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Does the Genetic Diversity of Macuilillo, *Oreopanax xalapensis* (Araliaceae), Change Along Successional Gradients of the Montane Cloud Forest?

Farah Z. Vera-Maloof[1], **Lorena Ruiz-Montoya**[1] and **Neptalí Ramírez-Marcial**[1]

Abstract

Forest regeneration after human-induced or natural disturbances results in a sequential replacement of species, starting with colonizing of pioneer species that modifies the physical conditions of the environment, which later allows the establishment of mid- and late-successional species. Whether the forest succession implies a reduction of population size of tree species might be expected a less genetic diversity in populations inhabiting oldest succession stages. We aimed to study whether genetic diversity of Macuilillo, *Oreopanax xalapensis* (a understory tree), decreases in advanced age-classes and in late successional of montane cloud forest at Huitepec Ecological Reserve (Chiapas, Mexico). We counted individuals from three age classes: seedlings, saplings, and young reproductive individuals in nine plots along three successional stages (early-, middle-, and late-successional forests). Seven enzyme systems (SOD, AP, FUM, G6PDH, GOT, GPI, and G3PDH) were used to obtain common genetic diversity; Φ-*statistics* and a Bayesian model were used to infer population structure. At the early-successional stage, *O. xalapensis* had a bigger population size and significantly high numbers of seedlings. In general, among the successional stages, the genetic diversity of *O. xalapensis* was moderate to high ($H_e = 0.25–0.46$); the saplings presented the highest expected genetic diversity (H_e). Populations were genetically structured by age-classes but not by successional stages. The successional stages affected the demographic dynamics with a mild impact on the genetic structure across age-classes of *O. xalapensis*. The scenic and recreational conservation strategies implemented in this forest have effectively allowed for population development of *O. xalapensis* and provide some explanation of genetic diversity at early-succession stage.

Keywords
forest succession, forest conservation, forest genetic diversity, genetic conservation, tropical montane cloud forest, tree demography

Introduction

The montane cloud forest (MCF) is a very important ecosystem due to its high biological diversity and ecosystem services (Kappelle & Brown, 2001; Scatena, Bruijnzeel, Bubb, & Das, 2011). Over the past few decades, the MCF has suffered a large reduction and fragmentation around the world as a consequence of its transformation into grasslands, cultivated areas, and human settlements (González-Espinosa, Meave, Lorea-Hernández, Ibarra-Manríquez, & Newton, 2011). The conservation of MCF ecosystems is important not only for the biodiversity within them but also to maintain their environmental services as regulators of local climate and as sources of livelihood for many indigenous

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communities (Toledo-Aceves, Meave, González-Espinosa, & Ramírez-Marcial, 2011).

The Huitepec Ecological Reserve (HER) in Chiapas, Mexico, has an important forest remnant of MCF, which has been maintained as a conservation area since 1985. Since then, it has been excluded from any type of forestry activity and has been used for recreational purposes only (Ramírez-Marcial, Ochoa-Gaona, González-Espinosa, & Quintana-Ascencio, 1998). Prior to HER being declared a protected area, firewood and oak timber were extracted from the forest, and sheep would frequently graze on the land. This management of the land promoted a mosaic of successional conditions that included open areas, secondary shrub vegetation, early- and mid-successional forests, and some relicts of late-successional vegetation (Ramírez-Marcial et al., 1998). Due to the conservation actions, the forest has recover, which implies a process of sequential replacement of species, establishment of some species and local extinction of others, which in turn modifies the conditions of the physical environment (Chazdon et al., 2007; Kayes, Anderson, & Puettman, 2011; Pandolfi, 2008). For example, the abundance of understory tree species in an MCF dominated by *Quercus* and *Podocarpus* species is higher under late-successional conditions than in early- or mid-successional conditions in northern Chiapas (Ramírez-Marcial, González-Espinosa, & Williams-Linera, 2001). Thus, some tree species may be present along broad successional gradients but their dominance may be less (Martínez-Ramos, Álvarez-Buylla, & Sarukhán, 1989) as well as its genetic diversity (Hamrick, Godt, & Gonzales, 2006; Vellend & Geber, 2005).

Few studies have addressed patterns of genetic diversity of tree species in Mexican MCF ecosystems (Newton, Allnut, Dvorak, Del Castillo, & Ennos, 2002; Newton et al., 2008; Rowden et al., 2004). Genetic studies of MCF tree species suggest that, in general, their populations are genetically differentiated by geographical isolation and geological history (Boehmer, 2011; Newton, Allnutt, Gillies, Lowe, & Ennos, 1999; Newton et al., 2002). However, the extent of genetic diversity attained during forest recovery has not been reported previously. Current restoration and conservation of MCF could be enhanced with a better understanding of the pattern of genetic diversity during forest recovery.

In the recent decade, our knowledge of plant succession in the tropical MCF has increased (Del Castillo, 2015; Trujillo-Miranda, Toledo-Aceves, López-Barrera, & Gerez-Fernández, 2018); however, the genetic diversity of some taxa present throughout all stages of succession, such as *Oreopana xalapensis*, is still poorly understood. The forest regeneration after human-induced or natural disturbances results in a sequential replacement of species, starting with colonizing of pioneer species that modifies the physical conditions of the environment, which later allows the establishment of mid- and late-successional species (Del Castillo, 2015). Biotic and abiotic conditions are in constant change in earlier successional stages than in later stages, thus the environment is less predictable and do not provide condition to the specializations (Wehenkel, Corral-Rivas, & Hernández-Díaz, 2011). Besides, the abundances of some species decrease as succession advance, due to intolerance to environment promoted in mature forest, for example, more shadow and humidity (Ramírez-Marcial et al., 2001). Thus, it can be expected a reduction of the genetic diversity as succession progress in species which are less abundant in mature forest than early stages of secondary succession. This trend has been observed in *Rhododendron simsii* and *Vaccinium carlesii*, understory shrub species from China subtropical forest (Hahn, Michalski, Fischer, & Durka, 2017). In contrast, Lumibao, Gaskill, Flood, and Mclachlan (2016) did not observe a significant reduction in genetic diversity of *Tsuga canadensis*, a dominant late-successional conifer, in secondary forest at eastern North America. These studies highlight that genetic diversity in tree species associated to forest succession should be further explored to our best knowledge of tree communities development and for improving the management of forests (Wehenkel et al., 2011).

The aim of our study was to improve our knowledge about the genetic diversity in a tree species inhabiting a forest where human disturbances ceased in the last three decades. Specifically, we address the following question: Are there significant differences in population sizes and genetic diversity of tree species across ages or successional stages in a MCF? We used *O. xalapensis* (Kunth) Decne. & Planch. (Araliaceae) as a biological model because it is a common species in the understory forest layer in many chronosequences of the MCFs in Mesoamerica, and it has recently been evaluated as *near threatened* due to habitat loss by forest fragmentation (González-Espinosa et al., 2011). We assessed these questions by counting individuals of three age classes in three MCF successional stages: early-, mid- and late-successional, following the classification of Ramírez-Marcial et al. (1998); and we estimated the genetic diversity and differentiation among ages-classes and successional stage using superoxide dismutase, acid phosphatase, fumarate hydratase, glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate, and glucose-6-phosphate isomerase enzyme system as genetic molecular markers previously established for this species (Ruiz-Montoya, Correa-Vera, Alfaro-González, Ramírez-Marcial, & Verónica-Vallejo, 2011). This genetic tool is useful for revealing genetic patterns, especially because specific DNA-markers have not yet been developed in *O. xalapensis*. We know that there is less abundance of *O. xalapensis* in late-successional than early stage (Quintana-Ascencio, Ramírez-Marcial, González-Espinosa, & Martínez-Icó, 2004) and we assumed that late-successional represent a predictably environment resulting in some kind of specialization, thus we expect a low genetic diversity in the late successional than early stage, and similar genetic diversity among age stage assuming random or mixed mating.

Methods

Study Species

O. xalapensis is a medium-sized tree and can be up to 20 m tall and 1 m in diameter at breast height (DBH) (Figure 1). This tree species has a broad geographic distribution from Mexico to Central America (Pennington & Sarukhán, 2005) and is typically found in old-growth forest conditions in MCFs in Mexico (Quintana-Ascencio & González-Espinosa, 1993). *O. xalapensis* is abundant in the remnants of MCF of Chiapas, but its habitats are being severely threatened by deforestation (González-Espinosa et al., 2011). It is an evergreen tree and flowering occurs during fall and winter (late November to early February), and fruiting occurs from April to May. The fruits are dispersed by birds (mainly *Catharus ustulatus* and *Turdus rufitorques*) as well as some small rodents (Ruiz-Montoya et al., 2011).

Study Site

We studied the population of *O. xalapensis* located in the HER at San Cristóbal de Las Casas, Chiapas, Mexico (Figure 2). HER is a relatively small private reserve (136 ha) with fairly extensive forest cover located on the east and northeast side of the HER, with an altitude ranging from 2,230 to 2,710 m asl (Ramírez-Marcial et al., 1998). It is composed of a series of ridges with steep slopes (40%–60%) and has a subhumid climate with abundant summer rainfall. The mean annual temperature is 14°C–15°C and the mean annual rainfall is 1,200 mm. The secondary montane oak forest is dominant from the lower to middle slopes of the mountain, and the primary MCF is dominant at the top portion (Ramírez-Marcial et al., 1998).

Successional Stages

We considered three MCF successional stages: early-, mid-, and late-successional, following the classification by Ramírez-Marcial et al. (1998). The early-successional stage (ESS) is secondary forest, found in the lowlands in the north and north-east region of the reserve, which has an abundance of *Quercus* spp. stumps indicating previous timber extraction (Ramírez-Marcial et al., 2001). An herbaceous layer with mostly perennial small plants, climbers, ferns, shrubs, and tree seedlings characterizes the ESS. The canopy is discontinuous, low (6–8 m), and dominated by oaks (*Quercus* spp.) with diameters greater than 30 cm, most of which resulted from individual sprouts. The mid-successional stage (MSS) is located along a narrow band around the base of the hill located between 2,330 and 2,460 m asl in the eastern and northern part of the HER. The MSS is characterized by scattered adults (25–30 m in height) and an abundance of seedlings and saplings of trees, and includes a sparse medium stratum (8–15 m in height), and a low stratum (4–7 m in height). The forest floor is generally more heterogeneous in plant cover and topography and receives more direct light than the ESS (Quintana-Ascencio et al., 2004). The late-successional stage (LSS), the primary forest, covers the upper portion of the hill and part of the western and north-westernmost sector of the HER. Vegetation is well preserved and there is no evidence of forest extraction, but some fallen trees are present due to strong winds

Figure 1. Some morphological characteristics of *Oreopanax xalapensis* Decne. & Planch. (a) Adult trunk, (b) leaves and branches, (c) preadult flowering, (d) preadult fruiting, and (e) seedling.

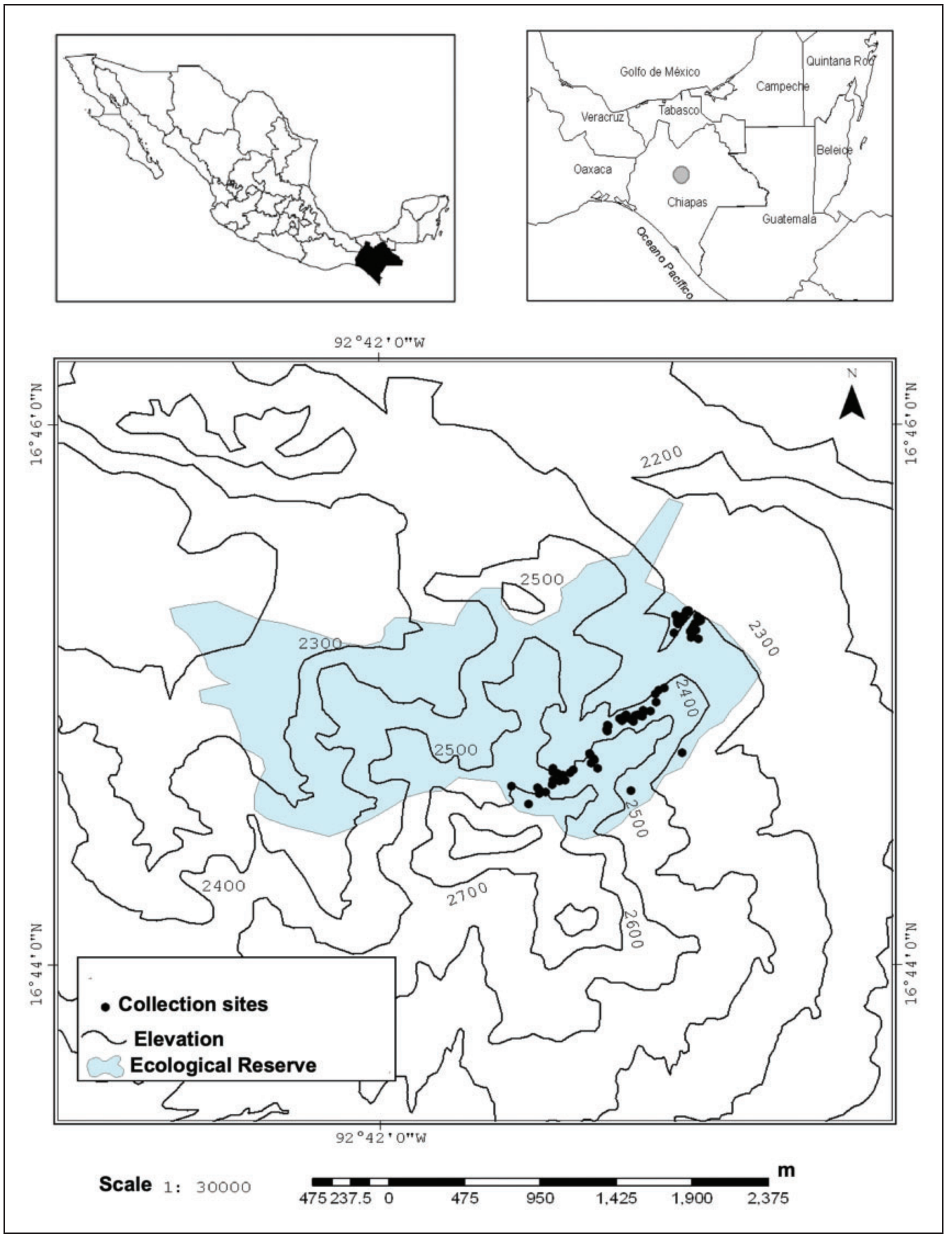

Figure 2. Location of the sampled sites of *Oreopanax xalapensis* at Huitepec Ecological Reserve, Chiapas, Mexico.

and storms. The canopy of LSS is mainly dominated by *Quercus ocoteifolia*, *Clethra chiapensis*, *Persea americana*, and *Cleyera theoides*, with maximum heights of 30 to 35 m (Ramírez-Marcial et al., 1998).

Population Size

We counted individuals of *O. xalapensis* of three age-classes: seedlings, saplings, and reproductive individuals, in 30 circular plots of 100 m^2 in each successional stage (3,000 m^2 total sampled area in each stage) from May to September 2009. We considered seedlings as individuals that were less than 30 cm height. The saplings were nonreproductive individuals ranging from 30 to 150 cm height and basal stem diameters <5 cm. Reproductive individuals were plants that were more than 150 m in height with reproductive structures (flowers, fruit). A chi-square test (χ^2) was used to compare frequencies of these age-classes among successional stages (Sokal & Rohlf, 1995).

Genetic Analysis

To record genetic data until 20 samples per age-class per successional stage were achieved. Leaves were gathered from individual randomly selected across the 30 plots per successional stage. Leaves were preserved in liquid nitrogen until they could be analyzed via cellulose acetate electrophoresis following the methods of Hebert and Beaton (1989). This genetic tool is useful for revealing genetic patterns, especially because of specific DNA markers have not yet been developed in *O. xalapensis*. For every individual, approximately 1 cm^2 of leaf material was macerated in a 3:1 mixture of extraction buffer solution YO: Vegetative extraction buffer II, obtained from Yeh and O'Malley (1980) and Cheliak and Pitel (1984), respectively. This mixture gave the best result of reproducibility and clarity of enzyme mark. Enzymes were extracted from the supernatant after centrifuging at 13,000 rpm for 4 min. Electrophoresis was done using cellulose acetate and two buffer solutions: citrates (CAAMP) and trizma glycine (TG) (Hebert & Beaton, 1989). We evaluated locus and alleles of these seven enzymes: superoxide dismutase (SOD, enzyme commission number EC 1.15.1.1, buffer CAAMP), acid phosphatase (AP, EC 3.1.3.2, CAAMP), fumarate hydratase (FUM, EC 4.2.1.2, CAAMP), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49, CAAMP), aspartate transaminase (GOT, EC 2.6.1.1, TG), glyceraldehyde-3-phosphate (G3PDH, EC 1.2.1.12, TG), and glucose-6-phosphate isomerase (GPI, EC 5.3.1.9, TG). Loci and alleles were identified according to the distance they migrated on a cellulose acetate; we obtained one loci with two alleles for all of the enzymes. Once established electrophoresis condition, we start the essay to record data genetic until got 20 samples per age-class per successional stage. In case a sample was unsuccessful, three repetitions were attempted before it was discarded. Therefore, the number of samples was variable by locus, age-classes and successional stages on range from 9 to 20. We estimated genetic diversity parameters as a percentage of polymorphic loci (P), observed (H_o), and expected (H_e) heterozygosity using GeneAlex software (Peakall & Smouse, 2006). A locus was considered to be polymorphic when the most common allele frequency was minor than or equal to 0.95. To estimate reduction in average heterozygosity due to inbreeding (inbreeding coefficient), the following equation was used: $f = 1 - H_o/H_e$, (Hedrick, 2000) and deviations from zero were tested by using chi-square analysis (χ^2) with the formula $\chi^2 = f^2 N$, where N is sample size with one degree of freedom (Hedrick, 2000). A significant deviation from Hardy–Weinberg Equilibrium (HWE) was evaluated for every locus within each group (ages and successional stages) and over all loci using a Fisher's combined test (Sokal & Rohlf, 1995). We tested allelic frequency heterogeneity across age-classes and successional stages by a combined test of replicated goodness-of-fit test (G-statistic; Sokal & Rohlf, 1995). The association of H_e and H_o with age-classes and successional stages was analyzed using a contingency table and tested using χ^2.

Structure of the *O. xalapensis* population in HER was determined by an analysis of molecular variance (Excoffier, Smouse, & Quattro, 1992) using GenAlex (Peakall & Smouse, 2006) with 1,000 iterations. We obtained fixation coefficients hierarchically which can be understood as the correlation of alleles (Excoffier et al., 1992) for age-classes drawn from the whole *O. xalapensis* sample ($\Phi_{ac/t}$), for age-classes within whichever of successional stages ($\Phi_{ac/ss}$), for successional stages relative to the all sample ($\Phi_{ss/t}$), for individuals relative to age-classes (Φ_{is}), and all sample (Φ_{it}).

To determine differentiation in genetic population structure across successional stages and age-classes without having previously defined group, we used the Bayesian approach implemented in the program Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). Structure determined probabilistically the number of *true* populations assumed to be in Hardy–Weinberg and linkage equilibrium between loci. The Structure program gave a parameter *K*-cluster obtained by simulations sampled with a Markov chain Monte Carlo (MCMC) algorithm. For this analysis, we included all samples at once and selected the admixture and the correlated frequencies model. The number of *K* clusters was determined by the methods of Evanno, Regnaut, and Goudet (2005), which was based on the second-order difference of the probabilities of *K* populations. We used 30,000 iterations and 30,000 MCMC for $K = 9$ populations. Once established the number of K-clusters, we plotted each of the individual membership scores by age-classes per successional stage.

Results

Population Size

Age-classes frequency of *O. xalapensis* was significantly different among successional stages ($\chi^2{}_{df,\ 4} = 97.8$, $p < .001$; Figure 3). The greatest number of *O. xalapensis* individuals was found in ESS, where frequency of seedlings was notably higher than saplings or reproductive individuals. In MSS and LSS, the more abundant size category was reproductive individuals. Seedlings were noticeably lower in LSS than in the other successional stages (Figure 3).

Genetic Diversity

On average, the observed number of alleles (N_a) was 2.0. Percentage of polymorphic loci was 100% for all populations except for seedlings from ESS and MSS (71%) (Table 1). The highest H_o value was observed in saplings from ESS (0.608) and MSS (0.562) (Table 1).

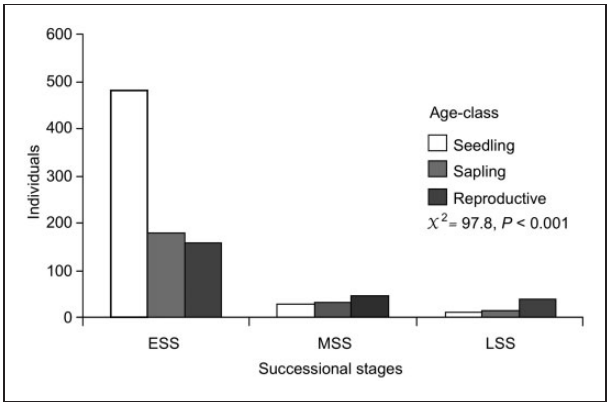

Figure 3. Age-class distribution of *Oreopanax xalapensis* inhabiting different successional stages at the Cerro Huitepec Ecological Reserve (Chiapas, Mexico). ESS = early-successional stage; MSS = mid-successional stage; LSS = late-successional stage.

The greatest value for H_e was found in sapling populations from ESS (0.464), and in the reproductive populations from ESS (0.465) and LSS (0.459).The lowest H_e value was observed in seedlings in ESS (0.299) and MSS (0.330) (Table 1). Negative values of f were recorded for seedlings (from −0.069 to −0.305) and saplings (from −0.100 to −0.348) in the three successional stages (Table 1). These results indicate an excess of heterozygotes in these groups; however, none of the values were significantly distinct from zero (Table 1). The frequency of observed genotypes was distinct from what was expected under the HWE for the majority of loci and age-classes for each successional stage. The combined test was significant, indicating a general bias of genotype frequency from HWE (Table 1).

Hierarchical analysis of molecular variance showed significant genetic difference among age-classes within successional stages ($\Phi_{ac/ss} = 0.175$) and within whole sample ($\Phi_{ac/t} = 0.163$). There were not significant genetic

Table 1. Estimators of Genetic Diversity (±1 Standard Error) for Different Age-Classes of *Oreopanax xalapensis* Inhabiting Successional Stages of Montane Cloud Forest in the Huitepec Ecological Reserve (Chiapas, Mexico).

		Age-classes			
SS	Estimator	Seedling	Sapling	Reproductive	Total
ESS	N	18.9 ± 0.5	17.9 ± 1.1	19.6 ± 0.2	56.3 ± 1.4
	N_a	1.7 ± 0.2	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	H_o	0.388 ± 0.134	0.608 ± 0.089	0.336 ± 0.056	0.448 ± 0.053
	H_e	0.299 ± 0.086	0.464 ± 0.021	0.465 ± 0.013	0.468 ± 0.014
	f	$-0.305 \pm .166^{ns}$	-0.348 ± 0.173^{ns}	0.252 ± 0.125^{ns}	0.472 ± 0.015^{ns}
	P	71.43	100	100	100
	HW	33.06^{*}	54.78^{*}	29.92^{*}	86.52^{*}
MSS	N	18.3 ± 0.9	17.9 ± 1.8	19.6 ± 0.2	55.7 ± 2.0
	N_a	1.7 ± 0.2	2.0 ± 0.0	2.0 ± 0.0	2 ± 0.0
	H_o	0.348 ± 0.137	0.562 ± 0.087	0.283 ± 0.033	0.391 ± 0.074
	H_e	0.330 ± 0.086	0.440 ± 0.023	0.449 ± 0.043	0.493 ± 0.007
	f	-0.076 ± 0.257^{ns}	-0.286 ± 0.147^{ns}	0.311 ± 0.096^{ns}	0.207 ± 0.144^{ns}
	P	71.43	100	100	100
	HW	47.51^{*}	40.19^{*}	32.56^{*}	83.75^{*}
LSS	N	15.9 ± 1.4	16.6 ± 1.2	19.4 ± 0.3	51.9 ± 1.8
	N_a	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
	H_o	0.405 ± 0.098	0.385 ± 0.091	0.311 ± 0.090	0.360 ± 0.081
	H_e	0.392 ± 0.050	0.369 ± 0.038	0.459 ± 0.021	0.437 ± 0.028
	f	-0.069 ± 0.179^{ns}	-0.100 ± 0.187^{ns}	0.331 ± 0.190^{ns}	0.157 ± 0.174^{ns}
	P	100	100	100	100
	HW	32.85^{*}	33.97^{*}	32.85^{*}	44.99^{*}
Total	N				18.206 ± 0.348
	N_a				1.937 ± 0.031
	H_o				0.403 ± 0.033
	H_e				0.407 ± 0.017
	F				-0.022 ± 0.062^{ns}
	P				100

SS = successional stage; N = average number of individuals analyzed; Na = average number of different alleles; H_o = observed heterozygosity; H_e = unbiased expected heterozygosity; f = fixation index; ESS = early-successional stage; MSS = mid-successional stage; LSS = late-successional stage; $HW = \chi^{2.}$ for combined Hardy–Weinberg equilibrium test; ns = not significant.
$^{*}p < .001$.

Table 2. Hierarchical Analysis of Molecular Variance of *Oreopanax xalapensis* Populations From the Huitepec Ecological Reserve (Chiapas, Mexico) and Estimated Variance Component (VC), Percentage Distribution of Variance (V) Among and Within Samples, and Endogamy Coefficient.

	AMOVA					
Source	*df*	SS	MS	VC	V (%)	Φ-statistics
Among successional stages	2	25.51	12.75	0.00	0	$\Phi_{ss/t} = -0.012^{ns}$
Among age-classes (per successional stage)	6	96.78	16.13	0.35	17	$\Phi_{ac/ss} = 0.175^{*}$
Among individuals	171	349.33	2.04	0.38	19	$\Phi_{ac/t} = 0.163^{*}$
Within individuals	180	231.50	1.29	1.29	64	$\Phi_{is} = 0.227^{*}$
Total	359	703.11		2.02	100	$\Phi_{it} = 0.353^{*}$

$\Phi_{ss/t}$ = endogamy coefficient of successional stage in relation to all sample; $\Phi_{ac/ss}$ = endogamy coefficient of age-class in relation to successional stage; $\Phi_{sc/t}$= endogamy coefficient of age-class in relation to all sample; Φ_{is} = endogamy coefficient of individual relative to age-class; Φ_{it}= endogamy coefficient of individual relative to total sampling; ns = not significant; AMOVA = analysis of molecular variance; SS = successional stage; MS = mid-successional.
$^{*}p < .001$.

differences among successional stages ($\Phi_{ss/t} \approx 0$, since the value obtained is negative) (Table 2). Significant levels of inbreeding within and total samples were also found (Φ_{is}, Φ_{it}). Three *K* genetic clusters were inferred by the Bayesian multilocus analysis (Figure 4(a)). Each one of the nine age-classes/successional stage groups displayed different genetic proportions from each inferred cluster (Figure 4(b)–(j)). LSS seedlings genetically were associated with the Cluster 3 (Figure 4(b)); seedlings in MSS have an admixture with Clusters 1 and 2 (Figure 4 (e)), and 80% of the ESS seedlings were associated with the Cluster 2 (Figure 4(h)). The genetic in saplings were similar across successional stages with the highest proportion of saplings being associated with the Cluster 3 (Figure 4(c), (f), and (i)). The reproductive individuals from ESS and MSS have and admixture with Clusters 2 and 3, while the individuals of LSS has a membership score of 41% to Cluster 2 (Figure 4(d), (g), and (j)).

Discussion

Population Size

We recorded different population sizes of *O. xalapensis* across successional stages of MCF at HER. Their populations were largest in ESS, with a significantly high number of seedlings, suggesting that the conservation strategy implemented provided favorable conditions to recruitment of seedlings and a potential population growth of *O. xalapensis*. The greatest seedling recruitment occurred in ESS, likely due to the greater amount of available sunlight, which favored *O. xalapensis* germination (Quintana-Ascencio et al., 2004). In the ESS inter- and intraspecific competition is high and microenvironmental conditions may vary greatly (Galindo-Jaimes, González-Espinosa, Quintana-Ascencio, & García-Barrios, 2002), therefore few seedlings can reach the juvenile and oldest size. We observed many young individuals (height <1 m, DBH <3 cm) with reproductive structures, suggesting that more individuals might contribute to the seedling population in ESS than in MSS or LSS. A decline in the number of seedling and juvenile individuals as the population grows older has been observed previously in this species in the same area (Quintana-Ascencio et al., 2004). Accordingly, fewer saplings and reproductive individuals were recorded in ESS. In contrast, in the advanced successional stages (MSS and LSS), the seedlings were very low in numbers, as were the saplings and reproductive individuals.

Genetic Diversity and Structure

Despite the significant differences in demographic structure by succession stage and decrement of population size as succession advance, the genetic diversity in *O. xalapensis* was homogeneously distributed across succession stage, indicating a good genetic recovery capacity of *O. xalapensis* in the ESS related to late stages.

The high genetic diversity of *O. xalapensis* (measured as H_e and polymorphism) contrasted with the low genetic diversity documented for angiosperms ($H_e = 0.169$, Hamrick, Godt, & Sherman-Broyles, 1992) and with some temperate tree species such as *Acer saccharum* ($H_e = 0.30$; Baucom, Estill, & Cruzan, 2005), *Alnus rubra* ($H_e = 0.11$, Xie, El-Kassaby, & Ying, 2002), and *Nothofagus* spp (Vergara, Gitzendanner, Soltis, & Soltis, 2014). Genetic diversity of tree species is associated with their longevity, mating system, and their dispersal mode (Hamrick & Loveless, 1986; Hamrick et al., 1992; Jordano, García, Godoy, & García-Castaño, 2007; Shea, 2007). In addition, temporal environmental suitability on a wide geographic scale could explain the genetic diversity pattern that was observed. The mating system of *O. xalapensis* is unknown. The *f* values suggest random or mixed mating, which may contribute to

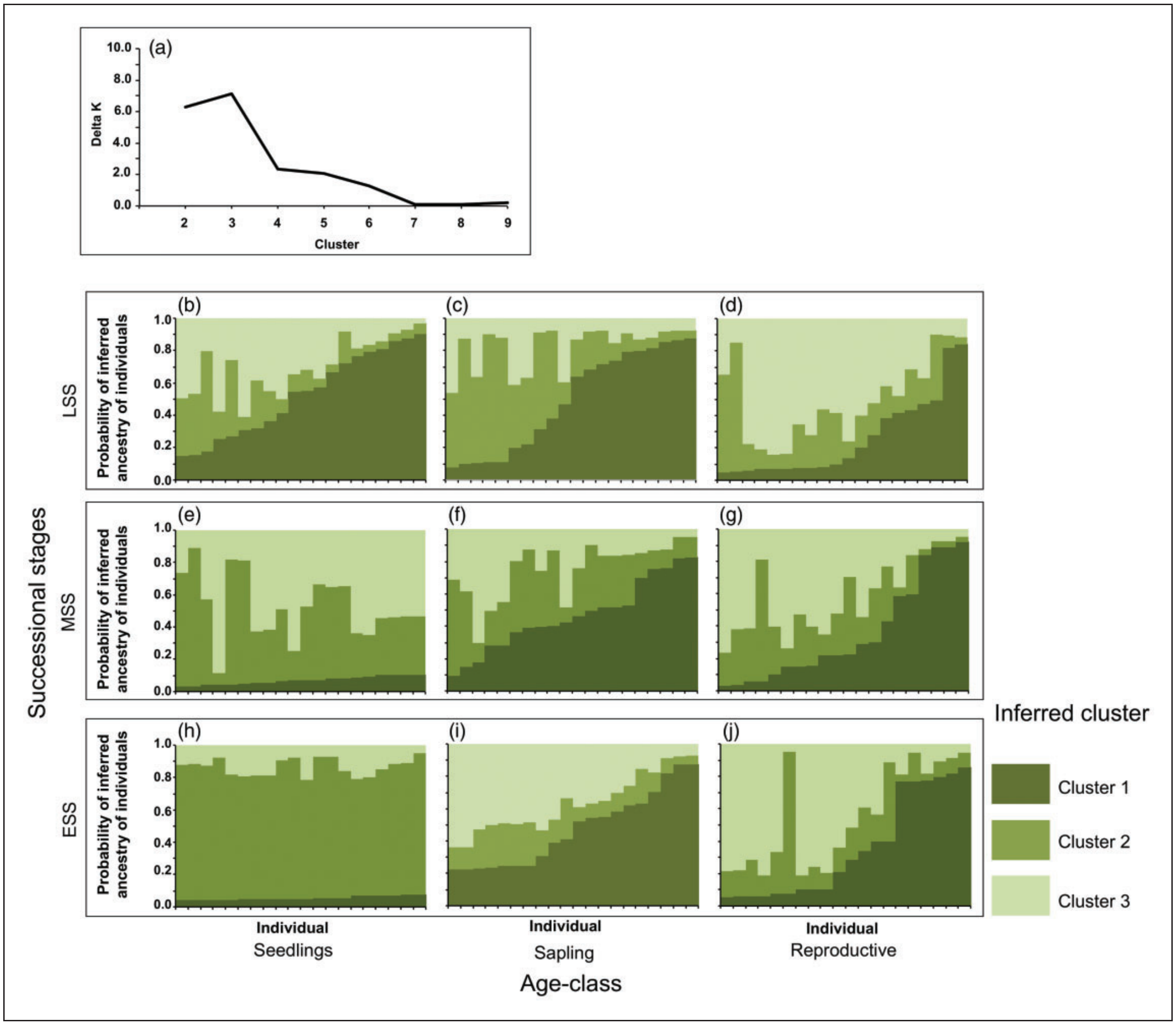

Figure 4. Bayesian cluster analysis of *Oreopanax xalapensis* computed by Structure software 2.3.4 with $K = 3$ to age-classes and successional stage. In each graph, the individuals are represented by a vertical line broken into K segments whose length is proportional to the estimated memberships in the three inferred clusters. Delta K analysis results following Evanno et al. (2005) are shown on the top chart.

preserving genetic diversity within and across successional stages. However, considering an arrangement hierarchical of samples, some level of endogamy was found, reflecting possible reproduction between related individuals. It is probably that part of population is product of a self-pollination, especially in LSS where number of reproductive individuals was less. The samples of *O. xalapensis* from ESS, MSS, and LSS were taken at a fine scale geographic distribution (around 350 m lineal distance), inferring that the seeds are easily dispersed among and between the different successional stages, which likely contributes to the genetic homogenization among populations (Sezen, Chazdon, & Holsinger, 2005). Seed dispersal in *O. xalapensis* is highly mediated by mobile animals such as birds, bats, and small rodents (Ruiz-Montoya et al., 2011), which may increase the gene flow, thereby preventing genetic differentiation (Hamrick, Murawski, & Nason, 1993). Therefore, we were unable to establish the relationship between population size and genetic diversity across successional stage. At a larger geographical scale (>20 km), *O. xalapensis* display differentiation among populations of the Highland of Chiapas, mainly driven by fragmentation of MCF (Ruiz-Montoya et al., 2011).

We found genetic structure related to age-classes, with the population of sapling presenting the highest genetic diversity as H_o or H_e. We recognize a possible sample bias in the reproductive individuals because we were unable to sample the oldest trees (>30 cm DBH) in

LSS because their canopy was out of reach. Instead, we decided to take samples from trees with 5 to 20 cm DBH with reproductive structures (flowers and fruit) in all successional stages. However, we are confident that the sampled reproductive individuals represent a cohort of adults, with potential or realized contributions to seedling populations. Bayesian analysis on structure shows three possible genetic population or genetics clusters. The hypothetical genome of three clusters was distributed among all sampled, but in some of them predominated one cluster, indicating a restricted genetic interchange between samples. However, considering the biology of *O. xalapensis*, the genetic structure might be associated to correlation spatial due to the fine geographic scale of the study, and the demographic change along successional stage also. The differentiation among age-classes of tree species has been related to assortative-mating (Goncalves, García, Heuertz, & Martínez-Santiago, 2019) and could also result from differences in survival rate of age-classes. Random or mixed mating is possible in *O. xalapensis* (on base of *f* values), even thus part of the seedlings could be offspring from a low number of adult tree, promoting reduced diversity and differentiation respect to advanced ages. On the other hand, the intraspecific competition may prevent that seedlings reach the juvenile age and these ones does not reach oldest size too. This limited transition from seedling to adult reduces the genetic diversity in both way random and by natural selection favoring genetic differentiation among age-class.

It is important note that enzymatic systems used provided value information; however, they showed low variability limiting to obtain contemporary patterns of genetic diversity and structure accordingly. Further studies should carry out using higher polymorphic markers, to reveal clearly patterns and evolutionary process involved in populations of *O. xalapensis* inhabiting in different forest ages.

In conclusion, our study suggests that *O. xalapensis* overcame any demographic and genetic impacts from timber extraction that occurred 50 years ago. The demographic and genetic resilience of *O. xalapensis* rely on its early reproduction (about 5 years old), individual longevity, and probably its extensive pollen and seed flow. The demographic dynamics in each successional stage is associated with forest age and has mild impact on the genetic structure across ages-classes of *O. xalapensis* in Huitepec locality.

Conservation Implications

First of all, it can be pointed out that the incipient forest with a canopy dominated by oaks offers humidity and light conditions which are favorable for the development of seedlings and juveniles of *O. xalapensis* (Quintana-Ascencio et al., 2004). It is highly probable that *O. xalapensis* reach maturity under these conditions. Second, the successional development without forest exploitation activities allows for the recovery and conservation of the genetic diversity of *O. xalapensis*, and with it, its evolutionary potential. Based on our results, it can be suggested that seeds from reproductive individuals from any of the successional stages can be used as seed sources for the enrichment of degraded MCFs of Chiapas. In the particular case of the HER site, our results of high genetic diversity and low genetic structures throughout the successional stages of the forest can be taken as indicators of the efficiency of conservation strategies, since ESS and MSS show similar genetic diversity to LSS. These results indicate that while the conservation strategy was initially for recreational purposes, it has also improved the recovery of the abundance and genetic diversity of *O. xalapensis* in areas with logging in the past in HER. However, this result cannot be generalized to other MCF species. Therefore, we suggest that genetic and demographic studies should be conducted on other MCF species, particularly those that are known to be at risk due to their restricted distribution and low density (e.g., González-Espinosa et al., 2011). Studies on genetic diversity can help ensure that restoration and conservation programs lead to higher levels of genetic diversity within the ecosystem, especially for endangered MCF species. This study showed that the continuity of *O. xalapensis* populations in HER is safely assured as long as the logging or habitat destruction is avoided. Unfortunately, worldwide MCF is continuously being transformed into pastures, cultivation areas, and human settlements (González-Espinosa et al., 2011). This transformation decreases the habitat of *O. xalapensis* by fragmenting and isolating populations. The continuation of this trend may lead to the possible extinction of many MCF species, especially *O. xalapensis*. Therefore, it is urgently needed to maintain the diversity and ecological functionality of HER, to establish measures to maintain the integrity of the MCF in order to recover and enrich the forests previously degraded from human activity.

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