MOLECULAR ECOLOGY

Molecular Ecology (2013) **22**, 6048–6059 doi: 10.1111/mec.12551

Relative importance of pollen and seed dispersal across a Neotropical mountain landscape for an epiphytic orchid

TYLER R. KARTZINEL,* RICHARD P. SHEFFERSON* and DORSET W. TRAPNELL†

*Odum School of Ecology, The University of Georgia, 140 East Green Street, Athens, GA 30602, USA, †Department of Plant Biology, The University of Georgia, Athens, GA 30602, USA

Abstract

Populations of many species are isolated within narrow elevation bands of Neotropical mountain habitat, and how well dispersal maintains genetic connectivity is unknown. We asked whether genetic structure of an epiphytic orchid, *Epidendrum firmum*, corresponds to gaps between Costa Rican mountain ranges, and how these gaps influence pollen and seed flow. We predicted that significant genetic structure exists among mountain ranges due to different colonization histories and limited gene flow. Furthermore, we predicted that pollen movement contributes more to gene flow than seeds because seeds are released into strong winds perpendicular to the narrow northwest–southeast species distribution, while the likely pollinators are strong fliers. Individuals from 12 populations and three mountain ranges were genotyped with nuclear microsatellites (nDNA) and chloroplast sequences (cpDNA). Genetic diversity was high for both markers, while nDNA genetic structure was low (F_{STn} = 0.020) and cpDNA structure was moderate (F_{STc} = 0.443). Significant cpDNA barriers occurred within and among mountain ranges, but nDNA barriers were not significant after accounting for geographic distance. Consistent with these contrasting patterns of genetic structure, pollen contributes substantially more to gene flow among populations than seed (m_p/m_s = 46). Pollinators mediated extensive gene flow, eroding nDNA colonization footprints, while seed flow was comparatively limited, possibly due to directional prevailing winds across linearly distributed populations. Dispersal traits alone may not accurately inform predictions about gene flow or genetic structure, supporting the need for research into the potentially crucial role of pollinators and landscape context in gene flow among isolated populations.

Keywords: chloroplast DNA, gene dispersal, genetic structure, microsatellites, pollinator

Received 14 February 2013; revision received 30 September 2013; accepted 2 October 2013

Introduction

Patterns of genetic variation predicted on the basis of a species' natural history and dispersal biology are often discordant with empirical genetic data (Yu *et al*. 2010). Such discord suggests that context-dependent processes often influence patterning of genetic variation. Integrating historical and ecological knowledge with genetic data provides valuable insights into context-dependent processes and can help distinguish between alternative hypotheses about the potential roles of genetic drift and biotic or abiotic selection pressures in speciation (Coyne & Orr 2004). Additional insights from geographically concordant patterns of genetic variation among species can help identify climate refugia, ancient migration routes and landscape barriers (Avise 2000). Thus, knowledge about patterns of genetic variation is critical for comparing and ultimately predicting the relative importance of processes that shape biodiversity.

Central American mountain biogeography is characterized by high plant diversity and naturally discontinuous species distributions in narrow elevational habitat bands within geologically distinct cordilleras (i.e. mountain ranges; Coates & Obando 1996; Gentry & Dodson 1987). Genetic insights into historical processes that

Correspondence: Tyler R. Kartzinel, Fax: (706) 542 4819; E-mail: tylerk@princeton.edu

produced current distributions of species and population connectivity within these habitats are valuable for anticipating future consequences of environmental change, as mountain habitats shift upward and become increasingly narrow and isolated due to climate warming and deforestation (Ponce-Reyes *et al.* 2012). For example, paleodistribution modelling of multiple Central American tree species suggests that changes in distribution reflect climatic oscillations (Cavender-Bares *et al.* 2011; Poelchau & Hamrick 2011). Thus, populations currently isolated in mountain habitats may have been more contiguous in the lowlands during cooler periods, but may experience increasing isolation as gaps between suitable habitats widen in warmer climates.

Population genetics provides powerful tools for understanding genetic connectivity of populations. Genetic structure is the nonrandom distribution of genetic variation among populations (Wright 1951). If historical factors strongly influenced colonization of populations on different mountain ranges and/or genetic connectivity among populations in spatially isolated mountain habitats, significant genetic structure should occur among populations on different mountain ranges. In northwestern Costa Rica, for example, geographically similar patterns of genetic structure have been reported for numerous low-elevation plant taxa, suggesting that gene flow was limited by multiple barriers of various strengths in this geologically active landscape that has experienced periods of rapid climate change (e.g. Poelchau & Hamrick 2012 and references therein). However, taxa occurring at higher elevations have been largely ignored, which is surprising considering two major historical factors that probably contributed to the concordant genetic structure relate to mountains: (i) colonization of volcanic mountain islands before completion of the Central American isthmus in the Pliocene (~3 Mya) and (ii) intense volcanic activity coinciding with Pleistocene climate shifts as the Guanacaste mountain range was formed (~0.6 Mya; Cavender-Bares *et al.* 2011; Coates & Obando 1996). Although such geological events have been consequential for Central American flora, genetic variation in taxa within the biologically diverse mountains remains largely unexplored.

Gene dispersal in plants is mediated by pollen and seeds, which are often dispersed in different seasons by different vectors. These mechanisms can be distinguished in most angiosperms because biparentally inherited nuclear DNA (nDNA) is dispersed by both pollen and seeds, while maternally inherited chloroplast DNA (cpDNA) is dispersed only by seed (Petit *et al.* 2005). Seed dispersal contributes to both the colonization of new populations and gene flow among established populations, while pollen only contributes to gene flow. Although diploid seeds disperse twice as many genes as haploid pollen, pollen movement contributes more to gene flow in most species (Petit *et al.* 2005).

Long-distance dispersal is critical for colonization of, and gene flow among, isolated mountain populations (e.g. Shafer *et al.* 2010). Prevailing winds influence both the spatial distribution of Central American mountain biomes (Coen 1983) and wind-mediated plant propagule dispersal among habitat patches (Nathan & Muller-Landau 2000). The trade winds push moist Caribbean air over the northwest–southeast-oriented mountain ranges, causing moisture gradients descending from the continental divide (cloud forest) into the rain shadow on the Pacific facing slopes (humid premontane and lower montane wet forest; Coen 1983). Winds are stronger during the dry season (December–March) than the rainy season (April–August; Coen 1983). In Costa Rica, dry season winds from the Caribbean are perpendicular to the relatively linearly arranged mountains. On the Pacific slope, winds consistently blow from the east in northern Costa Rica (i.e. Guanacaste and Tilarán mountain ranges) and from the southwest in southern Costa Rica (i.e. Talamanca mountain range) where taller and wider mountains cause a wind vortex with a horizontal axis, known as a rotor (Coen 1983). Weaker rainy season winds are convection-driven, with more variable directionality (Coen 1983). Thus, the seasons in which pollen and seeds are dispersed may affect gene flow, particularly when winds influence the movement of plant propagules and/or their animal vector.

Pollen and seed movement depend on dispersal vectors, which can be biotic or abiotic. Orchids, which are among the largest plant families (≥26 000 species) and comprise >14% of Costa Rica's flora (Hammel *et al.* 2004; WCSP 2013), produce many tiny, wind-dispersed seeds with potential for long-distance movement (Arditti & Ghani 2000). This characteristic is thought to increase genetic connectivity among orchid populations relative to other perennial herbs with wind-dispersed seeds (Hamrick & Godt 1996; Phillips *et al.* 2012). Wind tunnel experiments (Murren & Ellison 1998), seed fall traps (Jersáková & Malinová 2007), genetic parentage analysis (Jacquemyn *et al.* 2007) and fine-scale genetic structure (Trapnell *et al.* 2004) suggest that most orchid seeds settle locally. However, even low rates of long-distance dispersal can be critically important to gene flow and reducing genetic structure among populations (Nathan 2006; Yu *et al.* 2010). Thus, isolated orchid populations might be expected to exhibit high genetic connectivity. In contrast, gene flow by pollen may be less effective because orchids aggregate pollen into a few pollinia (≤8) that singly sire fruits and require animal dispersers with potentially limited efficacy or foraging

ranges (Tremblay *et al.* 2005). Nevertheless, orchid pollen movement can be greater than seed movement at some spatial scales (Trapnell & Hamrick 2005; Trapnell *et al.* 2013). Thus, the exceptional diversity of orchid floral morphology, and correspondingly diverse pollinators, highlights the importance of both ecological and geographic information for predictions about pollen flow. Because gene-flow estimates frequently contrast with predictions based on the plant dispersal traits, and because both pollen and seeds mediate gene flow, genetic comparison of pollen and seed dispersal is essential for elucidating the relative importance of these two dispersal mechanisms (Yu *et al.* 2010). Furthermore, data on orchid genetic variation are needed to resolve long-standing debates about orchid population differentiation, including the potential prominence of genetic drift, long-distance seed dispersal and/or natural selection by pollinators (Phillips *et al.* 2012).

We evaluate the historic influence of mountain ranges and dispersal on the genetic connectivity of an epiphytic orchid, *Epidendrum firmum*, in Costa Rica. *Epidendrum firmum* occurs in humid premontane (bmh-P) and lower montane wet forest (bmh-MB) Holdridge life zones (~860–1400 mASL; T. R. Kartzinel, personal observation; herbarium records). Habitat corridors between mountains that are circumscribed by these life zones narrow to <2.0 km in some places. Although pollinators and mating system of *E. firmum* are unknown, its pale flower, broad lip and long nectar tube suggest moth pollination. The floral morphology is similar to related species in the *Epidendrum difforme* species complex (Dressler 1993), which are self-incompatible and moth-pollinated (Goss 1977). Pollinators of this species complex include tiger moths (superfamily Noctuoidae, family Erebidae = Arctiidae; Zahiri *et al.* 2011) and possibly sphinx moths (family Sphingidae; Dodson & Frymire 1961; Goss 1977). The high diversity and mobility of common tropical tiger moths in disturbed habitats suggests some are unaffected by disturbance (Hilt & Fiedler 2005). Tropical sphinx moths are strong fliers, and some Costa Rican species do not appear to establish home ranges (Haber & Frankie 1989). Like many moth-adapted species, *E. firmum* blooms concurrently with increases in moth abundance at the onset of the rainy season (Haber & Frankie 1989), when wind speed and directionality are not pronounced (May–August). Seeds, however, are released into strong dry season winds (February–March).

We ask whether significant genetic structure reflects the isolation of populations in separate mountain ranges, and how this influences pollen and seed flow among populations in a narrow band of mountain habitat. We use putatively neutral, nontranscribed nDNA and cpDNA markers to test two hypotheses: (i) significant genetic structure corresponds to the spatial separation of mountain ranges, and (ii) pollen flow significantly exceeds seed flow between mountain ranges. We expect significant genetic structure between mountain ranges for both markers, reflecting limited pollen and seed flow that has perpetuated the genetic signature of colonization. Furthermore, we expect pollen flow significantly exceeds seed flow because the relevant guilds of moth pollinators include strong fliers, whereas seeds are dispersed by strong winds oriented perpendicular to the narrow mountain habitat. If pollinators homogenize genetic variation while seed dispersal is more limited among isolated orchid populations, this would represent a context-dependent reversal of patterns expected from knowledge about dispersal traits.

Methods

Study species and sampling

Epidendrum firmum Rchb.f. 1866, in the subtribe Laeliinae, is a member of the *Epidendrum difforme* species complex, which is subject to taxonomic revision (Dressler 1993). However, we are confident of our identification of *E. firmum* based on current taxonomic understanding (F. Morales, personal communication). *Epidendrum firmum* occurs in the Pacific slope rain shadow of Central America from Nicaragua to Panama (Dressler 1993). Although *E. firmum* has a restricted range and high habitat specificity, it can be locally abundant. Individuals grow in clusters of up to 60 stems, although usually much fewer, which typically bear 2–4 flowers that each produce four pollinia.

Leaf tissue was collected in May 2009–July 2010 from 12 populations across Costa Rica (21–67 plants/population; mean = 42.5; Fig. 1; Table S1, Supporting information), snap-frozen in liquid nitrogen and taken to the University of Georgia for analysis. Populations comprised ≥3 trees hosting *E. firmum* within 1–2 ha, selected to capture local-, regional- and species-level genetic variation from Rincon de la Vieja in the north to Chirripó in the south. Populations were separated by 0.4–241.5 km (mean = 65.0; Fig. 1). One population was from primary forest (ERP), while others were from disturbed pastures and roadsides, where *E. firmum* colonized cultivated trees including *Sapium glandulosum*, *Acnistus arborescens* and *Cupressus lusitanica*. The presence of populations on remnant trees and in nearby forest suggests that these were the seed sources for colonization of younger trees.

Genetic data acquisition

Nuclear microsatellite (nDNA) and chloroplast sequence (cpDNA) data were obtained to compare pat-

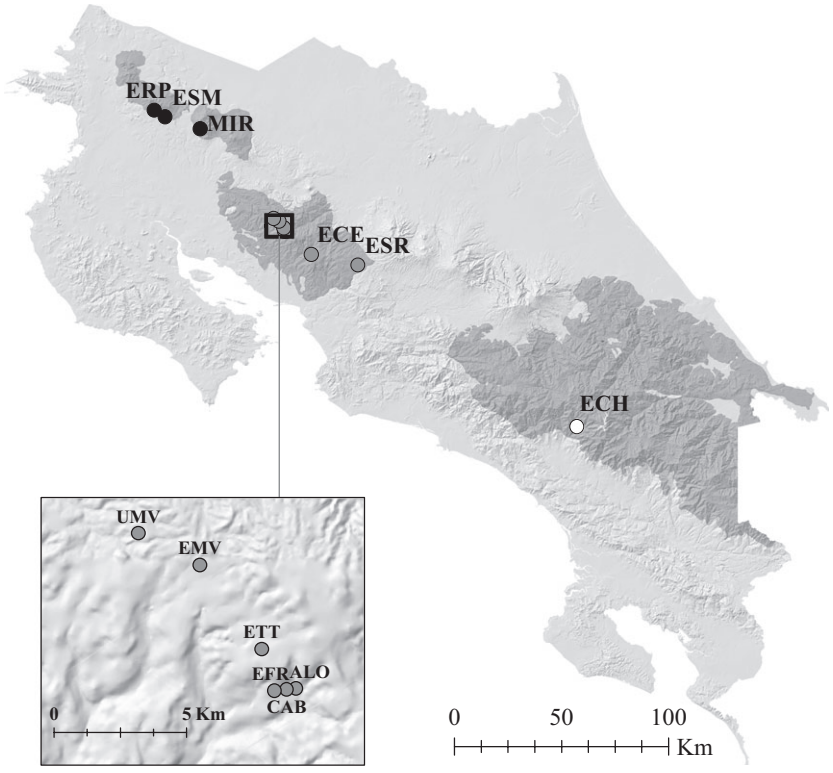

Fig. 1 Geographic locations of the 12 *Epidendrum firmum* populations in Costa Rica. The three mountain ranges are indicated with dark shading and populations from each mountain range are shaded differently (black circles = Guanacaste; grey = Tilarán; white = Talamanca).

terns of genetic variation. Genomic DNA was extracted with a Qiagen Plant DNeasy mini kit (Qiagen). Eight polymorphic microsatellite loci developed for *E. firmum* (Kartzinel *et al*. 2012) were used to genotype 510 samples (Appendix S1, Supporting information). Three loci were removed from analysis due to potential stutter or null alleles (Appendix S1, Supporting information). *Epidendrum firmum* can reproduce clonally, and while repeated collection of genets was avoided, some duplicate multilocus genotypes within host trees could not be distinguished by known cpDNA haplotype. As the probability of identity calculated in GenAlEx v.6.2 (Peakall & Smouse 2006) was always low ($<1.5 \times 10^{-8}$), duplicate genotypes that could have inadvertently originated from one genet were omitted from analysis, reducing the data set to 441 genotypes.

To assess cpDNA variation, we sequenced two noncoding cpDNA regions using primers 3′trnG^{UUC}–5′ trnG2G and rps16F–rps16R (Shaw *et al*. 2005) from 248 samples (11–28/population; mean = 21; Appendix S2, Supporting information). Sequences were edited and assembled in Sequencher 4.9 (GeneCodes Corp.) and aligned with ClustalW (Larkin *et al*. 2007). The concatenated sequence alignment spans 1370 bp.

Genetic diversity analyses

Nuclear genetic diversity measures include the average number of alleles/locus (A), effective number of alleles/locus (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e) and the occurrence and frequency of private alleles as calculated in GenAlEx v.6.2 (Peakall & Smouse 2006). Inbreeding coefficients (f) and allelic richness (AR) were calculated in FSTAT v.2.9.3 (Goudet 1995). Mean AR was calculated based on the rarefaction of each locus in each population to the smallest number of samples with full data/locus (11 samples/population; 15 samples/mountain range; 424 overall). Departure from Hardy–Weinberg equilibrium (HWE) was identified with exact tests in Genepop v.4.0.10 (Raymond & Rousset 1995; Rousset 2008).

For estimates of cpDNA diversity, we recoded INDELs and poly-A regions as biallelic SNPs. The number of haplotypes (H), private haplotypes (PH), haplotype diversity (HD) and segregating sites (S) were calculated in DnaSP 5.10 (Librado & Rozas 2009).

nDNA genetic structure analyses

We calculated pairwise and overall genetic differentiation, F_{STn} (θ; Weir & Cockerham 1984), and tested for significance with 1000 random permutations of genotypes among samples in FSTAT (Goudet 1995). Weir and Cockerham's θ was chosen because it adjusts for variation in population sample sizes. We compared F_{STn} to a similar metric that incorporates microsatellite mutation processes, R_{ST}, using Arlequin v.3.1 (Slatkin 1995; Excoffier *et al*. 2005). Alternative metrics to measure

genetic differentiation in data sets with many alleles/locus have been suggested, including G'_{ST} (Hedrick 2005) and D (Jost 2008). However, these metrics are less sensitive to population genetic processes when mutation rates are high relative to migration rates, and therefore may provide less information about processes contributing to genetic variance among population groups (Whitlock 2011). A Mantel test for isolation by distance (IBD) was performed by correlating linearized pairwise $F_{STn}/(1-F_{STn})$ with geographic distances (Rousset 1997) and evaluating significance with 999 permutations in GenAlEx. Because study populations were linearly distributed, geographic distances were not transformed. To identify populations with similar nDNA variation, principal components analysis (PCA) was performed on pairwise F_{STn} in GenAlEx, assuming negative values = 0.

cpDNA genetic structure analyses

Analysis of molecular variation (AMOVA) was used to estimate pairwise and overall cpDNA differentiation (F_{STc}). Significance was tested in Arlequin with 1000 permutations, applying the Tamura–Nei nucleotide substitution model because haplotypes were not assumed to be an equivalent number of mutational steps from one another (Nei & Li 1979; Excoffier *et al.* 2005). A Mantel test for IBD correlated linearized pairwise $F_{STc}/(1-F_{STc})$ with geographic distances and evaluated significance with 999 permutations in GenAlEx. In one case, this transformation was undefined because $F_{STc} = 1$ (Table S3, Supporting information; results). Thus, to perform a Mantel test for IBD using a transformed genetic distance matrix, we assumed this comparison was equal to the next largest pairwise F_{STc} observed. To further characterize cpDNA genetic structure, a median-joining haplotype network was constructed in NETWORK v.4.6 (Fluxus Technology Ltd; Bandelt *et al.*, 1999).

Identification of genetic discontinuities

Genetic discontinuities may occur among hierarchical groups of populations, and nDNA and cpDNA patterns may differ. While our primary hypothesis was that significant genetic structure reflects mountain ranges, we explored alternative hypotheses based on geographic locations with the strongest nDNA and cpDNA discontinuities as identified using BARRIER v.2.2 (Manni *et al.* 2004). BARRIER creates a map using GPS coordinates and employs Monmonier's algorithm to identify the largest genetic distances between neighbouring populations based on a matrix of genetic distances. To find bootstrap support for the single strongest barrier, we produced 1000 pairwise F_{STn} and F_{STc} genetic distance matrices with an ARLEQUIN batch file after resampling individual genotypes within populations with replacement in R v.2.15.1 (R Core Development Team 2010). Groups of populations separated by barriers we considered nontrivial (>10% bootstrap support) were further investigated.

Correspondence of nDNA and cpDNA genetic structure to potential barriers was evaluated using hierarchical AMOVAs and partial Mantel tests. Populations were grouped into: (i) three mountain ranges (our primary hypothesis) or (ii) four groups based on cpDNA discontinuities revealed by BARRIER, which suggested an additional discontinuity between northern and southern Tilarán (below). In hierarchical AMOVAs, we used the locus-by-locus F_{ST} estimator for nDNA and the Tamura–Nei distances for cpDNA, with 1000 permutations to calculate P-values (ARLEQUIN; Excoffier *et al.* 2005). We evaluated significance of hierarchical genetic structure with among-group variation (F_{CT}). Because these hierarchical groupings are spatially structured, we performed partial Mantel tests (Smouse *et al.* 1986) to test whether groupings reflect significant genetic structure after accounting for geographic distance. The response matrix comprised pairwise F_{STn} or F_{STc} values. A model matrix was constructed with groups coded as 0 (same group) or 1 (different group). A third matrix comprised geographic distances. Unlike the above Mantel tests for IBD, this tests a more general hypothesis of association between F_{ST} and population groupings after accounting for geographic distance. A significant result after Bonferroni correction indicates all processes measured by F_{ST}, including long-distance dispersal or population expansion, are associated with groupings after removing partial effects of distance. Groupings would not be significant if gaps between mountain ranges do not impose greater genetic barriers than the spatial separation of populations. Partial Mantel tests were run with 9999 permutations in *vegan* 2.0.4 in R (Oksanen *et al.* 2012). Having only one Talamanca population for analysis is suboptimal because imbalanced group sizes reduce statistical power (Luo & Fox 1996), but we only found one Talamanca population of this rare species.

Pollen vs. seed-mediated gene flow

We estimated the relative contribution of pollen and seeds to gene flow at multiple spatial scales by calculating pollen to seed migration ratios (m_p/m_s; Petit *et al.* 2005), using samples for which both nDNA and cpDNA data were available ($N = 225$). Pollen and seed disperse biparentally inherited nDNA, while maternally inherited cpDNA is dispersed only by seeds in most

angiosperms (Ennos 1994; Mogensen 1996), including directly investigated orchids (Cafasso *et al.* 2005). We calculated $m_p/m_s = [2(1/F_{STc}-1)-(1/F_{STn}-1)]/(1-1/F_{STc})$, where F_{STn} is Weir and Cockerham's θ and F_{STc} is calculated using haplotype frequencies in AMOVA (Petit *et al.* 2005). While this equation can include heterozygote deficit (F_{IS}) corrections, a simplifying assumption ($F_{IS} = 0$) may be applied with little change in the ratio (Petit *et al.* 2005). In an outcrossing hermaphroditic plant with strict maternal inheritance of cpDNA, expected F_{STc} can be calculated from observed F_{STn}: $F_{STc} = 6\ F_{STn}/(2 + 4\ F_{STn})$, assuming equal pollen and seed flow (Hamilton & Miller 2002). We tested the null hypothesis of equal pollen and seed flow using 95% confidence intervals surrounding expected and observed F_{STc} values, which were generated by bootstrapping. As these statistics depend on assumptions of Wright's island model (Hamilton & Miller 2002), we conservatively excluded ECH from analysis because this population is considerably distant and unidentified intermediate populations could cause estimation error.

Results

Genetic diversity

High nDNA and cpDNA diversity occurred within and among populations. Five nuclear microsatellite loci yielded 11–27 alleles/locus (Table 1). Mean diversity measures included allelic richness (AR) = 8.8 (range = 7.4–9.4), effective alleles/locus (A_e) = 7.3 (5.5–8.6), H_o = 0.785 (0.721–0.848) and H_e = 0.834 (0.795–0.867; Table 1). All populations except ETT and EFR had private alleles (1–3; mean = 1.58), as did each mountain range (Guanacaste = 7, Tilarán = 16, Talamanca = 3; Table 1). Genetic diversity was similar across mountain ranges, although more alleles/locus (A) and private alleles (PA) occur in Tilarán, where sample size was largest (Table 1). Within mountain ranges, mean PA/population was 2.3 (Guanacaste), 1.1 (Tilarán) and 3.0 (Talamanca; Table 1). Northern- and southern-most populations had the most PA (ERP frequency = 0.068, N loci = 2; ECH frequency = 0.069, N loci = 3; Table 1).

Table 1 Summary of nuclear microsatellite and chloroplast sequence diversity

	Nuclear microsatellites									Chloroplast DNA sequences					
Population	*N*	A	A_e	AR	PA	Freq.	H_o	H_e	*f*	*N*	S	H	PH	Freq.	HD
Guanacaste															
ERP	11	7.4	5.5	7.4	2	0.068	0.836	0.795	−0.004	11	3	3	0	0.000	0.473
ESM	48	14.4	8.2	9.4	2	0.010	0.790	0.858	0.090***	22	12	5	3	0.182	0.615
MIR	41	12.8	7.1	8.7	3	0.023	0.800	0.830	0.050	21	4	3	0	0.000	0.186
Mean	33.3	11.5	6.9	8.5	2.3	0.034	0.809	0.828	0.045	18	6.3	3.7	1.0	0.061	0.425
Pooled	100	17.0	8.5	10.5	7	0.008	0.799	0.855		54	12	7	4	0.667	0.587
Tilarán															
UMV	33	12.4	7.2	8.8	2	0.015	0.721	0.840	0.156***	21	2	3	1	0.048	0.529
EMV	43	14.2	7.9	9.0	1	0.012	0.808	0.837	0.047	21	0	1	0	0.000	0.000
ETT	40	12.2	6.4	8.2	0	0.000	0.784	0.817	0.053***	28	1	2	0	0.000	0.254
ALO	51	14.6	7.8	9.2	1	0.010	0.741	0.845	0.132***	25	5	5	1	0.120	0.767
CAB	46	14.0	7.9	9.0	1	0.011	0.816	0.849	0.050*	22	5	5	1	0.046	0.775
EFR	50	12.6	6.4	8.0	0	0.000	0.723	0.815	0.124***	22	6	7	2	0.091	0.792
ECE	47	14.4	8.6	9.4	3	0.014	0.848	0.867	0.032	25	2	3	0	0.000	0.157
ESR	14	10.0	7.1	9.1	1	0.036	0.829	0.815	0.020	13	0	1	0	0.000	0.000
Mean	40.5	13.1	7.4	8.8	1.1	0.012	0.784	0.836	0.077	22.1	2.6	3.4	0.6	0.092	0.409
Pooled	324	19.0	8.9	10.5	16	0.005	0.780	0.859		177	8	10	7	0.322	0.704
Talamanca															
ECH	17	9.8	7.3	8.7	3	0.069	0.722	0.836	0.167**	17	16	9	9	1.000	0.904
Overall															
Mean	36.8	12.4	7.3	8.8	1.6	0.022	0.785	0.834	0.076	20.7	4.7	3.9	1.4	0.124	0.454
Total	441	21.0	9.1	21.0	19	0.005	0.783	0.865		248	28	23	17	0.444	0.816

N, sample size after removal of likely clones. For nDNA: A, average number of alleles/locus; A_e, effective number of alleles/locus; AR, allelic richness; PA, private alleles; Freq., average frequency of private alleles/locus; H_o, observed heterozygosity; H_e, expected heterozygosity; and *f*, inbreeding coefficient. For cpDNA: S, segregating sites; H, haplotype number; PH, private haplotypes; Freq., average frequency of private haplotypes; and HD, haplotype diversity.

Significant departures from Hardy–Weinberg equilibrium (HWE), after Bonferroni correction, are indicated by *$P < 0.05$, **$P < 0.005$ and ***$P < 0.0005$.

Twenty-three cpDNA haplotypes were identified with eight SNPs in the 620-bp aligned *trnG* intron region, and seven SNPs, three INDELs and three poly-A regions in the 771-bp aligned *rps16* region. Populations varied in mean haplotype number (H) 3.92 (1–9), diversity (HD) = 0.454 (0.000–0.904) and private haplotypes (PH) = 1.4 (0–9; Table 1). Each mountain range had a high number and frequency of PH (Guanacaste PH = 4, frequency = 0.667; Tilarán PH = 5, frequency = 0.322; Talamanca PH = 9, frequency = 1.000). Some haplotypes unique to a mountain range occurred in multiple populations (Guanacaste = 1, Tilarán = 2; Table 1). Four Guanacaste haplotypes were shared with northern Tilarán, two of which were also shared with southern Tilarán (Fig. 2). However, most haplotypes were confined to one mountain range and only one of the five common Guanacaste and Tilarán haplotypes was shared at intermediate frequencies (Fig. 2). All Talamanca haplotypes were private. Seven of these nine haplotypes differed by ≤6 mutations from haplotypes on other mountain ranges. This is equivalent to the distance between the most distantly related haplotypes within other mountain ranges (Fig. 2). One Guanacaste and two Talamanca haplotypes were separated by >6 mutations (Fig. 2), contributing to a relatively high number of segregating sites in populations where they occurred (ESM, ECH; Table 1).

nDNA genetic structure

Partitioning of nuclear variation among populations was low (F_{STn} = 0.020; $P \leq 0.001$). When ECH was omitted, F_{STn} decreased, but remained significant (F_{STn} = 0.016; $P \leq 0.001$). Mean pairwise F_{STn} = 0.026 (0.016–0.059; Table S3, Supporting information) was also low. Similar results were obtained when accounting for microsatellite mutation rates (R_{ST} = 0.029). Low mean pairwise F_{STn} occurred in Guanacaste (ESM = 0.017) and Tilarán (ECE = 0.016; Table S3, Supporting information). Mean F_{STn} was greatest for ECH, nearly two times greater than the next greatest population (ESR = 0.031, ECH = 0.059; Table S3, Supporting information). PCA clustered Guanacaste and Tilarán populations together (Fig. S1, Supporting information). Six pairwise F_{STn} comparisons for populations from the same mountain range were not significant (Guanacaste = 2, Tilarán = 4), but all comparisons of populations among mountain ranges were significant (Table S3, Supporting information). There was significant IBD (r = 0.735, R^2 = 0.54, $P \leq 0.022$; Fig. S2a, Supporting information), which persisted with omission of ECH (r = 0.354, R^2 = 0.125, $P \leq 0.036$; Fig. S2b, Supporting information).

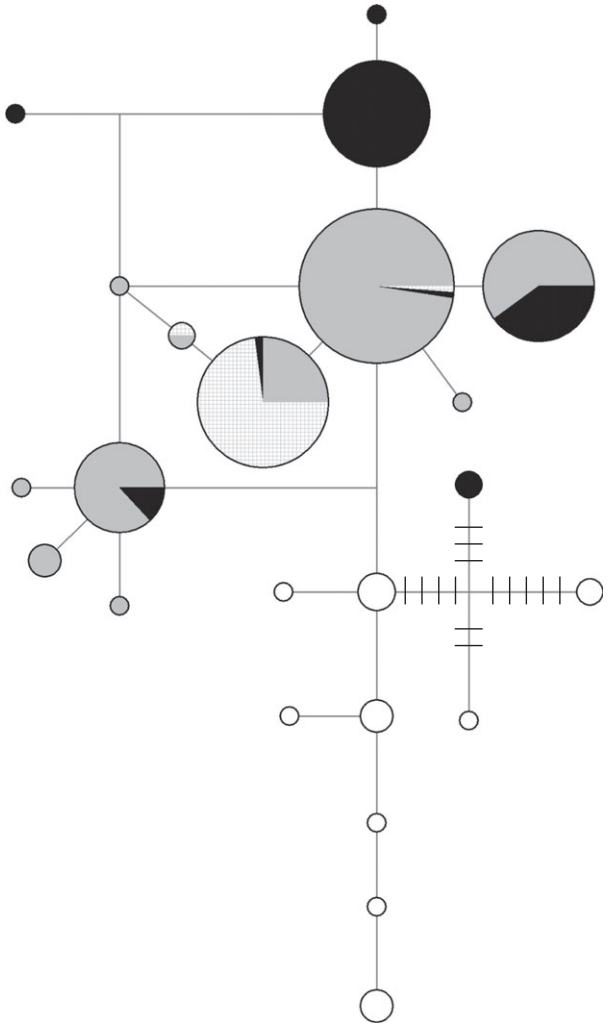

Fig. 2 Chloroplast haplotype network. Nodes are shaded to indicate the proportion of individuals from each mountain range sharing a haplotype, with northern and southern Tilarán populations distinguished (black circles = Guanacaste; grey = northern Tilarán; grey hatches = southern Tilarán; white = Talamanca). Lines represent one mutational step, and cross-hatches represent additional mutational steps between haplotypes.

cpDNA genetic structure

There was moderate cpDNA genetic structure (F_{STc} = 0.443; $P \leq 0.000$), which decreased but remained significant when omitting ECH (F_{STc} = 0.411; $P \leq 0.000$). Mean pairwise F_{STc} = 0.432 (0.276–0.609; Table S3, Supporting information). Excluding ECH decreased mean pairwise F_{STc} for all populations except ESR, which was geographically closest to ECH (Table S3, Supporting information). Mean pairwise F_{STc} was lowest in San Luis populations in northern Tilarán (ALO = 0.286; CAB = 0.294; EFR = 0.276; Table S3, Supporting information) and highest in southern Tilarán (ECE = 0.611;

ESR = 0.602; Table S3, Supporting information). Six pairwise F_{STc} values within the same mountain range were not significant (Guanacaste = 1; Tilarán = 5), but all comparisons between mountain ranges were significant (Table S3, Supporting information). No IBD occurred when ECH was included ($r = 0.029$, $R^2 = 0.001$, $P \leq 0.379$; Fig. S2c, Supporting information) or omitted from analysis ($r = 0.183$, $R^2 = 0.033$, $P \leq 0.131$; Fig. S2d, Supporting information). This was expected because centrally located EMV and ESR were fixed for different haplotypes (pairwise $F_{STc} = 1.0$).

Identification of genetic discontinuities

BARRIER revealed nDNA and cpDNA discontinuities in different locations. Nuclear data showed a discontinuity separating ECH from other populations (bootstrap support = 100%), but no barrier between Guanacaste and Tilarán. Chloroplast data revealed discontinuities between neighbouring mountain ranges (Guanacaste–Tilarán bootstrap support = 54.2%; Tilarán–Talamanca bootstrap support = 26.3%) and a barrier within Tilarán separating northern (UMV, EMV, ETT, ALO, CAB, EFR) and southern (ECE, ESR) populations (bootstrap support = 19.1%). This bootstrap evaluated support for the single strongest barrier, although multiple barriers may have biological importance. Medium to low bootstrap support for cpDNA discontinuities suggested the within-Tilarán discontinuity is comparable with the Tilarán–Talamanca discontinuity.

The additional genetic discontinuity within Tilarán was further evaluated. Our hypothesis of genetic structure among three mountain ranges was supported by hierarchical nDNA AMOVAs, although F_{CT} was low (0.012; $P \leq 0.001$; Table S4a, Supporting information). Significant support for low nDNA structure was also found for the four groups generated by BARRIER cpDNA analysis (i.e. Guanacaste, Talamanca, northern Tilarán, southern Tilarán; $F_{CT} = 0.009$; $P \leq 0.001$; Table S2b, Supporting information). Hierarchical cpDNA AMOVAs showed higher and significant genetic structure for three groups separated by mountain ranges ($F_{CT} = 0.363$; $P \leq 0.002$; Table S4c, Supporting information) and four groups identified by BARRIER (i.e. Guanacaste, Talamanca, northern Tilarán, southern Tilarán; $F_{CT} = 0.442$; $P \leq 0.000$; Table S4d, Supporting information). Although suboptimal, AMOVA for the discontinuity identified between ECH and all other populations is not reported due to the imbalance in available data.

Partial Mantel tests were consistent with a lack of nDNA genetic barriers among mountain ranges and the cpDNA barrier within Tilarán. After accounting for partial effects of geographic distance, F_{STn} was not significantly associated with mountain ranges ($P = 0.916$) or groupings based on cpDNA BARRIER analysis ($P = 0.830$). This suggested that gaps between mountain ranges and the barrier within Tilarán were not associated with greater F_{STn} than accounted for by geographic distance. Thus, the barrier separating ECH may simply reflect its substantial geographic isolation. After accounting for geographic distance, F_{STc} was also not significantly associated with mountain ranges ($P = 0.363$). However, the four population groupings based on BARRIER (i.e. Guanacaste, Talamanca, northern Tilarán, southern Tilarán) were significant after accounting for partial effects of distance ($r = 0.675$; $P \leq 0.001$). Thus, barriers both within and among mountain ranges influenced F_{STc}.

Pollen vs. seed-mediated gene flow

Pollen movement contributed significantly more to gene flow than seed dispersal at most spatial scales, with $m_p/m_s = 46.3$ among all populations except ECH and ranging from 0.03 among San Luis populations in northern Tilarán to 111.12 between Guanacaste and Tilarán (Table 2). Only at small spatial scales within two northern Tilarán localities did m_p/m_s not differ significantly from 1.0 (Table 2). However, results with relatively few populations should be interpreted with caution because type II error may be common when few populations and loci are included (Petit *et al.* 2005).

Discussion

Orchids tend to have low genetic structure compared with other plant taxa, consistent with high rates of gene flow associated with their capacity for long-distance seed dispersal and foraging ranges of their pollinators (Hamrick & Godt 1996; Phillips *et al.* 2012). However, for *Epidendrum firmum*, we predicted significant nDNA and cpDNA discontinuities among mountain ranges, due to biogeographic history and prevailing wind patterns that are perpendicular to the distribution of populations, thus limiting seed-mediated gene flow and pollinator movement. Contrary to our hypothesis, only cpDNA variation reflected significant genetic barriers. Although genetic diversity was high and genetic structure was low for both nDNA and cpDNA, our data suggest relatively limited seed-mediated gene flow, but extensive pollen movement between mountain ranges and among most populations within this narrow band of habitat. These results highlight the importance understanding not only the dispersal biology and natural history of species but the context-dependent processes that shape their evolutionary trajectory.

A low ratio of pollen vs. seed movement (i.e. m_p/m_s) is expected in epiphytic orchids where pollinator

Table 2 Partitioning of nuclear (F_{STn}) and chloroplast (F_{STc}) variation for pollen to seed migration ratios (m_p/m_s)

Groups	*N*	F_{STn}	F_{STc}	m_p/m_s
Between Guanacaste and Tilarán mountain ranges	2 (225)	0.004 (0.003–0.005)	0.312 (0.247–0.358)	111.124*
Among populations within Guanacaste and Tilarán	11 (225)	0.019 (0.015–0.023)	0.483 (0.448–0.564)	46.313*
Among populations within Guanacaste	3 (53)	0.023 (0.009–0.043)	0.364 (0.119–0.609)	22.320*
Among populations within Tilarán	8 (172)	0.019 (0.013–0.023)	0.424 (0.391–0.516)	36.018*
Among populations within northern Tilarán	6 (134)	0.019 (0.012–0.026)	0.217 (0.169–0.336)	12.309*
Between populations within southern Tilarán	2 (38)	0.016 (0.009–0.021)	0.000[†] (−0.029–0.080)	n.a.
Between populations within Rincon de la Vieja (ERP, ESM)	2 (32)	0.010 (−0.010–0.023)	0.318 (0.163–0.596)	44.140*
Among populations within Monteverde (UMV, EMV, ETT)	3 (67)	0.025 (0.011–0.037)	0.152 (0.045–0.385)	4.968
Among populations within San Luis (ALO, CAB, EFR)	3 (67)	0.011 (0.003–0.019)	0.025 (0.004–0.157)	0.032

Data are presented in descending hierarchical spatial scales: between mountain ranges with samples pooled within each range, among all populations, among populations within mountain ranges, among populations within Tilarán subregions and among populations within three localities. N = number of populations or pooled groups (number of samples with both nDNA and cpDNA data available). Separate 95% confidence intervals surrounding F_{STn} and F_{STc} are shown within parentheses. The 95% confidence intervals surrounding F_{STn} were used to predict F_{STc} value ranges corresponding to the null hypothesis that $m_p/m_s = 1.0$, where *indicates the predicted and observed confidence intervals do not overlap and m_p/m_s significantly differs from 1.0.
[†]Negative F_{STc} interpreted to be zero. The m_p/m_s equation is undefined if $F_{STc} = 0$.

movements are confined to their foraging ranges while numerous tiny seeds are released from the canopy into the wind currents and potentially transported long distances (Arditti & Ghani 2000; Nathan & Muller-Landau 2000; Tremblay *et al.* 2005; Phillips *et al.* 2012). However, in *E. firmum,* the m_p/m_s ratio is much greater at broad geographic scales than values reported for other orchids (e.g. 1.43–14.25; Pinheiro *et al.* 2011; Squirrell *et al.* 2001; Trapnell & Hamrick 2004; Trapnell *et al.* 2013) and the median of 183 species surveyed (17; Petit *et al.* 2005). Because all orchids have wind-dispersed dust seeds, this unusual finding likely results from the nature of its pollinator and context-dependent processes. These data illustrate that the spatial distribution of populations, differing dispersal mechanisms and extrinsic abiotic factors, can lead to strongly asymmetric pollen and seed flow patterns. Linear species distributions can contribute to limited gene-flow patterns, particularly among the patchily distributed populations that are characteristic of habitat specialists (Wright 1943; Loveless & Hamrick 1984). Long-distance seed-mediated gene flow may be relatively rare for *E. firmum* because its seeds are carried perpendicular to its linearly distributed habitat by strong winds. Strong-flying pollinators appear to be more effective gene-flow vectors because *E. firmum* pollination occurs before the dry season when winds are less directional and relentless. Only at local scales (Monteverde and San Luis), where air currents may be more consistently variable, are pollen and seed-mediated gene flow equally important.

Gene flow (i.e. between established populations) always decreases genetic structure, while colonization by few founders or founders from few sources often increases genetic structure (Hamrick & Nason 1996). We suggest that significant cpDNA structure among mountain ranges reflects a genetic footprint of colonization of volcanic islands prior to completion of the Central American isthmus (~3 Mya) and/or the upslope migration of previously contiguous populations with climatic warming since the last glacial maximum (~21 000 YBP). Although genetic structure can also arise from isolation and genetic drift, these factors may be less important for *E. firmum*, as with other investigated orchid species, as evidenced by the low overall and pairwise F_{ST} values (Phillips *et al.* 2012). *Epidendrum firmum* displays a pattern similar to that observed in another Neotropical epiphytic orchid at a smaller spatial scale, where nDNA and cpDNA revealed strong population founder effects while nDNA genetic structure showed little differentiation among populations as a result of extensive sphinx moth-mediated pollen flow (Trapnell *et al.* 2013). Paleodistribution modelling has helped elucidate the historic role that Pliocene and Pleistocene isolation, colonization and range shifts played in the current distribution and genetic composition of lowland tropical tree species, and could similarly improve our insights about higher elevation species (Cavender-Bares *et al.* 2011; Poelchau & Hamrick 2011).

Contemporary environmental changes could alter levels and patterns of genetic variation. Because pollen flow was historically substantial at multiple spatial scales, changes in pollinator movement patterns would probably result in increased genetic isolation, structure and drift, causing the loss of genetic variation. While relatively low seed flow occurred among mountain ranges, it was substantial at the smallest spatial scales. Habitat fragmentation and loss of recruitment sites

could thus be particularly consequential for local colonization and seed-mediated gene flow. In the highly fragmented San Luis population EFR, for example, small subpopulations occur on young *Sapium glandulosum* trees, which were probably colonized by multiple founders as evidenced by the presence of multiple cpDNA haplotypes (Table 1; T. R. Kartzinel, personal communication). Furthermore, m_p/m_s is <1.0 among San Luis populations (separated by <1.0 km), which may reflect populations returning to migration–drift equilibrium with equal pollen and seed migration and decreasing population structure (Hamilton & Miller 2002). Proximity of these second-growth trees to remnant populations probably facilitated colonization by genetically diverse individuals (Trapnell *et al.* 2013). Thus, the value of local remnant habitats and populations, which serve as reservoirs of propagules and genetic diversity for the natural recolonization of *E. firmum* populations over short spatial and temporal scales, cannot be overstated.

This study illustrates the value of examining both nuclear and chloroplast genetic variation for developing insights into historical processes. Asymmetric pollen and seed movement in *E. firmum* resulted from a combination of biotic and abiotic factors. Future research should consider the differing influences that the physical environment and biotic and factors may have on gene flow by pollen and seed, as the direction and magnitude of these effects may be context-dependent. Orchids, with their varied pollination strategies and tiny, wind-dispersed seeds, are valuable models for exploring such context dependency.

Acknowledgements

We thank K. Kellett, A. Jenks and B. Haines for field assistance; A. Salazar and F. Morales for species identification; J.L. Hamrick, R. Blanco, F. Camacho and F. Campos for permit assistance; C. Carrigan, B. Hooper and T. Halle for laboratory help; land owners and officials for facilitating access; J.L. Hamrick, S.M. Chang, J.P. Wares and four anonymous reviewers for helpful comments. Funding was provided by NSF (DEB 1110307; T.R.K. and D.W.T.), UGA Graduate School, Sigma Xi, Tinker Foundation, The Explorer's Club, Lewis and Clark Fund; Odum School of Ecology (T.R.K.); and UGA office of the Vice President for Research (D.W.T.).

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This manuscript resulted from the dissertation of T.R.K. who collected samples, conducted laboratory work, analysed data and wrote the manuscript. All authors participated intellectually, contributed resources and refined the manuscript.

Data accessibility

Microsatellite data and DNA sequence alignment: doi:10.5061/dryad.8r652. DNA sequences: GenBank Accession nos JX998189–JX998684.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Location and characteristics of populations.

Table S2 Genotypic disequilibrium between pairs of loci overall and within populations.

Table S3 Pairwise and mean F_{STn} (below diagonal) and F_{STc} (above diagonal).

Table S4 Hierarchical AMOVAs for nDNA (a, b) and cpDNA (c, d).

Fig. S1 The first two PCA axes for pairwise F_{STn} (a) including and (b) excluding population ECH.

Fig. S2 Correlations demonstrating isolation by distance for linearized pairwise F_{STn} against distance (km), with (a) or without (b) including ECH. Correlations demonstrating the lack of isolation by distance for linearized pairwise F_{STc} and distance (km) with (c) or without (d) including ECH.

Appendix S1 Nuclear microsatellite data acquisition and quality control.

Appendix S2 Chloroplast sequence data acquisition and quality control.