Influence of Pleistocene Glacial/Interglacial Cycles on the Genetic Structure of the Mistletoe Cactus *Rhipsalis baccifera* (Cactaceae) in Mesoamerica

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Abstract

Phylogeographical work on cloud forest-adapted species provides inconsistent evidence on cloud forest dynamics during glacial cycles. A study of *Rhipsalis baccifera* (Cactaceae), a bird-dispersed epiphytic mistletoe cactus, was conducted to investigate genetic variation at sequence data from nuclear [internal transcribed spacer (ITS), 677 bp] and chloroplast (*rpl32-trnL*, 1092 bp) DNA for 154 individuals across the species range in Mesoamerica to determine if such patterns are consistent with the expansion/contraction model of cloud forest during glacial cycles. We conducted population and spatial genetic analyses as well as gene flow and divergence time estimates between 24 populations comprising the distribution of *R. baccifera* in Mexico and Guatemala to gain insight of the evolutionary history of these populations, and a complementary species distribution modeling approach to frame information derived from the genetic analyses into an explicit paleoecological context. The results revealed a phylogeographical break at the Isthmus of Tehuantepec, and high levels of genetic diversity among populations and cloud forest areas. Despite the genetic differentiation of some *R. baccifera* populations, the widespread ITS ribotypes suggest effective nuclear gene flow via pollen and population differentiation shown by the *rpl32-trnL* suggests more restricted seed flow. Predictions of species distribution models under past last glacial maximum (LGM) climatic conditions and a significant signal of demographic expansion suggest that *R. baccifera* populations experienced a range expansion tracking the conditions of the cloud forest distribution and shifted to the lowlands with population connectivity during the LGM.

Subject areas: Population structure and phylogeography

Key words: Cactaceae, epiphytes, Mesoamerica, Mexico, Pleistocene, Rhipsalis, refugia
conditions during the Pliocene and climate changes during Pleistocene glaciations that promoted the expansion, contraction, and divergence of populations (Jaramillo-Correa et al. 2009; Ruiz-Sanchez and Ornelas 2014).

More recently, repeated cycles of cloud forest contraction and expansion due to Pleistocene climatic cycling has shaped genetic divergence in the region, producing a common phylo-

graphical break at the Isthmus of Tehuantepec (González et al. 2011; Gutiérrez-Rodríguez et al. 2011; Ornelas et al. 2013; Rodríguez-Gómez et al. 2013). However, plant species that presumably colonized northern Mesoamerica from South America are little studied. Four studies have explored the phy-

logeography of widespread, cloud forest-adapted plant species across northern Mesoamerica. High levels of genetic variation and weak geographical structuring were inferred for bird-dis-

persed *Podocarpus matudae* (Podocarpaceae) and *Pulicaria patifo-

lia* (Rubiaceae) (Ornelas et al. 2010; Gutiérrez-Rodríguez et al. 2011) and wind-dispersed *Liquidambur styraciflua* (Altingiaceae) (Ruiz-Sanchez and Ornelas 2014), whereas high levels of genetic variation and strong genetic structure were inferred for wind/gravity dispersed *Moussonia detpeana* (Gesneriaceae) (Ornelas and González 2014). Furthermore, comparative tests of simultaneous diversification revealed that the phyloge-

ographical breaks in several codistributed cloud forest-adapted taxa occurred as multiple vicariant events at different times (Ornelas et al. 2013). However, the detailed comparative phy-

logeographical data necessary to assess such hypotheses are still deficient, particularly for the flora of Mesoamerican cloud forest (Ramírez-Barahona and Eguiarte 2013), which so far characteristic epiphytes have been overlooked.

Based on the knowledge on precipitation during the last glacial maximum (LGM), and hence on the fragmented distribution of cloud forests, Ramírez-Barahona and Eguiarte (2013) proposed 2 demographic models for cloud forest-adapted plant species based on opposite precipitation regimes. The dry refugia model posits that populations of the cloud forest-adapted species were displaced and compressed into refugia by the opposing effects of aridity and cooling during the LGM and, subsequently, these populations expanded and recollected the species former range at the onset of more humid and warm conditions. In contrast, the moist forests model contends that changes in precipitation had a minor effect on the continuity of forest cover, and the unchanging humidity conditions favored downslope range expansion, little to no demographic growth, and population connectivity during the cold glacial periods, followed by the fragmentation into high altitude populations during warm interglacials (Ramírez-Barahona and Eguiarte 2013).

Here, we used nuclear and plastid DNA sequences of the mistletoe cactus *Rhipsalis baccifera* (J.S. Mueller) Stearn. (Cactaceae) to explicitly test geological and historical climatic change scenarios for cloud forest taxa in Mesoamerica and to identify patterns of population structure that may reflect the evolutionary history of the species. The bird-dispersed, epiphytic cactus lives on woody plants and, as a consequence, its distribution range could be influenced by geographical host range changes and potentially related to the availability of suitable host trees that expanded their distribution during glacial periods and then contracted during the interglacial periods (Jaramillo-Correa et al. 2009). Within a region, epiphytic mistletoe cacti tend to be found on a subset of tree species (Andrade and Nobel 1997), and they generally have tendencies for certain habitats or substrates (de la Rosa and Briones 2010). However, its associations with host plant species are loose, indicating that these mistletoe cacti do not require certain conditions for establishment (i.e., radiation, humidity, and characteristics of the tree bark). If epiphytes have a non specialized dependence on their hosts, the genetic structuring might not be influenced directly by the geographic structuring of host populations but the result of climatic fluctuations throughout the Pleistocene that altered the distribution of suitable habitat leading to variation in population continuity and isolation. As a consequence, its current distribution range might have resulted from colonization and recolonization events determined by climatic changes and historically unrelated to the availability of suitable host trees.

In this study, we used a broad geographical sampling of populations of *R. baccifera* DNA sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) in combination with a noncoding plastid DNA region (rpl32-trnL) from the chloroplast genome (cpDNA). As the chloroplast genome is maternally inherited in most angios-

perms, cpDNA sequences may provide a picture of evolutionary haplotype relationships of seed-mediated gene flow compared with the ITS sequences reflecting a combination of both pollen and seed movement (Ornelas and González 2014). Specifically, we assessed the pattern of genetic diversity of *R. baccifera* within and between conspecific populations in Mesoamerica, and determined whether its genetic divergence is geographically structured according to climatic scenarios and historical fragmentation of cloud forest distribution. The geographical isolation by the Isthmus of Tehuantepec might have led to reduced gene flow and account for much of the differentiation between populations on either side of the isthmus because of their distinctly unsuitable environmental conditions relative to cloud forest habitats favored by these epiphytes. If the species experienced a range expansion tracking the conditions of the cloud forest distribution and shifted to the lowlands with population connectivity during the LGM, as predicted by the moist forests model, we could detect a signif-

icant increase in the overall effective population size dat-

ing to sometime during the LGM when sudden demographic expansion occurred and descent to lower elevations allowed populations to gene flow (hypothesis 1). If the populations of the species were displaced to the lowlands and compressed into refugia without population connectivity during the LGM, as predicted by the dry refugia model, gene flow between iso-

lated populations from different refugia, and habitat suit-

ability would be limited, and populations from different refugia would thus become differentiated (hypothesis 2). Given the wide host and geographical ranges of *R. baccifera*, and that large-scale seed recruitment patterns produced by frugivorous birds that consume and eventually disperse mistletoe cactus seeds might have led to increased gene flow, the geographical genetic structure created by cloud forest isolation before and during the Pleistocene could be obscured.
Methods

Study System

The genus Rhipsalis Gaertner (Cactaceae) is mainly a New World epiphytic cactus distributed from Mexico to South America on a wide range of host trees (Bravo-Hollis 1987; Calvente et al. 2005, 2011a). In contrast to other epiphytic cacti, Rhipsalis is distinguished by its cylindrical leafless up to 1 m long stems hanging from the crevices, trunks, or branches of evergreen and deciduous trees (Bravo-Hollis 1987; Kress 1989). It is the largest genus in the tribe Rhipsalideae with c. 36–37 species distributed in 5 subgenera: Calamorhipsalis K.Schum. (3 spp.), Epallagogonium K.Schum. (7 spp.), Erythrorhipsalis A.Berger (8 spp.), Phyllarthrorhipsalis Buxb. (12 spp.), and Rhipsalis (6 spp.) (Barthlott and Taylor 1995; Hunt 1999; Calvente et al. 2005). The majority of species that belong to this genus (29 spp.) are endemic to Brazil, with several species presenting very restricted distribution ranges within the country (Calvente et al. 2005, 2008, 2011b). The affinities among these subgenera based on stem shape had been recently evaluated using molecular phylogenetic data (Calvente et al. 2011a, 2011b). These studies suggest that a subdivision of the genus in 3 main groups would be more appropriate based on flower traits: Rhipsalis s.l. ("core Rhipsalis"), Erythrorhipsalis, and Calamorhipsalis s.l. (="sunken pericarpel" clade). The "core Rhipsalis" clade includes all species previously included in Rhipsalis subg. Phyllarthrorhipsalis and Rhipsalis subg. Rhipsalis, plus R. pentalpiptera (subg. Epallagogonium). While Rhipsalis subg. Phyllarthrorhipsalis is monophyletic, Rhipsalis subg. Rhipsalis is polyphyletic. Intra-specific phylogenetic relationships and delimitation of some of the polymorphic Rhipsalis species remain unresolved (Calvente et al. 2011b; Korotkova et al. 2011).

Rhipsalis baccifera is naturally distributed from Mexico to South America (Bravo-Hollis 1987). Its distribution in the Caribbean, FL, and in the tropics of the Old World is thought to be the result of relatively recent human-based introductions (Cota-Sánchez and Bomfim-Patrício 2010). The species show considerable polymorphism and has been divided into 7 subspecies (Barthlott and Taylor 1995). Some authors recognize morphological variants occurring from eastern Mexico to northern South America (the Chocó-Darién biogeographical region) as R. baccifera baccifera, and R. baccifera Clever, and R. cassutha Gaertn. as syn. of R. baccifera (Barthlott and Taylor 1995). It is a representative species of the Mesoamerican cloud forests, where epiphytes constitute 30% of species present (Rzedowski 1996). Plants produce small, hermaphroditic self-compatible flowers that are presumably pollinated by insects, and many globose long-lasting fleshy fruits containing black seeds embedded in a transparent and whitish sticky mucilaginous pulp that resembles Phoradendron mistletoes (Bravo-Hollis 1987), a specialized seed dispersal system. The seeds are dispersed by a variety of birds across its distribution range (Guaraldo et al. 2013), the most important in Mesoamerica being common bush-tanager (Chlorospingus ophthalmicus) Du Bus, Emberizidae) and yellow-throated euphonia (Euphonia xirundinaceae Bonaparte, Thraupidae) (Cruz-Angón and Greenberg 2005; Guaraldo et al. 2013; J.F. Ornelas, unpublished data).

Sample Collection and DNA Sequencing

Stem tissue samples were collected from 154 individuals on a wide variety of host species in 24 populations throughout the species range in cloud forests of Mexico and Guatemala, ranging from almost sea level to 1624 m above sea level (Supplementary Figure S1 online; Supplementary Table S1 online). Target sampling localities were chosen based on localities of occurrence data acquired from Mexican herbarium records. Fifteen populations were sampled from 5 disjunct cloud forest areas along the Sierra Madre Oriental (nSMO, eSMO, sSMO), 5 in the Sierra de Los Tuxtlas region (TUX), 1 in the Sierra Madre de Chiaapas, and 2 in Guatemala. The population from Chiaapas was pooled with Guatemala populations (GUAT). Most populations collected have an accompanying herbarium voucher that is deposited at the Xalapa Herbarium of the Instituto de Ecología, AC (XAL). To prevent the effect of sampling clonal and vegetative growth, only one R. baccifera plant was sampled per individual host tree. Leaf tissue samples were preserved in silica gel desiccant until DNA extractions were performed. We additionally obtained ITS and rp32-trnL sequences from the GenBank of Drosophila simulans, Phlegra iaanthus, and various species of Lepismium, Schismberga, Hatria, and Rhipsalis from Calvente et al. (2011a) to be used for sequence alignment and as outgroups.

Total genomic DNA was extracted from silica-dried material using a modified 2×cteyl trimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle 1987). Amplification of the ITS region of the nrDNA was conducted with primers ITS17SE and ITS26SE (Sun et al. 1994), whereas for rp32-trnL (UGAG) region we used rp32 and trnL-UAG (Shaw et al. 2007). The 25 µL polymerase chain reaction (PCR) mix contained 5 µL of 5× buffer (Promega, Madison, WI), 3.5 µL MgCl2 (25 mM), 2 µL dNTPs mix (8mM), 0.5–1 µL each primer (10 µM), 0.15 µL Taq polymerase (5U/µL) (Promega), 0.2–0.5 µL of BSA (Promega), 1.5–3 µL of template DNA, and finally dH2O added to bring to volume. PCRs of ITS consisted of an initial denaturing at 94 °C for 4 min, followed by 10 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min, and a final step of 72 °C for 7 min. Chloroplast rp32-trnL (UGAG) amplifications used the following profile: initial denaturing at 80 °C for 5 min, followed by 10 cycles of 95 °C for 45 s, 60 °C for 45 s, 65 °C for 2 min, and a final step of 65 °C for 5 min. PCR products were purified with the QIAquick kit (Qiagen, Valencia, CA) and sequenced in both directions to check the validity of the sequence data using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). The products were analyzed on a 310 automated DNA sequencer (Applied Biosystems) at the Instituto de Ecología, AC sequencing facility, or at Macrogen, Seoul, South Korea. Finally, sequences were assembled using Sequencer 4.9 (Gene Codes, Ann Arbor, MI) and then were manually aligned with SE-AL 2.0a111 (http://tree.bio.ed.ac.uk/software/seal). All sequences used in this study have been submitted to GenBank (Accession nos. KM886469–KM886566, KM986672–KM986828).

Relationships Among Haplotypes

To infer genealogical relationships among haplotypes, statistical parsimony networks for the ITS and rp32-trnL data sets
were constructed as implemented in TCS 1.2.1 (Clement et al. 2000), with the 95% connection probability limit and treating gaps as single evolutionary events. Loops were resolved following the criteria given by Pfenninger and Posada (2002).

**Divergence Time Estimation**

We used Bayesian inference (BI) to estimate divergence time inferred on separate gene trees for the ITS and plastid rpl32-trnL sequences. The ingroup comprised all nrDNA or plastid cpDNA sequences of *R. baccifera* and sequences downloaded from GenBank of species representing *Rhapisida, Hatiora, Lepismium, Schilhmberia, Pereskia*, and *Pfeiffera* from Calvente et al. (2011a) used as multiple outgroups. Phylogenetic reconstruction was performed in BEAST 1.6.1 (Drummond and Rambaut 2007) using the uncorrelated lognormal relaxed molecular clock and the closest nucleotide substitution model under the Akaike information criterion, HKY+G+I for the ITS and GTR+G for the rpl32-trnL data sets), suggested by jMODELTEST 0.1.1 (Posada 2008). The tree prior model was set using a coalescent approach assuming constant population size or population expansion. We constrained Cactoidea, Rhipsialideae, *Rhapisida*, and *R. baccifera* to be monophyletic based on Edwards et al. (2005), Arakaki et al. (2011) and Calvente et al. (2011a). For temporal calibration of the root node of the tree, we used as secondary calibration the age estimated for the Cactaceae estimated at 35 Ma [normal distribution, mean 35, standard deviation (SD) 2.6, range 40.1–29.9 Ma] based on Arakaki et al. (2011). For the Cactoidea, Rhipsialideae, and *Rhapisida* crown groups, the ages of 24.4 Ma (normal distribution, mean 24.4, SD 1.0, range 26.36–22.44 Ma), 16 Ma (normal distribution, mean 16, SD 2.2, range 20.31–11.69 Ma), and 10 Ma (normal distribution, mean 10, SD 2.5, range 14.9–5.1 Ma) were assigned as secondary calibrations, respectively, according to divergence time estimates by Arakaki et al. (2011) based on fossil calibrations. Three independent 10^7 generation runs were performed with random starting trees, sampling every 1000 generations. Based on these results, data were analyzed using TRACER 1.6.0 (http://beast.bio.ec.ac.uk/tracer) to assess convergence and effective sample size (ESS cut off values >200) of each parameter, the first 10% trees per run were discarded as burn-in, and the remaining samples were combined in LOGCOMBINER 1.6.1 (http://beast.bio.ec.ac.uk/LogCombiner) and summarized as a maximum clade credibility tree with mean divergence times and 95% highest posterior density (HPD) intervals of age estimates in TREANNOTATOR 1.6.1 (http://beast.bio.ed.ac.uk/TreeAnnotator). Finally, these results summarized in a single tree were visualized in FIGTREE 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

**Population Indices and Geographic Structure of Populations**

Population diversity for unordered (*b*, *b*) and ordered haplotypes (*s*, *v*) and differentiation (*G*<sub>ST</sub>, *N*<sub>ST</sub>) parameters were estimated using PERMUT 1.0 (Pons and Petit 1996). Significant differences between *N*<sub>ST</sub> and *G*<sub>ST</sub> parameters were tested with 10 000 permutations. If *N*<sub>ST</sub> is significantly larger than *G*<sub>ST</sub>, this could indicate that the haplotypes found in a given population are phylogenetically closely related (Pons and Petit 1996). We also calculated haplotype diversity (*h*), nucleotide diversity (*τ*) and pairwise comparisons of *F*<sub>ST</sub> values between populations and groups in ARLEQUIN 3.01 (Excoffier et al. 2005) with 1000 permutations.

To determine whether or not populations are structured by cloud forest area, 3 analyses of molecular variance (AMOVAs; Excoffier et al. 1992) were performed based on pairwise differences using ARLEQUIN with populations treated as 1) a single group to determine the amount of variation partitioned among and within populations, and 2) grouped into 5 groups according to cloud forest fragmented distribution or 3) grouped into east and west of the Isthmus of Tehuantepec (Supplementary Figure S1 online; Supplementary Table S1 online). AMOVAs were performed using the Tamura and Nei model with a gamma shape parameter of 0.438 from the ITS and 0.286 from the rpl32-trnL matrices, and 10 000 permutations to determine the significance of each AMOVA.

We also conducted a spatial analysis of molecular variance (SAMOVA) as implemented in SAMOVA 1.0 (Dupanloup et al. 2002) to reconstruct groups of locations that are geographically homogeneous and genetically differentiated from each other, maximizing the proportion of total genetic variance due to differences among groups of locations (*F*<sub>CT</sub>). The most likely number of groups (K) was determined by repeatedly running SAMOVA with 2–7 groups (Supplementary Table S1 online; Supplementary Figure S1 online) and choosing those partitions with a maximum *F*<sub>CT</sub> value, as suggested by Dupanloup et al. (2002). We explored K-values with 10 000 simulated annealing simulations for each K. All analyses were performed separately for each marker.

**Demographic History**

The demographic history of each *R. baccifera* group was determined by means of neutrality tests and mismatch distributions carried out in ARLEQUIN. To test whether populations evolve under neutrality, Fu’s *F*<sub>T</sub> test (Fu 1997) and Tajima’s D (Tajima 1989) were calculated, and mismatch distributions (Harpending 1994) were calculated using the sudden expansion model of Schneider and Excoffier (1999) with 1000 bootstrap replicates. The validity of the sudden expansion assumption was determined using the sum of squares differences (SSD) and Harpending’s raggedness index (Hri; Harpending 1994), both of which are higher in stable, non-expanding populations (Rogers and Harpending 1992).

The demographic history of each *R. baccifera* group (east and west of the Isthmus of Tehuantepec; see Results) was also inferred with Bayesian skyline plots (Drummond et al. 2005) of changes in effective population size (*N*<sub>e</sub>) through time in BEAST. We chose a HKY substitution model with empirical base frequencies, a strict clock model, and a piecewise-linear coalescent Bayesian skyline tree prior with 5 starting groups. Two independent runs of 20 million generations each were run, with trees and parameters sampled every 1000 iterations, with a burn-in of 10%. Results of each run were visualized using TRACER to ensure that stationarity and
convergence had been reached, and that the ESS were higher than 200.

We used the computer program IMa (Hey and Nielsen 2007) on R. baccifera genetic groups (see Results) to estimate the time of divergence ($t$) between populations, effective number of migrants per generation ($m_1$ and $m_2$), and the effective population size of the ancestral ($q_A$) and descendant populations ($q_1$ and $q_2$). The isolation-with-migration (IM) model (Hey and Nielsen 2004) is appropriate to estimate parameters for 2 descendant populations that have diverged recently from the ancestral population and that may be sharing haplotypes as a result of gene interchange. We began with multiple runs of 10 000 steps (following 100 000 iterations as burn-in) to assess mixing and to fine-tune the parameter space. We then conducted the simulation for a burn-in of 1 million generations and 20 million steps, under the HKY model of sequence evolution. Three independent runs were performed with different seed numbers to guarantee convergence of samples (Hey and Nielsen 2004). We considered that the analyses had converged upon a stationary distribution if the independent runs had similar posterior distributions and the ESS for each parameter was at least 50. We report the mean parameter estimates of 3 runs and the 90% HPD intervals of each parameter. Because an appropriate chloroplast nucleotide substitution rate for R. baccifera has not been calibrated, we used the geometric mean of 0.002193 s/s/y of the mean mutation rates of 1.3×10^{-8} s/s/y for ITS and 3.7×10^{-8} for rpl32-trnL inferred by BEAST assuming constant population size to estimate the effective population sizes of each genetic group. The mutation rates were converted to per locus rate by multiplying by the fragment length in base pairs, and calculated for the different seed numbers to guarantee convergence of samples. After removing variables that exhibited a strong correlation (Spearman’s rank correlation >0.8) and had the lower loadings on first principal component (PC1), 14 variables (BIO2, BIO4–8, BIO10–16, BIO19) were used to generate the SDM model under current climatic conditions using MAXENT with the default parameters for convergence threshold (10^{-5}) and 500 iterations. We evaluated model performance using the area under the curve (AUC) values of the threshold independent receiving operating characteristic curve (ROC). Resulting distributions were projected with ArcView 3.2 (ESRI, Redlands, CA). To explore distributions earlier in time, the models under current climatic conditions of R. baccifera were projected to past climatic scenarios using the same BIO variables, at the LGM (c. 21 000 years before present) and Last Interglacial (LIG; 140–120 000 years before present). Past climate layers were also drawn from the WorldClim webpage for 2 LGM past climate scenarios developed by the Paleoclimate Modeling Intercomparison Project Phase II (Braconnot et al. 2007): the Community Climate System Model (CCSM; Collins et al. 2004) and the Model for Interdisciplinary Research on Climate (MIROC; Hasumi and Emori 2004), and the LIG (Otto-Bierlesner et al. 2006). Both CCSM and MIROC climate models simulate climatic conditions as they are calculated to have been for the LGM, with a stronger temperature decrease assumed in CCSM compared to MIROC (Otto-Bierlesner et al. 2007). Therefore, SDMs for present distribution, at LGM (CCSM and MIROC) and at LIG for R. baccifera are contrasted. Elevational and range changes of the predicted areas of cloud forest under current and past conditions were contrasted by extracting data from pixels with values greater than 75% of occurrence and using an elevation digital model of Mexico and Central America (http://www.inegi.org.mx).

Results

Relationships Among Haplotypes

The aligned ITS data set for R. baccifera (677 bp) yielded 22 ribotypes among 98 sequences (Supplementary Table S2).
The statistical parsimony network revealed 2 most frequent ribotypes, R1 and R4 (Figure 1). Samples from the SMO, TUX and Chiapas and Guatemala (GUAT) shared the most frequently recovered ribotype (R1) found in 50.5% of the individuals and in 75% of the sampled populations, and forms the core of the network topology. Ribotype R4 followed a similar geographic trend. Ribotypes connected to R1 by more than one step include 1 group located in the northern nSMO, consisting of 2 ribotypes (R2 and R3) private to Aquismón, one of the northernmost populations, ribotypes 5–15 shared by populations located in the central and southern Sierra Madre Oriental, and related ribotypes (17–19) found in 1 of the 2 southernmost populations in Guatemala. Ribotype R22 was connected to R1 by more than one step and corresponds to the sample from Brazil taken from Calvente et al. (2011a). The second group connected to R1 by one step contained R4, the second most frequent recovered ribotype (19.2% of individuals, 50% of populations), and related R13 and R14 found in Sierra de Los Tuxtlas region, R15 and R16 private to Ocozocuautla, Chiapas, and R20 and R21 located exclusively in 1 of the 2 populations in Guatemala (Figure 1; Supplementary Table S2 online).

The aligned rpl32-trnL data set for *R. baccifera* (1092 bp) yielded 17 haplotypes with 154 samples (Supplementary Table S2 online). Statistical parsimony retrieved a well-resolved network, in which 2 haplogroups could be distinguished (Figure 2). The most frequent (55.8% of the individuals and 54.1% of the sampled populations) haplotype (H1) forms the core of the SMO haplogroup, from Gómez Farías, Tamaulipas (nSMO) to Santa María Chayotepec, Oaxaca (sSMO); it was not retrieved in the TUX and GUAT populations. Haplotypes connected to H1 by one step consists of haplotypes exclusively found in the northernmost populations of SMO (H2 and H3), H4 and H6 located in populations in central Veracruz (cSMO), and H10 private to Sierra de Los Tuxtlas. The second most frequent (34.4% of the individuals and 62.5% of the sampled populations) and widespread haplotype (H7) forms the core of the second group of haplotypes distributed from Aquismón, Hidalgo (nSMO) to Patutul, Guatemala (Figure 2; Supplementary Table S2 online). Haplotypes connected to H7 by one or more steps consists of private haplotypes located in the northern (H5), central (H8 and H9) and southern (H11) populations of SMO, H12 located in Ocozocuautla, Chiapas, H13–15 in Guatemala, H16 in Colombia, and H17 to Brazil from Calvente et al. (2011a).

**Divergence Time Estimation**

The BEAST analysis grouped all the *R. baccifera* samples forming a well-supported monophyletic group. Within this clade, the relationships among haplotypes or ribotypes were poorly resolved, because only samples east of the Isthmus of Tehuantepec and Sierra de Los Tuxtlas formed a clade with some samples west of the isthmus nested within them. Diversification of ITS ribotypes in *R. baccifera* appears to have begun in the Pleistocene (constant population size = 2.42...
Ma, population expansion = 2.18 Ma) with a split between
R. baccifera and R. teres estimated at 2.84 Ma (95% HPD
3.90–1.15 Ma) assuming constant population size and 2.65
Ma (95% HPD 3.98–1.22 Ma) assuming population expansion.
However, these nodes were not supported (PP < 0.65; tree not shown).
Similarly, diversification of plastid rpb2-trnL haplotypes in R. baccifera
begun in the Pleistocene (constant population size = 2.12 Ma, 95% HPD
2.86–1.11 Ma, PP = 1.0; population expansion = 2.18 Ma, 95% HPD 3.08–
1.08 Ma, PP = 1.0) with a split between R. baccifera and R. teres
estimated at 2.32 Ma (95% HPD 3.19–1.27 Ma, PP = 0.99)
assuming constant population size and 3 Ma (95% HPD
3.45–1.24 Ma, PP = 0.99) assuming population expansion
(Supplementary Figure S2 online). The mean substitution rate
taken using the relaxed molecular clock for the
Rhipsalis node was 1.3 × 10⁻⁹ s/s/y (95% HPD: 3.3 × 10⁻⁹ to
0.0 × 10⁻⁹ s/s/y) for ITS and 3.7 × 10⁻⁹ s/s/y for rpb2-trnL.
(95% HPD: 5.0 × 10⁻⁹ to 0.3 × 10⁻⁹ s/s/y). The mutation estimates
are similar to those of other estimates in plants.

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Populations

differentiation among populations based on ITS variation
(GST = 0.056, SE = 0.0469) indicated that R. baccifera is not
genetically subdivided. Genetic diversity (hT = 0.749, SE
0.555; vT = 0.750, SE = 0.1395) across all populations was
slightly higher than the average within-population (hS = 0.707,
SE = 0.0593; vS = 0.724, SE = 0.1567). PERMUT analysis
showed that NST (0.035, SE NA) was significantly lower
(P < 0.05) than that for Gst, indicating no phylogeographical
structuring. In contrast, differentiation among populations
based on rpb2-trnL variation (Gst = 0.635, SE = 0.0878)
indicated that R. baccifera is highly subdivided. Genetic diversity
(bT = 0.588, SE = 0.0246; vT = 0.586, SE = 0.0743) across
all populations was much higher than the average within-
population (hS = 0.214, SE = 0.0564; vS (0.263, SE = 0.0888).
However, the Nst = 0.551 (SE = 0.1037) and Gst values
were not statistically different (P > 0.05).

Pairwise comparisons of FST values were not significant
for ITS when groups of populations are compared, whereas
for rpb2-trnL, most comparisons were high and signifi-
cant (Table 1). When groups separated by the Isthmus of
Tehuantepec were compared, pairwise comparisons of FST
values were high and significant for both data sets (Table 1).

The AMOVA results showed that 49.6% of the genetic
variation for the ITS and 52.7% for the rpb2-trnL was
explained by differences within populations and 50%
and 47%, respectively, by differences between popula-
tions when all locations were treated as a single group
(Table 2). Differences between groups were not significant
in the ITS data set (Table 2). The AMOVA for the
rpb2-trnL revealed population structure, with highest FCT
value (FCT = 0.49) obtained when populations are grouped
as separated by the Isthmus of Tehuantepec (Table 2).
When sampling sites were grouped by cloud forest area, a
significant but smaller proportion of the variation (45.9%) was attributed to differences between groups. Nucleotide diversity (π) was low for both data sets (Supplementary Table S3 online).

SAMOVA results revealed significant \( F_{CT} \) values for groups between \( K = 2 \) and \( K = 5 \), with the highest \( F_{CT} \) values for \( K = 2 \) (Table 2). When \( K = 2 \), the spatial genetic analysis identified one group formed by populations east of the Isthmus of Tehuantepec (Ocozocuautla in Chiapas, San Salvador, and San Julian in Guatemala) and the other one encompassing the populations located west of the isthmus (Sierra Madre Oriental and Sierra de Los Tuxtals region). The same 2 groups were identified in both the ITS and \( rpb2-trnL \) data sets. However, when \( K = 3 \), \( F_{CT} \) is smaller than \( K = 2 \), and an additional increase in the number of \( K \) led to a dissolution of group structure.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pairwise comparisons of ( F_{ST} ) values of ITS (above the diagonal) and ( rpb2-trnL ) (below the diagonal) among populations of ( Rhipsalis baccifera ) corresponding to cloud forest areas and groups of haplotypes separated by the Isthmus of Tehuantepec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nSMO</td>
</tr>
<tr>
<td>nSMO</td>
<td>—</td>
</tr>
<tr>
<td>cSMO</td>
<td>−0.0009</td>
</tr>
<tr>
<td>sSMO</td>
<td>0.6270</td>
</tr>
<tr>
<td>TUX</td>
<td>0.3493</td>
</tr>
<tr>
<td>GUAT</td>
<td>0.5650</td>
</tr>
<tr>
<td>East</td>
<td>—</td>
</tr>
<tr>
<td>West</td>
<td>—</td>
</tr>
</tbody>
</table>

Significant values at \( P < 0.05 \) after Bonferroni’s correction (\( P < 0.005 \)) are shown in bold.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Results of AMOVA and SAMOVA models on ( Rhipsalis baccifera ) populations with no groups defined ( a ) priori (a), by cloud forest area (b), and by populations separated by the Isthmus of Tehuantepec (c), SAMOVA ( K = 2 ) groups (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>** ITS **</td>
</tr>
<tr>
<td></td>
<td>df</td>
</tr>
<tr>
<td>a. No groups defined</td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>18</td>
</tr>
<tr>
<td>Within populations</td>
<td>78</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
</tr>
<tr>
<td>b. Cloud forest area</td>
<td></td>
</tr>
<tr>
<td>Among groups</td>
<td>4</td>
</tr>
<tr>
<td>Among populations within</td>
<td>14</td>
</tr>
<tr>
<td>Within populations</td>
<td>78</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
</tr>
<tr>
<td>c. Isthmus of Tehuantepec</td>
<td></td>
</tr>
<tr>
<td>Among groups</td>
<td>1</td>
</tr>
<tr>
<td>Among populations within</td>
<td>17</td>
</tr>
<tr>
<td>Within populations</td>
<td>78</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
</tr>
<tr>
<td>d. SAMOVA ( K = 2 )</td>
<td></td>
</tr>
<tr>
<td>Among groups</td>
<td>1</td>
</tr>
<tr>
<td>Among populations within</td>
<td>17</td>
</tr>
<tr>
<td>Within populations</td>
<td>78</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
</tr>
</tbody>
</table>

n.s., not significant (\( P > 0.05 \)), \(* P < 0.05\), \(* * P < 0.001\), \(* * * P < 0.0001\).
population west of the Isthmus of Tehuantepec but the population east of the Isthmus was relatively stable over time (Figure 3).

I Ma results are summarized in Table 3 and Figure 4. Results are reported as the average of 3 runs of mean parameter estimates and the 90% HPD intervals of each parameter. Based on the mutation rates of $2.19 \times 10^{-9}$ s/y inferred by BEAST, the eastern population size ($N_E$) is estimated to be higher than population sizes of ancestral and western populations (Table 3; Figure 4). We obtained single narrow peaks for the posterior probability distributions of all parameters, however, the tail of the posterior distribution of the effective population size of the population east of the Isthmus and migration parameters did not reach zero (did not converge) over a large range of priors (Figure 4). Divergence time between populations separated by the isthmus was estimated at $\approx 400$ KYA. Again, a peak at the left hand side of the posterior distribution of $t$ is observed, with a long right-hand tail over which the probability distribution remains flat. This means that this parameter value needs to be interpreted with caution because parameter values have essentially zero probability for most of the posterior distribution and the only values that are well supported are those less than 200 KYA (Figure 4). When testing for migration following the split between populations, migration in both directions of the Isthmus is almost zero (Table 3; Figure 4).

Palaeodistribution Modeling

The SDMs yielded a good fit for the current geographic distribution of *Rhipsalis baccifera* ($AUC = 0.964, P < 0.05$), indicating adequate model performance (Figure 5a). Although there was some over prediction in certain geographic areas (at the Isthmus of Tehuantepec that were predicted by the model with a high probability), the modeled distribution closely matches the current known distribution of *R. baccifera*. Our predictions under LGM conditions revealed that conditions of suitable habitat potentially expanded their distribution to the lowlands, and with a low probability into the Yucatan Peninsula applying both CCSM and MIROC simulations (Figure 5b-d). The comparison of the distribution of *R. baccifera* between present and LIG past climatic conditions model suggests that present distribution represents a northern expanded area occupied by the species at LIG (Figure 5e). Overall, SDMs suggest that suitable habitat for *R. baccifera* experienced range expansion under past LGM climatic conditions. Compared to current conditions, the 2 LGM (CCSM and MIROC) models suggest that the distribution of cloud forest expanded into lowland regions, 124 and 243 m, respectively, and 161 and 280 m compared to LIG conditions. CCSM and MIROC predicted a wider distribution of cloud forest during the LGM (65 and 27% of the present day distribution).

Discussion

Downslope Expansion and Population Connectivity during LGM

We found clear population genetic structure in *R. baccifera*, with differentiation between groups of populations separated by the Isthmus of Tehuantepec. Despite the genetic differentiation of some *R. baccifera* populations in Mesoamerica, there are widespread haplotypes in both the *ITS* and *rpl32-trnL* markers. In particular, the lower variation found in *ITS* and the presence of widespread haplotypes in the region are suggestive of effective nuclear gene flow via pollen (Smith et al. 2009). In contrast, the population differentiation of *R. baccifera* shown by the plastid *rpl32-trnL* data suggests that seed flow has been more restricted in some cases.

Our results from the $F_{ST}$, AMOVA and SAMOVA analyses suggest fairly high levels of genetic diversity among populations and cloud forest areas of *R. baccifera*, especially when considering the cpDNA. This is not consistent with expectations of the leading edge expansion model in which post-glacially colonized regions have reduced levels of genetic variation and large geographic areas mainly harboring a single haplotype (Hewitt 2000). We observed that most frequent
Table 3 Results of isolation with migration model (IMa) for the splits between groups of populations of *Rhipsalis baccifera* separated by the Isthmus of Tehuantepec (east vs. west)

<table>
<thead>
<tr>
<th>Model parameter estimates</th>
<th>( q_E )</th>
<th>( q_W )</th>
<th>( q_A )</th>
<th>( t )</th>
<th>( m_{E \rightarrow W} )</th>
<th>( m_{W \rightarrow E} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>30.668</td>
<td>0.986</td>
<td>3.555</td>
<td>1.667</td>
<td>0.2445</td>
<td>0.0921</td>
</tr>
<tr>
<td>HPD90L0</td>
<td>0.377</td>
<td>0.175</td>
<td>0.015</td>
<td>0.011</td>
<td>0.0003</td>
<td>0.0025</td>
</tr>
<tr>
<td>HPD90H0</td>
<td>75.805</td>
<td>1.797</td>
<td>10.189</td>
<td>4.641</td>
<td>0.4468</td>
<td>4.2242</td>
</tr>
</tbody>
</table>

### Demographic parameter estimates

<table>
<thead>
<tr>
<th>( N_E )</th>
<th>( N_W )</th>
<th>( N_A )</th>
<th>( t )</th>
<th>( N_{m_{E \rightarrow W}} )</th>
<th>( N_{m_{W \rightarrow E}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>180 108</td>
<td>5795</td>
<td>20 878</td>
<td>430 938</td>
<td>0.00004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00035</td>
</tr>
<tr>
<td>HPD90L0</td>
<td>2217</td>
<td>1028</td>
<td>88</td>
<td>3014</td>
<td>5.10 \times 10^{-8}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.25 \times 10^{-7}</td>
</tr>
<tr>
<td>HPD90H0</td>
<td>445 180</td>
<td>10 558</td>
<td>59 837</td>
<td>1 199 397</td>
<td>0.00007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00071</td>
</tr>
</tbody>
</table>

Model parameters indicate estimates without use of molecular rate of evolution for 6 parameters (IMa output values). Demographic rates represent parameters scaled to rates of molecular evolution. Values are averages of 3 runs of mean parameter estimates and the 90% HPD intervals of each parameter: effective population sizes \( (q) \), migration rates \( (N_{m}, \text{migrants per generation}) \), estimated time since divergence \( (t, \text{years}) \).

Haplotypes were retrieved in most cloud forest areas of *R. baccifera*, and significant evidence of population expansions was found in both cpDNA and nrDNA sequences of *R. baccifera*. BSPs, negative values of Tajima’s \( D \), Fu’s \( F_{S} \) and the results of mismatch distribution for both the nuclear and plastid markers suggest that east, west and both populations of the species have experienced recent demographic expansion, most likely during the LGM. Predictions of SDMs under past LGM climatic conditions and the significant signal of demographic expansion suggest that *R. baccifera* populations experienced a range expansion tracking the conditions of the cloud forest distribution and shifted to the lowlands with population connectivity during the LGM, as predicted by the moist forests model (hypothesis 1; Ramírez-Barahona and Eguiarte 2013). In addition, Bayesian skyline plots detected a significant increase in the overall effective population size dating to sometime during the LGM, when sudden demographic expansion occurred and descent to lower elevations allowed populations to gene flow. However, patterns of high genetic diversity among different populations do not fully support this hypothesis. The distribution of haplotype diversity suggests that the low genetic structure observed among cloud forest areas is due to population connectivity across low-elevation barriers. Additionally, we would hypothesise that during glacial periods, populations of *R. baccifera* expanded to the lowlands, gene flow was extensive, and habitats in which populations had gone extinct during the interglacials were probably re-colonized. Throughout interglacial periods, populations fragmented and contracted as they moved up in elevation with the warming climate and genetic divergence commenced across cloud forest areas dissected by mountains and low-elevation barriers. Alternatively, mountains provide a stable cloud forest habitat through glacial periods, in which populations of *R. baccifera* persisted in situ ever since LIG.

Despite the appeal of the contraction/expansion model of populations of cloud forest-adapted species throughout glacial-interglacial climate cycles by shifting elevation, there is little evidence for a common demographic pattern (Ramírez-Barahona and Eguiarte 2013). Ornelas et al. (2010) analyzed the phylogeographical structure of codistributed *P. matudae* (Podocarpaceae) with *R. baccifera* in cloud forests of Mexico and Guatemala, and found no evidence of range expansions. In contrast, Gutiérrez-Rodríguez et al. (2011) found evidence of demographic expansions in groups of haplotypes separated by the Isthmus of Tehuantepec using cpDNA sequences of also codistributed *P. padifolia* (Rubiaceae). Their results support a history of rapid population growth from ancestral populations with small size. However, the authors do not test their results against palaeoecological data and thus, it remains unclear as to whether their results fully support the expansion/contraction model of populations and its demographic consequences. More recently Ruiz-Sánchez and Ornelas (2014) analyzed the phylogeographical structure of *L. styriaciflua* (Altingiaceae), a deciduous tree with a disjunct distribution between the deciduous forest of the southeastern USA and the Mesoamerican cloud forest, and found signals of demographic expansion of Mesoamerican populations and habitat suitability connected and expanded to lower coastal areas under LGM climatic conditions. An alternative scenario for the contraction/expansion model of populations of cloud forest-adapted species is persistence throughout glacial–interglacial climate cycles. The spatial structuring of plastid variation in Begonia heracleifolia (Begoniaceae) provides support for the persistence of populations in situ during dry periods in the Pleistocene (Twyford et al. 2013a, 2013b), or the high genetic differentiation with genetic structuring of populations in Moussonia depeana (Gesneriaceae), in which populations remained geographically isolated primarily within the current fragmented distribution of the cloud forest, without population connectivity and range expansion to the lowlands, and the divergence events for geographically persistent and structured populations with limited gene flow traced back to the LGM or earlier (Ornelas and González 2014). The observed inconsistency between demographic...
haplotypes were found in the different R. baccifera collection sites they differ from each other by only one mutation as revealed by the haplotype network and the low values of nucleotide diversity in all populations, suggesting recent formation of the haplotypes. The combination of relatively low-to-high values of genetic diversity (h) and low values of nucleotide diversity (π), the non-significant differences between the $N_{ST}$ and $G_{ST}$ values, as well as the star-like shape of the haplotype network, where haplotypes differed from each other by few mutations and most are connected to a most common haplotype, indicate rapid population growth from ancestral populations with small effective population size (Avise 2000). This is supported by the results of the isolation with migration (IMa) analyses, which revealed that the ancestral population size was smaller than 1 of the 2 descendant populations. Furthermore, the results of the cpDNA haplotype network revealed that haplotypes from the Sierra Madre Oriental and Sierra de Los Tuxtlas are closely related and form a group distributed on the west side of the Isthmus of Tehuantepec, whereas the haplotypes private to Chiapas and Guatemala constitute a second group, distributed on the east side of the isthmus, suggesting genetic isolation between the 2 geographic areas. This pattern of genetic differentiation was also supported by the SAMOVA (K = 2) and the AMOVA between groups separated by the isthmus, which revealed a significant level of differentiation ($F_{CT} = 0.49$). Because chloroplast DNA in most angiosperms is maternally inherited, the patterns of gene flow of R. baccifera found in this study reflect patterns of seed dispersal. The IMa results revealed no migration across the Tehuantepec isthmus in both directions, suggesting that haplotype sharing among R. baccifera localities is not the result of ongoing gene flow across the isthmus but retention of ancestral polymorphisms with not enough time for lineage sorting and thus to genetically differentiate.

Divergent ITS paralogues could explain the conflict between the ITS data set and the rpl32-trnL results. However, we did not encounter any evidence of ITS paralogues either through multiple bands or explicit ambiguity in the chromatograms. Even though, it is possible that divergent ITS paralogues may have been amplified in this study, including pseudo genes and recombinants as previously found for other cacti (Harpke and Peterson 2006), other phylogenetic studies with different cacti using ITS did not encounter evidence of paralogy or mention evidence of paralogues (Arias et al. 2003; Griffith and Porter 2009; Calvente et al. 2011a). Future studies employing a broader sampling may shed light on the evolution of ITS in Cactaceae as a whole. Given this, the higher phylogeographical structure and support obtained by the analyses of the rpl32-trnL data set, we choose to consider the rpl32-trnL results as representing the best phylogeographical estimate of R. baccifera in Mesoamerica. As cpDNA is maternally inherited in flowering plants, the difference between ITS and rpl32-trnL results reflects higher levels of pollinator-mediated gene flow than seed dispersal (Smith et al. 2009).

**Phylogeographical Structure and the Isthmus of Tehuantepec**

The phylogeographical structure found in R. baccifera suggests that two lineages occupy geographic regions separated by the

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**Figure 4.** Means of posterior probability distributions of parameters calculated in 3 IMa runs using the mutation rate calculated in BEAST. (a) $q_{W-E}$, (b) $q_{E-W}$, (c) $q_A$ are the west and east of the Isthmus of Tehuantepec and the ancestral population sizes, respectively ($q \times 1000$); (d) $N_{M_{W-E}}$, (e) $N_{M_{E-W}}$ are migrants per generation west-to-east and east-to-west, respectively; (f) $t$ is the time as divergence ($t \times 1000$ years).
Isthmus of Tehuantepec. Our phylogeographical analyses highlight the importance of the Isthmus of Tehuantepec in driving isolation and genetic divergence between $R. bacifera$ populations separated by the isthmus. Several phylogeographical studies have indicated a genetic break at the isthmus in species of amphibians and reptiles (Mulcahy et al. 2006; Castoe et al. 2009), rodents (Sullivan et al. 2000; León-Paniagua et al. 2007), birds (Cortés-Rodríguez et al. 2008a, 2013; Barrera-Guzmán et al. 2012; González et al. 2011; Rodríguez-Gómez et al. 2013), and plants (Gutiérrez-Rodríguez et al. 2011), but in some cases such a break is not clear (Cortés-Rodríguez et al. 2008b; Navarro-Sigüenza et al. 2008; Vázquez-Miranda et al. 2009; Arbeláez-Cortés et al. 2010; Ornelas et al. 2010). A comparative phylogeographical study detected several pulses of divergence among 10 cloud forest-adapted taxa at the Isthmus of Tehuantepec (Ornelas et al. 2013), indicating that the genetic divergence observed across different lineage pairs likely occurred variously during different temporal windows. Another study based on mitochondrial DNA examined several bird species with distributions on either side of the Isthmus and concluded that fluctuations in the climate of montane habitats had fractured the bird fauna during the late Pliocene and Pleistocene (Barber and Klicka 2010). However, other studies have found that the Isthmus of Tehuantepec has not acted as a barrier for dispersal given the lack of genetic differentiation between populations of other highland bird species (Navarro-Sigüenza et al. 2008; Arbeláez-Cortés et al. 2010) or lowland species (Cortés-Rodríguez et al. 2008b; Vázquez-Miranda et al. 2009). The lowlands of the Isthmus of Tehuantepec have been under the influence of climatic changes and shifts of montane forests repeatedly over the last million years, creating an apparent barrier to gene flow (Barber and Klicka 2010; González et al. 2011; Ornelas et al. 2013). However, it has acted as a connector for other species (Gutiérrez-Rodríguez et al. 2011; Rodríguez-Gómez et al. 2013) in which divergence occurred with gene flow. It is clear that a comparative approach of further genetic studies dealing with codistributed species living within ecologically narrow forest communities such as cloud forests with distinct ecologies along with species palaeodistribution modeling, might prove useful in uncovering general ecological response patterns of species to climate change, particularly precipitation. It is possible that the strong divergence across the Isthmus of Tehuantepec could be swamping out patterns of divergence among populations on either side of the Isthmus in some of the analyses, and that the

Figure 5. Results from the MAXENT analyses showing species distribution models for $Rhipsalis bacifera$ (a) at present, (b) Last Glacial Maximum (LGM, MIROC, 21 ka), (c) Last Interglacial (LIG, 140–120 ka), and (d) Last Glacial Maximum (LGM, CCSM, 21 ka). The output of MAXENT consists of grid maps with each cell having an index of suitability between 0 and 1. Low values (light grey) indicate that the conditions are unsuitable for the species to occur, whereas high values (dark grey, black) indicate that the conditions are suitable. There is clear evidence that populations are connected during glacial cycles. Note that for maps b and d projections extend out into the ocean because of changes in sea levels.
phylogeographical break separating populations masks other processes that are occurring, as suggested by the high levels of genetic diversity among different populations (significant divergence in $F_{ST}$ values of cpDNA among the majority of cloud forest areas; see also Gutiérrez-Rodríguez et al. 2011; Ornelas and González 2014). The occurrence of widespread plastid haplotypes in some presumably recently re-colonized areas (i.e., post-glacial population expansion) of the range of $R. baccifera$ is consistent with the hypothesis of effective seed dispersal episodes, in which the mobility of bird dispersal agents has erased the genetic structure predicted by the dry refugia model within groups of populations separated by the Isthmus. Although large-scale seed recruitment patterns produced by frugivorous birds that consume and eventually disperse mistletoe cactus seeds might have led to increased gene flow, seed germination experiments suggest that $R. baccifera$ can colonize a wide range of light conditions in the plant canopy but substrate humidity could limit seed germination within the cloud forest (Zotz and Hietz 2001; de la Rosa and Briones 2010). Thus, geologic and climate-driven processes implicated in the fragmentation of the Mesoamerican cloud forests and, consequently the distribution of potential host species at a larger geographical scale, as well as selection pressures imposed by seed dispersers, could have influenced the distribution of the genetic variation among populations of $R. baccifera$. If the historical fragmentation of cloud forest patches or selection reduced gene flow among $R. baccifera$ populations, isolation may lead to genetic divergence and ultimately allopatric speciation.

**Supplementary Material**


**Funding**

Consejo Nacional de Ciencia y Tecnología (CONACYT, 61710); Departamento de Biología Evolutiva, Instituto de Ecología (20030/10563 to J.F.O.). CONACYT (224634 to F.R.G.).

**Acknowledgments**

We thank Luis M. García-Feria, Eduardo Ruiz-Sanchez, Antonio Vázquez, Clementina González, Tomahit Velázquez, Victoria Sosa, Pablo Carrillo, Alejandro Espinosa de los Monteros, Ismael Guzmán Valdivieso, Juan Manuel Pech, Luis Mendoza, María Teresa Mejía Saules, Cristina Bárcenas, Ninfa Portilla, Salvador González and Carla Gutiérrez-Rodríguez for laboratory and field assistance; Pierre Mokondoko for technical support on niche modelling; and J.J. Vega (Universidad San Carlos de Guatemala) and A. Lu MacVean (Universidad del Valle de Guatemala) for providing help with the loans of material. Permission to conduct our fieldwork was granted by the Mexican government (INFE, SEMARNAT, SGPA/DGVS/0208/07) and Universidad del Valle de Guatemala herbarium (UVAL) collecting permits. Amanda Zellmer, Christopher I. Smith, and an anonymous reviewer provided helpful comments for improving earlier versions of this manuscript.

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Received February 11, 2014; First decision May 14, 2014; Accepted December 18, 2014

Corresponding Editor: Christopher Smith