcz. (0.0041–0.0059) based on ITS 1-2 and ITS 3-4. Studies conducted on species inhabiting ecosystems like those in REBISO but using other molecular markers reported low to moderate genetic diversity levels, measured as heterozygosity values by microsatellites (H_o) of 0.253 in *Dendropanax arboreus* (Figuroa-Esquivel et al., 2010) and 0.154 in *Manilkara zapota* (González-Hernández et al., 2012).

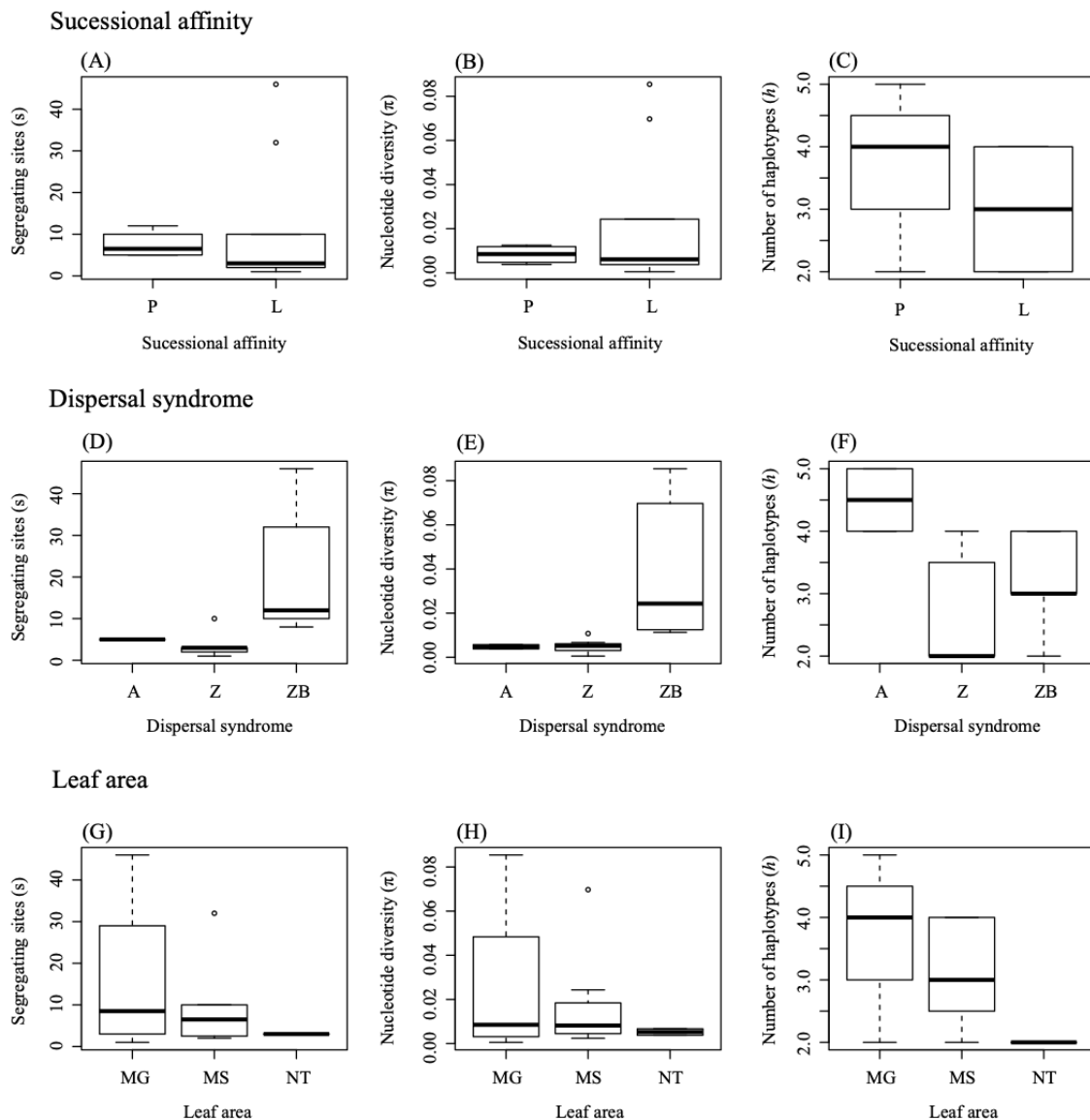


FIGURE 2. Genetic diversity of functional/ecological groups of tree species inhabiting the humid tropical forest of Selva El Ocote Biosphere Reserve, southeast Mexico.

(A) successional affinity, L = late-successional, P = pioneer. (B) dispersal syndrome, A = anemochory, Z = zoochory, B = barochory, ZB = zoochory and barochory. (C) leaf area, MG = megaphyll (>180 cm²), MS = mesophyll (45 cm² – 180 cm²), NT = notophyll (20 cm² – 45 cm²) (Raunkiaer, 1934; Webb, 1959).

According to our results, two dispersal syndromes are related to higher genetic diversity: zoochory/barochory. Using enzymes as genetic markers, Hamrick et al. (1993) showed that species with barochore seeds, whose propagules are deposited near the mother plant, exhibit greater genetic structure than those with zoochore seeds or

fruits. Zoochore seeds display higher mobility than barochore seeds, implying greater dispersal, a weaker genetic structure, and lower genetic diversity at the species level due to homogenization through gene flow (Frankham et al., 2002; Hedrick, 2000).



TABLE 2. Kruskal-Wallis test to analyze the genetic diversity of the nuclear ribosomal DNA ITS-1 region in 14 tree species inhabiting the humid tropical forest of Selva El Ocote Biosphere Reserve, southeast Mexico, in relation to functional and ecological traits.

Kruskal-Wallis	<i>d.f.</i>	χ^2	<i>P</i> -value
π - Succession	1	0.080	0.773
π - Dispersal	2	9.037	0.011
π - Area	2	0.543	0.762
<i>h</i> - Succession	1	1.793	0.180
<i>h</i> - Dispersal	2	4.387	0.111
<i>h</i> - Area	2	3.843	0.146
<i>s</i> - Succession	1	0.731	0.392
<i>s</i> - Dispersal	2	8.775	0.012
<i>s</i> - Area	2	1.021	0.600

The predictive variables considered were successional affinity (Succession), dispersal syndrome (Dispersal), and leaf area (Area). *s* = segregating sites, π = nucleotide diversity, *h* = number of haplotypes, *d.f.* = degrees of freedom. Relationships with statistical significance are shown in bold.

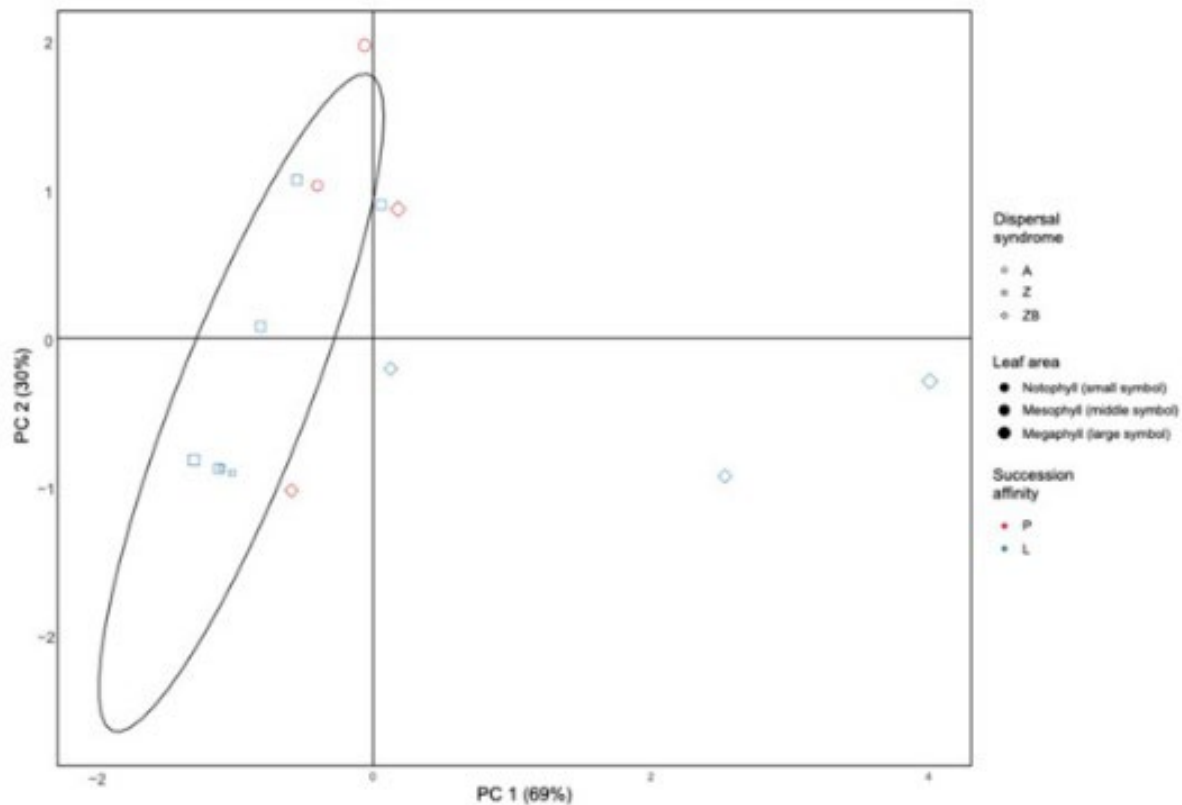


FIGURE 3. Principal Component Analysis on genetic diversity parameters estimated based on the nuclear ribosomal DNA ITS-1 region of 14 tree species inhabiting the humid tropical forest of Selva El Ocote Biosphere Reserve, southeast Mexico.

The shape depicts the dispersal syndrome; the color represents the succession affinity; The symbol size indicates the leaf area. A = anemochory, Z = zoochory, B = barochory, Z/B = zoochory and barochory, P = pioneer, L = late-successional

Since exclusively barochore species did not fall in our sampling, we cannot discern whether species dispersed by gravity have higher or lower genetic diversity than those dispersed solely by zoochory. However, we found that species with both dispersal syndromes (zoochory and barochory) showed the highest nucleotide diversity, likely due, at least in part, to the expected low gene flow levels for both dispersal syndromes (Hamrick et al., 1993; Holderegger, 2006); we do not rule out that the relationship identified is an effect of the species that make up the group in particular and not necessarily the dispersion syndrome of these, so it is suggested to include more species in subsequent studies that evaluate this issue.

Anemochory also allows for wide-ranging dispersal. The two anemochore species included in our study, *Heliocarpus appendiculatus* and *Cordia alliodora* (R. & P.) Oken showed nucleotide diversity values of the same order of magnitude as most zoochore species studied. This suggests a consistent relationship, i.e., greater dispersal capacity is associated with lower genetic diversity for some tropical species within REBISO (Ruiz-Montoya et al., 2021).

Dispersal maintains the gene flow that determines the assembly of communities (Soons et al., 2017; Zhu et al., 2019) and reduces the effect of genetic drift, a process that governs in part the evolution of ITS (Kirk & Freeland, 2011; Palstra & Ruzzante, 2008). This is probably the reason why we found significant differences only between the functional groups formed according to the dispersal syndrome. Adaptive genetic variation associated with the dispersal syndrome could be identified using, for example, Single Nucleotide Polymorphisms (SNPs) (Andrews & Luikart, 2014).

Our results did not reveal different levels of genetic diversity related to the successional affinity. Leaf area could be related to successional affinity since shade-tolerant species are typical of late-successional-stage forests and usually have a larger leaf area (Kohyama et al., 2003; Poorter & Bongers, 2006); therefore, these species could have high levels of genetic diversity. Nevertheless, this pattern was not evidenced in the present study.

Implications of the findings

The findings of this study indicate that REBISO maintains levels of genetic diversity that must be conserved to ensure the survival of tree species populations and communities. Therefore, issues requiring urgent monitoring and management are habitat fragmentation and deforestation due to changes in land use to agricultural activities (Manzanilla-Quñones & Aguirre-Calderón, 2017), as these affect the abundance and composition of biotic communities (Fahrig, 2003). Halting deforestation is essential for preserving the genetic diversity of tropical tree species in the REBISO and conserving the size of populations and gene flow between populations, as this facilitates functional connectivity (Spear et al., 2016; Worthy & Swenson, 2019; Zambrano et al., 2019) and improves ecosystem resilience (Thomas et al., 2014).

CONCLUSIONS

We detected a high genetic diversity in the 14 tree species of REBISO sub-humid tropical forest, and an association between genetic diversity and dispersal syndrome, whereas the other ecological traits showed no relationship, because of this our hypothesis was only accepted for dispersal. The relationships observed in this study need to be further investigated since only neutral processes were indirectly surveyed by ITS, and it is very likely that ecological traits have been under selective pressure. Thus, the use of adaptive loci and a larger number of species in each functional and ecological group is necessary. In addition, other factors may contribute more strongly to genetic diversity (e.g., life forms).

We detected a high genetic diversity in the 14 tree species of REBISO sub-humid tropical forest, and an association between genetic diversity and dispersal syndrome, whereas the other ecological traits showed no relationship, because of this our hypothesis was only accepted for dispersal. The present study is first to explore the genetic diversity of tree species and its relationship with the attributes evaluated, aspects that contribute to understanding the key factors that govern biological



communities and represent indicators of ecosystem functioning, all of which can be useful for the conservation of vulnerable ecosystems, such as humid tropical forests.

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