

Three-dimensional fine-scale genetic structure of the neotropical epiphytic orchid, *Laelia rubescens*

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Abstract

Epiphytic plants occupy three-dimensional space, which allows more individuals to be closely clustered spatially than is possible for populations occupying two dimensions. The unique characteristics of epiphytes can act in concert to influence the fine-scale genetic structure of their populations which can, in turn, influence mating patterns and other population phenomena. Three large populations of *Laelia rubescens* (Orchidaceae) in the Costa Rican seasonal dry forest were sampled at two levels of intensity to determine: (i) whether individual clusters contain more than one genotype, and (ii) the spatial distribution and fine-scale genetic structure of genotypes within populations. Samples were assayed for their multilocus allozyme genotypes and spatial autocorrelation analyses were performed. High levels of genetic diversity, high genotypic diversity and low among-population variation were found. In the larger clusters, multiple genets per cluster were common with discrete clusters containing up to nine genotypes. Spatial autocorrelation analyses indicated significant positive genetic structure at distances of ≤ 45 cm. This result is likely due to the formation of discrete clusters by vegetative reproduction, as well as the establishment of sexually derived progeny within and near maternal clusters.

Keywords: allozymes, clonal growth, Orchidaceae, spatial autocorrelation

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Introduction

An epiphytic habit is characteristic of ~30 000 vascular plant species, with the highest diversity of taxa occurring in the Americas (Madison 1977). Within the Orchidaceae, an estimated 20 000 species have adopted this growth form. Most vascular epiphytes, including epiphytic orchids, are limited to tropical and subtropical regions (Dressler 1981). The vast majority of epiphytic orchids are animal-pollinated and have tiny wind-dispersed seeds (Dressler 1981). Epiphytes enjoy a unique suite of environmental advantages as well as challenges. Probably most importantly, they can compete better for sunlight than terrestrial herbaceous plants (Dressler 1981). Because there is almost constant air movement within the tree canopy an epiphyte can tolerate direct sunlight without overheating. Epiphytes can also better avoid

predation, attract pollinators and capitalize on the wind to disperse their seeds (Dressler 1981). However epiphytes, particularly those in drier habitats, must also cope with the challenges of obtaining adequate moisture and nutrients.

Epiphytic plants also differ from their terrestrial counterparts by being distributed in three dimensions. As a result, an individual plant can be surrounded by more individuals than would be possible in two dimensions. This has interesting ramifications for ecological processes such as competition, but may also affect the spatial distribution of genetic variation within populations of epiphytes. For example, the genetic structure of an epiphytic species may differ between vertical and horizontal planes within the tree depending on the prevailing mode of reproduction. Horizontal structure would be represented by a higher degree of relatedness among plants on the same branch, whereas vertical structure would be observed if there was more relatedness among orchids on different branches lying in the same vertical plane. If clonal spreading is the primary mechanism of reproduction we would expect to see more

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genetic structure within branches than among branches. If, however, the primary mode of reproduction is sexually derived seeds, the level of fine-scale genetic structure will be determined by the pattern of seed dispersal. Even with panmictic pollen dispersal, if seed dispersal is localized, family structure will exist. If seeds are gravity dispersed, genetic structure would be evident along a vertical plane with increasing structure towards the lowest branches.

Fine-scale genetic structure is defined as the nonrandom distribution of genetically similar individuals *within* populations (McCauley 1997). In plants, fine-scale genetic structure is influenced by clonal reproduction, mating systems, patterns of seed dispersal and colonization, population density of established plants (Hamrick & Nason 1996) and local selection. Patterns of seed dispersal and colonization tend to have the greatest impact on the spatial distribution of genetic diversity within plant populations (Nason & Hamrick 1997). If the propagules that colonize new sites come from many source populations, levels of genetic diversity within newly colonized sites will be high. In contrast, if colonists come from a single source population, genetic diversity within populations will be more restricted and genetic variation among populations will be higher.

Our overall objective was to determine the fine-scale genetic structure of the epiphytic orchid, *Laelia rubescens*, within three sizable populations located in the Costa Rican seasonally dry tropical forest. Fine-scale genetic structure within populations is of interest because the spatial arrangement of related individuals within populations provides insights into seed dispersal patterns. Also, because pollination is often between near neighbours, an understanding of fine-scale genetic structure may allow predictions concerning the likelihood of selfing or biparental inbreeding. By examining three populations we can determine whether the spatial scale and magnitude of genetic structure is homogeneous across populations. A population is here defined as all *L. rubescens* individuals occupying a single host tree. *Laelia rubescens* spreads clonally with each pseudobulb (inflated stem tissue) giving rise to one or two new pseudobulbs per year. Each pseudobulb bears one, or occasionally two, leaves leading to the formation of clusters. These clusters can become quite large, consisting of 100 or more pseudobulbs. More specifically, our objectives were to determine: (i) whether each orchid cluster constitutes a single genetic individual or whether clusters are comprised of multiple genotypes; and (ii) what the spatial distribution and fine-scale genetic structure of orchid genotypes are within a population. We predict that due to clonal growth each *L. rubescens* cluster represents one or at most two genotypes in cases where adjacent clusters have spread and merged together giving the appearance of a single distinct cluster. Clusters may also grow outward; over time, the original pseudobulbs in the middle may die and fall away leaving two or more spatially distinct but genetically identical clusters

within close proximity. Thus, closely adjacent clusters on a branch may have identical genotypes but clusters on different branches would represent different genotypes. We further posited that, owing to the long-distance dispersal potential of the tiny wind-dispersed seeds, populations are established by multiple colonization events and that there is substantial subsequent seed immigration (i.e. gene flow), both of which should produce little fine-scale genetic structure other than that generated by clonal growth.

Materials and methods

Study species

Laelia rubescens Lindley is a neotropical, perennial epiphyte that ranges from Mexico to Panama (Williams & Allen 1980) and prefers dry habitats below 800 m (Mora de Retana & Atwood 1992). Its bisexual flowers are exclusively animal-pollinated with hummingbirds believed to be the primary pollinators (D.W. Trapnell, personal observation). Intraflower pollination is not possible but pollination between flowers within the same genet (geitonogamy) occurs. Sticky pollen grains aggregate to form distinct pollinia, each containing enough pollen grains to fertilize every ovule within a flower. As a result, fruit capsules contain seeds pollinated by a single pollen donor and represent full-sib progeny arrays. Each fertilization event results in hundreds of thousands of tiny wind-dispersed seeds (each seed is ~0.4 mm in length). Seeds possess no endosperm but instead have large internal air spaces that allow them to float in the air column (Arditti & Ghani 2000). Their size and morphology together facilitate long-distance dispersal. Orchid seeds may also be transported in birds' feathers and animal fur (Arditti & Ghani 2000). Once established on a suitable substrate, *L. rubescens* grows clonally with each fleshy pseudobulb capable of producing one or two new pseudobulbs per year. Each pseudobulb bears one, and sometimes two, thick leathery leaves. Clusters can become quite large over time and possess 100 or more pseudobulbs. Each pseudobulb produces only one inflorescence that bears as many as 15–20 showy pink flowers (Halbinger & Soto 1997; D.W. Trapnell, personal observation).

Site description

The research site was located in the Pacific lowlands of northwest Costa Rica, in the Tempisque River basin of Guanacaste Province. *Laelia rubescens* grows on a variety of host trees (Trapnell & Hamrick, in prep.) that occur in habitats ranging from primary forests to highly human-modified landscapes. In less disturbed forests *L. rubescens* is widely dispersed with relatively few individuals per tree. It is in open pasture that it reaches its greatest abundance. When the tropical dry forest was cleared for pasture, often one or

more shade trees were left standing. These isolated trees have large spreading canopies and it is on these host trees that *L. rubescens* is most abundant, with populations of 350 or more clusters. These trees support several other epiphytic species that occur in densities of a few individuals per tree. These include two orchids (*Brassavola nodosa* and *Encyclia fragans*), one bromeliad (*Tillandsia schiedeana*) and a cactus (*Hylocereus costaricensis*). The area is classified as a seasonally dry tropical forest characterized by semi-deciduous trees and a 6-month dry season (December–May). Study populations are located on Hacienda Solimar (latitude 10°17'; longitude 85°08'), a privately owned 2000 ha cattle ranch characterized by isolated trees and small groups of trees in multiple open pastures. Hacienda Solimar became highly disturbed with the establishment of cattle pastures during the mid-1950s. This pattern is typical of many of the dry forest regions of Costa Rica (Sader & Joyce 1988).

Sampling

To understand the distribution of genetic variation within *L. rubescens* populations, sampling was performed at two levels:

Sampling level I. The most intense sampling was performed to determine the complete genotypic composition of individual clusters and to address the question of whether clusters consist of one or more genets. At this level, eight large, discrete clusters located in two trees were selected. Clusters varied in size, possessing between 14 and 96 leaves. Every leaf within these eight clusters was sampled and assayed for its multilocus allozyme genotype. Leaf location within clusters was not mapped.

Sampling level II. The second, less intense but more comprehensive sampling was undertaken to elucidate the fine-scale genetic structure of orchids within three populations (i.e. trees). Here, every cluster within the three populations was sampled but only a limited number of leaves per cluster were collected. Three trees separated by distances of 388–1218 m and that supported 51, 75 and 251 discrete clusters, respectively, were selected. Every cluster within each population was mapped in three-dimensional space by recording the distance and angle from the centre of the tree trunk to the beginning of each cluster. Height was measured with a marked pole or a clinometer where necessary. One leaf was collected from every small cluster (< 12 cm diameter). Where clusters exceeded 12 cm in diameter, a leaf sample was collected approximately every 12 cm. The multilocus allozyme genotype of each leaf was determined. At this level of sampling, in cases where multiple leaves within a cluster possessed identical genotypes, redundant genotypes were removed from subsequent genetic diversity and spatial autocorrelation analyses.

Enzyme extraction and electrophoresis

Samples of leaf tissue were snap frozen in liquid nitrogen within a few hours of collection and were stored in an ultra-cold dry shipper. Samples were sent to the University of Georgia where they were crushed in chilled mortars using a pestle, liquid nitrogen and a pinch of sea sand to disrupt cellular compartmentalization. Enzymes were extracted from the tissue with a polyvinylpyrrolidone-phosphate extraction buffer (Mitton *et al.* 1979). The resulting slurry containing crude protein extract was absorbed onto 4 × 6 mm wicks punched from Whatman 3 mm chromatography paper. Wicks were stored in microtest plates at –70 °C until used for electrophoresis. Wicks were placed in horizontal gels composed of 10% potato starch and electrophoresis was performed. Seven enzyme stains in three buffer systems resolved ten putative polymorphic loci. The enzymes stained and the 10 polymorphic loci identified (in parentheses) for each of the three buffer systems were: (i) system 8: diaphorase (DIA1), fluorescent esterase (FE1, FE2) and triosephosphate isomerase (TPI1); (ii) system 10: UTP-glucose-1-phosphate (UGPP1); (iii) system 11: malate dehydrogenase (MDH1, MDH3), 6-phosphogluconate dehydrogenase (6-PGD1, 6-PGD2) and phosphoglucomutase (PGM2). All buffer and stain recipes were adapted from Soltis *et al.* (1983) except DIA and UGPP, which were taken from Cheliak & Pitel (1984) and Manchenko (1994), respectively. Buffer system 8 is a modification of buffer system 8 as described by Soltis *et al.* (1983). Banding patterns were consistent with those expected for each enzyme system (Weeden & Wendel 1989). Between two and four alleles were observed at each polymorphic locus.

Genetic analyses

Levels of allozyme diversity were estimated for data collected at sampling level II using a computer program designed by Loveless & Schnabel. Measures of genetic diversity were mean number of alleles per polymorphic locus, AP ; effective number of alleles per locus, A_E ; and genetic diversity, H_E ($= 1 - \sum p_i^2$, where p_i is the frequency of the i th allele; Nei 1973), which is the proportion of loci heterozygous per individual under Hardy–Weinberg expectations. Pooled values for these parameters were calculated by pooling data from the three populations. Within-population values were calculated for each population and then averaged across all three populations.

Observed heterozygosity (H_O) was compared with Hardy–Weinberg expected heterozygosity (H_E) for each polymorphic locus in each population by calculating Wright's fixation indices using $F = (H_E - H_O)/H_E$ (Wright 1922). Deviations were tested for significance using $\chi^2 = F^2N(a - 1)$; $df = a(a - 1)/2$ where N is the total number of individuals analysed and a is the number of alleles at the locus (Li & Horvitz 1953).

Variation among populations was estimated using Nei's (1973) measures of genetic diversity. Total genetic diversity (H_T), mean genetic diversity within populations (H_S) and mean genetic diversity among populations (D_{ST}) such that $H_T = H_S + D_{ST}$, were determined for each polymorphic locus. The proportion of genetic variation among populations (G_{ST}) was calculated for each polymorphic locus using $G_{ST} = D_{ST}/H_T$ (Nei 1973). Heterogeneity in allele frequencies among populations was tested by χ^2 (Workman & Niswander 1970). Genotypic diversity was calculated for each population using the complement of Simpson's index corrected for finite samples with the equation: $D = 1 - \sum\{[n_i(n_i - 1)]/[N(N - 1)]\}$ where n_i is the number of individuals of genotype i and N is the population size (Pielou 1969; Peet 1974).

Spatial autocorrelation analyses

The data gathered from sampling level II were analysed for spatial autocorrelation to determine if there is fine-scale genetic structure within populations. Because we are interested in the effect of propagule dispersal on the distribution of genetic variation within populations and not clonal growth, only one individual of each multilocus genotype within a cluster was utilized in the analyses. A pairwise comparison of genetic relatedness of this subset of individuals with respect to spatial distance separating those individuals within populations was used to determine if there is spatial autocorrelation between multilocus genotypes and distance within each of the three populations. Heywood (1991) showed that Wright's (1922) coefficient of relationship, ρ , is a multilocus equivalent to the autocorrelation coefficient, Moran's I (Moran 1950). An algorithm designed by J.D. Nason (Loiselle *et al.* 1995; Kalisz *et al.* 2001) uses a multilocus estimator of kinship to summarize genetic correlations between individuals over a range of distance intervals. The coefficient of co-ancestry calculated (f_{ij}) measures genetic relatedness between paired individuals and allows examination of genetic structure at any spatial scale.

To estimate coefficients of co-ancestry, the genotype of an individual at a locus is described as 1.0, 0.5 or 0.0 depending on whether that individual possesses 2, 1 or 0

copies of the allele (Heywood 1991). For each distance interval, the mean f_{ij} is calculated for all possible pairs of individuals separated by that distance. If a population is in Hardy-Weinberg equilibrium and has no spatial genetic structure, the expected f_{ij} value is 0. In the absence of inbreeding or population genetic subdivision, values of $f_{ij} = 0.125$ would be expected for half-sib comparisons, whereas $f_{ij} = 0.250$ would be expected for full-sib comparisons and parent-offspring comparisons.

Nason's algorithm was used to estimate the coefficient of co-ancestry between all possible pairs of individuals within each of the three mapped populations. This parameter was compared with spatial distances in three-dimensional space between each pair. The shortest distance interval utilized was 0–7.5 cm. Although leaves within larger clusters were sampled every 12 cm, often there were discrete adjacent clusters that were closer to one another than 12 cm. The coefficient of co-ancestry was also calculated within clusters possessing multiple genotypes for every such cluster within each tree.

The significance of the estimated f_{ij} values was tested by using randomized procedures to construct a confidence interval about the null hypothesis of no spatial genetic structure. We performed $N - 1 = 399$ simulation trials with $\alpha = 0.05$. Thus a 95% confidence envelope of simulated genetic structure statistics, assuming no genetic structure, was constructed.

Results

Genetic diversity

Ten putative polymorphic loci were resolved (*DIA1*, *FE1*, *FE2*, *TPI1*, *UGPP1*, *MDH1*, *MDH3*, *6-PGD1*, *6-PGD2* and *PGM2*). There was no evidence of linkage. Estimates of genetic diversity pooled across all populations were high with 2.60 alleles per polymorphic locus and an effective number of alleles per locus (A_E) of 1.41 (Table 1). Mean total genetic diversity at polymorphic loci was relatively low ($H_T = 0.210$) indicating that allele frequencies were skewed at several loci. Most of this variation occurs within populations ($H_S = 0.206$).

Table 1 Summary of allozyme variation at 10 polymorphic loci for three populations of *Laelia rubescens*. Variation is described by proportion of all loci that are polymorphic (P; if a second allele is observed at a locus it is considered polymorphic), mean number of alleles per polymorphic locus (AP), mean number of alleles per locus (A), mean effective number of alleles per locus (A_E), mean observed heterozygosity (H_O) and mean expected heterozygosity (H_E). Standard deviations are shown in parentheses. Gene frequency data are available from the first author upon request

Population	P (%)	AP	A	A_E	H_O (s.d.)	H_E (s.d.)
Tree 465	60.0	2.33	1.80	1.33	0.215 (0.034)	0.185 (0.070)
Tree 467	100.0	2.50	2.50	1.39	0.189 (0.022)	0.201 (0.072)
Tree 468	90.0	2.22	2.10	1.39	0.194 (0.031)	0.210 (0.066)
Population level mean	83.3	2.35	2.13	1.37	0.199 (0.017)	0.199 (0.040)
Pooled	100.0	2.60	2.60	1.41	—	0.210

Table 2 Distribution of genotypes. Probability of identity is the probability of having identical genotypes within a population and is based on allele frequencies within that population (Paetkau *et al.* 1998)

	Tree 465	Tree 467	Tree 468
Number of spatially or genetically distinct individuals	92	257	137
Number of unique genotypes	41	114	80
Probability of identity	0.020	0.010	0.009
Expected number of clusters with the same genotype	1.8	2.6	1.2
Observed number of genotypes found in multiple clusters	18	43	23
Genotypic diversity (D)	0.974	0.991	0.990

Consequently, the proportion of genetic variation among populations was low ($G_{ST} = 0.016$). Despite the low G_{ST} value, private alleles were found in two populations (one and four alleles, respectively; Slatkin 1985). Tests for heterogeneity in allele frequencies among populations of *L. rubescens* indicate that 7 of the 10 polymorphic loci were significantly ($P < 0.05$) different among the three populations.

Laelia rubescens displayed high levels of genetic diversity at the population level with mean values of $AP = 2.35$, $A_E = 1.37$ and $H_E = 0.199$ (Table 1). The observed mean heterozygosity (H_O) was the same as the expected mean value (H_E). Fixation indices (F) were significantly different from the Hardy–Weinberg expected values in 13% (4 of the 30) of the chi-square tests. Based on chance alone, one would expect 5% (1.5 of the 30 tests) to be significantly different from the expected Hardy–Weinberg value. The mean F value across all polymorphic loci was 0.085, suggesting that there is a slight excess of homozygotes in these populations. Mean genotypic diversity (D) was 0.985, ranging from 0.974 to 0.991 (Table 2), indicating that, although most clusters had unique multilocus genotypes, there were also genotypes that occurred in more than one cluster.

Spatial genetic structure

For the eight orchid clusters where every leaf was assayed for its multilocus genotype (sampling level I), multiple genotypes were found in six of the eight clusters (Table 3). Clusters were comprised of 14–95 leaves and between one and five distinct multilocus genotypes. The second smallest cluster (15 leaves) contained five genotypes, whereas the largest cluster (95 leaves) had only two multilocus genotypes. For the more extensive sampling scheme (level II), the generality of multiple genotypes per cluster was confirmed. We found that 18% of the clusters (64 of 357) in the three populations possessed multiple genotypes (Table 4). A maximum of nine genotypes was observed in two clusters. Genotypes *within* a cluster differ from one another by an average of 2.17 loci, whereas a random sample of 12 clusters per population showed a mean difference of 2.68 loci *among* clusters, suggesting that somatic mutation is not responsible for the genotypic variation observed within clusters. In the

Table 3 Genotypic composition of eight large clusters as shown by comprehensive sampling of every leaf

Cluster I.D.	A	B	C	D	E	F	G	H
Number of leaves	14	15	18	29	33	41	48	95
Number of genotypes	1	5	1	5	5	5	3	2

Table 4 Observed frequency of clusters with multiple genotypes as shown by limited sampling per cluster

Number of genotypes within a cluster	1	2	3	4	5	6	7	8	9
Number of clusters	293	36	11	7	6	2	0	0	2

Table 5 Kinship coefficient values (f_{ij}) for the shortest distance intervals in the three populations

Distance interval (cm)	Tree 465	Tree 467	Tree 468
7.5	0.060*	0.065*	0.201*
15.0	0.086*	0.062*	0.125*
22.5	0.084*	0.050*	0.109*
45.0	−0.003	0.029*	0.051*
67.5	0.017	0.016	0.013
90.0	0.026	−0.001	0.033

*Significant values at $\alpha = 0.05$.

three populations we observed 18, 43 and 23 genotypes that occurred in more than one cluster (Table 2). Based on the probabilities of having identical genotypes within populations (0.020, 0.010 and 0.009; Paetkau *et al.* 1998), we would have expected only 1.8, 2.6 and 1.2 genotypes in more than one cluster (Table 2).

Spatial autocorrelation analyses (sampling level II) showed significant positive genetic structure in all three populations. Maximum coefficient of co-ancestry values (f_{ij}) = 0.086, 0.065 and 0.201 were found in trees 465, 467 and 468, respectively (Table 5). We found significant positive autocorrelation among individuals located up to 22.5 cm

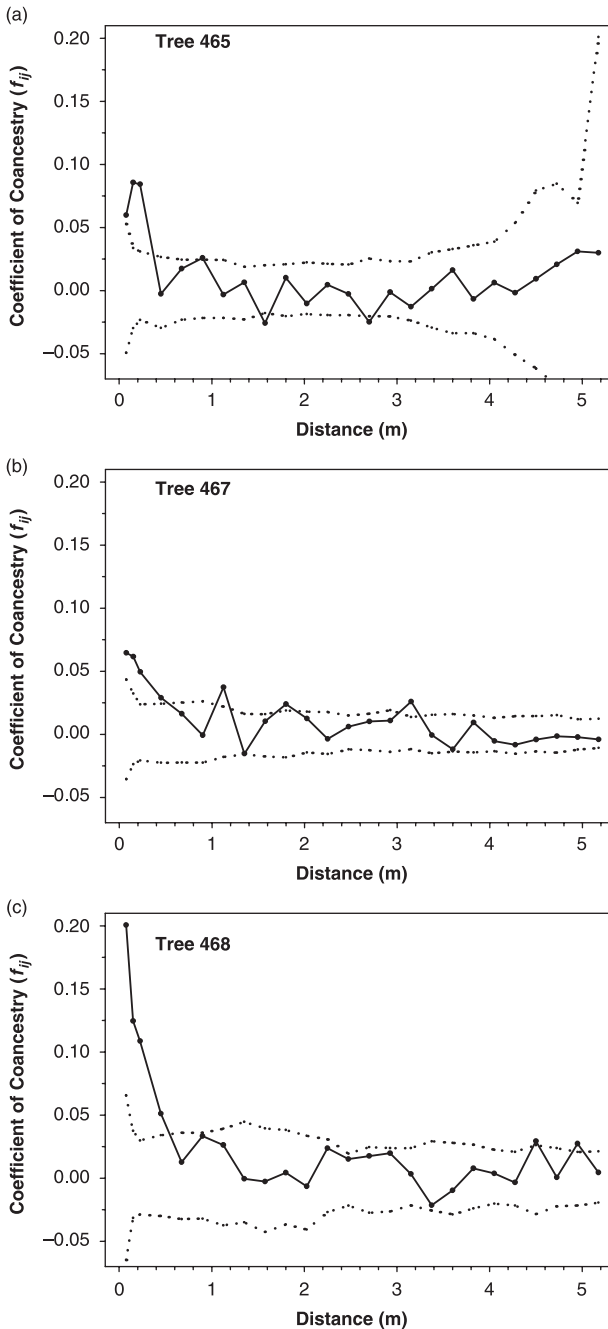


Fig. 1 Correlograms of estimated co-ancestry values (f_{ij}) for trees (A) 465 (B) 467 and (C) 468. The dashed lines represent a 95% confidence interval about the null hypothesis of no genetic structure.

apart in tree 465 and up to 45 cm apart in trees 467 and 468 (Table 5; Fig. 1). When the coefficient of co-ancestry was calculated within every cluster with multiple genotypes (all redundant genotypes within a cluster were removed), mean f_{ij} values were 0.153, 0.106 and 0.135 in trees 465, 467 and 468, respectively. These are approximately the values expected for half sibs.

Discussion

Laelia rubescens possesses moderately high levels of genetic variation at both the pooled and population level indicating that there is sufficient genetic diversity to allow an informative analysis of fine-scale genetic structure (clonal exclusion probabilities of 0.974, 0.991 and 0.990 for the three trees). The among-population genetic variation ($G_{ST} = 0.016$) for these three populations is considerably lower than the mean found for plants in all life history categories (Hamrick & Godt 1989) probably due to the scale of the study. The relatively small distances separating the three host trees probably allows high rates of gene flow among these populations (Trapnell & Hamrick, in review.).

Because of its clonal growth pattern, it was assumed *a priori* that discrete clusters scattered along the tree trunk and branches each represented a single genetically uniform individual. We also anticipated that some clusters might consist of at most two genotypes in cases where adjacent, genetically distinct clusters expanded vegetatively and merged, giving the appearance of a single large, discrete cluster (D.W. Trapnell, personal observation). These expectations were not supported by the data, however. With both sampling schemes we commonly found multiple genotypes within a cluster. With the intensive sampling of eight large clusters (sampling level I), 75% of the clusters contained 2–5 distinct multilocus genotypes per cluster. Furthermore, with sampling level II, 18% of the clusters possessed multiple genets with up to nine genotypes detected in large, physically discrete clusters. There are two explanations for the differences observed between the two sampling schemes. First, the less intensive sampling (level II), conducted throughout all three populations, probably missed many multiple genotypes because leaves were only sampled every 12 cm in the larger clusters. Second, this less intensive sampling includes *all* of the very small clusters that consist of a single genotype. The data indicate that multiple genotypes are not the result of somatic mutation within a single genetic individual. Rather, this pattern likely represents the deposition and preferential establishment of sexually derived progeny in existing clusters. Because of the harsh moisture and nutrient availability conditions characteristic of the epiphytic growth habit, particularly in a seasonal tropical dry forest, well-established clusters may act as nurse plants for the seedlings. Clusters retain moisture that would otherwise be lost to evaporation and act as a trap collecting mineral-rich dust from the wind. An additional consideration is that mature clusters have an established mycorrhizal association. Because the tiny seeds contain no endosperm, germination and seedling development are impossible without an association with endomycorrhizal fungi (Dressler 1981). When seeds disperse and settle on a substrate lacking *L. rubescens* the expectation is that the necessary mycorrhizal fungi will often be absent making

germination impossible. It could also be argued that the presence of multiple genotypes within clusters is due to the simultaneous establishment of several seeds within a 'safe site'. Although possible, this scenario is not consistent with the observation of significant genetic relatedness within clusters as it is likely that trees would be colonized by long-distance dispersal from several source populations. The absence of significant relatedness beyond 45 cm supports this conclusion.

Bidirectional growth along a branch and subsequent die-off of the oldest pseudobulbs in the middle should produce separate but adjacent clusters that would represent multiple ramets of the original parent plant. This prediction was supported by our analyses. There was a much higher occurrence of identical genotypes in distinct clusters (18–43) than would be expected (1–3) based on the probability of identity within these populations. Many of these represented multiple ramets of a single genet. However clusters with identical genotypes were not always contiguous. Some of this can be explained by clusters that are sloughed off the tree with some of its bark. This occurs relatively frequently (D.W. Trapnell, personal observation) and provides an opportunity for a portion of the cluster to re-establish on the lower branches.

The spatial scale and magnitude of fine-scale genetic structure were similar in all three populations. Spatial autocorrelation analyses revealed significant fine-scale genetic structure within populations at distances of ≤ 45 cm in three-dimensional space with no significant structuring beyond that. This suggests that there are similar factors shaping all three populations even though the number of clusters per tree varied by a factor of five. The results further suggest that most of the genetic structure exists on the horizontal plane (i.e. within branches) rather than the vertical plane (i.e. among branches) because branches are typically separated by more than half a metre. Maximum coefficient of co-ancestry values (f_{ij}) in the three populations ranged from 0.065 to 0.201. Values of $f_{ij} = 0.125$ would be expected for half-sib comparisons, whereas $f_{ij} = 0.250$ would be expected for full-sib or parent–offspring comparisons. The variability in relatedness observed in the three populations may be a reflection, in part, of the fact there were more small clusters in trees with lower f_{ij} values which would result in only one leaf per cluster being sampled. Populations with a higher proportion of large clusters, where multiple leaves per cluster were sampled, would facilitate detection of higher relatedness values.

There are several factors that can explain the high levels of spatial genetic structure at the smallest distance intervals within these populations. The characteristic clonal growth pattern of this species and the bidirectionality of spreading along and around branches sometimes give rise to adjacent, physically discrete clusters with identical genotypes. This leads to genetic structure within branches (i.e. horizontal

plane). It is thus possible to have small neighbouring clusters more closely spaced relative to one another than different genotypes within a single large cluster. The significant spatial genetic structuring observed at the smallest distance intervals within the three populations is also due to high positive f_{ij} values of genotypes within clusters, which also contributes to structure within branches. This result could not be due to vegetative reproduction because all clonal replicates within a cluster were removed from these analyses. The observed pattern is consistent with localized seed dispersal and recruitment around maternal plants (Kalisz *et al.* 2001) as well as the finding of multiple genotypes within clusters. Genetic structuring will be further enhanced if there is correlated dispersal and recruitment of full-siblings from a single fruit (Schnabel *et al.* 1998), such as characterize *L. rubescens* capsules. However, the absence of significant population structuring beyond distance intervals of less than half a metre suggests that individuals that founded these populations originated from multiple source populations rather than from a single or a few original colonists. In addition, following the original colonization events there probably has been continuous immigration and the establishment of additional genotypes. This pattern, together with the low among population genetic variation, would further suggest high rates of gene flow via seeds in this epiphytic orchid.

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This research is a part of Dorset Trapnell's doctoral dissertation. She uses molecular markers to understand the evolutionary factors that shape patterns of genetic variation in natural populations. This work reflects her deep interest in tropical epiphytic plants and particularly the Orchidaceae. Her dissertation advisor is Jim Hamrick whose focus is on the evolution of natural plant populations with an emphasis on the genetic structure of populations and on those evolutionary factors that influence the development and maintenance of genetic structure. John Nason is interested in the population and conservation genetics of plants and their associated insect herbivores and pollinators.
