

# Mating patterns and gene flow in the neotropical epiphytic orchid, *Laelia rubescens*

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## Abstract

Understanding mating patterns and gene movement in plant populations occupying highly disturbed landscapes is essential for insights into their long-term survival. We used allozyme genetic markers to examine mating patterns and to directly measure pollen flow in the Central American epiphytic orchid, *Laelia rubescens*. Study populations were located in disturbed, seasonally dry tropical forest in Costa Rica. Every flowering individual within 15 populations and 12–18 seedlings from each maternal individual were genotyped over two reproductive seasons. Strict correlated mating by orchids produces full-sib progeny arrays from which the multilocus diploid genotype of the pollen parent can be inferred. These paternity analyses produced detailed quantitative estimates of pollen movement within and among populations of this species. Although our data illustrate that mating patterns vary spatially and temporally among trees, among pastures, and between years, overall patterns were surprisingly consistent. Thirty-four per cent of the capsules produced in both years resulted from gene flow events. Where pollen parents were identified, pollen moved mean distances of 279 m and 519 m in 1999 and 2000 respectively and a maximum documented distance of 1034 m. A substantially larger floral display in 2000 corresponded to a marked increase in pollen dispersal distances. Smaller populations, which more closely resembled those in undisturbed forest, had higher rates of gene flow than the large populations that characterize disturbed sites. We predict the occurrence of greater gene flow between low-density populations occupying undisturbed habitats.

**Keywords:** paternity analysis, pollen dispersal, dry forest

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## Introduction

Patterns of mating and gene movement strongly influence the spatial and temporal distribution of genetic diversity within populations and species. Unlike evolutionary factors that cause the genetic divergence of populations (e.g. local adaptation, genetic drift), outcrossing mating systems and gene flow among populations increase effective population sizes and genetically homogenize spatially distinct populations (Slatkin 1985a; Ellstrand 1992), preserving genetic diversity and reducing inbreeding. Gene flow also introduces novel genetic variation into existing populations (Slatkin 1985a). The dispersal and establishment of genes into foreign gene pools (i.e. gene flow, Endler 1977) is a key evolutionary factor shaping the genetic composition of species

(Ellstrand & Marshall 1985; Hamrick 1987). It is especially important to understand the extent of gene flow in anthropogenically fragmented landscapes as population sizes are often greatly reduced relative to predisturbance conditions.

Landscape level disturbance of natural habitats has unfortunately become the norm rather than the exception. With increased habitat destruction and landscape disturbance, once continuous populations become fragmented which may lead to isolation, genetic drift, inbreeding, and ultimately the loss of genetic variation. Genetic diversity and its maintenance are crucial for long-term species survival, because it increases the likelihood that species will have the genetic resources to survive future environmental fluctuations. Developing insights into patterns of gene movement in anthropogenically altered landscapes is essential to understanding the long-term survival potential of affected taxa.

Seasonally dry tropical forests are particularly vulnerable to disturbance as they are ideally suited for cattle

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ranching and other forms of agriculture. In Costa Rica, the once continuous tropical dry forest, located in the Pacific lowlands of Guanacaste Province, underwent wholesale clearing for cattle ranching in the 1950s. This has resulted in a highly disturbed and fragmented landscape with natural vegetation only occurring along streams or escarpments and in fragments that vary in size and degree of isolation. Such landscapes provide an excellent context in which to study the genetic connectivity of remnant populations (e.g. Apsit *et al.* 2001; White *et al.* 2002). The extent and duration of disturbance experienced by these landscapes necessitate a better understanding of mechanisms that shape the evolutionary trajectories of species occupying these fragments. As a result of worldwide human-induced disturbances, it is no longer sufficient to exclusively study species occupying pristine habitats.

We use paternity exclusion analyses to describe breeding patterns within and among populations of the epiphytic orchid, *Laelia rubescens* Lindley, in the dry forest of Costa Rica, to directly measure contemporary pollen flow, and to determine minimum pollen immigration distances. Specifically, we ask how much pollen-mediated gene flow occurs among populations (a population = all individuals on a single host tree) within pastures, among pastures within a circumscribed area, or from outside of the study site? Over what distances does pollen immigration occur? Is there a relationship between flower number within a population and estimated gene flow rates? Finally, are direct gene flow estimates derived from paternity analyses consistent with estimates based on genetic structure among populations? Our expectation is that most current pollen movement is within populations and that there is a high incidence of geitonogamous selfing. We expect relatively little long-distance pollen flow (i.e. beyond 200 m) with most immigrant pollen originating from populations within pastures. We further posit that populations with more flowering individuals experience less gene flow.

## Materials and methods

### Study organism

*Laelia rubescens* Lindley (Orchidaceae) is a neotropical, long-lived perennial epiphyte ranging from Mexico to Panama (Williams & Allen 1980) in dry habitats below 800 meters (Mora de Retana & Atwood 1992). Its bisexual flowers are exclusively animal-pollinated with hummingbirds believed to be the primary pollen vectors (D.W. Trapnell, pers obs). Intraflower pollination is not possible but geitonogamous pollinations occur. Orchids are well suited for paternity analyses because their pollen grains are aggregated in cohesive, sticky packets (pollinia) that are transported as a unit by an animal vector. Each *L. rubescens* flower has eight pollinia. A pollinium possesses enough pollen grains to fertilize every

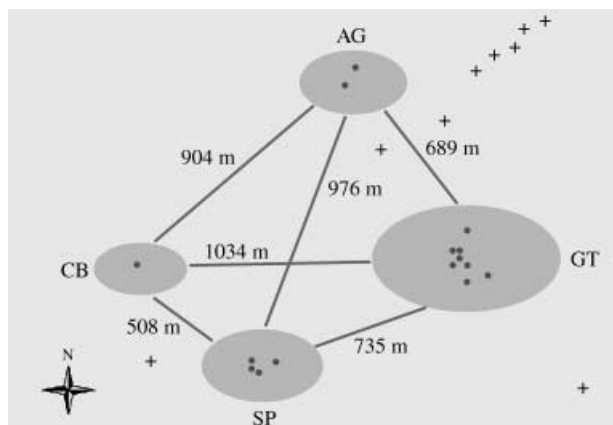
ovule in a recipient flower. As a result, individual capsules contain seeds pollinated by a single pollen donor and represent full-sib progeny arrays. This attribute allows inference of the diploid genotype of the pollen parent, facilitating direct measurement of gene flow and detailed descriptions of mating patterns within populations. Such strict correlated mating is only known in orchids (Gentry & Dodson 1987; Nilsson 1992), milkweeds, mimosoid legumes, and some tropical figs. Each fertilization event results in hundreds of thousands of tiny wind-dispersed seeds. Once established on suitable substrate, *L. rubescens* grows clonally with each fleshy pseudobulb (inflated stem tissue) producing one or two new pseudobulbs/year. Each pseudobulb produces one inflorescence that bears as many as 20 showy, pink flowers (Halbinger & Soto 1997; D.W. Trapnell, pers obs). Clusters can possess 100 or more pseudobulbs and produce multiple inflorescences simultaneously. Anthesis (January to March) is over an extended period during the dry season. A single inflorescence has been observed to mature up to 11 capsules (D.W. Trapnell, pers obs).

### Study site

The research site is 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), occurring in habitats ranging from primary forests to highly human-modified landscapes. In less disturbed forests, *L. rubescens* is widely dispersed with relatively few clusters per tree. When the tropical dry forest was cleared for pastures, often one or more shade trees were left. These isolated trees typically have large spreading canopies and it is on these 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 seasonally dry tropical forest characterized by semideciduous trees and a six-month dry season (December to May). Study populations are located at Hacienda Solimar (10°17' N and 85°08' W), a privately owned 2000-hectare cattle ranch characterized by isolated trees and small groups of trees in multiple pastures. Hacienda Solimar was established during the mid-1950s and represents a pattern of disturbance typical of many dry forest regions of Costa Rica (Sader & Joyce 1988).

### Sampling and seed culture

Four large pastures at Hacienda Solimar (AG, CB, GT, and SP), separated by 508 m to 1034 m, were selected (Fig. 1).



**Fig. 1** Map of the four study pastures showing distance in meters between their midpoints. Black circles within the shaded ovals represent the locations of *L. rubescens* populations. The + symbols represent known populations of *L. rubescens* that were not sampled.

Typically, second growth forest, ranging in width from 10 to 50 m, occupied stream courses and fence rows between pastures. Study pastures had one, two, four, and eight trees respectively supporting populations of *L. rubescens* (Fig. 1). A population is comprised of all clusters of *L. rubescens* growing within a single host tree. There were 15 populations (i.e. trees) each containing four to 102 clusters of *L. rubescens*. Viable capsules were produced in nine of the 15 populations in 1999 and 11 in 2000, located in the same three pastures in both years (Table 1). The spatial distribution and structure of orchids within the landscape at Hacienda Solimar allowed direct estimates of gene flow in a spatial hierarchy (i.e. within trees, among trees within pastures and among pastures).

At the end of the 1998 flowering season all old inflorescences within the 15 host trees were removed so that individuals flowering during 1999 could be identified. In April and May of 1999, all mature capsules (211) and subtending maternal leaf material were collected. Subtending leaf

**Table 1** Types of matings that produced capsules during 1999 and/or 2000. In column one, the two letters designate the pasture while numbers represent the host tree. Totals for the pastures and the years represent weighted averages based on the number of capsules per tree

Pasture and tree	Year	Fruit number	Within tree		Within pasture G.F.	Among pasture G.F.	Unknown G.F.
			Selfing	Outcrossing			
SP-461	1999	6	0%	17%	50%	0%	33%
	2000	5	20%	40%	20%	20%	0%
SP-462	1999	53	15%	60%	2%	6%	17%
	2000	181	6%	70%	0%	6%	18%
SP Total	1999	59	14%	56%	7%	5%	18%
	2000	186	6%	69%	1%	6%	18%
AG-468	1999	10	10%	70%	0%	0%	20%
	2000	49	19%	51%	6%	8%	16%
AG-469	1999	13	8%	23%	54%	0%	15%
	2000	1	100%	0%	0%	0%	0%
AG Total	1999	23	9%	43%	31%	0%	17%
	2000	50	20%	50%	6%	8%	16%
GT-473	1999	30	0%	76%	0%	7%	17%
	2000	41	22%	46%	10%	5%	17%
GT-474	1999	2	0%	50%	0%	50%	0%
	2000	7	0%	14%	72%	14%	0%
GT-475	1999	1	0%	0%	100%	0%	0%
	2000	3	0%	0%	34%	33%	33%
GT-476	1999	0	0%	0%	0%	0%	0%
	2000	5	20%	0%	0%	0%	80%
GT-477	1999	3	0%	33%	67%	0%	0%
	2000	25	16%	16%	12%	44%	12%
GT-478	1999	0	0%	0%	0%	0%	0%
	2000	6	0%	17%	33%	0%	50%
GT-480	1999	12	25%	41%	17%	0%	17%
	2000	6	0%	50%	33%	17%	0%
GT Total	1999	48	6%	63%	10%	6%	15%
	2000	93	15%	30%	18%	17%	20%
Overall Total	1999	130	10%	56%	12%	5%	17%
	2000	329	11%	55%	6%	10%	18%

tissue from every pseudobulb that flowered but failed to produce capsules was also collected as these individuals represented possible pollen donors. Upon completion of sampling in 1999, all inflorescences were removed as before. During April of 2000 a second reproductive period was similarly sampled with 376 mature capsules collected. Leaf samples from maternal plants and all possible pollen donors were analysed for their multilocus allozyme genotypes.

Seeds were germinated under sterile conditions. Because nearly all capsules had partially dehisced, it was necessary to sterilize the seeds to kill fungal spores prior to placing the seeds in culture. Seeds were extracted, sterilized in 10% bleach solution for 10 minutes, rinsed with autoclaved deionized water for 10 minutes, and cultured on autoclaved germination medium (G & B Orchid Laboratory Mother Flask Medium IV) under sterile conditions. Once the seeds germinated and roots emerged, seedlings were transferred to sterile growth medium (G & B Orchid Laboratory Replate Flask Medium IV). Seedlings were kept in a growth chamber maintained at approximately 30 °C under constant light. Seeds from viable capsules had high germination rates. However, some cultures were lost to fungal contamination by spores that were highly resistant to multiple sterilization attempts. Upon reaching approximately 1.5–2 cm, after 8–14 months, 12–18 seedlings per capsule were assayed for allozymes.

#### *Enzyme extraction and electrophoresis*

Leaf tissue from maternal plants and all possible pollen donors within each of the 15 populations were snap frozen in liquid nitrogen within a few hours of collection and stored in an ultra-cold dry shipper. Samples were sent to the University of Georgia and were crushed in chilled mortars with a pestle, liquid nitrogen, and a pinch of sea sand to disrupt cellular compartmentalization. Seedlings were crushed, without liquid nitrogen or sea sand. Enzymes were extracted 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 10% potato starch gels and electrophoresis was performed. Seven enzyme stains in three buffer systems resolved 10 putative polymorphic loci. Enzymes stained and the 10 polymorphic loci identified (in parentheses) for each of the three buffer systems were: system 8-: diaphorase (DIA1), fluorescent esterase (FE2), and triosephosphate isomerase (TPI1), system 10: fluorescent esterase (FE1), UTP-glucose-1-phosphate (UGPP1), 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 diaphorase and UTP-glucose-1-phosphate 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 & Wendell 1989). Between two and four alleles were observed at each polymorphic locus. Seedling allozyme expression was comparable or superior to that of adult leaf tissue.

#### *Data analyses*

Correlated mating in orchids greatly facilitates paternity exclusion analyses to directly estimate pollen flow rates and patterns (e.g. see Broyles *et al.* 1994 and Nason *et al.* 1996). Because fertilization results in a singly sired progeny array, the multilocus diploid genotype of the pollen donor was inferred by visually comparing the multilocus genotype of the maternal plant (acquired directly for each inflorescence) with genotypes of 12 full-sib progeny from an individual capsule. Where there was ambiguity, an additional six progeny were analyzed (i.e. 18 total). Multilocus genotypes of all possible pollen donors within an individual tree and pasture were determined by genotyping all flowering individuals within each pasture. If the inferred paternal genotype did not match a possible pollen donor within the tree, the capsule must have resulted from immigrant pollen. For pollen originating within the 15 study populations, actual pollen movement distances were recorded for each population and pasture (Fig. 1). With the number of genotypes detected at the 10 polymorphic allozyme loci resolved, it was possible to distinguish 1 749 600 distinct genotypes. Because our data (Trapnell *et al.* 2004) show that these populations have only a slight excess of homozygotes, we assumed Hardy–Weinberg equilibrium to calculate an exclusion probability of 0.986 (i.e. the probability of obtaining two individuals with the same genotype is 0.014). Estimates of apparent pollen flow obtained from these analyses are minimum estimates of total gene flow at the individual tree level, as it is possible that genotypes identified within a population could also occur outside this area. However, because of the very high exclusionary power of the full-sib analyses we expect apparent and total gene flow rates to be similar, unlike the situation when multilocus immigrant gametes are identified.

Although we are aware of the problems with comparing indirect (historical) and direct (current) estimates of gene flow (Bossart & Prowell 1998; Whitlock & McCauley 1999) we determined the values with each approach to test if they are qualitatively consistent (i.e. high, intermediate, or low pollen flow). Two indirect methods of measuring historical gene flow among populations within species were employed using genetic structure data. Number of gene

migrants per generation was estimated using  $Nm = (1 - G_{ST})/4G_{ST}[(k - 1)/k]^2$ , where  $k$  is the number of populations (Wright 1951; Crow & Aoki 1984) and the  $G_{ST}$  values were based on allele frequencies of pollen donors within each of the 15 populations. The second estimate was based on mean frequencies of private alleles (i.e. alleles found only in one population) and does not assume neutral alleles (Slatkin 1985b; Barton & Slatkin 1986). The number of migrants per generation ( $N_{em}$ ) was found directly for each population by  $N_{em} = N_e/2(a/b)$ , where  $N_e = 1/\sum[1/2(x_i + y_i)]^2$ ,  $x$  is the proportion of male gametes contributed by individual  $i$  to total capsule production within the population,  $y$  represents the proportion of female gametes contributed by individual  $i$  to total capsule production within the population (Muona & Harju 1989; Kjaer 1996),  $a$  = number of capsules with pollen donors outside the population and  $b$  = total number of capsules produced within the population. This was divided by two to properly account for the haploid nature of pollen. Direct  $N_{em}$  values were averaged across all populations for each year and compared to indirect  $Nm$  values based on observed genetic structure.

As floral display can affect pollinator movement patterns we examined if there was a relationship between the number of flowers produced in a population (i.e. tree) and the estimated rate of pollen immigration by graphing estimated pollen flow rates versus the number of inflorescences.

## Results

In 1999, the 15 study populations produced 1884 inflorescences, all of which represent possible pollen donors. Of these, 149 (7.9%) produced 211 capsules located in nine populations. Single inflorescences produced up to four capsules. An estimated 1.4 % of all flowers produced mature capsules. In 2000, these populations produced 2575 inflorescences of which 211 (8.2%) produced 376 capsules from 11 populations. Fruit bearing inflorescences produced up to 11 capsules each. An estimated 1.8 % of all flowers set fruit in 2000. Seedlings from 130 capsules (range of zero to 53 capsules/population; mean = 12) in 1999 and 329 capsules (range of one to 181 capsules/population; mean = 30) in 2000 survived fungal contamination and were used in the paternity analyses.

Genetic differentiation among populations was low ( $G_{ST} = 0.031$  and  $0.052$  in 1999 and 2000 respectively). Despite low  $G_{ST}$  values, a single private allele was found in each of three populations (CB, AG-468, and AG-469) and two were found in a fourth (GT-473) in 1999. In 2000 one private allele was detected in each of two populations (CB and AG-469) and two were observed in a third population (AG-468). Tests for significant heterogeneity in allele frequencies among the 15 *L. rubescens* populations indicate that 90%

and 100% of the polymorphic loci were significant in 1999 and 2000 respectively ( $P < 0.05$ ).

Comprehensive sampling allowed us to determine the multilocus genotype of every flowering individual (i.e. every possible pollen donor) within these 15 populations while strict correlated mating allowed us to identify the diploid genotype of the pollen source for every capsule. Paternity of a capsule by multiple pollen donors, evidenced by a paternal contribution of more than two alleles at one of the four loci with three or more alleles, was not observed in any of the progeny arrays. Five mating types were identified: (1) geitonogamous selfing; (2) within tree outcrossing; (3) gene flow events (i.e. pollen originating outside of the target population) between populations (i.e. trees) within the same pasture, on a scale of tens of meters; (4) gene flow events between pastures, on a scale of hundreds of meters; (5) gene flow originating from unidentified sources. This latter group resulted either from unsampled individuals located within the study site (relatively few individuals; Fig. 1) or from individuals located outside the study area (the majority).

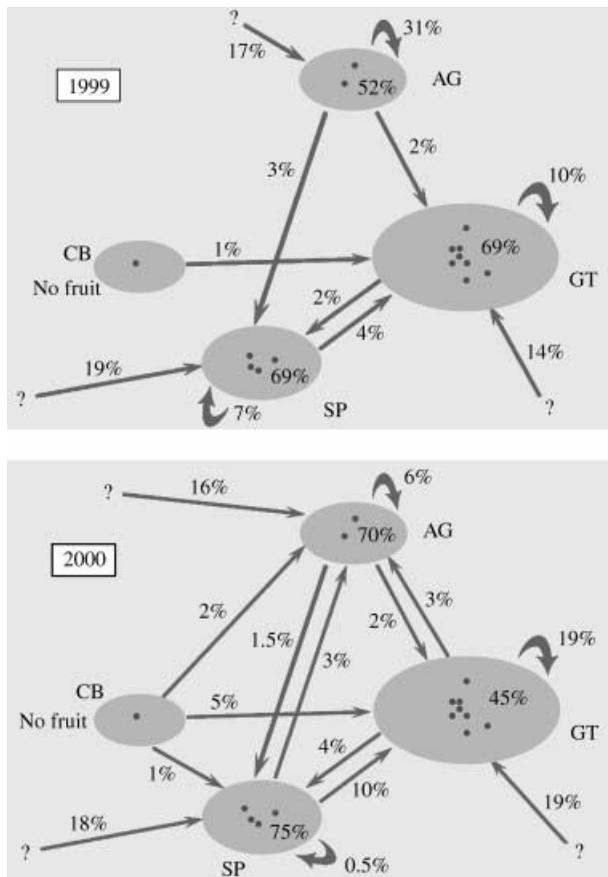
No capsules were produced in the fourth pasture (CB) during either year. Geitonogamy accounted for 10% (6% to 14%) of fertilization events in three pastures in 1999 and 11% (6% to 20%) in 2000 (Table 1). With this level of selfing, the equilibrium inbreeding coefficient was 0.05 which indicates that *L. rubescens* is predominantly outcrossing (Hamrick & Godt 1989). Within population outcrossing was observed for 56% (43%–63%) of the capsules produced in 1999 and 55% (30%–69%) in 2000 (Table 1). Gene flow events explained 34% (30%–48%) of the 1999 fertilizations and 34% (25%–55%) of the 2000 pollinations (Table 1; Fig. 2).

When only gene flow events among populations are examined, 36% (22% to 64%) of pollen originated from different populations within the same pasture in 1999 and 19% (2% to 33%) in 2000 (Table 2). Pollen flow between pastures was responsible for 14% (0%–20%) of the 1999 capsules resulting from gene flow and 28% (26%–32%) of the 2000 gene flow capsules (Table 2). Pollen originated from an unidentified source in 50% (36%–61%) of the 1999 gene flow events and 53% (35%–72%) of the 2000 gene flow events (Table 2). Pollen movement among populations averaged 279 m (64 m to 437 m) in 1999 and 519 m (440 m to 719 m) in 2000 (Table 2).

Closer examination of all types of gene movement in two trees illustrates the observed patterns. In tree 468, located in pasture AG, 70% of the capsules in 2000 were sired by pollen donors within the tree (i.e. selfing and within tree outcrossing), 6% by orchids in another tree within the same pasture, and 8% by orchids in a different pasture (Table 1). Pollen from unidentified sources was responsible for 16% of the capsules (Table 1). In tree 462, located in SP, 75% of pollen in 1999 originated within populations, 2% came from a different population in the same pasture, 6% came

**Table 2** Source of gene flow events for all populations that produced fruit in 1999 and 2000. The two letters in column one designate the pasture. Average distances were calculated for within and among pasture gene flow only. Totals for the two years represent weighted averages based on the number of capsules per tree

Pasture	Number of Gene Flow Fruit	Within Pasture Gene Flow	Among Pasture Gene Flow	Unknown Gene Flow	Average Distance
<i>1999</i>					
SP	18	22%	17%	61%	437 m
AG	11	64%	0%	36%	64 m
GT	15	33%	20%	47%	330 m
Total	44	36%	14%	50%	279 m
<i>2000</i>					
SP	46	2%	26%	72%	719 m
AG	15	20%	27%	53%	522 m
GT	51	33%	32%	35%	440 m
Total	112	19%	28%	53%	519 m



**Fig. 2** Patterns of gene movement in 1999 and 2000. Percentages shown within the shaded ovals represent the mean proportion of within population pollinations in that pasture. Values next to curved arrows represent the proportion of fruit resulting from pollen movement among trees within that pasture. Arrows connecting pastures indicate the proportion of pollen originating from another pasture. Arrows originating with a question mark indicate the percentage of fruit with an unknown pollen parent.

from different pastures, and 17% originated from unknown sources (Table 1). The same population in 2000 had 76% of the pollen from within the tree, 0% from different populations within the pasture, 6% from other pastures, and 18% from unidentified donors (Table 1). These three examples illustrate how similar patterns of pollen flow are in different populations and in the same population in consecutive years.

Genetic structure data from 1999 and 2000 gave estimates of 7.82 and 4.58 migrants per generation, respectively. Slatkin's (1985b) private allele approach provided estimates of 3.68 and 2.60 migrants. When direct measures were employed, and all populations with capsules were considered, there were 1.93 (0.95–4.29 within populations) migrants in 1999 and 2.34 (0–5.47 within populations) in 2000. Mean effective population size for populations with 10 or more capsules was 15.8 in 1999 and 24.3 in 2000 (Trapnell & Hamrick, in prep). Mean effective population size for populations with fewer than 10 capsules was 4.4 in 1999 and 4.6 in 2000. The mean effective population size over these two reproductive periods was 19.5 for populations with  $\geq 10$  fruit and 4.5 for populations with  $< 10$  capsules (Trapnell & Hamrick, in prep).

The number of inflorescences within populations had a strong influence on the percentage of capsules resulting from gene flow (Fig. 3). In smaller populations, a much higher percentage of the pollen originated in another population. In large populations with larger overall floral displays, a lower percentage of fertilizations resulted from gene flow events. When pollinators visit a large population they apparently remain long enough to facilitate multiple within population pollinations.

## Discussion

Inference of the diploid genotype of each pollen parent from full-sib progeny arrays within individual capsules,

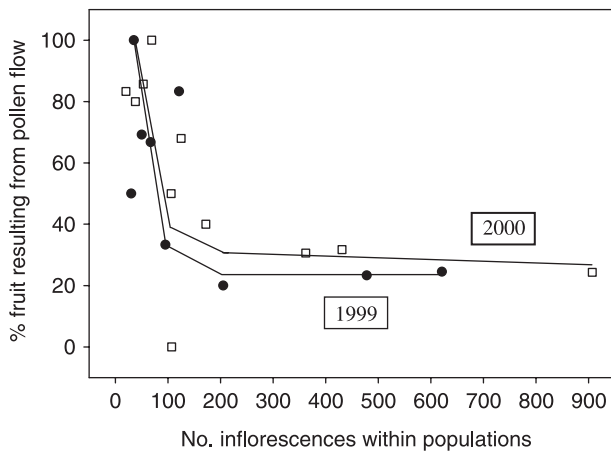


Fig. 3 Pollen flow relative to floral display within populations in 1999 (circles) and 2000 (open squares).

coupled with the comprehensive genotyping of each potential pollen donor within the 15 study populations, has produced one of the most detailed descriptions of pollen movement available for any plant species. Fragmented landscapes are particularly well suited for paternity analyses because all possible pollen donors within a fragment can be identified and pollen originating beyond the fragment has moved across a known minimum distance. Contrary to our expectation that most pollen movement occurs within populations, the results clearly demonstrate that gene flow via pollen is important in *Laelia rubescens* populations and that hummingbird-mediated pollen flow is not hindered by landscape discontinuities (Webb & Bawa 1983). Slightly over one third of the capsules produced in 1999 and 2000 resulted from gene flow events with documented pollen dispersal across hundreds of meters. In forest trees, pollen and seeds are released at greater heights than the propagules of terrestrial herbaceous plants and are thus dispersed over longer distances. Therefore higher rates of gene flow are expected for trees than terrestrial herbaceous plants with similar modes of pollination and seed dispersal (Hamrick & Nason 2000). Herbaceous epiphytes, however, should enjoy some of the dispersal advantages of trees; greater visibility to pollinators and the ability to capitalize on wind for seed dispersal (Dressler 1981). Hamrick & Nason (2000) report that pollen immigration for multiple tree species often exceeded 25% over distances of several hundred meters. In *L. rubescens*, pollen immigration was greater than 20% over several hundred meters. Species with high rates of gene flow should have relatively little genetic structure among geographically separated populations, a result that is consistent with the low  $F_{ST}$  (0.088; Trapnell & Hamrick 2004) values observed among Costa Rican populations of *L. rubescens*. This value is more similar to the mean for long-lived woody species (0.084) than for herbaceous plants

(0.305; Hamrick & Nason 2000). Thus, *L. rubescens* appears to have the increased propagule dispersal advantages of forest trees. *Laelia rubescens* is not an unusual orchid; an epiphytic habit is characteristic of approximately 20 000 orchid species most of which are animal-pollinated with wind-dispersed seeds (Dressler 1981).

Our data demonstrate that pollen flow varies spatially and temporally among populations, among pastures, and between years. Much of the variation among pastures is related to the number of populations per pasture as well as differing population sizes within host trees. Linhart (1973) has shown that pollen dispersal patterns are strongly influenced by the foraging behavior of the hummingbird vectors. A summary of all matings shows that the percentage of capsules resulting from geitonogamy, within population outcrossing, and gene flow were virtually identical between years (Table 1) despite substantial differences in floral display and capsule production. In contrast, sources of immigrant pollen varied between years with 36% and 19% from within pastures, 14% and 28% among pastures and 50% and 53% from unidentified sources. As a result, mean pollen movement distances increased from 1999 to 2000 (279 m vs. 519 m). Thus, while the overall pattern of gene movement was consistent between years, there was clearly a difference in the interpopulation distances that pollen was transported. This suggests that the larger floral displays in 2000 led to more pollinator movement between pastures, further supporting the observation that pollinators fly long distances over disturbed habitats to reach flowering populations (White *et al.* 2002; Hamrick & Apsit 2004).

There are two possible sources of unidentified pollen donors. Within the area circumscribed by the four pastures there are several trees with a few unsampled *L. rubescens* clusters (Fig. 1). However, the quantitatively more likely sources of unknown pollen are moderate to large populations of *L. rubescens* located outside of the study area. Our data indicate that pollinators travel distances beyond 1000 m, as evidenced by pollen movement from pasture CB to GT (Figs 1 and 2). By considering the area within 1000 m of the four pastures, a large number of additional potential pollen donors are within the range of pollinator movement. We estimate that, within the area surrounding each pasture, we sampled 40% to 60% of the potential pollen donors.

There were large differences in floral display and fruit set between the two years. In 1999 the number of inflorescences (1884) and capsules (211) were not inconsequential. However, in 2000 there was a 37% increase in the number of inflorescences (2575) and a 78% increase (376 capsules) in fruit set. Fruit set relative to the total estimated number of flowers produced increased 29% from 1.4% to 1.8%. Sampling two consecutive years that differed substantially in reproductive output allowed clearer insights into the mating patterns of this species.

That floral display is a factor affecting pollen movement was confirmed by comparing the percentage of capsules resulting from gene flow to the number of inflorescences within populations (Fig. 3). In smaller populations, a much higher percentage (40%–100%) of the pollen originated from another population. Within large populations, a lower percentage of fertilizations (c. 25%) resulted from gene flow. Apparently, once pollinators arrive at a large population they remain long enough to facilitate multiple within population pollinations. Our results are consistent with theoretical predictions (Pyke 1984; Ellstrand & Elam 1993; Cresswell & Osborne 2004) and empirical data from other species (milkweed species — Broyles *et al.* 1994; the tropical tree *Swietenia humilis* — White *et al.* 2002). Although our data indicate greater long-distance visitation to trees with increased floral display in 2000, the overall rate of gene flow was identical in the two years (34%). While trees with fewer flowers have a higher proportion of immigrant pollen, populations with more inflorescences have a higher absolute number of gene flow events. An explanation for this seeming paradox is the probability that when hummingbirds visit larger populations there are multiple inflorescences flowering simultaneously allowing more intrapopulation pollination. In trees with fewer inflorescences there was less overlap in flowering so a higher proportion of fruit result from inter-population pollinations. *Laelia rubescens* populations within relatively undisturbed forests in Guanacaste are smaller and produce fewer inflorescences than populations in disturbed habitats. As a result, there might be higher rates of gene flow between populations in undisturbed habitats. Our expectation is supported by Linhart's (1973) observations that hummingbird-pollinated plants growing in dense stands along forest edges experienced more restricted pollen movement than small, widely scattered populations, resulting from the trap-lining behavior of their pollinators. Inbreeding was also more likely where there is a higher density of inflorescences (Linhart 1973). A detailed examination of mating patterns within populations from undisturbed landscapes should clarify this issue.

Direct measures of pollen immigration ( $N_{em} = 1.93$  and  $2.34$ ) are less than the indirect estimates obtained from  $G_{ST}$  ( $Nm = 7.82$  and  $4.58$ ) and Slatkin's (1985b) private alleles approach ( $Nm = 3.68$  and  $2.60$ ). However, the direct measure only considers realized pollen-mediated gene flow while gene movement via both pollen and seeds is included in the indirect estimates. The tiny, wind-dispersed seeds of *L. rubescens* are potentially capable of long-distance dispersal. In a separate study (Trapnell & Hamrick 2004), we found that pollen-mediated nuclear gene migration is 1.40 times greater than seed-mediated migration among locations in Costa Rica but that this ratio did not differ significantly from 1.00 ( $m_p/m_s$ ; Ennos 1994). This is one of the lowest ratios found in any plant examined and

suggests that seed dispersal is an equally important component of gene movement in this species. If migration via seeds were included in these direct measures,  $N_{em}$  values would be more consistent with the indirect measures. This concordance suggests that contemporary gene flow rates are a reasonable estimate of historical rates of gene flow in these populations.

Previous investigations of gene flow in tropical plants have focused on trees with animal-mediated pollen movement (e.g. Hamrick & Murawski 1990; Boshier *et al.* 1995; Chase *et al.* 1996; Nason *et al.* 1996; Dawson *et al.* 1997; Nason & Hamrick 1997; Loveless *et al.* 1998; Apsit *et al.* 2001; White *et al.* 2002). In most cases, pollen flow rates were > 25% and documented immigrant pollen movement exceeded a few hundred meters. Only a few tropical trees have pollen immigration distances > 1000 m. Three species of *Ficus* (pollinated by species-specific wasps) had pollen flow > 1000 m with suggested dispersal distances of 6–14 km (Nason & Hamrick 1997). In *Swietenia humilis* (pollinated by small butterflies, bees and other insects) direct pollen flow measures documented dispersal distances > 4.5 km (White *et al.* 2002). The wind-pollinated pioneer tree, *Cecropia obtusifolia*, may have experienced pollen immigration levels that exceeded 10 km (Kaufman *et al.* 1998). Our data extend our understanding of gene flow in the tropics to a herbaceous epiphyte. Collectively, these studies show that pollen moves over greater distances and at higher rates than expected in fragmented populations and may be higher than in natural populations. Some of these investigations, including ours, suggest that fragments with fewer individuals experience higher rates of gene flow (Broyles *et al.* 1994; Nason & Hamrick 1997; White *et al.* 2002). In general populations of plant taxa examined to date have had levels of pollen flow that are sufficient to prevent or reduce inbreeding and genetic drift. This suggests that small fragments have conservation value as stepping stones that contribute to maintaining the genetic connectivity of populations (Nason & Hamrick 1997; White *et al.* 2002). The primary determinant of pollen flow between isolated fragments is pollinator behavior and, as White *et al.* (2002) point out, all pollinators have a distance threshold beyond which they cannot travel. Thus the degree to which landscape fragmentation affects different plant species will be highly variable (e.g. Aldrich & Hamrick 1998). It is essential to these and other species, which may not have the means for such high levels of gene flow, that habitat fragments are preserved and not permitted to become further isolated to the point that gene flow among populations is restricted.

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This research is a part of Dorset Trapnell's doctoral dissertation. She uses molecular markers to understand evolutionary factors that shape patterns of genetic variation in natural populations and the consequences of habitat disturbance. This work reflects her deep interest in tropical epiphytes and particularly the Orchidaceae. Her dissertation advisor is Jim Hamrick whose focus is on the genetics and 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; natural selection, the mating system and pollen and seed dispersal patterns.

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