MOLECULAR ECOLOGY

Molecular Ecology (2013) **22**, 3680–3692 doi: 10.1111/mec.12338

Genetic inference of epiphytic orchid colonization; it may only take one

DORSET W. TRAPNELL,* J. L. HAMRICK,* CAITLIN D. ISHIBASHI* and TYLER R. KARTZINEL†
Department of Plant Biology, University of Georgia, Athens, Georgia 30602, USA, †Odum School of Ecology, University of Georgia, Athens, GA 30602, USA

Abstract

Colonization of vacant habitat is a fundamental ecological process that affects the ability of species to persist and undergo range modifications in continually shifting landscapes. Thus, understanding factors that affect and limit colonization has important ecological and conservation implications. Epiphytic orchids are increasingly threatened by various factors, including anthropogenic habitat disturbance. As cleared areas (e.g. pastures) are recolonized by suitable host trees, the establishment and genetic composition of epiphytic orchid populations are likely a function of their colonization patterns. We used genetic analyses to infer the prevailing colonization pattern of the epiphytic orchid, *Brassavola nodosa*. Samples from three populations (i.e. individuals within a tree) from each of five pastures in the dry forest of Costa Rica were genotyped with neutral nuclear and chloroplast markers. Spatial autocorrelation and hierarchical genetic structure analyses were used to assess the relatedness of individuals within populations, among populations within pastures and among populations in different pastures. The results showed significant relatedness within populations (mean $r = 0.166$) and significant but lower relatedness among populations within a pasture (mean $r = 0.058$). Our data suggest that colonization of available habitats is by few individuals with subsequent population expansion resulting from *in situ* reproduction, and that individuals within a tree are not a random sample of the regional seed pool. Furthermore, populations within a pasture were likely colonized by seeds produced by founders of a neighbouring population within that pasture. These results have important ramifications for understanding conservation measures needed for this species and other epiphytic orchids.

Keywords: *Brassavola nodosa*, genetic structure, Orchidaceae, pollen flow, seed dispersal

Received 23 February 2012; revision received 20 March 2013; accepted 26 March 2013

Introduction

Habitat disturbance and anthropogenic alteration of landscapes have become nearly ubiquitous features of contemporary environments. Throughout the Earth's history, natural phenomena have caused habitat perturbations, but during recent decades, human-mediated landscape modifications have far outpaced natural habitat disturbance and are responsible for putting many species at risk, either directly or indirectly (Whitmore 1997). However, abandonment of anthropogenically disturbed landscapes provides an opportunity to study colonization patterns and early stages of habitat restoration. Colonization is a fundamental ecological process that affects the ability of all species to persist and undergo range shifts and/or expansion in continually shifting landscapes. Understanding the mechanisms that affect colonization is therefore of critical importance at a time of widespread habitat degradation and rapid climate change. As the original colonizing species arrive in abandoned clearings and establish, they alter the landscape in a variety of ways, including providing suitable habitat for subsequent colonists. Epiphytic plants, which are a major contributor to overall biodiversity in the Neotropics both directly (Richards 1957;

Correspondance: Dorset W. Trapnell, Fax: +706 542 1805;
E-mail: dorset@plantbio.uga.edu

Madison 1977; Gentry & Dodson 1987; Küper *et al.* 2004) and indirectly (Ozanne *et al.* 2003), are an excellent exemplar of this phenomenon. Only when suitable host trees are available can epiphytes colonize the landscape. Thus, the number and heterogeneity of colonizing trees within a landscape will shape the spatial distribution and composition of epiphytic species (Raventós *et al.* 2010). As cleared areas, such as pastures, are recolonized by suitable host trees, the establishment of epiphyte populations, and their genetic composition, will be a function of patterns of seed dispersal and colonization, in particular, the number and source of founding individuals (Slatkin 1977; Wade & McCauley 1988; Whitlock & McCauley 1990). Understanding factors that affect and limit colonization by epiphytic plants can provide valuable insights into colonization processes and may have important conservation implications for these major constituents of Neotropical biodiversity.

Guanacaste Province, Costa Rica, is characterized by seasonal dry forest, much of which was cleared for cattle ranching in the last century (Sader & Joyce 1988). Subsequent to clearing, many pastures have been colonized by a variety of tree species, including *Crescentia alata* (Bignoniaceae). These trees provide suitable habitat for epiphyte colonization, including several orchid species (Yeaton & Gladstone 1982; DW Trapnell and JL Hamrick, personal observation). Orchids are major contributors to the Neotropical epiphyte flora (Schuettpelz & Trapnell 2006). One of the more common orchids in this habitat is *Brassavola nodosa* (Janzen 1983; DW Trapnell and JL Hamrick, personal observation), the focus of this study. To investigate prevailing colonization patterns of this long-lived, epiphytic perennial, we used genetic analyses of multiple populations in a hierarchical spatial context. A population is defined as all the *B. nodosa* individuals within a tree. A distinctive genetic signature is often evident within and among recently established populations that reflect the manner in which seeds disperse, colonize open habitats and found new populations, and whether subsequent population growth primarily results from continued seed immigration (e.g. Erickson *et al.* 2004) or the recruitment of progeny of founding individuals. Thus by examining genetic relatedness within recently colonized populations, we can infer processes of colonization and subsequent population expansion (Pardini & Hamrick 2008).

To elucidate colonization patterns in this epiphytic orchid, we used biparentally inherited neutral nuclear markers to genotype individuals from fifteen *B. nodosa* populations and examined levels of genetic relatedness at multiple spatial scales. Maternally inherited chloroplast markers were also employed to gain additional insights into the number of original colonists. Autocorrelation analyses were used to quantify genetic relatedness among individuals and the nonrandom spatial distribution of genetically related individuals within and among populations. If a site was colonized by few individuals, and an increase in the number of individuals within the population results primarily from the founders' progeny, we should find significant genetic relatedness within populations and limited genetic relatedness among populations. If a site was colonized by many seeds from multiple sources and/or subsequent recruitment results from continued seed immigration, we should not find significant genetic relatedness within populations. Specific questions addressed are (i) what is the pattern of seed dispersal and colonization by the epiphytic orchid, *B. nodosa*, in trees that have colonized neglected pastureland and (ii) subsequent to initial founding, do increases in population density result primarily from continued long-distance seed dispersal or from the deposition of seeds produced by founding individuals? These two scenarios should produce very different levels of genetic relatedness among individuals within populations.

Materials and methods

Study species

Brassavola nodosa (Linnaeus) Lindley is a long-lived, perennial, epiphytic and lithophytic orchid in subfamily Epidendroideae that occurs naturally from Mexico to Panama, the West Indies and northern South America (Ames & Correll 1985). It occurs below 600 m in seasonal dry forest, often in the salt spray zone of coastal habitats (Janzen 1983; DWT and JL Hamrick, personal observation). It is relatively common and grows on many tree species (DW Trapnell, personal observation) including several mangrove species (Schemske 1980; Murren & Ellison 1996, 1998). It has sympodial growth, and each individual typically produces one new shoot with a single leaf each year (Murren & Ellison 1998) although sometimes multiple shoots arise from the previous year's growth. Only the individual's youngest shoot produces an inflorescence whose terminal raceme bears 1–7 flowers (Murren & Ellison 1998) that open simultaneously and remain open for 10–34 days if unfertilized (Murren & Ellison 1996). The fragrant, greenish white flowers attract night-pollinating hawk moths (Sphingidae; Dressler 1981). In Costa Rica, hawk moths are reported to transport *B. nodosa* pollen over long distances (Janzen 1983). *Brassavola nodosa* pollen grains are aggregated in eight pollinia, each possessing enough pollen grains to fertilize every ovule in a receptive flower. Thus, as is true for most orchids, fertilization results in full-sibling progeny arrays. Although *B. nodosa* is self-compatible, a pollinator is required to transfer

pollinia (Schemske 1980). An estimated 300 000 seeds/capsule are produced and dispersed by wind (Yeaton & Gladstone 1982; Murren & Ellison 1998).

Study site and sampling

The study site is in the Pacific lowlands of northwest Costa Rica, in the lower Tempisque River basin of Guanacaste Province. The area is classified as seasonally dry tropical forest characterized by semideciduous trees and a 6-month dry season (December–May). As has been typical for much of the tropical dry forest in Guanacaste since the 1950s (Sader & Joyce 1988), the area is highly disturbed from forest conversion to pastureland. *Brassavola nodosa* is one of four common orchid species found growing on deciduous trees in Guanacaste (Janzen 1983). Our primary research site (PVR) consisted of four pastures (A–D) situated along the road between Bagaces and Palo Verde National Park (Fig. 1). Pastures were separated by 0.18–7.11 km, and each pasture had at least three trees (e.g. A-1, A-2 and A-3) supporting populations of *B. nodosa*. A population is defined as all *B. nodosa* individuals growing within a tree. All twelve trees were *Crescentia alata* (Bignoniaceae), the Calabash or Jicaro tree. Each population was mapped, and leaf samples were collected from a mean of 42 individuals per population (range of 9–56), for a total of 498 samples. Care was taken to sample individuals separated by at least 60 cm. All orchids within pasture C and D populations were sampled, while a subset of individuals within A and B populations (~100–175 individuals/population) were sampled. Distances among populations in pasture A = 16.4–21.0 m (mean 18.8 m), pasture B = 53.5–236.0 m (mean 157.5 m), pasture C = 24.0–51.7 (mean 34.6 m) and pasture D = 56.3–81.0 m (mean 69.1 m). For comparison, collections were also made from three populations of *B. nodosa*, growing in *C. alata*, located in a pasture near Horizontes Experimental Forest (HEF), 46 km northwest of PVR. The *C. alata* supporting *B. nodosa* populations in HEF appear to be older than the PVR trees, each containing ~200 orchids. Thus, these *B. nodosa* populations may be older than the PVR populations. Samples were collected from 25, 29 and 28 individuals, respectively, from trees separated by 21–73 m (mean 55.3 m).

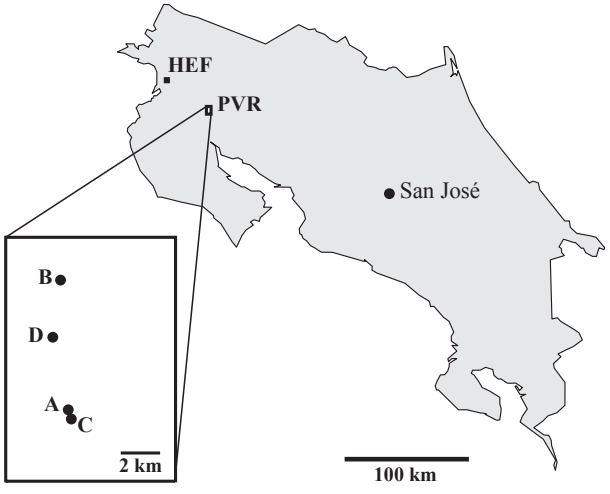

Fig. 1 Geographic locations of the research sites (PVR and HEF), and PVR pastures A, B, C, and D in Guanacaste Province, Costa Rica.

Enzyme extraction and electrophoresis

Leaves were snap frozen in liquid nitrogen within a few hours of collection and transported to The University of Georgia in an ultra-cold dry shipper. Leaves were crushed in chilled mortars with a pestle, liquid nitrogen and a pinch of sea sand to disrupt cellular compartmentalization. Enzymes were extracted from the tissue with an extraction buffer (Wendel & Parks 1982) and 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 starch gels (10%), and electrophoresis was performed. Six gel-electrode buffer combinations and 11 enzyme systems resolved one monomorphic locus and 18 putative polymorphic loci (i.e. more than one allele per locus). Enzymes stained and polymorphic loci (in parentheses) for each buffer system were: (1) buffer system 4: 6-phosphogluconate dehydrogenase (*6Pgd-1*, *6Pgd-2*), phosphoglucoisomerase (*Pgi-2*) and shikimic dehydrogenase (*Skdh*); (2) buffer system 6: fluorescent esterase (*Fe-2*, *Fe-3*); (3) buffer system 7: aspartate aminotransferase (*Aat-1*, *Aat-2*) and diaphorase (*Dia-1*); (4) buffer system 8: triosephosphate isomerase (*Tpi-1*, *Tpi-2*); (5) buffer system 10: fluorescent esterase (*Fe-1*); phosphoglucomutase (*Pgm-1*) and UTP-glucose-1-phosphate (*Ugpp-1*); and (6) buffer system 11: isocitrate dehydrogenase (*Idh-1*), malate dehydrogenase (*Mdh-1*, *Mdh-3*) and phosphoglucomutase (*Pgm-2*). All buffer and stain recipes were adapted from Soltis *et al.* (1983) except *Ugpp* (Manchenko 1994), *Aat* and *Dia* (Cheliak & Pitel 1984). Buffer system 8- is a modification of buffer system 8 as described by Soltis *et al.* (1983). Banding patterns were consistent with diploidy and Mendelian inheritance patterns expected for each enzyme system (Weeden & Wendell 1989) with two to six alleles per polymorphic locus.

Chloroplast markers

A subsample of individuals (7–12 individuals/population; mean = 9.6) assayed for nuclear variation were

also analysed for chloroplast (cpDNA) diversity. Total genomic DNA was extracted from allozyme wicks using a modified cetyltrimethyl ammonium bromide (CTAB) (Doyle & Doyle 1987) protocol. Two noncoding regions of the cpDNA were selected for amplification; the *rpl32-trnL* intergenic spacer (primers rpL32-F/ trnL$^{(UAG)}$; Shaw *et al.* 2007) in the small single-copy region with an alignment length of 708 bp and the *trnG* intron (primers 3′trnG^{UUC}/5′trnG2G; Shaw *et al.* 2005) in the large single-copy region, which has an alignment length of 643 bp. The 12.5 μL PCRs consisted of 1× ThermoPol Buffer [New England Biolabs (NEB)], 1 mM $MgCl_2$ (Sigma), 12.5 μg bovine serum albumin, 0.25 mM of each dNTP (NEB), 0.1 μM of each primer, HPLC-grade water, genomic DNA template and 0.5 units *Taq* DNA polymerase (ThermoPol, NEB). PCRs were performed with an Eppendorf thermal cycler as follows: initial denaturation at 80 °C for 5 min, followed by 35 amplification cycles (denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min and extension at 65 °C for 4 min), a final extension cycle 65 °C for 4 min, holding at 4 °C until samples were removed and storage at 4 °C.

Amplicons were electrophoresed, along with a Quick-Load 100-bp DNA Ladder (#N0467S, NEB), on 1.5% agarose gels and visualized. Amplicons were purified using ExoSAP-IT (USB, Cleveland, OH, USA) with reaction conditions of 37 °C for 45 min, followed by 80 °C for 15 min. Sequencing reactions were performed using an ABI Prism BigDye Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) under reaction conditions of 96 °C for 15 s, followed by 25 cycles of 96 °C for 10 s and 68 °C for 2 min. Samples were prepared for genotyping with an ethanol/EDTA/ sodium acetate precipitation, in accordance with the BigDye Terminator v3.0 Cycle Sequencing Kit protocol. Fragments were resuspended in 10 μL of Hi-Di formamide and analysed on an ABI 3730 Automated DNA Sequencer (Applied Biosystems). Sequences were assembled using SEQUENCHER 4.2 (Gene Codes 2000) and aligned using CLUSTALW (Thompson *et al.* 1994).

Genetic diversity analyses

Nuclear genetic diversity measures were estimated using LYNSPROG, designed by M.D. Loveless and A.F. Schnabel. Measures of genetic diversity were percent polymorphic loci, *P*; total number of alleles, A_T; and genetic diversity, H_e (proportion of loci heterozygous/individual under Hardy–Weinberg expectations; Nei 1973). Pooled values for these parameters were calculated by pooling data from all populations and for the three populations within each pasture. Individual population values were calculated for each population and then averaged across all populations. Allele frequency heterogeneity among populations was tested by the chi-square method of Workman & Niswander (1970). Observed heterozygosity (H_o) was compared with Hardy–Weinberg expected heterozygosity (H_e) for each polymorphic locus in each population by calculating Wright's inbreeding coefficient (*F*, Wright 1922, 1951). Deviations from Hardy–Weinberg expectations were tested for significance using $\chi^2 = F^2N$ $(a - 1)$; d.f. = 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). The Bonferroni correction for multiple comparisons was applied using FSTAT (Goudet 2001). Partitioning of variation among populations and pastures was estimated according to Nei's (1973) measures (i.e. G_{ST}) of genetic diversity as well as AMOVA using GENALEX 6.41 (Peakall & Smouse 2006).

The effective number of cpDNA haplotypes per population was calculated as $C_e = 1/\Sigma p_i^2$ where p_i is the frequency of the ith haplotype with the chloroplast treated as a single locus. Partitioning of cpDNA variation (G_{STc}) among populations and pastures was also determined.

The nDNA and cpDNA G_{ST} values among PVR populations were used to estimate the relative contribution of pollen (m_p) and seed dispersal (m_s) to overall gene flow as,

$$m_p/m_s = [2(1/F_{STc} - 1) - (1/F_{STn} - 1)]/(1 - 1/F_{STc})$$

where F_{STc} is cytoplasmic variation among sites, and F_{STn} is nuclear variation among sites (Petit 1992; Petit *et al.* 1992). Because this statistic reveals a relative relationship between F_{ST} values, it should render the assumption of genetic and demographic equilibrium within populations less important. When pollen and seed migration rates are identical, cpDNA haplotypes in an outcrossing hermaphroditic plant with strict maternal chloroplast inheritance should show a smaller effective population size and three times as much fixation as nuclear alleles, as F_{STn} approaches zero (Hamilton & Miller 2002). Under the null hypothesis of equal pollen and seed flow rates, the expected difference between chloroplast and nuclear F_{ST} values was calculated by $F_{STc} = 6\ F_{STn}/(2 + 4\ F_{STn})$ (Hamilton & Miller 2002). Significance of the difference between expected F_{STn}, due to different effective population sizes of the two genomes, and observed F_{STn} values was tested with a 95% confidence interval generated by bootstrapping procedures.

Genetic relatedness analyses

Within population, multilocus genotypes (MLGs) were compared for the presence of duplicate genotypes. The

expected number of pairs of individuals with identical MLGs based on chance alone, for each population, was calculated by ½ $[N \times (N - 1)] \times PI_m$ where N represents the number of individuals sampled within the population, and PI_m is the probability of identity as calculated by Peatkau & Strobeck (1994) and equals $1 - P_E$ (exclusion probability). Where the observed number of duplicate genotypes within a population exceeded the expected number, only one individual with that genotype was included in the genetic relatedness analyses.

Relatedness was quantified using pairwise comparisons of genetic similarity of individuals with respect to the spatial distance separating those individuals, relative to individuals drawn randomly from a reference population, using GenAlEx 6.41 (Smouse & Peakall 1999; Peakall & Smouse 2006). The autocorrelation coefficients calculated represent genetic relatedness of individuals relative to a random background. Significance of autocorrelation values was tested by constructing a two-tailed 95% confidence interval around the null hypothesis of no relatedness (i.e. $r = 0$) by performing 999 random permutations of genotypes among geographic locations. In a typical spatial genetic structure analysis, genetic relatedness of pairs of individuals is plotted against the spatial distance separating paired individuals (e.g. Trapnell *et al.* 2004; Barbará *et al.* 2008; Chung *et al.* 2011; Juárez *et al.* 2011). However, our analyses are not concerned with comparing pairwise relatedness of individuals within populations with the spatial distance separating those individuals. Rather, we are interested in the *mean* pairwise relatedness of individuals within a population compared to the mean relatedness of individuals between populations and between pastures. As a result, we employ three distance 'classes': mean pairwise relatedness among individuals within populations (within tree = WT), mean pairwise relatedness among individuals within different trees within a pasture (among trees = AT) and mean pairwise relatedness among individuals in different pastures (among pastures = AP).

If background relatedness in the reference population is high (e.g. half- or full-sib progeny arrays), relatedness of neighbouring individuals would be similar to relatedness between two individuals randomly selected from the focal population (Pardini & Hamrick 2008). High relatedness is particularly likely in orchids because (i) they have correlated mating, whereby seeds within a fruit are full-siblings (Gentry & Dodson 1987; Nilsson 1992) and (ii) localized seed dispersal and recruitment of full-sib progeny arrays have been documented in another Neotropical dry forest epiphytic orchid, *Laelia rubescens* (Trapnell *et al.* 2004). To control for the possibility of high relatedness of individuals within trees and within pastures, we examined relatedness patterns across the study area, where a random sample of the reference population, consisting of individuals sampled across the landscape, should have less genetic similarity. This permits accurate insights regarding the founding, and subsequent growth, of populations. Thus autocorrelation of *B. nodosa* was calculated for the full PVR data set at three distance intervals: WT, AT and AP. The first distance interval included pairwise comparisons of individuals within each of the 12 focal trees (WT) and was determined with twelve separate analyses where relatedness was calculated for pairs of individuals within each PVR population relative to a reference population consisting of individuals in the remaining eleven PVR populations. Another twelve analyses assessed pairwise relatedness between individuals within pairs of trees within a pasture (AT) relative to a reference population consisting of the remaining ten PVR populations. Lastly, pairwise comparisons of individuals located in different pastures (AP) were determined with six pairwise analyses measuring the relatedness of individuals from each focal pasture with individuals in one of the other pastures, relative to a background consisting of the remaining two pastures. In each case, this was accomplished by assigning artificial spatial coordinates to all individuals. While the composition of the reference population varied for each analysis, genetic differences among the reference populations were slight: for example, among the six, AP reference populations $G_{STn} = 0.008$. The three HEF populations were analysed similarly, with a reference population of 54 samples collected primarily from a site ~12 km away, near Liberia. To test the relationship between relatedness (r) values and distances between pairs of trees within each pasture, a linear regression analysis was performed utilizing all 15 populations.

Effective number of seed parents

Autocorrelation coefficient values generated by GenAlEx were used to estimate the effective number of seed parents (N_{es}) in each population. Inherent in these estimates are four assumptions: (i) seed parents within a population are unrelated, (ii) seed parents produce equal numbers of progeny that become established within the natal population, (iii) pollen parents represent a random sample of the pollen pool in the landscape (i.e. outside the natal population) and (iv) progeny produced by each seed parent are half-sibs with an r value of 0.250. Although orchids produce fruit consisting of full-sib progeny arrays and are capable of selfing, typically via geitonogamy, we assumed half-sibship to account for the production of multiple fruit by an individual within and among years. Based on a matrix of relatedness of progeny from pairs of maternal

individuals where relatedness among seeds from individual A is 0.250, but relatedness between seeds produced by A and B is zero, we estimated the effective number of seed parents as:

$$r = \frac{N_{es}}{\frac{1}{2}N_{es}(N_{es}+1)} \times 0.250, \quad \text{thus} \quad N_{es} = \frac{2(0.250)}{r} - 1$$

Linear regression analyses were performed to test the relationship between N_{es} values and the number of cpDNA haplotypes within populations, as well as between N_{es} and the effective number of haplotypes within trees.

Results

Nuclear genetic diversity

Eighteen putative polymorphic loci and one monomorphic locus were resolved with a maximum of six alleles per locus, for a total of 65 alleles. Nine private alleles (i.e. found in one population) were observed in five populations: A-1 (four alleles), C-1 (2), C-2 (1), H-4 (1) and H-5 (1). Twelve alleles were found exclusively in the four PVR pastures while five alleles were observed exclusively in HEF. Tests of heterogeneity in allele frequencies among populations indicate that 17 of the 18 polymorphic loci were significant ($P < 0.005$). Mean genetic diversity measures within populations were percent polymorphic loci (P) = 69.9% (47.4–89.5), total number of alleles (A_T) = 43 (33–52), observed heterozygosity (H_o) = 0.247 (0.208–0.298) and expected genetic diversity (H_e) = 0.238 (0.202–0.282). Pastures A (P = 89.5%, A_T = 55) and C (P = 89.5%, A_T = 54) had higher pooled values for P and A_T than pastures B (P = 73.7%, A_T = 45) and D (P = 73.7%, A_T = 47). A similar pattern was not seen for H_e. Pooled species level values are P = 94.7%, A_T = 65 and H_e = 0.264. After Bonferroni correction in FSTAT (Goudet 2001), 2.5% (5 of 198) of F_{IS} estimates were significantly different (adjusted $P < 0.00018$) from Hardy–Weinberg expected values; four were positive suggesting a deficiency of heterozygotes and one was negative indicating an excess of heterozygotes. Mean F_{IS} across all polymorphic loci was −0.009 suggesting a slight excess of heterozygotes. The proportion of genetic variation (G_{STn}) among all fifteen populations of *Brassavola nodosa* was 0.058 and significant (P = 0.000), with G_{STn} = 0.034 (P = 0.000) among the five pastures and G_{STn} = 0.024 (P = 0.002) among populations within pastures (Table 1). Within PVR, G_{STn} = 0.050 (P = 0.000) among the twelve populations, of which 0.027 (P = 0.000) was among the four pastures and 0.023 (P = 0.003) was among populations within pastures (Table 1). Because G_{STn} and AMOVA values revealed similar patterns and significance, only G_{STn} values are presented.

Table 1 Partitioning of nuclear (G_{STn}) and chloroplast (G_{STc}) genetic variation among *Brassavola nodosa* populations, pastures and sites

	G_{STn}	G_{STc}
Among all populations	0.058**	0.342**
Among all pastures	0.034**	0.206**
Among populations within pastures	0.024**	0.136*
Between PVR and HEF sites	0.012**	0.079**
PVR site		
Among populations within PVR site	0.050**	0.301**
Among pastures within PVR site	0.027**	0.160**
Among populations within PVR pastures	0.023**	0.141*
Between pastures A and B	0.010*	0.074*
Between pastures A and C	0.021**	0.011
Between pastures A and D	0.021**	0.121**
Between pastures B and C	0.026**	0.046
Between pastures B and D	0.030**	0.293**
Between pastures C and D	0.018**	0.151*
Among populations within pasture A	0.021*	0.023
Among populations within pasture B	0.020*	0.257*
Among populations within pasture C	0.033*	0.258
Among populations within pasture D	0.034	0.074
HEF site		
Among populations within pasture H	0.017	0.193

PVR, Palo Verde road site and HEF, Horizontes Experimental Forest road site.
*Indicates significance at $P < 0.05$ and ** indicates significance at $P < 0.01$.

Chloroplast genetic diversity

The 114 samples from PVR assayed for cpDNA variation yielded seven haplotypes, with a mean of 2.67 per population [range = one (D-2) to five (C-2); Table 2]. The number of haplotypes observed in each pasture was three (B), four (A and D) and six (C). Pastures C and D each had unique haplotypes (two and one, respectively) and a fourth haplotype occurred only in pastures A and C, which are <200 m from one another. The three most common haplotypes were found in all four pastures, although not in all trees. The effective number of haplotypes/population in PVR ranged from 1.00 (D-2) to 4.57 (C-2) with a mean = 2.08 (Table 2). Genetic structure (G_{STc}) values among all populations and among PVR populations were similar (0.342 and 0.301, respectively; Table 1) and significant (P = 0.000). At both scales, partitioning of genetic variation among pastures contributed more to overall G_{STc} than the partitioning among populations within pastures (Table 1). In PVR, G_{STc} = 0.160 (P = 0.000) among the four pastures and was variable between pairs of pastures, rang-

ing from 0.011 (A and C) to 0.293 (B and D) and was significant ($P < 0.05$) for four of the six pairwise comparisons (A and B, A and D, B and D, C and D; Table 1). Partitioning of haplotype variation among trees within PVR pastures ranged from 0.023 (A) to 0.258 (C), but was only significant in B (0.257; $P = 0.014$). Pairwise G_{STc} values were positively (r =0.532), although nonsignificantly, correlated with the mean distance separating populations.

Among the 15 populations, pollen and seed movement did not differ significantly ($m_p/m_s = 4.25$; $P > 0.05$). However, among PVR populations, pollen flow contributed significantly more to overall gene flow than seed movement ($m_p/m_s = 5.39$; $P < 0.05$). This was also the case among populations within pastures B (14.95; $P < 0.05$) and C (8.19; $P < 0.05$); populations in B were separated by the largest distances (53.5–236.0 m).

Genetic relatedness

Duplicate genotypes were observed in populations B-1, B-3 and C-2, consisting in each case of a single pair of genetically identical individuals. As probabilities of identity were low (0.0001 for B-1 and B-3, and 0.0000 for C-2), the genetically identical individuals were treated as clones and only one ramet per genet was included for the relatedness analyses. Sample sizes of each population and within each distance interval were sufficiently large (i.e. $\geq$36 pairs; Vekemans & Hardy 2004) to render statistically robust results (Table 3). Significant relatedness was observed within every tree, except H-4, with r values ranging from 0.003 (H-4) to 0.350 (C-3; Table 3). Mean within tree relatedness varied among the four PVR pastures with the lowest mean autocorrelation value occurring in pasture A (0.107) and the highest mean values occurring in pastures B (0.245) and D (0.244; Table 3; Fig. 2). Mean within population relatedness for the four PVR pastures was 0.199, while the mean r value within the three HEF populations was significantly lower (0.031; $P < 0.01$). Relatedness was also significant in all but three comparisons between populations within the same pasture (C-1/C-3, H-3/H-4 and H-3/H-5), but was generally lower than within populations, with values ranging from −0.135 (B-1 and B-3) to 0.243 (B-2 and B-3; Table 3). Mean among tree within pasture values also varied greatly among the four PVR pastures (Table 3; Fig. 2). Pasture B had the lowest among tree relatedness ($r = 0.005$) while pasture D had the highest (0.166; Table 3). Mean among tree within pasture values across the four PVR pastures was 0.070, much lower than the mean within tree r value. The mean among tree value for HEF was 0.014 (Table 3; Fig. 2). Relatedness between most pairs of PVR pastures was significant and negative (mean $r = -0.031$; Table 3).

A linear regression analysis of the relationship between relatedness and distances separating pairs of trees within all five pastures was significantly negative ($r = -0.566$; $P < 0.05$). When only the PVR pastures were tested, the negative correlation was higher ($r = -0.616$; $P < 0.05$).

Table 2 The estimated effective number of *Brassavola nodosa* seed parents (N_{es}) per population based on nuclear data as well as the observed and effective number of chloroplast haplotypes

Populations	N_{es}	Observed # cpDNA haplotypes	Effective # cpDNA haplotypes
Palo Verde road (PVR) site			
A-1	5.9	4	2.81
A-2	2.6	2	1.95
A-3	3.5	3	2.63
Mean	4.0	3.00	2.46
B-1	1.8	3	2.32
B-2	0.7	2	1.72
B-3	0.9	2	1.66
Mean	1.1	2.33	1.90
C-1	3.5	3	1.81
C-2	2.4	5	4.57
C-3	0.4	2	1.60
Mean	2.1	3.33	2.66
D-1	1.8	3	1.59
D-2	0.6	1	1.00
D-3	1.0	2	1.25
Mean	1.2	2.00	1.28
PVR mean	2.09	2.67	2.08
Horizontes Experimental Forest (HEF) site			
H-3	11.5	2	1.88
H-4	165.7	2	1.28
H-5	8.8	2	1.98
HEF mean	62.0	2.00	1.71
Overall mean	14.1	2.53	2.00

Effective number of seed parents

The estimated effective number of seed parents per PVR population varied from 0.4 (C-3) to 5.9 (A-1; Table 2). Within the PVR site, pasture A had the highest mean effective number of seed parents ($N_{es} = 4$) while pastures B and D each had a mean N_{es} close to one. The HEF populations had many more seed parents with a mean N_{es} of 62.0 (Table 2). The effective number of founders (N_{es}) in PVR was highly correlated with the percentage of polymorphic loci (P; $r = 0.906$; $P < 0.01$) and the number of alleles (A_T; $r = 0.869$; $P < 0.01$) within each of the PVR populations but not H_e ($r = 0.401$; $P > 0.05$). The HEF populations were not included in

Table 3 Spatial autocorrelation coefficients (*r*) and the number of pairs of individuals in brackets for each distance class for *Brassavola nodosa*

		r values (pairs of individuals)			
	N	Within trees	Between trees within pastures	Among pastures	
Palo Verde road (PVR) site					
A-1	51	0.072 (1275)*	A-2 and A-3	0.049 (2550)*	A and B	−0.026 (23864)*
A-2	51	0.137 (1275)*	A-1 and A-3	0.080 (2550)*	A and C	−0.060 (18088)*
A-3	50	0.112 (1225)*	A-1 and A-2	0.056 (2601)*	A and D	0.005 (10184)*
Mean		0.107		0.062		−0.027
B-1	51	0.178 (1275)*	B-2 and B-3	0.243 (2809)*	B and A	−0.026 (23864)*
B-2	53	0.291 (1378)*	B-1 and B-3	−0.135 (2703)*	B and C	−0.025 (18683)*
B-3	53	0.265 (1378)*	B-1 and B-2	−0.093 (2703)*	B and D	−0.083 (10519)*
Mean		0.245		0.005		−0.045
C-1	52	0.111 (1326)*	C-2 and C-3	0.039 (660)*	C and A	−0.060 (18088)*
C-2	55	0.146 (1485)*	C-1 and C-3	0.008 (624)	C and B	−0.025 (18683)*
C-3	12	0.350 (66)*	C-1 and C-2	0.090 (2860)*	C and D	0.003 (7973)
Mean		0.202		0.046		−0.027
D-1	49	0.176 (1176)*	D-2 and D-3	0.238 (81)*	D and A	0.005 (10184)*
D-2	9	0.310 (36)*	D-1 and D-3	0.130 (441)*	D and B	−0.083 (10519)*
D-3	9	0.245 (36)*	D-1 and D-2	0.130 (441)*	D and C	0.003 (7973)
Mean		0.244		0.166		−0.025
PVR mean		0.199		0.070		−0.031
Horizontes Experimental Forest (HEF) site					
H-3	25	0.040 (300)*	H-4 and H-5	0.022 (812)*	—	—
H-4	29	0.003 (406)	H-3 and H-5	0.006 (700)	—	—
H-5	28	0.051 (378)*	H-3 and H-4	0.014 (725)	—	—
HEF mean		0.031		0.014	—	—
Overall mean		0.166		0.058	—	—

N, population sample size.
*Indicates significance at $P < 0.05$.

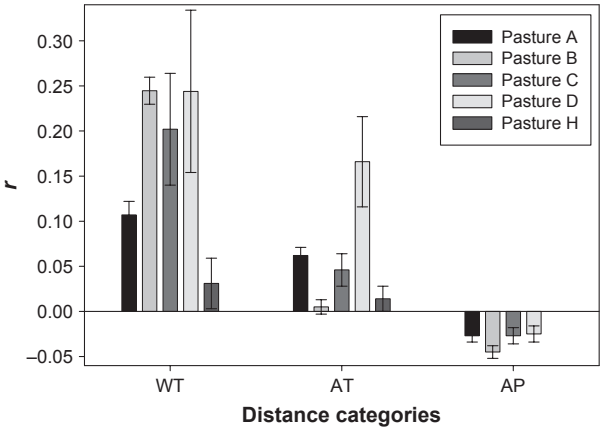

Fig. 2 Mean levels of genetic relatedness (*r*) of individuals within and among fifteen populations of *Brassavola nodosa* at different spatial scales. WT = mean relatedness among individuals within tree crowns; AT = mean relatedness among individuals within the crowns of different trees within the pasture; AP = mean relatedness among individuals in different pastures. Error bars represent the most conservative per pasture (i.e. largest) 95% confidence interval around the null hypothesis of no genetic structure.

these analyses as PVR and HEF represent different geographic locations, and our genetic data indicate that HEF and the four PVR pastures were founded by individuals from different source populations based on the number of unique alleles (5 and 12, respectively). The relationship between N_{es} and the number of cpDNA haplotypes per PVR population is significantly positive ($r = 0.644$; $P < 0.05$).

Discussion

Our objective was to infer colonization processes by the epiphytic orchid, *Brassavola nodosa*, from patterns of genetic diversity within and among its populations. This study clearly demonstrates that populations of *B. nodosa* at PVR were founded by very few individuals, and that subsequent population growth results primarily from recruitment of progeny produced by founding individuals. The data further suggest that seeds produced by founders of the first population to establish in a pasture were responsible for colonizing other trees within that pasture.

Nuclear genetic diversity (P and A_T) within the PVR populations was significantly positively correlated with estimates of the effective number of founding individuals (N_{es}). Correlation between H_e and N_{es} was also positive but nonsignificant. This is not surprising as Nei *et al.* (1975) demonstrated that small founding populations affect A_T and P more than H_e. Nuclear genetic diversity is lowest in the three small populations (C-3, D-2, D-3) that also have some of the highest relatedness values (0.350, 0.310 and 0.245, respectively) indicating that these populations were recently founded by very few individuals. The cpDNA haplotype data support this with one (D-2) and two (C-3, D-3) haplotypes observed (Table 2). We argue that their low genetic diversity is due to these populations being in the early stages of population expansion after founding rather than the number of individuals analysed (every individual in these populations was sampled). There is no reason to believe that the very high r values seen for these three populations are an artefact of small population size. Thus, we conclude that both low genetic diversity and high genetic relatedness in these three trees are due to population expansion from one or two colonists.

Among the 15 populations, there was little nDNA genetic structure ($G_{STn} = 0.058$) that is less than reported for the epiphytic orchid *Catasetum viridiflavum* (0.10) across 16 sites spanning ~8.5 km (Murren 2003) and the mean reported for 16 orchid species (0.087) surveyed by Hamrick & Godt (1996). Of the among population genetic structure, 59% (0.034) occurs among pastures and 41% (0.024) occurs among populations within pastures (Table 1). Most among population genetic structure is attributable to the 12 PVR trees ($G_{STn} = 0.050$) rather than heterogeneity between the PVR and HEF sites (separated by 46 km; $G_{STn} = 0.012$; Table 1) even though 17 alleles (i.e. 40%) were not shared between these two regions. However, these values are similar to F_{STn} values reported for another epiphytic orchid, *Laelia rubescens*, which grows in sympatry with *B. nodosa* (Trapnell & Hamrick 2004; Hamrick & Trapnell 2011) and suggest extensive long-distance gene flow among populations.

Chloroplast genetic structure was substantially higher than G_{STn}, consistent with the reduced effective population size of uniparentally inherited organelles. The G_{STc} value among PVR pastures is similar to that reported for *L. rubescens* among pastures in the lower Tempisque basin (0.181; Trapnell & Hamrick 2004), which is unsurprising considering the similarity of these sympatric species. Genetic variation among pastures contributed more to the overall values than G_{STc} among populations within pastures (Table 1). It is also interesting to note that pasture D, which lies between B and A/C, is the most genetically dissimilar (Fig. 1). The G_{STc} values among trees within pastures were positively correlated, although nonsignificantly, with the mean spatial distances between trees.

Pollen and seed movement among the four PVR pastures did not differ significantly ($m_p/m_s = 4.86$; $P > 0.05$) and is similar to *L. rubescens* (3.92; $P > 0.05$) among pastures in the lower Tempisque basin (Trapnell & Hamrick 2004), but contrary to the terrestrial *Epidendrum fulgens* in the Amazonian rainforest (14.25; Pinheiro *et al.* 2011). However, among PVR populations, pollen movement contributed significantly more to overall gene flow than seed dispersal ($m_p/m_s = 6.18$; $P < 0.05$). This was also the case among populations within pastures B (14.95; $P < 0.05$) and C (8.19; $P < 0.05$); populations in B were separated by the largest distances (53.5–236.0 m).

Autocorrelation analyses revealed significant relatedness within populations (mean $r = 0.166$), with one exception (H-4), and much lower values between pairs of populations within pastures (mean $r = 0.058$; Table 3; Fig. 3) that were significant for all but three pairs of trees located in pastures C and H. Five populations (B-2, B-3, C-3, D-2 and D-3) had relatedness values that exceeded, or were comparable to, values expected for half-siblings (0.250). Three of these trees appeared to be younger indicating that their orchid populations, consisting of 9–12 individuals (C-3, D-2 and D-3), were also younger. Thus, individuals within these five populations may represent a higher proportion of full-siblings relative to half-siblings. These high autocorrelation coefficients suggest that new populations are colonized by very few individuals. This is supported by estimates of the effective number of seed parents per population at the PVR site where all but one tree (A-1) was estimated to have had less than four founders (Table 2). While the number of cpDNA haplotypes/population represents the minimum number of founding individuals, estimated N_{es} per population was significantly positively correlated with the observed number of haplotypes.

Our data further indicate that subsequent population growth in the PVR pastures results primarily from *in situ* reproduction and establishment of progeny of the original colonists. Although orchids produce tiny seeds that are potentially capable of long-distance wind movement (Dressler 1981; Arditti & Ghani 2000), many seeds may settle within a few metres of the maternal plant [e.g. *L. rubescens* (Trapnell *et al.* 2004) and *Orchis purpurea* (Jacquemyn *et al.* 2007)]. Localized seed deposition near maternal plants has also been documented in the epiphytic bromeliad *Vriesea gigantea* (Paggi *et al.* 2010). Furthermore, colonists within trees are not a random sample of the regional seed pool as evidenced by higher relatedness between individuals occupying neighbouring trees relative to the reference population.

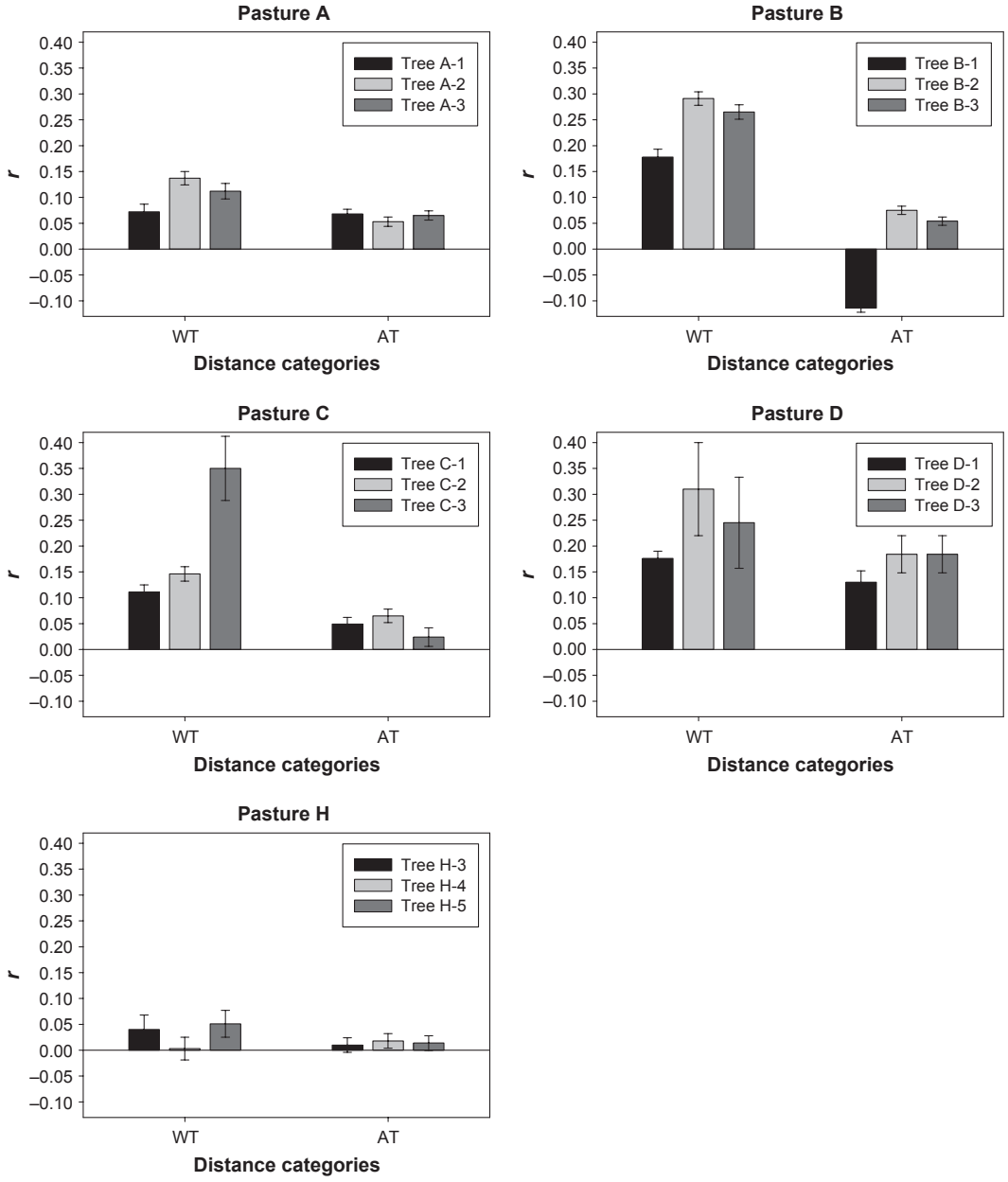

Fig. 3 Levels of genetic relatedness (*r*) of individuals within and among populations of *Brassavola nodosa* within each of the five pastures. WT = mean relatedness among individuals within tree crowns and AT = mean of pairwise relatedness among individuals in each tree with individuals in the other two trees within the pasture. The error bars represent a 95% confidence interval around the null hypothesis of no genetic structure.

Considering the potential for long-distance seed movement once seeds are released from the canopy and enter the air column, significant *r* values between neighbouring trees were unexpected. Two possible scenarios could explain this result: (i) populations within a pasture were colonized by propagules from the same source or (ii) populations were colonized by seeds produced by founders in a neighbouring population within the same pasture. The second scenario is supported by the significant negative correlation between the distance separating pairs of trees within a pasture and the relatedness of *B. nodosa* individuals in those populations, indicating that relatedness was higher between spatially closer populations.

Although the PVR and HEF sites possess similar levels of genetic diversity, relatedness within and between populations at these two sites differed markedly. Within population, relatedness was substantially

lower in HEF (0.003–0.051) than the PVR populations (0.072–0.350; Table 3). Between tree, values were also generally much lower in HEF (mean = 0.014) than PVR (mean = 0.070; Table 3; Fig. 3). Thus, N_{es} values within HEF trees were substantially higher. Together, this suggests that the HEF populations may be older, have experienced more reproductive events, and experienced higher rates of gene flow by continued seed and/or pollen immigration. The greater age of the HEF populations is corroborated by field observations of the sites as well as host tree sizes and appearances. PVR populations were more recently established and the data strongly suggest that all but two of the PVR trees were founded by less than four individuals and six populations were founded by only one or two colonists. The PVR and HEF populations illustrate that as populations mature, genetic relatedness within and between trees decreases due to a combination of factors: more reproductive events, greater seed shadow overlap of neighbouring populations, continued seed immigration and recruitment, and long-distance pollen dispersal.

The very high relatedness (r) within PVR populations but relatively low G_{STn} values among populations appears paradoxical at first glance. How can there be such widespread gene movement among populations and yet such high levels of relatedness within trees? Because biparentally inherited nDNA variation is dispersed via haploid pollen and diploid seeds, while maternally inherited cpDNA variation is dispersed exclusively by seeds, examination of both nuclear and chloroplast variation is useful for resolving this paradox. The low H_{es} values and the small number of haplotypes within populations clearly suggest that the PVR populations were colonized by very few seeds (in most cases ≤ 3), possibly from different sources. These data further indicate that most individuals within populations consist of progeny of these founders. However, the low G_{STn} values among trees and the m_p/m_s ratio that significantly exceeds 1.0 indicate that within PVR, most gene flow among populations has been mediated by pollen dispersal. Widespread pollen movement among populations by hawk moths has resulted in relative homogeneity of nDNA diversity. Nocturnal hawk moth pollinators in Costa Rica are reported by B. Haber to be strong fliers capable of transporting pollen over 10–15 km (Janzen 1983). Thus, in these young PVR populations, there has been limited colonization by immigrant seeds, but when these founders flower, they attract wide-ranging hawk moth pollinators and sample from the regional pollen pool.

Colonization of vacant habitats is a fundamental ecological process that is critically important to every species, regardless of Kingdom. Colonization of novel sites plays a central role in the ability of species to expand and/or shift their ranges in response to a fluctuating environment, such as habitat disturbance and climatic changes. The ability of species to effectively found new populations and modify their ranges has assumed a particular urgency with the accelerated rate of human-induced habitat fragmentation and climate change. Before effective conservation policies can be designed, it is critical to understand the processes affecting colonization and population growth. Effective colonization of *B. nodosa* populations in disturbed habitats can be enhanced by increasing the availability and proximity of suitable host trees. These data also illustrate the importance of hawk moth pollinators for gene flow among newly established populations of this species. Our results have important ramifications for understanding conservation and restoration measures needed for this species as well as other epiphytic orchid taxa.

Acknowledgements

The authors thank C. Carrigan, C. Deen and personnel from the Trapnell and Hamrick labs for their assistance as well as four anonymous reviewers for their helpful comments. Funding was provided by the University of Georgia Office of the Vice President of Research.

References

Ames OA, Correll DS (1985) *Orchids of Guatemala and Belize*. Dover, New York, New York.

Arditti J, Ghani AKA (2000) Tansley review no. 110 numerical and physical properties of orchid seeds and their biological implications. *New Phytologist*, **145**, 367–421.

Barbará T, Lexer C, Martinelli G, Mayo S, Fay MF, Heuertz M (2008) Within-population spatial genetic structure in four naturally fragmented species of a neotropical inselberg radiation, *Alcantarea imperialis*, *A. geniculata*, *A. glaziouana* and *A. regina* (Bromeliaceae). *Heredity*, **101**, 285–296.

Cheliak WM, Pitel JA (1984) Techniques for starch gel electrophoresis of enzymes from forest tree species. In: *Petawawa National Forestry Institute, Information Report P1-X-42*, pp. 1–49. Canadian Forestry Service, Agriculture, Chalk River, Ontario, Canada.

Chung MY, Nason JD, Chung MG (2011) Significant demographic and fine-scale genetic structure in expanding and senescing populations of the terrestrial orchid *Cymbidium goeringii* (Orchidaceae). *American Journal of Botany*, **98**, 2027–2039.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **19**, 11–15.

Dressler RL (1981) *The Orchids: Natural History and Classification*. Harvard University Press, Cambridge, Massachusetts.

Erickson DL, Hamrick JL, Kochert GD (2004) Ecological determinants of genetic diversity in an expanding population of the shrub *Myrica cerifera*. *Molecular Ecology*, **13**, 1655–1664.

Gene Codes (2000) *Sequencher Version 4.1.1 b1*. Gene Codes Corporation, Ann Arbor, Michigan.

Gentry AH, Dodson CH (1987) Diversity and biogeography of Neotropical vascular epiphytes. *Annals of the Missouri Botanical Garden*, **74**, 205–233.

Goudet J (2001) *Fstat, a program to estimate and test gene diversities and fixation indices (version 2.9.3)*. Available from http://www2.unil.ch/izea/softwares/fstat.html

Hamilton MB, Miller JR (2002) Comparing relative rates of pollen and seed gene flow in the island model using nuclear and organelle measures of population structure. *Genetics*, **162**, 1897–1909.

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **351**, 1291–1298.

Hamrick JL, Trapnell DW (2011) Using population genetic analyses to understand patterns of seed dispersal. *Acta Oecologica*, **37**, 641–649.

Jacquemyn H, Brys R, Vandepitte K, Honnay O, Roldán-Ruiz I, Wiegand T (2007) A spatially explicit analysis of seedling recruitment in the terrestrial orchid *Orchis purpurea*. *New Phytologist*, **176**, 448–459.

Janzen DH (1983) *Encyclia cordigera*. In: *Costa Rican Natural History* (ed. Janzen DH), pp. 240, 283 & 633. The University of Chicago Press, Chicago, Illinois.

Juárez L, Montana C, Ferrer MM (2011) Genetic structure at patch level of the terrestrial orchid *Cyclopogon luteoalbus* (Orchidaceae) in a fragmented cloud forest. *Plant Systematics and Evolution*, **297**, 237–251.

Küper W, Kreft H, Nieder J, Köster N, Barthlott W (2004) Large-scale diversity patterns of vascular epiphytes in Neotropical montane rain forests. *Journal of Biogeography*, **31**, 1477–1487.

Li CC, Horvitz DG (1953) Some methods of estimating the inbreeding coefficient. *American Journal of Human Genetics*, **5**, 107–117.

Madison M (1977) Vascular epiphytes: their systematic occurrence and salient features. *Selbyana*, **2**, 1–13.

Manchenko GP (1994) *Handbook of Detection of Enzymes on Electrophoretic Gels*. CRC Press, Ann Arbor, Michigan.

Murren CJ (2003) Spatial and demographic population genetic structure in *Catasetum viridiflavum* across a human-disturbed habitat. *Journal of Evolutionary Biology*, **16**, 333–342.

Murren CJ, Ellison AM (1996) Effects of habitat, plant size, and floral display on male and female reproductive success of the Neotropical orchid *Brassavola nodosa*. *Biotropica*, **28**, 30–41.

Murren CJ, Ellison AM (1998) Seed dispersal characteristics of *Brassavola nodosa* (Orchidaceae). *American Journal of Botany*, **85**, 675–680.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the USA*, **70**, 3321–3323.

Nei M, Maruyama T, Chakraborty R (1975) Bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Nilsson LA (1992) Orchid pollination biology. *Trends in Ecology and Evolution*, **7**, 255–259.

Ozanne CMP, Anhuf D, Boulter SL *et al.* (2003) Biodiversity meets the atmosphere: a global view of forest canopies. *Science*, **301**, 183–186.

Paggi GM, Sampaio JAT, Bruxel M *et al.* (2010) Seed dispersal and population structure in *Vriesea gigantea*, a bromeliad from the Brazilian Atlantic Rainforest. *Botanical Journal of the Linnean Society*, **164**, 317–325.

Pardini EA, Hamrick JL (2008) Inferring recruitment history from spatial genetic structure within populations of the colonizing tree *Albizia julibrissin* (Fabaceae). *Molecular Ecology*, **17**, 2865–2879.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Peatkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bean populations. *Molecular Ecology*, **3**, 489–495.

Petit RJ (1992) *Polymorphisme de l'ADN chloroplastique dans un complexe d'especes: les chenes blancs europeens. Approche de genetique des populations*. Thesis, University of Paris XI, Orsay, France.

Petit RJ, Kremer A, Bacilieri R, Ducousso A, Zanetto A (1992) Structuration genetique chez les chenes blancs europeens. In: *Complexe d'especes, flux de genes et ressources genetiques des plantes* (ed. Lavoisier C), pp. 155–163. Bureau des Resources Genetiques, Paris, France.

Pinheiro F, de Barros F, Palma-Silva C, Fay MF, Lexer C, Cozzolino S (2011) Phylogeography and genetic differentiation along the distributional range of the orchid *Epidendrum fulgens*: a neotropical coastal species not restricted to glacial refugia. *Journal of Biogeography*, **38**, 1923–1935.

Raventós J, Mujica E, Wiegand T, Bonet A (2010) Analyzing the spatial structure of *Broughtonia cubensis* (Orchidaceae) populations in the dry forests of Guanahacebibis, Cuba. *Biotropica*, **43**, 173–182.

Richards PW (1957) *The Tropical Rain Forest*. Cambridge University Press, Cambridge, UK.

Sader SA, Joyce AT (1988) Deforestation rates and trends in Costa Rica, 1940–83. *Biotropica*, **20**, 11–19.

Schemske DE (1980) Evolution of floral display in the orchid *Brassavola nodosa*. *Evolution*, **34**, 489–493.

Schuettpelz E, Trapnell DW (2006) Exceptional epiphyte diversity on a single tree in Costa Rica. *Selbyana*, **27**, 65–71.

Shaw J, Lickey EB, Beck JT *et al.* (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, **92**, 142–166.

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*, **94**, 275–288.

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.

Soltis DE, Haufler CH, Darrow DC, Gastony GJ (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal*, **73**, 9–27.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence

alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **11**, 4673–4680.

Trapnell DW, Hamrick JL (2004) Partitioning nuclear and chloroplast variation at multiple spatial scales in the neotropical epiphytic orchid, *Laelia rubescens*. *Molecular Ecology*, **13**, 2655–2666.

Trapnell DW, Hamrick JL, Nason JD (2004) Three-dimensional fine-scale genetic structure of the neotropical epiphytic orchid, *Laelia rubescens*. *Molecular Ecology*, **13**, 1111–1118.

Vekemans X, Hardy OJ (2004) New insights from fine-scale genetic structure analyses in plant populations. *Molecular Ecology*, **13**, 921–935.

Wade MJ, McCauley DE (1988) Extinction and recolonization—their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.

Weeden NF, Wendell JF (1989) Genetics of plant isozymes. In: *Isozymes in Plant Biology* (eds Soltis DE & Soltis PS), pp. 46–72. Dioscorides Press, Portland, Oregon.

Wendel JF, Parks CR (1982) Genetic control of isozyme variation in *Camillia japonica* L. *Journal of Heredity*, **73**, 197–204.

Whitlock DE, McCauley DE (1990) Some population genetic consequences of colony formation and extinction—genetic correlations within founding groups. *Evolution*, **44**, 1717–1724.

Whitmore TC (1997) Tropical forest disturbance, disappearance and species loss. In: *Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented Communities* (eds Laurance WF & Bierregaard RO), pp. 3–12. University of Chicago Press, Chicago, Illinois.

Workman PL, Niswander JD (1970) Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics*, **22**, 24–49.

Wright S (1922) Coefficients of inbreeding and relationship. *The American Naturalist*, **56**, 330–338.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

Yeaton RI, Gladstone DE (1982) The pattern of colonization of epiphytes on Calabash trees (*Crescentia alata* HBK.) in Guanacaste Province, Costa Rica. *Biotropica*, **14**, 137–140.

D.W.T. and J.L.H. are interested in the evolutionary biology of natural plant populations and co-conceived the project, made all field collections and performed the nDNA genotyping. C.D.I. and T.R.K., graduate students working in the D.W.T. lab, obtained cpDNA haplotypes. D.W.T. performed all the analyses and wrote the manuscript while J.L.H. provided feedback and editing.

Data accessibility

Population locations, as well as allozyme genotype and chloroplast haplotype data: Dryad entry doi: 10.5061/dryad.m5105.