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Euglossine bees mediate only limited long-distance gene flow in a tropical vine

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Summary

1. Euglossine bees (Apidae: Euglossini) have long been hypothesized to act as long-distance pollinators of many low-density tropical plants. We tested this hypothesis by analyzing gene flow and genetic structure within and among populations of the euglossine bee-pollinated vine *Dalechampia scandens*.
2. Using microsatellite markers, we assessed historical gene flow by quantifying regional-scale genetic structure and isolation by distance among 18 populations, and contemporary gene flow by estimating recent migration rates among populations. To assess bee-mediated pollen dispersal on a smaller scale, we conducted paternity analyses within a focal population, and quantified within-population spatial genetic structure in four populations.
3. Gene flow was limited to certain nearby populations within continuous forest blocks, while drift appeared to dominate on larger scales. Limited long-distance gene flow was supported by within-population patterns; gene flow was biased towards nearby plants, and significant small-scale spatial genetic structure was detected within populations.
4. These findings suggest that, although female euglossine bees might be effective at moving pollen within populations, and perhaps within forest blocks, their contribution to gene flow on the regional scale seems too limited to counteract genetic drift in patchily distributed tropical plants. Among-population gene flow might have been reduced following habitat fragmentation.

Introduction

In light of ongoing pollinator declines (Vamosi *et al.*, 2006; Potts *et al.*, 2010; Gonzalez-Varo *et al.*, 2013), it is of great importance to understand the contribution of different kinds of pollinators to gene flow and genetic structuring of plant populations. Animal pollination is particularly common in the tropics (Ollerton *et al.*, 2011), where the great diversity of flowering plants exhibits an equally impressive diversity of pollination systems (Bawa, 1990; Armbruster, 2006). Following early suggestions that patches of low-density tropical plants would generally be genetically isolated, highly inbred, and prone to divergence through drift (Baker, 1959; Fedorov, 1966), diverse tropical forests have inspired much research into patterns of gene flow and genetic structure. Although long-distance gene flow appears common in many canopy trees (Bawa, 1992; Dick *et al.*, 2008), recent work suggests different patterns for understorey species (Lasso *et al.*, 2011; Surget-Groba & Kay, 2013; Twyford *et al.*, 2014), and little is known about the population genetics of woody vines or lianas (but see Foster & Sork, 1997; Gianoli *et al.*, 2016).

Euglossine bees (Apidae: Euglossini) are important pollinators of a large number of neotropical plants (Dressler, 1968; Janzen, 1971; Williams & Dodson, 1972; Armbruster & Webster, 1979), and several observations suggest that distinct patterns of genetic structure might be expected in species pollinated by these bees. First, euglossine bees are generally assumed to exhibit traplining, the habit of repeatedly visiting a regular sequence of flowers or patches (Janzen, 1971; Ackerman *et al.*, 1982; Dressler, 1982; Thomson *et al.*, 1997). Theoretical and empirical work has suggested that trapline foraging increases pollination distances relative to a pollinator randomly searching for resources (Ohashi & Thomson, 2009). Second, euglossine bees are thought to have large foraging ranges, and therefore contribute to long-distance gene flow among geographically isolated populations typical of many neotropical plants (Janzen, 1971). Janzen released *Eufriesea surinamensis* females at up to 23 km from their nest, and found that they would often return to their nest within hours (e.g. from 20 km in 65 minutes), suggesting that these bees have large foraging ranges. Given some pollen carry-over between flowers during extended foraging trips (Cresswell, 2006), rare long-distance pollination events might thus be expected. Since Janzen's pioneering experiments, the extraordinary movement ability of euglossine bees has been confirmed by radio telemetry (Wikelski *et al.*, 2010) and mark-recapture studies of male euglossines (e.g. Murren, 2002; Pokorny *et al.*, 2015). While these studies have shown that male euglossines can indeed travel across long distances in a short period of time, the movement patterns of

78 foraging female bees, which are bound to their nest, remain largely unknown (López-Uribe *et*
79 *al.*, 2008). Despite their suggested importance as 'long-distance pollinators', there are to our
80 knowledge no studies assessing the impact that euglossine bees have on the population-
81 genetic structure of the plant species they pollinate.

82 Most neotropical species of *Dalechampia* vines (Euphorbiaceae) are pollinated by
83 female euglossine bees (Armbruster, 1988; Armbruster, 1993a), which collect floral resins for
84 use in nest construction (Fig. 1; Armbruster, 1984). Species of *Dalechampia* therefore provide
85 excellent opportunities for better understanding the impact of euglossine pollination on gene
86 flow and genetic structuring of plant populations. Seed dispersal in *Dalechampia* occurs
87 through explosive dehiscence of capsules, whereby seeds are thrown only one to a few metres
88 from the maternal plant (Armbruster, 1982). Although some seed dispersal might occur by
89 water or wind, for example, contemporary gene flow at distances of more than a few metres
90 reflects to a large extent pollen transport by bees. Populations are often small and patchy,
91 suggesting that there might be a high degree of inbreeding and strong genetic drift. It is not
92 known, however, to what extent bees contribute to gene flow among populations, hence
93 counteracting the effect of drift. The widespread species *D. scandens* exhibits variation
94 among regions in mating system, and in pollinator species, paralleling the variation observed
95 in the genus as a whole (Armbruster 1985; 1993a). In north-western Costa Rica the primary
96 pollinators are female euglossine bees, providing a tractable study system for understanding
97 euglossine-mediated gene flow.

98 The hypothesis that female euglossine bees have large foraging ranges and act as long-
99 distance pollinators yields several testable predictions about the population-genetic structure
100 of euglossine bee-pollinated plants. First, contemporary gene flow should be detectable
101 among plant populations within the foraging ranges of individual bees (i.e. anything from a
102 few to, say, 20 kilometres for a female euglossine bee). Second, if gene flow is common
103 among neighbouring populations, this should produce a pattern of isolation by distance on the
104 regional scale (Hutchison & Templeton, 1999). To test these two predictions, we genotyped
105 individuals sampled from 18 *D. scandens* populations at 35 microsatellite loci. We assessed
106 historical gene flow by quantifying region-scale genetic structure and isolation by distance,
107 and contemporary gene flow by estimating recent migration rates among populations. To
108 complement these regional-scale analyses, we assessed patterns of gene flow at a smaller
109 scale by conducting paternity analyses within a focal population, and quantifying spatial
110 genetic structure within four populations.

Materials and Methods

Study species

Dalechampia scandens L. (Euphorbiaceae) is a species complex of twining vines occurring in disturbed habitats throughout most of the lowland Neotropics, from Mexico to Argentina (Armbruster, 1985). Unisexual staminate and pistillate flowers are aggregated into functionally bisexual blossom inflorescences, which are visited by resin-collecting female bees (Fig. 1). In our study area in north-western Costa Rica, the most abundant pollinators are female euglossine bees, including *Euglossa dilemma* (c. 11 mm; Fig. 1) and *Eufriesea* cf. *surinamensis* (17-19 mm). Some populations are pollinated also by female *Hypanthidium* (Megachilidae), but because of their smaller size (c. 6-7 mm) and lower abundance, we assumed that they contribute to gene flow within populations, but their contribution to gene flow among populations is negligible. In our focal population (S8), approximately 80% of observed pollinator visits were by female euglossine bees, and the remaining 20% were by *Hypanthidium*. No other pollinators have been observed on *D. scandens* in Costa Rica (Armbruster, 1988; Opedal *et al.*, 2016), although *Eulaema* sp. could be potential visitors. These observations lead us to suggest that any long-distance gene flow in this system would be mediated by female euglossine bees, although we cannot completely exclude the possibility that smaller bees are involved.

Dalechampia blossoms exhibit functional protogyny, with a female phase (stigmas receptive, anthers not dehisced) followed by a bisexual phase. Outcross pollination is most likely to occur during the female phase, while facilitated selfing appears to be common during pollinator visits to blossoms in the bisexual phase. Selfing rates estimated from progeny arrays in four of the study populations ranged from 0.51 to 0.84 (mean = 0.69), indicating a mixed mating system with a tendency towards selfing (Opedal *et al.*, 2016).

Population sampling and materials collection

Dalechampia scandens grows in patches of one or sometimes several intertwined individuals, and we treated patches as the unit of study to avoid repeated sampling of the same genotype (one leaf from each patch was included in the analyses). We collected leaf material from 4 – 23 patches (median = 13) in 18 populations in north-western Costa Rica (Fig. 2, Table 1), belonging to the 'large-glanded' variety of *D. scandens*. These populations represent most of the localities known from herbarium collections in this region, and we have searched

intensively for additional populations. Still, there are surely un-sampled populations which might act as stepping-stones for (multi-generational) gene flow among the study populations. Most populations occurred in scrublands or forest edges along gravel roads. Populations were generally small, ranging from less than five to approximately 100 flowering patches (Table 1). We collected leaves from all patches we could locate in small populations, and from ca. 15-20 patches in larger populations. The position of each patch was recorded with a handheld GPS receiver. If two clearly separate patches had the same coordinates, we estimated the between-patch distance in the field and manually adjusted the coordinates. Leaves were stored individually in paper envelopes and dried in silica gel. Between-population distances ranged from 1.8 km to 157 km, and the median distance between neighbouring populations was 8.4 km (mean = 11.7 km, range = 1.8 km – 36.5 km).

Microsatellite genotyping

DNA extraction and PCR amplification followed the procedures described in Falahati-Anbaran *et al.* (2013). Fragment sizes were determined with an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, US), and microsatellite alleles scored using GeneMapper 4.0 (Applied Biosystems). Thirty-five microsatellite markers for *D. scandens* (Falahati-Anbaran *et al.*, 2013; Falahati-Anbaran *et al.*, 2016) were initially screened in 258 field-collected leaf samples. The relatively large number of markers was chosen to compensate, to some extent, for the limited sample sizes in small populations. One plate (96 samples) was genotyped twice to assess genotyping and allele-scoring error. For the purpose of paternity analyses, offspring grown from seeds collected in the S8 population (144 offspring from 32 blossoms from 17 patches) were genotyped for a subset of 16 markers that were polymorphic in the population.

Analyses

Within-population summary statistics (allelic richness, effective number of alleles, expected and observed heterozygosities, inbreeding coefficients) were computed with GenAlEx 6.5 (Peakall & Smouse, 2006; Peakall & Smouse, 2012). Linkage disequilibrium (LD) among loci was estimated in Arlequin 3.5 (Excoffier & Lischer, 2010), and its significance tested by permutation tests with 10000 permutations. The proportion of loci in significant LD (P_D) was calculated based on the method of Stenøien and Såstad (1999).

Among-population differentiation and isolation by distance

The distribution of genetic variation in the study area was assessed by global and pairwise G'_{ST} values, computed with GenAlEx 6.5. G'_{ST} takes values between 0 and 1, and is a standardized measure of genetic differentiation that corrects for the high allelic diversity commonly observed at microsatellite loci (Hedrick, 2005). To facilitate comparison with previous studies, we also computed conventional G_{ST} values. To assess the relationship between geographic distance and genetic differentiation (isolation by distance), we regressed pairwise $G'_{ST}/(1- G'_{ST})$ ratios on the natural logarithm of geographic distances between populations (Rousset, 1997). A neighbour-joining tree based on Nei's genetic distance was constructed with Populations 1.2.31.

The genetic structure of the study populations was further assessed using the Bayesian clustering algorithm implemented in STRUCTURE 2.3.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). We initially ran 5 replicate MCMC runs for K (number of genetic clusters) ranging from 1 to 15 under the admixture model with allele frequencies assumed to be correlated across populations (assuming independent allele frequencies yielded similar results), and no prior information about sampling localities. Each chain ran for 100 000 iterations following 50 000 iterations as burnin. The initial analysis indicated that the number of genetic clusters was approximately 10, and we subsequently ran 10 replicate runs for K ranging from 6 to 15. Independent runs were aligned and summarized using the online CLUMPAK software (Kopelman *et al.*, 2015). The most likely number of genetic clusters was inferred by inspection of the posterior probability of the data under each value of K.

Inference of recent migration events

Recent migration events between populations were assessed with BayesAss 3.0 (Wilson & Rannala, 2003). The program uses Bayesian MCMC computation to estimate the posterior probability of each individual being a recent immigrant into the population where it was sampled, and its most likely geographical origin, provided that establishment in the present population occurred within the last few generations. The model ran for 10 million iterations with a burnin phase of 1 million iterations and a thinning interval of 9000 iterations, yielding 1000 posterior estimates. Convergence of MCMC runs were confirmed by investigation of posterior trace plots, and by running several replicate chains.

Paternity analyses

To investigate pollen flow between patches within populations, paternity analyses were conducted in the S8 population using the maximum-likelihood approach implemented in CERVUS 3.07 (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007). For the initial power simulation (establishment of critical Δ values), we set the proportion of candidate fathers sampled to 0.75 and the selfing rate to 0.51. The proportion of candidate fathers sampled was a crude guess based on field observations, while the selfing rate was set equal to the estimated selfing rate for the population (Opedal *et al.*, 2016). Strict and relaxed confidence levels were set to 95% and 80%, respectively. In the paternity analyses, we included the maternal genotypes and tested for selfing. In four cases where maternal and offspring genotypes mismatched, we took the conservative approach of treating the mother as unknown (these mismatches were most likely due to sampling fruits from different mothers within a patch).

Within-population genetic structure

Within-population spatial genetic structure (i.e. the decrease in relatedness between plants with increasing geographic distance) was assessed in four populations (S1, S2, S8 and S21) where genotype data were available for approximately 20 patches, which we considered a minimum sample size for this analysis. Pairwise kinship coefficients were computed in SPAGEDI 1.5 (Hardy & Vekemans, 2002), using the estimator of Ritland (1996), which has been shown to perform well for microsatellite data (Vekemans & Hardy, 2004). To quantify spatial genetic structure, we split the pairwise distances between patches into 10 distance classes, each comprising approximately 20 patch pairs, and regressed average pairwise kinship coefficients for each distance class on the natural logarithm of geographic distance. We used the regression slope, b_{log}, to compute the *Sp* statistic of Vekemans & Hardy (2004) as: $-b_{log}\,[1-\hat{F}_{(1)}]^{-1}$, where $\hat{F}_{(1)}$ is the average kinship coefficient between individuals in the first distance class (i.e. 'neighbouring' plants). The value of the *Sp* statistic increases with stronger spatial genetic structure, and because it is relatively insensitive to variation in sampling scale, it is useful for quantitative comparison of spatial genetic structure among populations and species (Vekemans & Hardy, 2004). The paternity analyses indicated that at least three out of 17 patches (16.7%) comprised more than one genetic individual. Because we sampled only one leaf per patch, the estimated strength of spatial genetic structure might be underestimated if patches often comprise several closely related individuals, and should thus be regarded as conservative.

Results

Microsatellite diversity

Thirty microsatellite markers were polymorphic across populations, with a total of 233 alleles (mean = 7.8 alleles per locus, range = 2 – 30). The estimated average genotyping and allele-scoring error rate per locus was 0.48%. Genetic diversity within populations was relatively low (mean H_E = 0.28, range = 0.11 – 0.43, Table 1), and the level of inbreeding was moderate to high (mean F_{IS} = 0.26, range = -0.20 – 0.58, Table 1).

Among-population genetic structure

More than half of the genetic variation in the study region occurred among populations [Global G'_{ST} (95% CI) = 0.56 (0.49, 0.64), G_{ST} = 0.40 (0.37, 0.44)]. Pairwise G'_{ST} values ranged from 0.025 to 0.758 (Supporting Information, Table S1). When we regressed $G'_{ST}/(1-G'_{ST})$ ratios on the logarithm of geographic distance, we found a weak pattern of isolation by distance (Mantel R = 0.175, P = 0.061, Fig. 3). The level of genetic differentiation tended to increase with increasing geographic distance up to approximately 20 km, after which there was no obvious relationship (type IV pattern, *sensu* Hutchison & Templeton, 1999).

The STRUCTURE analysis suggested that the samples belonged to at least eleven genetic clusters (the posterior probability of the data increased with increasing K, reaching an asymptote at K = 11 clusters). The assignment of populations to clusters remained similar for values of K near 11. Populations assigned to the same genetic cluster were generally close geographically (Fig. 2), suggesting localized gene flow. The distance between populations assigned to the same cluster ranged from 1.8 to 13.0 km (median = 6.97 km, the cluster comprising the S6 and S7 populations was excluded because it was unstable across STRUCTURE runs).

Recent migration rates

The strong genetic isolation of most populations was confirmed by the fact that most recent-migration-rate estimates were very low (Fig. 4), and all non-zero migration rates detected were unidirectional. The two highest estimated migration rates were detected from population S8 to S9 (separated by 5.5 km), and from S20 to S19 (separated by 3.3 km). The genetic connectivity of these two population pairs was further supported by the two lowest pairwise G'_{ST} values observed between any two populations (0.103 and 0.025, respectively, TableS1), and the populations being assigned to the same genetic clusters (Fig. 2). The estimated migration rate exceeded zero for four additional population pairs, all of which suggested gene

flow from larger source populations into small populations that apparently contained fewer than 10 individuals at the time of sampling (populations S4, S26, S12 and S22). These four estimates should be treated with caution, however, due to small sample size in the recipient populations, and because the 95% credible intervals overlapped with the 95% CIs of migration rates between very distant populations (Fig. 4), which we regard as noise (Wilson & Rannala, 2003).

Paternity analyses

The combined exclusion probability for the second parent (the probability of excluding an incorrect candidate father from paternity) was 98.3%. A single most likely father was assigned to 92 of the 144 offspring genotyped in the S8 population. The remaining offspring had either several likely candidate fathers (7 offspring), one most likely, but uncertain father (8 offspring), or no likely candidate father (37 offspring). The latter offspring were most likely sired by either an unsampled father within the population, or by a father from outside the population. Given that the migration-rate analysis suggested some gene-flow into the S8 population, we suspect that some of these pollination events indeed represent medium- to long-distance gene flow into the population. Among the offspring with paternity assigned, 76 were most likely the result of autogamous or geitonogamous selfing. After removing cases of uncertain paternity, and non-independent replicates (same father assigned to several offspring within a family), we ended up with 15 between-patch pollination events where paternity was assigned within the 80% certainty threshold. For these events, pollination distances (the distance between the maternal and the most likely paternal plant) ranged from 6.9 m to 156.8 m (mean = 50.6 m, median = 20.9 m, SD = 56.8 m, Fig. 5).

Within-population spatial genetic structure

Average kinship coefficients decreased significantly with increasing between-patch distance in all four populations considered, with the strength of spatial genetic structure (*Sp* statistic) ranging from 0.024 to 0.084 (Table 2).

Discussion

If female euglossine bees are effective long-distance pollinators, this should be detectable in the population-genetic structure of the plants they pollinate. Across north-western Costa Rica, we found that gene flow appears to occur between certain nearby populations of the

euglossine-bee pollinated vine *Dalechampia scandens*. However, we also found that the geographic distance between populations explained a very limited proportion of the genetic differentiation at the regional scale. Furthermore, pollen movement within a focal population was strongly biased towards nearby plants, resulting in small-scale spatial genetic structure. Overall, these findings are consistent with recent studies reporting locally restricted gene flow in tropical understorey plants (Lasso *et al.*, 2011; Surget-Groba & Kay, 2013; Theim *et al.*, 2014; Twyford *et al.*, 2014), and seem only partly consistent with the hypothesis that female euglossine bees are effective long-distance pollinators (Janzen, 1971). Nevertheless, there were a few exceptions to the overall pattern, suggesting that medium- to long-distance gene flow occurs between certain populations within continuous blocks of forest.

Mating system appears to be the principle determinant of population-genetic structure in plants (Hamrick & Godt, 1996; Nybom, 2004; Duminil *et al.*, 2007; Duminil *et al.*, 2009), but whether this relationship is caused primarily by reduced gene flow due to reduced cross-pollination or increased genetic drift resulting from reduced effective population sizes in selfing populations remains an open question (Duminil *et al.*, 2009). The overall level of genetic differentiation among *D. scandens* populations was high (global $G'_{ST} = 0.56$, $G_{ST} = 0.40$), and closer to the average reported for predominantly selfing species (0.42) than for mixed-mating species (0.26; Nybom, 2004). Because *D. scandens* populations are generally small, often comprising fewer than 50 flowering individuals, it seems likely that genetic drift and founder effects are important factors driving the divergence of these populations (e.g. Ellstrand & Elam, 1993). The relationship between geographic distance and genetic differentiation among populations (Fig. 3) resembles a 'Type IV' isolation-by-distance pattern (Hutchison & Templeton, 1999), that is a weak increase in genetic differentiation with increasing geographic distance among nearby populations (up to approximately 20 km), and thereafter a wide scatter of points with no further relationship between geographic distance and genetic differentiation. This pattern suggests that most historical gene flow has occurred between nearby populations, while drift dominates at larger geographic scales.

The results of the recent-migration-rate analysis were consistent with the localized gene flow inferred from pairwise G'_{ST} values and their relationship with geographic distance. Nevertheless, we did detect a few cases of apparent genetic connectivity between nearby populations, both of which involved population pairs within continuous blocks of forest that have had only limited disturbance, and that contain relatively dense populations of euglossine bees (Ø. H. Opedal & E. Albertsen, unpublished data). Gene flow between the two

populations within Palo Verde National Park (S8 and S9) is probably facilitated by the presence of scattered patches of plants along the gravel road connecting the two populations. In contrast, no signal of gene flow was detected between these populations and three populations located on roadsides within agricultural areas just outside Palo Verde (S1, S2, and S7), which were also assigned to different genetic clusters (Fig. 2). Although patches of plants occur also between these populations, it seems that bees travel more rarely to patches outside the continuous forest block in Palo Verde. Similarly, genetic connectivity appeared to be high between the S19 and S20 populations, two small populations separated by 3.3 km and almost 500 m in altitude within Diriá National Park. Thus, it appears that the critical distance limiting contemporary gene flow in this system is in the range of 3 to 10 km, and more fine-scale sampling of populations within this range would be desirable to obtain a more accurate estimate. These results further suggest that gene flow depends on habitat characteristics. Habitat fragmentation is characteristic of the Guanacaste region of Costa Rica, and although gene flow appears to occur among forest fragments on a small scale (Trapnell & Hamrick, 2005), our results suggest that fragmentation might have produced barriers to gene flow on the regional scale. We suspect this is due to euglossine bees largely avoiding disturbed agricultural areas (Brosi *et al.*, 2007; Ø. H. Opedal & E. Albertsen, unpublished data).

Most studies into the spatial ecology of euglossine bees have focused on males because they are readily attracted to chemical baits, and both mark-recapture (e.g. Murren, 2002; Pokorny *et al.*, 2015) and population-genetic studies (e.g. Dick *et al.*, 2004; López-Uribe *et al.*, 2014; Suni *et al.*, 2014) are therefore easy to implement. Although some observations suggest that both male and female bees establish traplines and remain in restricted areas for extended periods (Ackerman *et al.*, 1982; Armbruster, 1993b; López-Uribe *et al.*, 2008), it is generally thought that male and female euglossines differ in the size of their home range (Janzen, 1981). While provisioning brood cells, females are bound to their nest and make foraging trips from a central location. Although some of these foraging trips might be long, as suggested by Janzen (1971), it seems likely that most foraging is done locally if resources are readily available near the nest (e.g. within a single plant population, or a group of populations within a forest block). We cannot completely rule out the possibility, however, that female euglossine bees travel regularly among populations without effecting gene flow. Separating between these possibilities would require direct studies of bee movement, or comparative population-genetic studies of plants and bees.

Within the S8 population, we detected a limited number of between-patch pollination events. This was partly because about half the offspring produced in the population were most likely selfed, automatically reducing the opportunity for pollen flow both within and into the population. Still, euglossine or megachilid bees occasionally moved pollen over distances large enough to maintain genetic connectivity within the population. Indeed, the within-population estimate should be treated as a conservative estimate of average between-patch pollination distances, because the among-population analyses suggested that rare long-distance pollination events occur in this system. Pollination events between plants separated by hundreds of metres or even kilometres appear also to occur in other species with strong-flying pollinators. For example, bumblebees and hummingbirds readily moved pollen between source populations and experimental populations of *Delphinium nuttallianum* up to 400 m apart (Schulke & Waser, 2001), bumblebees and hawkmoths moved pollen between patches of *Aquilegia coerulea* up to 150 m apart (Brunet & Holmquist, 2009), and hummingbirds moved pollen among fragmented populations (host trees) of the epiphytic orchid *Laelia rubescens* (Trapnell & Hamrick, 2005). Because *D. scandens* is a small liana (woody vine), pollen dispersal distances are not directly comparable to either herbaceous species or large trees. Average between-patch pollination distances in *D. scandens* appear relatively large compared to most herbaceous species studied (4.4 m to 26.3 m; studies compiled by Whitehead *et al.*, 2015), but are perhaps shorter than for animal-pollinated trees, for which most estimates are in the range of hundreds of metres, and sometimes several kilometres (Dick *et al.*, 2008). This pattern could be explained by the low density of typical *D. scandens* populations, because pollinator flight distances tend to increase at lower plant densities (e.g. Levin & Kerster, 1969; Fenster, 1991), and perhaps by a trapline-foraging strategy of female euglossines. The bimodal distribution of between-patch pollination distances (Fig. 5) suggests that pollinator movements within populations might be non-random, and we can speculate that they differ between pollinator species (small *Hypanthidium* vs. larger euglossines).

Most pollination events, however, occurred between nearby plants, or within patches (including geitonogamous selfing). This pattern was supported by the observation of significant spatial genetic structure in all four populations considered. The average strength of spatial genetic structure (Sp = 0.044, range = 0.024 – 0.084) falls well within the range reported in other plant species (Vekemans & Hardy, 2004), and corresponds reasonably well with the average values reported for mixed-mating species (0.037), and for animal-pollinated

species (0.017). Very few estimates of genetic structure are available for woody vines, but the average estimate for *D. scandens* falls closer to the average for herbaceous plants (0.046) than for small and large trees (0.026 and 0.010, respectively; Vekemans & Hardy, 2004). Taken together, the within-population patterns are consistent with the limited evidence of long-distance gene flow, and suggest that the high selfing rates of most *D. scandens* populations limit the opportunity for pollen migration between populations.

Conclusions and future perspectives

Taking a multilevel approach, we have shown that although euglossine and megachilid bees were effective at moving pollen within a focal population of *D. scandens,* gene flow among populations appeared to be limited to certain nearby populations. Although foraging trips of female euglossine bees might occasionally involve several neighbouring *D. scandens* populations, most of the populations we studied were genetically highly differentiated, suggesting very limited historical gene flow. This pattern may result from the predominance of drift or limited opportunity for cross-pollination in these small populations experiencing moderate to high levels of selfing. Indeed, if rare gene-flow events occur between populations separated by tens of kilometres, either directly or through stepping-stone populations, the genetic effects of these events may be swamped by drift and therefore not easily detectable. To the best of our knowledge, this is the first large-scale investigation of population-genetic structure in a species pollinated primarily by female euglossine bees. To further understand the role of euglossine bees as long-distance pollinators, data from additional plant species are clearly needed. Little is known, for example, about how resin foraging compares to nectar foraging by female euglossines. Of particular interest are data from species pollinated by male euglossine bees, as is well known in orchids. Because males are not bound to a nest, they might indeed travel more often among plant populations. For example, male *Eulaema cingulata* appeared to move from the mainland to pollinate island populations of *Catasetum* orchids (Murren, 2002). Pollination by fragrance-collecting male euglossine bees has evolved independently several times in *Dalechampia* (Armbruster & Webster, 1979; Armbruster *et al.*, 1989; Armbruster *et al.*, 1992; Armbruster, 1993a), and a test of male vs. female euglossine bees as agents of gene flow could be conducted by comparing the population-genetic structure of sister species pollinated by male and female bees.

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Author Contributions

ØHO and CP initiated the study. ØHO, EA, WSA and RPB conducted field work. ØHO and MFA conducted laboratory work. ØHO analysed the data with contributions from MFA and HKS. ØHO wrote the manuscript with contributions from all authors.

Data Accessibility

Data available from the Dryad Digital Repository: doi:10.5061/dryad.34b84

References

Ackerman JD, Mesler MR, Lu KL, Montalvo AM. 1982. Food-foraging behavior of male Euglossini (Hymenoptera, Apidae) - Vagabonds or trapliners. *Biotropica*, 14: 241-248.

Armbruster WS. 1982. Seed production and dispersal in *Dalechampia* (Euphorbiaceae) - divergent patterns and ecological consequences. *American Journal of Botany*, 69: 1429-1440.

Armbruster WS. 1984. The role of resin in angiosperm pollination: ecological and chemical considerations. *American Journal of Botany*, 71: 1149-1160.

Armbruster WS. 1985. Patterns of character divergence and the evolution of reproductive ecotypes of *Dalechampia scandens* (Euphorbiaceae). *Evolution*, 39: 733-752.

Armbruster WS. 1988. Multilevel comparative analysis of the morphology, function, and evolution of *Dalechampia* blossoms. *Ecology*, 69: 1746-1761.

Armbruster WS. 1993a. Evolution of plant pollination systems - hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution*, 47: 1480-1505.

Armbruster WS. 1993b. Within-habitat heterogeneity in baiting samples of male euglossine bees - possible causes and implications. *Biotropica*, 25: 122-128.

Armbruster WS 2006. Evolutionary and ecological perspectives on specialization: from the arctic to the tropics. In: Waser NM, Ollerton J eds. *Plant-pollinator interactions: from specialization to generalization*. Chicago: University of Chicago Press, 260-282.

Armbruster WS, Herzig AL, Clausen TP. 1992. Pollination of 2 sympatric species of *Dalechampia* (Euphorbiaceae) in Suriname by male euglossine bees. *American Journal of Botany*, 79: 1374-1381.

Armbruster WS, Keller S, Matsuki M, Clausen TP. 1989. Pollination of *Dalechampia magnoliifolia* (Euphorbiaceae) by male euglossine bees. *American Journal of Botany*, 76: 1279-1285.

Armbruster WS, Webster GL. 1979. Pollination of 2 species of *Dalechampia* (Euphorbiaceae) in Mexico by euglossine bees. *Biotropica*, 11: 278-283.

Baker HG. 1959. Reproductive methods as factors in speciation in flowering plants. *Cold Spring Harbor Symposia on Quantitative Biology*, 24: 177-191.

Bawa KS. 1990. Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics*, 21: 399-422.

Bawa KS. 1992. Mating systems, genetic differentiation and speciation in tropical rain forest plants. *Biotropica*, 24: 250-255.

Brosi BJ, Daily GC, Shih TM, Oviedo F, Durán G. 2007. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology*, 45: 773-783.

Brunet J, Holmquist KGA. 2009. The influence of distinct pollinators on female and male reproductive success in the Rocky Mountain columbine. *Molecular Ecology*, 18: 3745-3758.

Cresswell JE 2006. Models of pollinator-mediated gene dispersal in plants. In: Harder LD, Barrett SC eds. *Ecology and evolution of flowers*. Oxford, UK: Oxford University Press, 83-101.

Dick CW, Hardy OJ, Jones FA, Petit RJ. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology*, 1: 20-33.

Dick CW, Roubik DW, Gruber KF, Bermingham E. 2004. Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae: Euglossini) revealed by comparative mitochondrial DNA phylogeography. *Molecular Ecology*, 13: 3775-3785.

Dressler RL. 1968. Pollination by euglossine bees. *Evolution*, 22: 202-210.

Dressler RL. 1982. Biology of the orchid bees (Euglossini). *Annual Review of Ecology and Systematics*, 13: 373-394.

Duminil J, Fineschi S, Hampe A, Jordano P, Salvini D, Vendramin GG, Petit RJ. 2007. Can population genetic structure be predicted from life-history traits? *American Naturalist*, 169: 662-672.

Duminil J, Hardy OJ, Petit RJ. 2009. Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evolutionary Biology*, 9: 177.

493 Ellstrand NC, Elam DR. 1993. Population genetic concequences of small population size - implications
494 for plant conservation. *Annual Review of Ecology and Systematics*, 24: 217-242.
495 Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population
496 genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10: 564-567.
497 Falahati-Anbaran M, Stenøien HK, Bolstad GH, Hansen TF, Pérez-Barrales R, Armbruster WS, Pélabon
498 C. 2016. Novel microsatellite markers for *Dalechampia scandens* (Euphorbiaceae) and closely
499 related taxa: application to studying a species complex. *Plant Species Biology*, DOI:
500 10.1111/1442-1984.12142.
501 Falahati-Anbaran M, Stenøien HK, Pélabon C, Bolstad GH, Perez-Barrales R, Hansen TF, Armbruster
502 WS. 2013. Development of microsatellite markers for the neotropical vine *Dalechampia*
503 *scandens* (Euphorbiaceae). *Applications in Plant Sciences*, 1: 1200492.
504 Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus
505 genotype data: Linked loci and correlated allele frequencies. *Genetics*, 164: 1567-1587.
506 Fedorov AA. 1966. The structure of the tropical rain forest and speciation in the humid tropics.
507 *Journal of Ecology*, 54: 1-11.
508 Fenster CB. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) I. Gene dispersal. *Evolution*,
509 45: 398-409.
510 Foster PF, Sork VL. 1997. Population and genetic structure of the west African rain forest liana
511 *Ancistrocladus korupensis* (Ancistrocladaceae). *American Journal of Botany*, 84: 1078-1091.
512 Gianoli E, Torres-Díaz C, Ruiz E, Salgado-Luarte C, Molina-Montenegro MA, Saldaña A, Ríos RS. 2016.
513 Woody climbers show greater population genetic differentiation than trees: insights into the
514 link between ecological traits and diversification. *Evolution*, DOI: 10.1111/evo.13073.
515 Gonzalez-Varo JP, Biesmeijer JC, Bommarco R, Potts SG, Schweiger O, Smith HG, Steffan-Dewenter I,
516 Szentgyoergyi H, Woyciechowski M, Vila M. 2013. Combined effects of global change
517 pressures on animal-mediated pollination. *Trends in Ecology & Evolution*, 28: 524-530.
518 Hamrick JL, Godt MJW. 1996. Effects of life history traits on genetic diversity in plant species.
519 *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 351:
520 1291-1298.
521 Hardy OJ, Vekemans X. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic
522 structure at the individual or population levels. *Molecular Ecology Notes*, 2: 618-620.
523 Hedrick PW. 2005. A standardized genetic differentiation measure. *Evolution*, 59: 1633-1638.
524 Hutchison DW, Templeton AR. 1999. Correlation of pairwise genetic and geographic distance
525 measures: Inferring the relative influences of gene flow and drift on the distribution of
526 genetic variability. *Evolution*, 53: 1898-1914.
527 Janzen DH. 1971. Euglossine bees as long-distance pollinators of tropical plants. *Science*, 171: 203-
528 205.
529 Janzen DH. 1981. Bee arrival at 2 Costa Rican female *Catasetum* orchid inflorescenses, and a
530 hypothesis on euglossine population structure. *Oikos*, 36: 177-183.
531 Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS
532 accommodates genotyping error increases success in paternity assignment. *Molecular*
533 *Ecology*, 16: 1099-1106.
534 Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. 2015. Clumpak: a program for
535 identifying clustering modes and packaging population structure inferences across K. *Mol*
536 *Ecol Resour*, 15: 1179-1191.
537 Lasso E, Dalling JW, Bermingham E. 2011. Strong spatial genetic structure in five tropical *Piper*
538 species: should the Baker-Fedorov hypothesis be revived for tropical shrubs? *Ecology and*
539 *Evolution*, 1: 502-516.
540 Levin DA, Kerster HW. 1969. Dependence of bee-mediated pollen and gene dispersal upon plant
541 density. *Evolution*, 23: 560-571.
542 López-Uribe MM, Oi CA, Del Lama MA. 2008. Nectar-foraging behavior of Euglossine bees
543 (Hymenoptera: Apidae) in urban areas. *Apidologie*, 39: 410-418.

López-Uribe MM, Zamudio KR, Cardoso CF, Danforth BN. 2014. Climate, physiological tolerance and sex-biased dispersal shape genetic structure of Neotropical orchid bees. *Molecular Ecology*, 23: 1874-1890.
Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7: 639-655.
Murren CJ. 2002. Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. *Journal of Ecology*, 90: 100-107.
Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, 13: 1143-1155.
Ohashi K, Thomson JD. 2009. Trapline foraging by pollinators: its ontogeny, economics and possible consequences for plants. *Annals of Botany*, 103: 1365-1378.
Ollerton J, Winfree R, Tarrant S. 2011. How many flowering plants are pollinated by animals? *Oikos*, 120: 321-326.
Opedal ØH, Albertsen E, Armbruster WS, Pérez-Barrales R, Falahati-Anbaran M, Pélabon C. 2016. Evolutionary consequences of ecological factors: pollinator reliability predicts mating-system traits of a perennial plant. *Ecology Letters*, DOI: 10.1111/ele.12701.
Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6: 288-295.
Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28: 2537-2539.
Pokorny T, Loose D, Dyker G, Quezada-Euan JJG, Eltz T. 2015. Dispersal ability of male orchid bees and direct evidence for long-range flights. *Apidologie*, 46: 224-237.
Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25: 345-353.
Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155: 945-959.
Ritland K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. *Genetical Research*, 67: 175-185.
Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, 145: 1219-1228.
Schulke B, Waser NM. 2001. Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*. *Oecologia*, 127: 239-245.
Stenøien HK, Såstad SM. 1999. Genetic structure in three haploid peat mosses (*Sphagnum*). *Heredity*, 82: 391-400.
Suni SS, Bronstein JL, Brosi BJ. 2014. Spatio-temporal genetic structure of a tropical bee species suggests high dispersal over a fragmented landscape. *Biotropica*, 46: 202-209.
Surget-Groba Y, Kay KM. 2013. Restricted gene flow within and between rapidly diverging Neotropical plant species. *Molecular Ecology*, 22: 4931-4942.
Theim TJ, Shirk RY, Givnish TJ. 2014. Spatial genetic structure in four understory *Psychotria* species (Rubiaceae) and implications for tropical forest diversity. *American Journal of Botany*, 101: 1189-1199.
Thomson JD, Slatkin M, Thomson BA. 1997. Trapline foraging by bumble bees: II. Definition and detection from sequence data. *Behavioral Ecology*, 8: 199-210.
Trapnell DW, Hamrick JL. 2005. Mating patterns and gene flow in the neotropical epiphytic orchid, Laelia rubescens. *Molecular Ecology*, 14: 75-84.
Twyford AD, Kidner CA, Ennos RA. 2014. Genetic differentiation and species cohesion in two widespread Central American *Begonia* species. *Heredity*, 112: 382-390.
Vamosi JC, Knight TM, Steets JA, Mazer SJ, Burd M, Ashman TL. 2006. Pollination decays in biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 956-961.
Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, 13: 921-935.

596 Whitehead MR, Linde CC, Peakall R. 2015. Pollination by sexual deception promotes outcrossing and
597 mate diversity in self-compatible clonal orchids. *Journal of Evolutionary Biology*, 28: 1526-
598 1541.
599 Wikelski M, Moxley J, Eaton-Mordas A, Lopez-Uribe MM, Holland R, Moskowitz D, Roubik DW, Kays
600 R. 2010. Large-range movements of neotropical orchid bees observed via radio telemetry.
601 *Plos One*, 5: e10738 (DOI: 10710.11371/journal.pone.0010738).
602 Williams NH, Dodson CH. 1972. Selective attraction of male euglossine bees to orchid floral
603 fragrances and its importance in long distance pollen flow. *Evolution*, 26: 84-95.
604 Wilson GA, Rannala B. 2003. Bayesian inference of recent migration rates using multilocus
605 genotypes. *Genetics*, 163: 1177-1191.

606

Table 1. Summary statistics and characteristics of study populations used to investigate gene flow and genetic structure in *Dalechampia scandens*.

Population	Abbr.	N	W	Size	n	N_A (SE)	N_E (SE)	H_O (SE)	H_E (SE)	F_{IS} (SE)	P_D
Bebedero	S1	10° 24' 32.6"	85° 09' 48.2"	M	19	2.000 (0.173)	1.496 (0.103)	0.112 (0.024)	0.250 (0.043)	0.575 (0.042)	0.26
Puente la Amistad	S2	10° 14' 30.4"	85° 15' 09.5"	XL	21	3.300 (0.346)	2.177 (0.219)	0.328 (0.043)	0.409 (0.050)	0.180 (0.033)	0.21
Paquera	S3	09° 49' 21.2"	84° 54' 28.4"	S	16	3.533 (0.364)	2.111 (0.218)	0.325 (0.039)	0.415 (0.044)	0.208 (0.039)	0.09
Santa Cruz	S4	10° 13' 03.6"	85° 31' 05.7"	XS	5	2.067 (0.179)	1.626 (0.122)	0.273 (0.053)	0.289 (0.047)	0.065 (0.071)	0.05
Murciélago	S6	10° 54' 02.7"	85° 43' 51.6"	M	19	2.800 (0.316)	1.855 (0.185)	0.176 (0.027)	0.338 (0.048)	0.421 (0.051)	0.23
Bagaces	S7	10° 26' 07.5"	85° 15' 24.3"	M	10	1.667 (0.175)	1.324 (0.088)	0.123 (0.032)	0.168 (0.041)	0.190 (0.058)	0.10
Palo Verde A	S8	10° 23' 12.4"	85° 19' 06.6"	L	23	2.800 (0.334)	1.875 (0.218)	0.236 (0.041)	0.325 (0.049)	0.248 (0.050)	0.10
Palo Verde B	S9	10° 20' 48.6"	85° 20' 55.0"	L	19	2.800 (0.344)	1.841 (0.187)	0.198 (0.032)	0.328 (0.048)	0.357 (0.052)	0.14
Punta Cacique	S11	10° 34' 11.9"	85° 41' 42.1"	M	12	1.500 (0.125)	1.246 (0.071)	0.119 (0.037)	0.140 (0.036)	0.166 (0.081)	0.12
Playa Matapalo	S12	10° 31' 39.3"	85° 45' 21.1"	S	4	1.300 (0.085)	1.180 (0.058)	0.075 (0.036)	0.107 (0.032)	0.278 (0.126)	0.00
Malpais	S13	09° 36' 23.9"	85° 07' 56.6"	S	13	2.367 (0.242)	1.684 (0.147)	0.141 (0.028)	0.300 (0.046)	0.526 (0.054)	0.23
Isla San Lucas	S16	09° 56' 58.3"	84° 54' 03.7"	S	13	2.967 (0.323)	1.973 (0.181)	0.356 (0.048)	0.392 (0.044)	0.115 (0.056)	0.12
Quebrada Seca	S18	09° 51' 26.2"	85° 20' 56.2"	S	13	2.033 (0.212)	1.524 (0.128)	0.111 (0.027)	0.232 (0.045)	0.453 (0.073)	0.22
PN Diriá	S19	10° 10' 23.9"	85° 35' 43.8"	S	9	2.900 (0.333)	2.073 (0.209)	0.368 (0.049)	0.398 (0.047)	0.094 (0.060)	0.11
Cerro Brujo	S20	10° 09' 56.1"	85° 37' 28.2"	S	7	2.967 (0.277)	2.202 (0.223)	0.325 (0.039)	0.428 (0.046)	0.188 (0.060)	0.07
Horizontes	S21	10° 42' 08.9"	85° 35.55.5	L	17	1.767 (0.157)	1.304 (0.088)	0.086 (0.019)	0.164 (0.037)	0.388 (0.059)	0.23
Playa Cabuyal	S22	10° 40' 33.6"	85° 38' 40.6"	XS	4	1.500 (0.115)	1.296 (0.072)	0.092 (0.028)	0.168 (0.038)	0.402 (0.092)	0.01
Playa Hermosa	S26	10° 34' 09.4"	85° 40.42.6	XS	5	1.500 (0.115)	1.302 (0.071)	0.210 (0.053)	0.172 (0.039)	-0.201 (0.082)	0.12
Average					12.7	2.320	1.672	0.203	0.279	0.261	0.13

Population size indicates the number of flowering patches, scored as: ≤5 (XS), 5-15 (S), 15-25 (M), 25-50 (L) and >50 (XL). Population-level estimates with standard errors over loci are given for the number of alleles per locus (N_A), the number of effective alleles per locus (N_E), observed and expected heterozygosities (H_O and H_E, respectively), and inbreeding coefficients (F_{IS}). P_D is the proportion of polymorphic loci in significant linkage disequilibrium.

607

Table 2. Fine-scale spatial genetic structure parameters in four *Dalechampia scandens* populations.

Population	ASD ± SE (mm)	n	Distance range (m)	b_{log} ± SE	$F_{(1)}$ ± SE	*Sp*
Bebedero (S1)	2.35 ± 0.15	19	278	-0.069 ± 0.013	0.176 ± 0.035	0.084
Horizontes (S21)	3.35 ± 0.17	17	532	-0.039 ± 0.011	0.060 ± 0.029	0.041
Puente la Amistad (S2)	3.41 ± 0.18	21	193	-0.025 ± 0.005	0.015 ± 0.012	0.025
Palo Verde A (S8)	4.50 ± 0.19	23	271	-0.024 ± 0.008	0.012 ± 0.021	0.024

Anther-stigma distances (ASD) were measured in the field. n is the number of patches sampled. b_{log} is the slope of the regression of average pairwise kinship coefficients on the logarithm of between-patch distance. $F_{(1)}$ is the average kinship coefficient of neighbouring patches, and *Sp* is calculated as $-b_{log}(1-F_{[1]})^{-1}$.

608

609 **Figure legends**

610 Figure 1. A female *Euglossa* hovering in front of a blossom inflorescence of *Dalechampia*
611 *scandens.* The bees collect resin secreted by the gland located above the male flowers for use
612 in nest construction. Notice the resin already collected on the hind tibiae of the bee. Due to
613 their extraordinary flight ability, euglossine bees have been suggested to forage over large
614 areas, and thus act as long-distance pollinators. Photo by E. Albertsen.

615 Figure 2. Map of *Dalechampia scandens* study populations in north-western Costa Rica and
616 their assignment to genetic clusters. The neighbour-joining tree (constructed independently
617 from the genetic-cluster analysis) indicates the phylogenetic relationships among the
618 populations, and is colour-coded according to the main genetic cluster assigned for each
619 population.

620 Figure 3. Relationship between pairwise geographic distance and genetic differentiation
621 among 18 populations of *Dalechampia scandens* in Costa Rica.

622 Figure 4. Migration rates (i.e. estimated proportion of individuals with migrant ancestry) with
623 95% credible intervals among 18 *Dalechampia scandens* populations. Each data point
624 represents the highest unidirectional migration rate between the two populations in each pair.

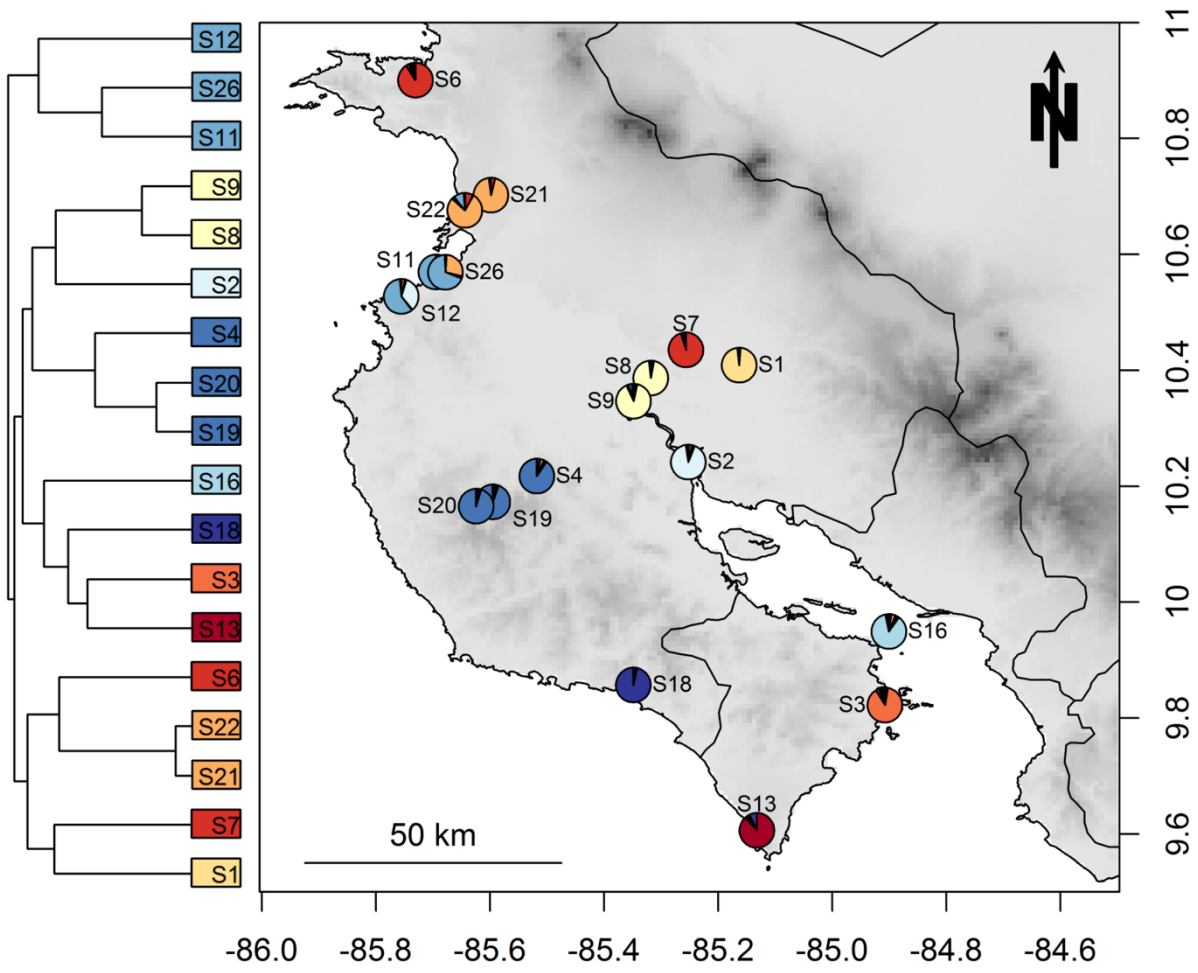
625 Figure 5. (a) Map of pollination events in the S8 population inferred from paternity analyses.
626 Each black circle represent a plant, and arrows represent the pollen movement from the most
627 likely father to the maternal plant. (b) Observed distribution of pollination distances.

628

629 **SUPPORTING INFORMATION**

630 **Table S1:** Pairwise G'_{ST} values among *Dalechampia scandens* populations in Costa Rica.

631


S12
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50 km
-86.0
-85.8
-85.6
-85.4
-85.2
-85.0
-84.8
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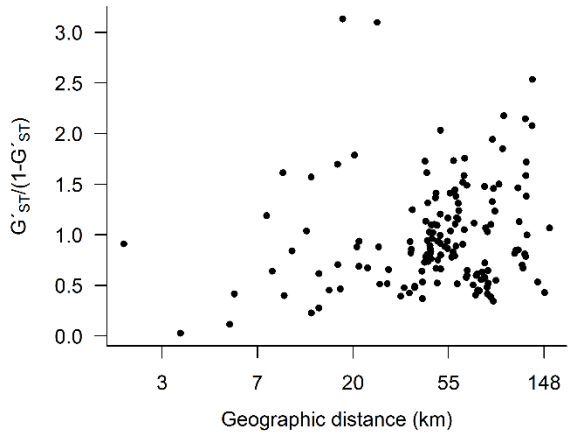
3.0
2.5
2.0
1.5
1.0
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0.0
G´ST/(1-G´ST)
3
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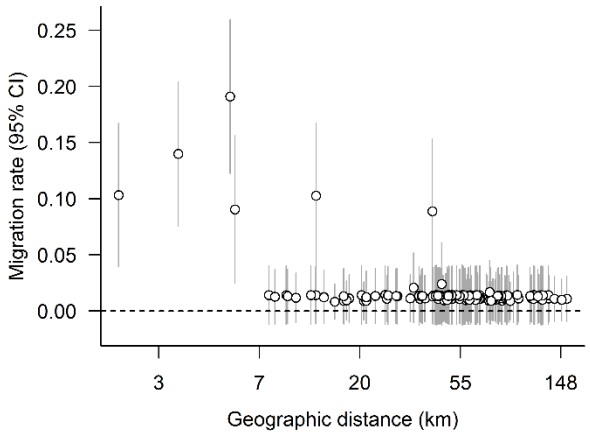
148
Geographic distance (km)

636

637


Migration rate (95% CI)
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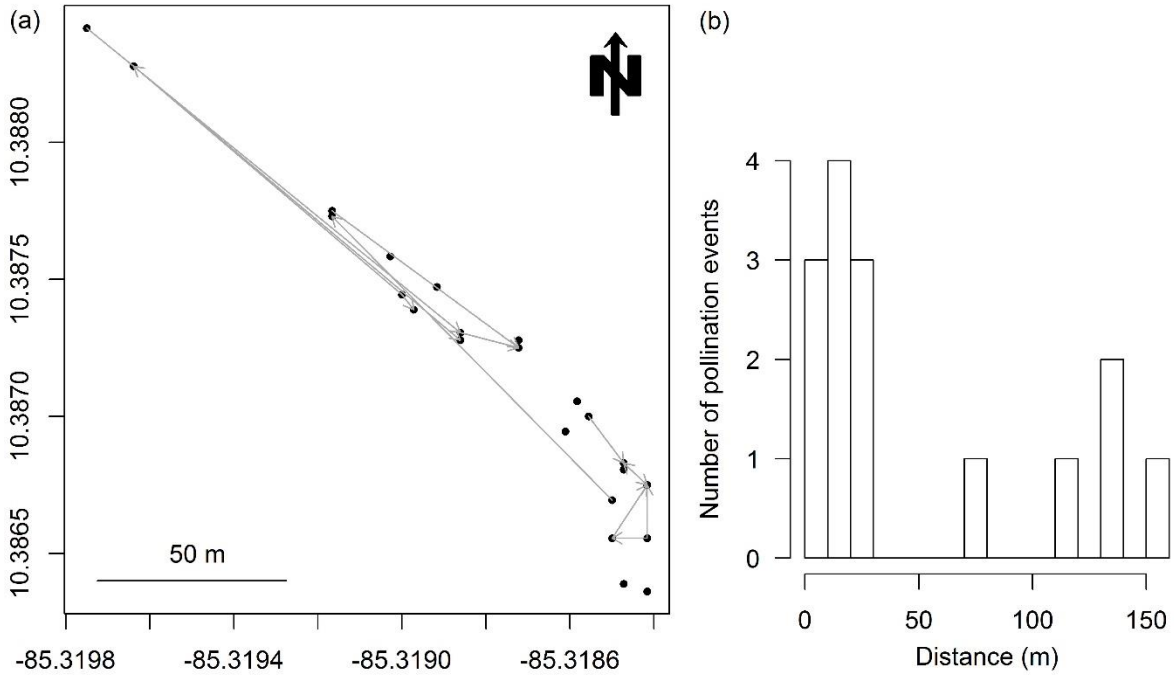
148
Geographic distance (km)

638

639


(a)
10.3880
10.3875
10.3870
10.3865
50 m
-85.3198
-85.3194
-85.3190
-85.3186
(b)
Number of pollination events
0
1
2
3
4
0
50
100
150
Distance (m)

640

641