4-24-2020

Investigating Drivers of Genetic Structure in Plants: Global, Regional and Local Scales

Diana Gamba-Moreno
University of Missouri-St. Louis, dlgtk5@mail.umsl.edu

Investigating Drivers of Genetic Structure in Plants: Global, Regional and Local Scales

Diana L. Gamba-Moreno

M.S. Biology: Ecology and Systematics, San Francisco State University, 2013
B.S. Biology (emphasis in Botany), Universidad del Valle, Cali, Colombia, 2010

A Dissertation Submitted to The Graduate School at the University of Missouri-St. Louis
in partial fulfillment of the requirements for the degree
Doctor of Philosophy in Biology with an emphasis in Ecology, Evolution, and Systematics

May
2020

Advisory Committee

Nathan Muchhala, Ph.D.
Chairperson

Robert Ricklefs, Ph.D.

Christine Edwards, Ph.D.

María del Carmen Ulloa, Ph.D.

Abstract

Genetic structure within and among plant populations is a critical component of plant biodiversity, informing local adaptation, conservation, and incipient speciation. However, its drivers remain poorly understood, especially across different spatial scales. In my dissertation I examined factors that affect plant population genetic structure at global, regional, and local scales. At the global scale, I performed a literature review of population genetic differentiation (F_{ST}) in seed plants based on a 337-species dataset with data on F_{ST} and species traits. Using phylogenetic multiple regressions, I found that F_{ST} is higher for tropical, mixed-mating, non-woody species pollinated by small insects, and lower for temperate, outcrossing trees pollinated by wind. At the regional scale, I tested the effect of flowering asynchrony on genetic divergence between conspecific subpopulations of understory flowering plants in the Andean biodiversity hotspot. I documented flowering phenology for nine species at two sites over one year and inferred population genetic parameters with a genome-wide genotyping approach termed 2b-RAD sequencing. I found that species with higher flowering asynchrony between their subpopulations also show greater genetic divergence. At the local scale, I examined the effect of insect vs. hummingbird pollination modes on the fine-scale spatial genetic structure (SGS) of understory plants in the Andes. I focused on six species for which I confirmed putative pollinators through fieldwork and used the same genotyping technique as above. I found that insect pollination results in a stronger pattern of spatial autocorrelation among closely related individuals, relative to hummingbird pollination. Finally, I

investigated the effect of animal pollination mode and latitudinal region on plant SGS, based on a 147-species global dataset. I found that pollination by small insects is significantly associated with stronger SGS relative to pollination by large insects and vertebrates, particularly in understory plants. Likewise, species from tropical regions have significantly greater SGS than species from temperate zones. Thus, factors that affect plant population genetic differentiation are also important for plant SGS. Overall, my findings shed light on the global drivers of genetic structure in plants, and point to important mechanisms for regional genetic divergence and local genetic connectivity in Andean flowering plants.

Keywords: 2b-RAD sequencing, population genetic differentiation, spatial genetic structure, Andes, flowering asynchrony, pollination mode, latitudinal region.

Table of contents

Chapter I: Global patterns of population genetic differentiation in seed plants

Running title: Drivers of genetic differentiation in plants

Diana Gamba, Department of Biology, University of Missouri at Saint Louis

dlgtk5@mail.umsl.edu

Nathan Muchhala, Department of Biology, University of Missouri at Saint Louis

muchhalan@umsl.edu

Author for correspondence: Diana Gamba; One University Boulevard, 223R Research Hall, Saint Louis, MO 63121, USA; dlgtk5@mail.umsl.edu; +1 (314) 702-0326.

In review: Molecular Ecology

Abstract: 233 words

Total word count (main text): 6436

Abstract

Evaluating the factors that drive patterns of population differentiation in plants is critical for understanding several biological processes such as local adaptation and incipient speciation. Previous studies have given conflicting results regarding the significance of pollination mode, seed dispersal mode, mating system, growth form, and latitudinal region in shaping patterns of genetic structure, as estimated by F_{ST} values, and no study to date has tested their relative importance together across a broad scale. Here we assembled a 337-species dataset for seed plants from publications with data on F_{ST} from nuclear markers and species traits, including variables pertaining to the sampling scheme of each study. We used species traits, while accounting for sampling variables, to perform phylogenetic multiple regressions. Results demonstrated that F_{ST} values were higher for tropical, mixed-mating, non-woody species pollinated by small insects, indicating greater population differentiation, and lower for temperate, outcrossing trees pollinated by wind. Among the factors we tested, latitudinal region explained the largest portion of variance, followed by pollination mode, mating system and growth form, while seed dispersal mode did not significantly relate to F_{ST}. Our analyses provide the most robust and comprehensive evaluation to date of the main ecological factors predicted to drive population differentiation in seed plants, with important implications for understanding the basis of their genetic divergence. Our study is the first that we are aware of to robustly demonstrate greater population differentiation in tropical regions.

Keywords: F_{ST}, life-hostory traits, latitudinal region, pollination mode.

Introduction

Understanding the factors that drive patterns of genetic variation among plant populations is central in biology because genetic diversity is the raw material on which evolution acts. Quantifying population differentiation, which is most frequently done using the fixation index F_{ST} (Wright,1951; see Holsinger & Weir, 2009; Meirmans & Hedrick, 2011 for a review of F_{ST} and related metrics), is important for understanding the first stages of allopatric speciation (Harvey, Singhal, & Rabosky, 2019; Templeton, 1981), as well as the basis of local adaptation (Leimu & Fischer, 2008; Linhart & Grant, 1996), and provides critical information for conservation genetics (Ellstrand, 1992; Ellstrand & Elam, 1993; Kramer & Havens, 2009). Life history traits are expected to influence population genetic structure in seed plants (Duminil et al., 2007; Hamrick & Godt, 1996; Loveless & Hamrick, 1984). However, previous studies have given conflicting results as to the importance of specific traits, such as pollination mode, seed dispersal mode, mating system, and growth form (e.g., Duminil et al., 2007; Hamrick & Godt, 1996), and only one study has compared patterns of F_{ST} variation between latitudinal regions (Dick, Hardy, Jones, & Petit, 2008). Furthermore, little is known about the relative importance of these factors. Below, we discuss prior evidence for each of these factors in turn, and then detail our approach to test them all together in a single analysis that also accounts for phylogenetic relatedness.

Pollination mode is predicted to affect population genetic structure, because pollen dispersal is critical to moving alleles between plant populations.

Previous reviews have lumped different pollination mutualists together as animal pollination and compared them to wind pollination (Hamrick, Godt, & Sherman-Broyles, 1992; Loveless & Hamrick, 1984), revealing that wind tends to reduce genetic structure. Although the idea has not been tested on a broad scale, it has long been thought that different types of animal pollinators should also lead to differences in population genetic structure due to differences in their movement patterns and pollen carry-over capacity (Castellanos, Wilson, & Thomson, 2003). In fact, direct measures of pollen dispersal reveal that volant vertebrates and large bees transport larger proportions of pollen from individual trees to longer geographic distances than small insects (Dick et al., 2008). Given these results, we predict that small insects restrict gene flow among plant populations and increase F_{ST}, compared to large insects, vertebrates, or wind.

Seed dispersal mode is also expected to influence plant population genetic structure because, like pollination mode, it directly affects the movement of alleles and thus gene flow among populations. Strong evidence suggests that limited dispersal increases fine-scale spatial genetic structure in plants (Gelmi-Candusso, Heymann, & Heer, 2017) and in other organisms (Aguillon et al., 2017), which in consequence might scale up and lead to greater population genetic structure (Hamrick & Trapnell, 2011). In fact, reviews of the allozyme literature suggest that seed dispersal by wind and ectozoochory results in lower F_{ST} than dispersal by gravity and endozoochory due to greater gene flow among populations from long distance dispersal events (Hamrick & Godt, 1996; Hamrick, Murawski, & Nason, 1993). However, Duminil et al. (2007) found that

dispersal mode was not a significant predictor of F_{ST}. The lack of consistency among studies encourages further work with larger sample sizes to fully understand the role of seed dispersal mechanisms on population genetic structure.

Unlike pollination and seed dispersal modes, the effect of mating system on plant population genetic structure has been well-established in previous broad-scale studies (Duminil et al., 2007; Loveless & Hamrick, 1984), which suggest that it is the most important predictor of F_{ST} variation. Mating system affects inbreeding, which lowers within-population variation, inflating between-population F_{ST} values (Charlesworth, 2003). Duminil, Hardy, and Petit (2009) found that the outcrossing rate and the inbreeding coefficient, which measures biparental inbreeding and selfing, are both significant predictors of F_{ST} in seed plants. Both selfing and inbreeding increase inbreeding depression and induce purging of deleterious alleles, reducing effective population size and increasing genetic drift, which can ultimately lead to fixation of different alleles in different populations (Angeloni, Ouborg, & Leimu, 2011; Wright, Ness, Foxe, & Barrett, 2008). In contrast, outcrossing increases gene flow within populations, potentially intensifying pollen-mediated gene flow among populations, which counteracts genetic drift and thus decreases population genetic structure (Duminil et al., 2009; Ellstrand, 2014).

Growth form is also an important predictor of population genetic structure. Broad-scale analyses (Duminil et al., 2009; Hamrick et al., 1992) have found strong associations between growth form and F_{ST}, with woody plants tending to

have lower F_{ST} than herbaceous plants. The mechanism that causes this association is unclear, however, and may actually be driven by correlations between growth form and other factors. For example, Duminil et al. (2009) found that growth form only affects F_{ST} indirectly, through its influence on outcrossing rate (t_m) and inbreeding coefficient (F_{IS}); woody growth form is associated with greater t_m and lower F_{IS}. However, Hamrick and Godt (1996) reviewed the allozyme literature for over 300 species and found that when considering outcrossing plants, woody plants show lower levels of F_{ST} than herbs, which suggests that growth form directly affects gene flow among populations, decreasing population genetic structure. This could be because in trees greater geographic distance is presumably required for genetic differences to be detected among populations than in herbs, given that trees are larger than herbs. Thus, when considered at similar geographic scales, we predict that herbs have populations with greater genetic differentiation than trees.

Finally, the latitudinal region in which a plant occurs could also affect its population genetic structure due to differences among regions in spatial and climatic landscapes. In general, geographic heterogeneity and seasonal asynchrony over short distances are considerably higher in the tropics than in the temperate zones (Esquerré, Brennan, Catullo, Torres-Pérez, & Keogh, 2019; Ricklefs, 1977; Stein, Gerstner, & Kreft, 2014), which may act to disrupt mating among conspecific subpopulations, and thus limit gene flow (Martin, Bonier, Moore, & Tewksbury, 2009; Quintero, González-Caro, Zalamea, & Cadena, 2014). Additionally, genetic drift could have a more prominent role in the tropics

than in the temperate zones, due to the fact that most species in the tropics occur at low population densities and thus should have lower effective population sizes than in temperate zones (Dick et al., 2008; ter Steege et al., 2013). In fact, although their sample size was limited and phylogenetic autocorrelation was not accounted for, Dick et al. (2008) found that tropical trees have on average higher F_{ST} values than temperate trees. Given all of the above effects, we predict that F_{ST} is higher in the tropics than in the temperate zones.

Previous studies have not included all of the aforementioned factors together when modeling patterns of population genetic structure in seed plants (Duminil et al., 2007; Hamrick et al., 1992; Hamrick & Godt, 1996; Loveless & Hamrick, 1984; Nybom & Bartish, 2000). Furthermore, the most thorough study of F_{ST} in seed plants was over a decade ago (Duminil et al., 2007) and thus could not take advantage of the wealth of population genetic studies published since then. Here we reviewed publications to assemble a 337-species database of seed plants with the goal of evaluating the factors predicted to best explain variation in plant population genetic structure. We focused on studies that used nuclear markers because their genetic structure should reflect both pollen and seed movement (due to biparental inheritance), unlike chloroplast markers, which only reflect seed movement (due to maternal inheritance) (McCauley, 1994). We examined five ecological factors, including pollination mode, seed dispersal mode, mating system, growth form, and latitudinal region, while controlling for phylogenetic autocorrelation. We also accounted for variables pertaining to the sampling scheme that have been shown to affect F_{ST} values for plants (Nybom &

Bartish, 2000) and other systems (Blasco-Costa & Poulin, 2013; Pascual, Rives, Schunter, & Macpherson, 2017; Riginos, Douglas, Jin, Shanahan, & Treml, 2011); namely, genotyping technique, distance between populations, and sample size. Using multiple regressions, we asked: (Q1) What set of life history traits promote population divergence in seed plants? (Q2) Do patterns of variation in F_{ST} differ between latitudinal regions? (Q3) What are the relative importance of these factors in explaining variation in F_{ST}?

Materials and methods

Data collection

We constructed an F_{ST} dataset through a systematic search in google scholar (key words: “genetic structure”, “population differentiation”, “population genetics”, “genetic diversity”, “population gene flow”) for articles published up until June 2018. The search yielded 356 peer-reviewed publications on seed plants for which measures of population genetic structure (F_{ST}) based on nuclear markers were available. When multiple studies reported F_{ST} values for the same species, we recorded the F_{ST} from the study with the largest geographic range, as this may better represent the genetic diversity found in the species (Cavers et al., 2005). By this criterion, we compiled a dataset that included 337 unique species. We extracted information for the predictor variables directly from the publications, and infrequently complemented this, where necessary, with information from peer-reviewed literature on the studied species (see Appendix S1 and Table S1 in Supporting Information). Predictor variables were included in

multiple regressions to explain variation in F_{ST} values (see section F_{ST} models). We included three factors that pertained to the sampling scheme of each study and that can potentially affect F_{ST} (Nybom, 2004; Nybom & Bartish, 2000): genetic marker used, maximum distance between populations, mean sample size per population. We used them to construct a null model to be compared against models with our factors of interest. Factors of interest consisted of five categorical variables with 2–4 levels: mating system (outcrossing, mixed-mating), growth form (non-woody, shrub, tree), pollination mode (large insects, small insects, vertebrates, wind), seed dispersal mode (animal, gravity, wind), and latitudinal region (tropics, sub-tropics, temperate). Below we explain the F_{ST} estimates and all eight factors used in this study in greater detail.

F_{ST} estimates

We collected F_{ST} and F_{ST} analogs as measures of genetic differentiation (Holsinger & Weir, 2009; Meirmans & Hedrick, 2011) which we collectively refer to F_{ST} throughout this paper. Assuming an island model of migration-drift equilibrium, Wright (1951) developed a theoretical framework for studying the gene frequency variation among subpopulations through the fixation indices, i.e. F-statistics. In this model, F_{ST} is the degree of gene differentiation among subpopulations for genes that have only two alleles. Nei (1973) expanded the model for polymorphic genes, and proposed G_{ST} as a measure of the gene diversity partitioned among subpopulations, relative to the total gene diversity of the population. Subsequently, Weir & Cockerham (1984) proposed a standard

measure of genetic structure θ based on Wright (1951). The statistic θ is estimated per and across loci, and represents the correlation of genes, or coancestry, among individuals in a given population. Excoffier, Smouse, and Quattro (1992) proposed AMOVA (Analysis of Molecular Variance) and corresponding statistic ϕ_{ST}; the proportion of genetic diversity partitioned among populations. Finally, Hedrick (2005) proposed a standardized measure of population differentiation, G'_{ST}, which accounts for the level of heterozygosity of the marker used for genotyping individuals ($G'_{ST}=G_{ST}overall/G_{ST}max$).

The most common statistic in our dataset was θ. When θ was reported per loci, we took the mean across loci as the global F_{ST} for that species. The AMOVA derived ϕ_{ST} was also common. Some studies reported both θ and ϕ_{ST}, in which case we used ϕ_{ST} as it likely better represents genetic structure among populations (Hey & Pinho, 2012). The statistics θ and ϕ_{ST} were, however, frequently almost equivalent. Another common measure was G_{ST}; when reported for multiple pairs of populations, we used the mean across all pairs. A few studies reported G'_{ST}. It was not possible to back-transform G'_{ST} to G_{ST} because such studies did not report the maximum possible G_{ST} in their data (Hahn, Michalski, Fischer, & Durka, 2016). Even though G'_{ST} potentially yields a higher value than G_{ST} (or θ and ϕ_{ST}) based on the same data (Hedrick, 2005; Meirmans & Hedrick, 2011), we still included G'_{ST} values, reasoning that any trend of variation in population genetic structure due to the variables here tested should still be present.

Molecular markers

F_{ST} values can be strongly affected by the genotyping technique implemented (Nybom, 2004; Nybom & Bartish, 2000; Meirmans & Hedrick, 2011), thus, we included this factor in our null model. In our database, the majority of studies used nuclear microsatellites (140 species), followed by allozymes (114 species). Fewer studies used dominantly inherited markers, including Amplified Fragment Length Polymorphism (60 species), Random Amplification of Polymorphic DNA (16 species), and Inter-Simple Sequence Repeat (7 species).

Distance between populations

Greater distance between populations should correspond to greater genetic differentiation based on an isolation by distance model (Wright, 1943). Thus, we also included in our null model the maximum distance between populations used in each study. We calculated this based on the coordinates of the two most distant populations. When this was not available, we used the scale bar of maps showing sampled populations. Distance varied from 0.01–9900 km (mean=703 ± 1077 SD).

Mean sample size per population

The maximum value that F_{ST} can take decreases when the within-population expected heterozygosity increases. Thus, a general concern is that large sample sizes are required because small samples can overestimate F_{ST}

(Holsinger & Weir, 2009; Kalinowski, 2005; Willing, Dreyer, & van Oosterhout, 2012). We accounted for this potential bias by including the mean sample size per population in our null model. Across the studies, this sample size ranged from 3 to 285 individuals per population, with an overall mean of 40.12 (± 44.9 SD).

Pollination mode

Species were coded as pollinated by wind, small insects, large insects, or vertebrates. Small insect pollinators included small Hymenoptera (i.e., *Trigona* and *Melipona* bees and wasps), Diptera (i.e., hoverflies and gnats), Coleoptera (i.e., small curculionids), Hemiptera (i.e. Anthocoridae and Miridae), and Thysanoptera (i.e., thrips). Large insects included large bees (i.e., honeybees, bumblebees, carpenter bees, euglossine bees) and Lepidoptera (i.e., hawk moths and yucca moths, monarch butterflies). We included honeybees in the large insect category based on evidence showing that honeybees have flying and pollen carry-over capacity similar to bumblebees (Cresswell, Bassom, Bell, Collins, & Kelly, 1995; Escaravage & Wagner, 2004). Vertebrates included bats, hummingbirds, and other nectarivorous birds such as honeyeaters and sunbirds. Some instances of vertebrate pollination were more generalized, with visitors including a combination of bats, birds, rodents, and/or marsupials.

Seed dispersal mode

Species were coded as dispersed by wind, animals, or gravity. Plants

adapted to wind dispersal presented fruits or seeds that were particularly light and/or winged. For those plants adapted to animal dispersal, exploratory analyses showed that different types of animal dispersal were not significantly different (results not shown). Thus, we kept the animal dispersal category broad, including plants with fruits or seeds dispersed by endo-, ecto-, or syn-zoochory. Plants with no adaptations for vector-mediated seed dispersal were coded as gravity dispersed. Based on the information reported in publications with F_{ST} and trait data, we did not find evidence of secondary movement of fruits or seeds by biotic agents. In some instances, however, water may play a secondary role in dispersing seeds that fall under mother plants, as in the mangrove species *Avicennia* spp. and *Rhizophora* spp., and for *Beta vulgaris* L., *Casuarina cunninghamiana* Miq., *Cocos nucifera* L., and *Primula nutans* Georgi, as well as for many forest trees after floods or inhabiting riparian sites (Levine & Murrell, 2003; Nilsson, Brown, Jansson, & Merritt, 2010).

Mating system

We coded species as selfing, mixed-mating, or outcrossing, as identified by the authors in each study. Selfing species included strictly autogamous species. They were rare (N=7) and not included in the final 337-species dataset, due to their low sample size. Mixed-mating species included those that undergo both outcrossing and selfing to some extent, through either autogamy or geitonogamy (Goodwillie, Kalisz, & Eckert, 2005). Outcrossing species included plants that are self-incompatible, unisexual (i.e. monoecious or dioecious), or

dichogamous hermaphrodites; i.e. either having the male reproductive organs come to maturity before the female organs (protandry), or vice versa (protogyny).

Growth form

Species were coded as trees, shrubs, or non-woody plants. Trees included woody plants >10m tall, typically with a single trunk coming from the base. Shrubs included upright woody plants <10 m tall, typically with one or several trunks coming from the base. We also included in the shrub category hemi-parasites and hemi-epiphytes. Non-woody plants included herbs, epiphytes, and non-woody climbers. Growth form of species was often linked to habitat in that many non-woody plants and shrubs occurred in the forest understory, while many trees occurred in the subcanopy and canopy. However, non-woody plants, shrubs, and trees also occurred in open habitats like prairies. We did not include habitat as an additional predictor in our models due to its high collinearity with growth form.

Latitudinal region

We recorded the geographic location of each study to create an additional categorical variable for latitudinal region. Species were coded as tropical, sub-tropical, or temperate. Tropical regions included sites between the tropics of Cancer and Capricorn (23.5° north and south of the equator, respectively), which are characterized by relatively low variation in daylight and temperature throughout the year, but with large environmental heterogeneity over short

distances. Sub-tropical regions included latitudes from 23.5° to 35° (north and south). These regions have climates similar to the tropics, but with more seasonal fluctuations. Temperate regions included latitudes greater than 35° north and south. These zones are characterized by a wide range of temperatures throughout the year, and by clearly marked seasonal changes.

Analytical framework

Analyses were performed in R (R Core Team 2018). Prior to model testing, we performed transformations of continuous data to improve normality of model residuals (details in Appendix S2). F_{ST} was transformed using Tukey's ladder of powers transformation (Tukey, 1970) with the function transformTukey from the R package rcompanion (Mangiafico, 2018). Continuous predictors were transformed using their natural logarithm. We also estimated correlations (Plackett, 1983) and evaluated multicollinearity issues (Acock & Stavig, 1979; Fox & Monette, 1992) among predictor variables (Appendix S3). The multicollinearity tests indicated that all predictors could be included together in a multiple regression (Table S2 and Table S3).

In order to calculate and subsequently perform models that correct for phylogenetic signal (Freckleton, Harvey, & Pagel, 2002), a species-level phylogeny (Fig. S1) was produced with the R package V.PhyloMaker (Jin & Qian, 2019). This package prunes a custom list of species from the latest and most complete mega-tree of vascular plants (Smith & Brown, 2018) (see Appendix S4 for details). We then assessed phylogenetic signal in categorical predictors with

Abouheif's (1999) method (Jombart, Balloux, & Dray, 2010; Pavoine, Ollier, Pontier, & Chessel, 2008), and in F_{ST} values with Pagel's (1999) λ (Molina-Venegas & Rodríguez, 2017; Revell, 2012) (Appendix S5). We found that closely related species tend to be more similar than expected by chance in their mating system, growth form, pollination mode, seed dispersal mode, latitudinal region and F_{ST}. The highest observed Moran's *I* was that of growth form, followed by pollination mode, latitudinal region, seed dispersal mode, and lastly mating system (Fig. S2). F_{ST} values were also phylogenetically autocorrelated (Pagel's λ=0.52, P<0.001 and Pagel's λ=0.53, P<0.001 for raw and transformed F_{ST} values, respectively). Given the high levels of phylogenetic signal, we implemented phylogenetically informed multiple regressions (Symonds & Blomberg, 2014) with the function 'phylolm' from the R package phylolm (Ho & Ané, 2014). For the fit of models, the likelihood of the parameters was calculated with a Brownian motion model of evolution (Ho & Ané, 2014) (Appendix S6).

Finally, for the categorical predictors with more than two levels we chose reference levels based on exploratory analyses with phylogenetic ANOVA and post-hoc tests (Garland, Dickerman, Janis, & Jones, 1993; Revell, 2012). We selected the level which mean was most different from that of other levels (Tables S4 and S5). Reference levels were as follow: trees for growth form, small insects for pollination mode, gravity for dispersal mode, and temperate for latitudinal region.

F_{ST} models

We began our phylogenetic multiple regressions analyses of factors affecting genetic structure by constructing a null model with the sampling-scheme variables. We sequentially added the life history traits to this null model, checking whether each addition improved model fit of a multiple regression based on Akaike Information Criterion (AIC) scores (Akaike, 1974). Mating system and growth form were added together as there is ample evidence of their effect on F_{ST} (Duminil et al., 2007; Hamrick & Godt, 1992). We then added pollination mode and seed dispersal mode, to check whether either, or both together, improved the previous model. After finding the best model explaining F_{ST} with life history traits (Q1), we compared this model to one that included latitudinal region as an additional factor (Q2). We assessed the variance explained by each model with the R package rr2 and the function 'R2.pred' (Ives, 2018; Ives & Li, 2018). We further evaluated the best-fit model through a backward stepwise model selection with the function 'phylostep' in the phylolm package. The functions 'phylostep' and 'phylolm' were congruent in finding the same best model.

We then evaluated the importance of each variable in this best-fit model (Q3). We used the R package rr2 and the function 'R2.lik' to obtain the unique contribution of each factor in terms of the amount of F_{ST} variance explained by comparing the best-fit model with a reduced model not including the factor of interest.

Results

Taxonomic scope and phylogeny

The 337 species were distributed in 210 genera, representing 96 families in 34 orders. The majority of species (268) belonged to the Eudicots, followed by 43 Monocots, 17 Magnoliids, and 9 Gymnosperms. The families Fabaceae (mostly *Acacia*; 8 species) and Fagaceae (mostly *Quercus*; 13 species) were particularly well represented, with 37 and 26 species respectively (Table S1). The resulting phylogeny had 337 tips and 311 nodes (Fig. S1). In other words, 92% of the phylogeny was resolved, and only 26 tips (8%) belonged to polytomies. These polytomies correspond to clades for which phylogenetic information remains scarce or unclear (Stevens, 2001 onwards): *Begonia* (Begoniaceae), *Alcantarea* and *Encholirium* (Bromeliaceae), *Streptocarpus* (Gesneriaceae), *Arceuthobium* (Santalaceae), *Magnolia* (Magnoliaceae), *Piper* (Piperaceae), *Psychotria* (Rubiaceae), *Acacia* (Fabaceae), and *Sorbus* (Rosaceae).

Life history traits that promote population divergence in seed plants (Q1)

Among phylogenetic multiple regressions with the four life history traits (models 1–4, Table 1), model 4 was the best-fit, indicating that mating system, growth form, pollination mode and seed dispersal mode all influence F_{ST} (AIC=–482.3). However, the performance of model 4 was almost indistinguishable from that of model 3 (ΔAIC=2.2), which only differed in the lack of the factor seed dispersal mode. Further evidence for the relative unimportance of seed dispersal mode can be seen in the fact that adding seed dispersal mode to model 1 (which

only has mating system and growth form) results in much less improvement of fit (models 2 vs. 1, ΔAIC=2.5) than adding pollination mode (models 3 vs. 1, ΔAIC=16.6).

Differences among latitudinal regions (Q2)

Adding the factor latitudinal region to models with the four life history traits notably increased fit to the data (models 5–7, AIC=–488.6 to –503.9, Table 1). This is particularly evident when comparing the best-fit models for each instance (models 4 vs. 6, ΔAIC=21.6). Model performance was indistinguishable for models 6 vs. 7 (ΔAIC=1), which only differed in the addition of seed dispersal mode. Finally, in models 5 and 7 the factor seed dispersal mode was no longer a significant predictor of F_{ST} (Table 1 and 2). Below we focus on results from model 7, as it is the most inclusive model of the factors we tested with the best fit to the data.

Figure 1 shows how the levels of each factor affect population differentiation as measured by F_{ST} values (after transformation). The effect of each factor is depicted after accounting for the effect of the other independent variables in model 7. For mating system, outcrossers tend to have lower population differentiation than mixed-mating plants (Fig. 1a). Trees tend to have significantly lower population differentiation relative to non-woody plants and shrubs, while the latter two growth forms did not differ between each other (Fig. 1b). Pollination by small insects leads to significantly greater differentiation compared to large insect, vertebrate and wind pollination, while the latter three

pollination modes did not differ between each other (Fig. 1c). Temperate zones have significantly lower F_{ST} values than tropics and subtropics, and the latter two regions did not differ from each other (Fig. 1e). Finally, seed dispersal mode was not a significant predictor of population genetic differentiation. F_{ST} values associated with gravity dispersal were highly variable, and although gravity dispersal results in higher F_{ST} values compared to wind dispersal, this difference was not significant. Animal dispersal also resulted in highly variable F_{ST} values that did not differ from other dispersal modes (Fig. 1d).

Most important factor for explaining F_{ST} (Q3)

Of all of the factors that we analyzed, latitudinal region explained the highest percent variation (7%), higher than the life history traits in model 7 (0.9–6%, Fig 1f). Of the life history traits, mating system and pollination mode had the highest independent contribution to the variation in F_{ST} values (6% each), followed by growth form (4%), while the contribution of dispersal mode was very low (0.9%) and not statistically significant (Fig. 1f).

Influence of variables in the null model

Variables in the null model were significant predictors of F_{ST} in all multiple regressions (Table 1) and in model 7 (Table S6). Distance had the highest independent contribution (8%), compared to genetic marker and mean sample size (4% each). In general, F_{ST} values become larger when the geographic scale of studies increases. In contrast, F_{ST} values decrease with larger mean sample

sizes of individuals per population. Codominant markers (microsatellites and allozymes) tend to underestimate F_{ST} values, while dominant markers (AFLP and RAPD) overestimate them. ISSR markers did not differ from others.

Discussion

Here we provide the most robust and comprehensive evaluation to date of factors driving population genetic differentiation in seed plants. We largely found support for our hypothesis of factors that significantly influence F_{ST} and several intriguing patterns emerge from our analyses. Overall, we found higher F_{ST} for tropical, mixed-mating, non-woody species pollinated by small insects, and lower F_{ST} for temperate, outcrossing, trees pollinated by wind. Latitudinal region was the most important predictor for F_{ST} relative to the others tested. Mating system and pollination mode had equal contributions for explaining F_{ST}. Growth form was also a key factor influencing F_{ST}, while seed dispersal mode was not important in our most inclusive model (Table 2, Fig. 1).

Influence of latitudinal region on F_{ST}

Population differentiation was higher in the tropics and subtropics than in temperate regions (Fig. 1e). This result supports the idea that patterns of local diversity, such as the partitioning of genetic diversity among plant populations, cannot be explained in isolation from the geographic and historic processes of each region (Ricklefs, 1987, 2004, 2006). Some factors that may contribute include regional differences in seasonality, macroevolution, and geography,

differences which have more generally been hypothesized to contribute to the latitudinal diversity gradient (i.e. increased species richness closer to the equator) (Mittelbach et al., 2007; Rolland, Condamine, Jiguet, & Morlon, 2014; Schemske, Mittelbach, Cornell, Sobel, & Roy, 2009). Below we discuss some of these ideas, including the 'asynchrony of seasons hypothesis' (ASH) (Martin et al., 2009), the 'time/area hypothesis' (Fine & Ree, 2006), and the 'niche conservatism hypothesis' (Kerkhoff, Moriarty, & Weiser, 2014).

One compelling explanation for the regional differences in F_{ST} is based on the idea that the tropics can have highly asynchronous rainfall patterns over small spatial scales (Martin et al., 2009). Given that most plants time their flowering to seasons (Crimmins, Crimmins, & Bertelsen, 2011; Gaudinier & Blackman, 2019), and that seasons are largely determined by rainfall in the tropics, small-scale differences in rainfall potentially disrupt gene flow and cause high population differentiation over short distances compared to the temperate zones. This is the aforementioned ASH, and our analyses support the prediction of higher population differentiation in the tropics. We note that the tropics and subtropics did not differ in F_{ST}, and that these regions have comparable climatic patterns (Sitnikov, 2009), thus the ASH may extend to subtropical regions.

Higher F_{ST} in the tropics/subtropics than in the temperate zones can also be due to the different history of plant lineages in each region. The 'time/area hypothesis' (Fine & Ree, 2006) and the 'niche conservatism hypothesis' (Kerkhoff et al., 2014) allude to the idea that tropical clades are older and tend to live in the same environments throughout their evolutionary history, while

temperate clades diversified more recently after switching to novel environments once cooling began in the Oligocene. Thus, most temperate species likely expanded their populations fairly recently post-glaciation (34 Mya), resulting in lower population differentiation due to recent gene flow maintaining cohesion. In contrast, tropical species may have been in the same place longer and their populations have had more time to isolate due to dispersal limitations and build up genetic differentiation (Kisel & Barraclough, 2010; Smith et al., 2014). Tropics and subtropics share strong floristic affinities (Sarmiento, 1972), which corresponds to the similar F_{ST} between them.

Finally, gene flow is likely more restricted in the tropics due to its heterogeneous orogeny and rich fluvial systems. Such geographic differences have also been hypothesized to contribute to the latitudinal diversity gradient (e.g., Smith et al., 2014; Wallace, 1854). This argument becomes particularly compelling in combination with the fact that temperature does not vary as extremely through the year in the tropics. Given this, different subpopulations would be expected to evolve narrower physiological niches that adapt them to particular altitudinal zones, and a similarly sized mountain would impose a greater barrier to dispersal, and thus to gene flow among subpopulations, in tropical than in temperate regions (Ghalambor, 2006; Janzen, 1967).

Thus, overall, our results are in line with hypotheses that suggest greater species diversity in the tropics is due to higher speciation rates rather than lower extinction rates. While the specific mechanisms differ, including those mentioned above and others (see Mittelbach et al., 2007), these hypotheses all posit greater

population-level differentiation that then scales up to faster speciation rates in a model of allopatric or parapatric speciation. Direct tests on the influence of population differentiation on speciation rates are necessary in order to establish that population differentiation is a rate-limiting step of the speciation process (Harvey et al., 2019). Such tests are scarce and have only focused on vertebrates, finding a positive association in New World birds (Harvey et al., 2017), and no association in Australian lizards (Singhal et al., 2018). We encourage similar tests in seed plants at a global scale. Nevertheless, ours is the first study that we are aware of to clearly document such a pattern of greater population differentiation in the tropics for seed plants (see Martin & McKay, 2004 for a study in vertebrates).

Influence of pollination mode on F_{ST}

We found that pollination mode plays a key role in population differentiation, contrary to the findings of the latest review of F_{ST} and species traits in seed plants (Duminil et al., 2007). Specifically, species pollinated by small insects have significantly higher F_{ST} than those with other pollination modes. This pattern is likely due to reduced gene flow among plant populations. In fact, small insects have a lower pollen carry-over capacity than bumblebees and vertebrates (Dick et al., 2008; Rhodes, Fant, & Skogen, 2017), and studies of pollinator movement show that euglossine bees, hawkmoths, and bats can all travel long distances, even across fragmented habitats (Brunet, Larson-Rabin, & Stewart, 2012; Finger, Kaiser-Bunbury, Kettle, Valentin, & Ghazoul, 2014;

Janzen, 1971; López-Uribe, Oi, & Del Lama, 2008; McCulloch et al., 2013; Skogen, Overson, Hilpman, & Fant, 2019). Our results show that wind, large insects, and vertebrates have homogenizing effects on plant F_{ST}, which are statistically indistinguishable. Taken together, these patterns suggest that plants pollinated by small insects might be more sensitive to habitat fragmentation; the inability of these pollinators to connect distant fragments may decrease genetic diversity within populations, and along with it the ability to adapt in response to anthropogenic change.

One important caveat is that the limited information on pollination systems for many species necessitated a relatively coarse-grained division of pollination mode into broad taxonomic groups. This approach overlooks potential behavioral differences within these groups. For instance, within the vertebrate pollination category, territorial hummingbirds likely move pollen much shorter distances than trap-lining hummingbirds (Betts, Hadley, & Kress, 2015; Ohashi & Thomson, 2009), and bats may carry pollen more efficiently (Muchhala & Thomson, 2010) and to longer distances than hummingbirds (Lemke, 1984, 1985; Tello-Ramos, Hurly, & Healy, 2015).

Influence of mating system on F_{ST}

Our results provide additional support for the idea that mating system is a strong predictor of F_{ST} (Fig. 1a), even in the presence of other factors (Duminil et al., 2007). Mating system associates with F_{ST} because any amount of inbreeding (through mixed-mating) increases homozygosity within a subpopulation, and

reduces its effective population size, leading to increased population structure due to genetic drift. In contrast, outcrossing maintains genetic cohesion within and among subpopulations, decreasing genetic drift and reducing population structure (Charlesworth, 2003). Because populations of mixed-mating species are often highly differentiated, they will likely have populations with unique genetic diversity. Accordingly, conservation efforts for them should maximize the number of populations protected to maximize genetic diversity to increase their chances to adapt to environmental change (Ellstrand & Elam, 1993).

Influence of growth form on F_{ST}

We found that trees have populations with significantly lower F_{ST} than both shrubs and non-woody plants (Fig. 1b). Even though most trees are outcrossing in our dataset, our results show that growth form contributes to the variation in F_{ST} independently from mating system, contrary to the findings of Duminil et al. (2007, 2009). The inherent difference in scale between growth forms may contribute to this pattern: a given geographic distance between subpopulations may restrict gene flow much more for an herb than for a tree. In fact, neighborhood size, i.e. the spatial extent of closely related individuals, is larger in trees than shrubs and herbs (Vekemans & Hardy, 2004). Furthermore, trees usually have greater longevity than shrubs and non-woody plants (Duminil et al., 2009), which may increase the chances of gene flow between tree subpopulations, more than for other growth forms. Finally, the fact that growth form and habitat are tightly linked may also contribute; many non-woody plants

and shrubs in our dataset occur in the forest understory, while many trees reach the canopy. Givnish (2010) and Theim, Shirk, and Givnish (2014) hypothesized that the understory imposes more limits to gene flow than the canopy because of the sedentary lifestyle of animal mutualists in the understory.

Seed dispersal and F_{ST}

Our results did not support the hypothesis that gravity-mediated seed dispersal increases population differentiation compared to wind or animal dispersal (Givnish, 2010) (Fig. 1d). This is in line with previous findings suggesting that the genetic structure of nuclear markers is largely driven by pollen flow (Petit et al., 2005; Sork, Nason, Campbell, & Fernandez, 1999; Skogen et al., 2019), and that the effect of seed dispersal is only detectable in the population genetic structure of chloroplast genes (Duminil et al., 2007). However, we note that gravity dispersal resulted in highly variable F_{ST} values, potentially due to unrecorded secondary seed vectors. F_{ST} values for animal dispersal were also highly variable, which suggests that different animals could have different effects on population differentiation. Thus overall, as with vertebrate pollination, we suspect that more fine-scaled classifications of dispersers may improve our understanding of their effects on plant population genetic structure. Testing this idea, however, requires more detailed data on animal dispersal modes, which can be difficult to characterize. For example, in our study many species have a mix of seed dispersers, including small to large mammals and birds (like most Arecaceae, Fabaceae, Fagaceae, Myrtaceae,

Sapotaceae, among others), making it difficult to assign plants to a disperser-specific taxonomic affiliation or foraging behavioral trait.

Considerations on model inference

Phylogenetic multiple regressions allowed us to evaluate the unique effect of each predictor on F_{ST} while correcting for phylogenetic autocorrelation, which had not been accomplished in previous broad-scale studies. Additionally, we note that after adding the factor latitudinal region, the scaling parameter that corrects for phylogenetic autocorrelation (λ fit in Table 1) became insignificant. This suggests that latitudinal region decreases the phylogenetic autocorrelation in the residuals modeled by our phylogenetic regressions (Freckleton, 2009). In fact, an alternative across-species multiple regression of model 7 (i.e., a linear model assuming phylogenetic independence) yielded identical results with indistinguishable fit to the data ($\Delta AIC=1.9$). We suspect that region captured important phylogenetic information in F_{ST} and species traits; within each regional species pool, lineages share strong biogeographic and phylogenetic affinities. Put another way, we think that regional affiliation is the most important underlying factor influencing F_{ST} values at a global scale, and when not included, phylogenetic signal becomes a proxy for latitudinal region due to the tendency for closely related species to occur in similar regions.

Future directions

Understanding how plant population genetic structure is affected by life

history traits can greatly improve management strategies for populations facing increasingly fragmented habitats due to human-accelerated global change. Our study reveals that gene flow is generally more limited in non-woody species pollinated by small insects, making them more susceptible to isolation and loss of genetic diversity. Thus, in order to preserve the largest amount of genetic diversity for species with such traits, conservation efforts should seek to maintain numerous subpopulations spanning a wide geographic extent. Future broad-scale studies of F_{ST} variation could provide more even greater insights for conservation by including population densities (Murawski & Hamrick, 1991; Sork et al., 1999), effects of habitat fragmentation (Aguilar, Quesada, Ashworth, Herrerias-Diego, & Lobo, 2008; Skogen et al., 2019), and the landscape context of populations (Sork et al., 1999).

Another avenue for future research involves linking patterns of genetic variation at different scales. Little is known about how factors that affect genetic patterns over fine spatial scales (i.e., within subpopulations) extend to genetic patterns over larger spatial scales (i.e., among subpopulations). Intuitively, species with greater fine-scale genetic structure (Loiselle, Sork, Nason, & Graham, 1995) should also have greater population genetic structure, but this has rarely been tested. For example, a recent review found greater fine-scale genetic structure in species with short-distance dispersers, than those dispersed by birds (Gelmi-Candusso et al., 2017), but it is unclear whether this difference would extend over larger distances. Overall, we expect that more comprehensive studies of ecological interactions, in combination with increasing amounts of

genetic data collected at various spatial scales will continue to improve our understanding of the factors that influence population genetic structure in seed plants.

Acknowledgements

We thank the researchers whose published data we used in this paper. Thanks to Robert Ricklefs, Christine Edwards, and Carmen Ulloa for advice in this study. Isabel Loza, Justin Baldwin and Sebastián Tello provided valuable help with statistical analyses. Many thanks to Justin Zweck, Justin Baldwin, Krissa Skogen, and to members of the Muchhala lab at the University of Missouri at Saint Louis for constructive discussions on a previous version of this manuscript. This research was supported by the Whitney Harris World Ecology Center at the University of Missouri at Saint Louis.

References

Abouheif, E. (1999). A method for testing the assumption of phylogenetic independence in comparative data. *Evolutionary Ecology Research*, *1*(8), 895–909.

Acock, A. C., & Stavig, G. R. (1979). A measure of association for nonparametric statistics. *Social Forces*, *57*(4), 1381–1386. doi: 10.1093/sf/57.4.1381

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., & Lobo, J. (2008). Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology*, *17*(24), 5177–5188. doi: 10.1111/j.1365-294X.2008.03971.x

Aguillon, S. M., Fitzpatrick, J. W., Bowman, R., Schoech, S. J., Clark, A. G., Coop, G., & Chen, N. (2017). Deconstructing isolation-by-distance: The genomic consequences of limited dispersal. *PLoS Genetics*, *13*(8), e1006911. doi: 10.1371/journal.pgen.1006911

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, *19*(6), 716–723. doi: 10.1109/TAC.1974.1100705

Angeloni, F., Ouborg, N. J., & Leimu, R. (2011). Meta-analysis on the association of population size and life history with inbreeding depression in plants. *Biological Conservation*, *144*(1), 35–43. doi: 10.1016/j.biocon.2010.08.016

Betts, M. G., Hadley, A. S., & Kress, W. J. (2015). Pollinator recognition by a keystone tropical plant. *Proceedings of the National Academy of Sciences*, *112*(11), 3433–3438. doi: 10.1073/pnas.1419522112

Blasco-Costa, I., & Poulin, R. (2013). Host traits explain the genetic structure of parasites: a meta-analysis. *Parasitology*, *140*(10), 1316–1322. doi: 10.1017/S0031182013000784

Brunet, J., Larson-Rabin, Z., & Stewart, C. M. (2012). The distribution of genetic diversity within and among populations of the rocky mountain columbine: The impact of gene flow, pollinators, and mating system. *International Journal of Plant Sciences*, *173*(5), 484–494. doi: 10.1086/665263

Castellanos, M. C., Wilson, P., & Thomson, J. D. (2003). Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution*, *57*(12), 2742–2752. doi: 10.1111/j.0014-3820.2003.tb01516.x

Cavers, S., Degen, B., Caron, H., Lemes, M. R., Margis, R., Salgueiro, F., & Lowe, A. J. (2005). Optimal sampling strategy for estimation of spatial genetic structure in tree populations. *Heredity*, *95*(4), 281–289. doi: 10.1038/sj.hdy.6800709

Charlesworth, D. (2003). Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *358*(1434), 1051–1070. doi: 10.1098/rstb.2003.1296

Cresswell, J. E., Bassom, A. P., Bell, S. A., Collins, S. J., & Kelly, T. B. (1995). Predicted pollen dispersal by honey-bees and three species of bumble-bees foraging on oil-seed rape: A comparison of three models. *Functional Ecology*, *9*, 829–841. doi: 10.2307/2389980

Crimmins, T. M., Crimmins, M. A., & Bertelsen, C. D. (2011). Onset of summer flowering in a ‘Sky Island’is driven by monsoon moisture. *New Phytologist*, *191*(2), 468–479. doi: 10.1111/j.1469-8137.2011.03705.x

Dick, C. W., Hardy, O. J., Jones, F. A., & Petit, R. J. (2008). Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology*, *1*(1), 20–33. doi: 10.1007/s12042-007-9006-6

Duminil, J., Fineschi, S., Hampe, A., Jordano, P., Salvini, D., Vendramin, G. G., & Petit, R. J. (2007). Can population genetic structure be predicted from life-history traits? *The American Naturalist*, *169*(5), 662–672. doi: 10.1086/513490

Duminil, J., Hardy, O. J., & Petit, R. J. (2009). Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evolutionary Biology*, *9*(1), 177. doi: 10.1186/1471-2148-9-177

Ellstrand, N. C. (1992). Gene flow by pollen: Implications for plant conservation genetics. *Oikos*, *63*(1), 77–86. doi: 10.2307/3545517

Ellstrand, N. C. (2014). Is gene flow the most important evolutionary force in plants? *American Journal of Botany*, *101*(5), 737–753. doi: 10.3732/ajb.1400024

Ellstrand, N. C., & Elam, D. R. (1993). Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics*, *24*(1), 217–242. doi: 10.1146/annurev.es.24.110193.001245

Escaravage, N., & Wagner, J. (2004). Pollination effectiveness and pollen dispersal in a *Rhododendron ferrugineum* (Ericaceae) population. *Plant Biology*, *6*(05), 606–615. doi: 10.1055/s-2004-821143

Esquerré, D., Brennan, I. G., Catullo, R. A., Torres-Pérez, F., & Keogh, J. S. (2019). How mountains shape biodiversity: The role of the Andes in biogeography, diversification, and reproductive biology in South America's most species-rich lizard radiation (Squamata: Liolaemidae). *Evolution*, *73*(2), 214–230. doi: 10.1111/evo.13657

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), 479–491.

Fine, P. V. A., & Ree, R. H. (2006). Evidence for a time-integrated species-area effect on the latitudinal gradient in tree diversity. *The American Naturalist*, *168*(6), 796–804. doi: 10.1086/508635

Finger, A., Kaiser-Bunbury, C. N., Kettle, C. J., Valentin, T., & Ghazoul, J. (2014). Genetic connectivity of the moth pollinated tree *Glionnetia sericea* in a highly fragmented habitat. *PLoS ONE*, *9*(10), e111111. doi: 10.1371/journal.pone.0111111

Fox, J., & Monette, G. (1992). Generalized collinearity diagnostics. *Journal of the American Statistical Association*, *87*(417), 178–183. doi: 10.1080/01621459.1992.10475190

Freckleton, R. P. (2009). The seven deadly sins of comparative analysis. *Journal of Evolutionary Biology*, *22*(7), 1367–1375. doi: 10.1111/j.1420-9101.2009.01757.x

Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *The American Naturalist*, *160*(6), 712–726. doi: 10.1086/343873

Garland, T., Dickerman, A. W., Janis, C. M., & Jones, J. A. (1993). Phylogenetic analysis of covariance by computer simulation. *Systematic Biology*, *42*(3), 265–292. doi: 10.1093/sysbio/42.3.265

Gaudinier, A., & Blackman, B. K. (2019). Evolutionary processes from the perspective of flowering time diversity. *New Phytologist*, nph.16205. doi: 10.1111/nph.16205

Gelmi-Candusso, T. A., Heymann, E. W., & Heer, K. (2017). Effects of zoochory on the spatial genetic structure of plant populations. *Molecular Ecology*, *26*(21), 5896–5910. doi: 10.1111/mec.14351

Ghalambor, C. K. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative and Comparative Biology*, *46*(1), 5–17. doi: 10.1093/icb/icj003

Givnish, T. J. (2010). Ecology of plant speciation. *TAXON*, *59*(5), 1326–1366. doi: 10.1002/tax.595003

Goodwillie, C., Kalisz, S., & Eckert, C. G. (2005). The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 47–79. doi: 10.1146/annurev.ecolsys.36.091704.175539

Hahn, C. Z., Michalski, S. G., Fischer, M., & Durka, W. (2016). Genetic diversity and differentiation follow secondary succession in a multi-species study on woody plants from subtropical China. *Journal of Plant Ecology*, rtw054. doi: 10.1093/jpe/rtw054

Hamrick, J. L., & Godt, M. J. W. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions: Biological Sciences*, *351*(1345), 1291–1298. doi: 10.1098/rstb.1996.0112

Hamrick, J. L., Godt, M. J. W., & Sherman-Broyles, S. L. (1992). Factors influencing levels of genetic diversity in woody plant species. In: Adams W.T., Strauss S.H., Copes D.L., Griffin A.R. (Eds.), *Population Genetics of Forest Trees. Forestry Sciences, vol 42*. (pp. 95–124). Berlin, Germany: Springer. doi: 10.1007/978-94-011-2815-5_7

Hamrick, J. L., Murawski, D. A., & Nason, J. D. (1993). The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio*, *107*(1), 281–297. doi:10.1007/BF00052230

Hamrick, J.L., & Trapnell, D. W. (2011). Using population genetic analyses to understand seed dispersal patterns. *Acta Oecologica*, *37*(6), 641–649. doi: 10.1016/j.actao.2011.05.008

Harvey, M. G., Seeholzer, G. F., Smith, B. T., Rabosky, D. L., Cuervo, A. M., & Brumfield, R. T. (2017). Positive association between population genetic differentiation and speciation rates in New World birds. *Proceedings of the National Academy of Sciences*, *114*(24), 6328–6333. doi: 10.1073/pnas.1617397114

Harvey, M. G., Singhal, S., & Rabosky, D. L. (2019). Beyond reproductive isolation: demographic controls on the speciation process. *Annual Review of Ecology, Evolution, and Systematics*, *50*(1), 75–95. doi: 10.1146/annurev-ecolsys-110218-024701

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution*, *59*(8), 1633–1638. doi: 10.1111/j.0014-3820.2005.tb01814.x

Hey, J., & Pinho, C. (2012). Population genetics and objectivity in species diagnosis: Population genetics and species diagnosis. *Evolution*, *66*(5), 1413–1429. doi: 10.1111/j.1558-5646.2011.01542.x

Ho, T. L., & Ané, C. (2014). A linear-time algorithm for gaussian and non-gaussian trait evolution models. *Systematic Biology*, *63*(3), 397–408. doi: 10.1093/sysbio/syu005

Holsinger, K. E., & Weir, B. S. (2009). Genetics in geographically structured populations: Defining, estimating and interpreting FST. *Nature Reviews Genetics*, *10*(9), 639–650. doi: 10.1038/nrg2611

Ives, A. R. (2018). R2s for correlated data: Phylogenetic models, LMMs, and GLMMs. *Systematic Biology*, *68*(2), 234–251. doi: 10.1093/sysbio/syy060

Ives, A. R., & Li, D. (2018). rr2: An R package to calculate R2s for regression models. *J. Open Source Software*, *3*(30), 1028. doi: 10.21105/joss.01028

Janzen, D. H. (1971). Euglossine bees as long-distance pollinators of tropical plants. *Science, New Series*, *171*(3967), 203–205. doi: 10.1126/science.171.3967.203

Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist*, *101*(919), 233–249. doi: 10.1086/282487

Jin, Y., & Qian, H. (2019). V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography*, ecog.04434. doi: 10.1111/ecog.04434

Jombart, T., Balloux, F., & Dray, S. (2010). Adephylo: new tools for investigating the phylogenetic signal in biological traits. *Bioinformatics*, *26*(15), 1907–1909. doi: 10.1093/bioinformatics/btq292

Kalinowski, S. T. (2005). Do polymorphic loci require large sample sizes to estimate genetic distances? *Heredity*, *94*(1), 33–36. doi: 10.1038/sj.hdy.6800548

Kerkhoff, A. J., Moriarty, P. E., & Weiser, M. D. (2014). The latitudinal species richness gradient in New World woody angiosperms is consistent with the tropical conservatism hypothesis. *Proceedings of the National Academy of Sciences*, *111*(22), 8125–8130. doi: 10.1073/pnas.1308932111

Kisel, Y., & Barraclough, T. G. (2010). Speciation has a spatial scale that depends on levels of gene flow. *The American Naturalist*, *175*(3), 316–334. doi: doi.org/10.1086/650369

Kramer, A. T., & Havens, K. (2009). Plant conservation genetics in a changing world. *Special Issue: Plant Science Research in Botanic Gardens*, *14*(11), 599–607. doi: 10.1016/j.tplants.2009.08.005

Leimu, R., & Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PLoS ONE*, *3*(12), e4010. doi: 10.1371/journal.pone.0004010

Lemke, T. O. (1984). Foraging ecology of the long-nosed bat, *Glossophaga soricina*, with respect to resource availability. *Ecology*, *65*(2), 538–548. doi: 10.2307/1941416

Lemke, T. O. (1985). Pollen carrying by the nectar-feeding bat *Glossophaga soricina* in a suburban environment. *Biotropica*, *17*(2), 107–111. doi: 10.2307/2388502

Levine, J. M., & Murrell, D. J. (2003). The community-level consequences of seed dispersal patterns. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 549–574. doi: 10.1146/annurev.ecolsys.34.011802.132400

Linhart, Y. B., & Grant, M. C. (1996). Evolutionary Significance of Local Genetic Differentiation in Plants. *Annual Review of Ecology and Systematics*, *2*, 237–277. doi: 10.1146/annurev.ecolsys.27.1.237

Loiselle, B. A., Sork, V. L., Nason, J., & Graham, C. (1995). Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, *82*(11), 1420–1425. doi: 10.1002/j.1537-2197.1995.tb12679.x

López-Uribe, M. M., Oi, C. A., & Del Lama, M. A. (2008). Nectar-foraging behavior of Euglossine bees (Hymenoptera: Apidae) in urban areas. *Apidologie*, *39*(4), 410–418. doi: 10.1051/apido:2008023

Loveless, M. D., & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics*, *1*, 65–95. doi: 10.1146/annurev.es.15.110184.000433

Mangiafico, S. (2018). rcompanion: functions to support extension education program evaluation. *R package version 2.0. 0*.

Martin, P. R., Bonier, F., Moore, I., & Tewksbury, J. (2009). Latitudinal variation in the asynchrony of seasons: implications for higher rates of population differentiation and speciation in the tropics. *Ideas in Ecology and Evolution*, *2*, 9–17. doi: 10.4033/iee.2009.2.3.n

Martin, P. R., & McKay, J. K. (2004). Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evolution*, *58*(5), 938–945. doi: 10.1111/j.0014-3820.2004.tb00428.x

McCauley, D. E. (1994). Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: implications for studies of gene flow in plants. *Proceedings of the National Academy of Sciences*, *91*(17), 8127–8131. doi: 10.1073/pnas.91.17.8127

McCulloch, E. S., Tello, J. S., Whitehead, A., Rolón-Mendoza, C. M., Maldonado-Rodríguez, M. C., & Stevens, R. D. (2013). Fragmentation of Atlantic Forest has not affected gene flow of a widespread seed-dispersing bat. *Molecular Ecology*, *22*(18), 4619–4633. doi: 10.1111/mec.12418

Meirmans, P. G., & Hedrick, P. W. (2011). Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources*, *11*(1), 5–18. doi: 10.1111/j.1755-0998.2010.02927.x

Mittelbach, G. G., Schemske, D. W., Cornell, H. V., Allen, A. P., Brown, J. M., Bush, M. B., … Turelli, M. (2007). Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters*, *10*(4), 315–331. doi: 10.1111/j.1461-0248.2007.01020.x

Molina-Venegas, R., & Rodríguez, M. Á. (2017). Revisiting phylogenetic signal; strong or negligible impacts of polytomies and branch length information? *BMC Evolutionary Biology*, *17*(1), 53. doi: 10.1186/s12862-017-0898-y

Muchhala, N., & Thomson, J. D. (2010). Fur versus feathers: pollen delivery by bats and hummingbirds and consequences for pollen production. *The American Naturalist*, *175*(6), 717–726. doi: 10.1086/652473

Murawski, D. A., & Hamrick, J. L. (1991). The effect of the density of flowering individuals on the mating systems of nine tropical tree species. *Heredity*, *67*(2), 167–174. doi: 10.1038/hdy.1991.76

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences*, *70*(12), 3321. doi: 10.1073/pnas.70.12.3321

Nilsson, C., Brown, R. L., Jansson, R., & Merritt, D. M. (2010). The role of hydrochory in structuring riparian and wetland vegetation. *Biological Reviews*, *85*(4), 837–858. doi: 10.1111/j.1469-185X.2010.00129.x

Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, *13*(5), 1143–1155. doi: 10.1111/j.1365-294X.2004.02141.x

Nybom, H., & Bartish, I. V. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, *3*(2), 93–114. doi: 10.1078/1433-8319-00006

Ohashi, K., & Thomson, J. D. (2009). Trapline foraging by pollinators: Its ontogeny, economics and possible consequences for plants. *Annals of Botany*, *103*(9), 1365–1378. doi: 10.1093/aob/mcp088

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, *401*(6756), 877–884. doi: 10.1038/44766

Pascual, M., Rives, B., Schunter, C., & Macpherson, E. (2017). Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PLOS ONE*, *12*(5), e0176419. doi: 10.1371/journal.pone.0176419

Pavoine, S., Ollier, S., Pontier, D., & Chessel, D. (2008). Testing for phylogenetic signal in phenotypic traits: New matrices of phylogenetic proximities. *Theoretical Population Biology*, *73*(1), 79–91. doi: 10.1016/j.tpb.2007.10.001

Petit, R. J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D., & Vendramin, G. G. (2005). Invited review: Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology*, *14*(3), 689–701. doi: 10.1111/j.1365-294X.2004.02410.x

Plackett, R. L. (1983). Karl Pearson and the Chi-Squared Test. *International Statistical Review / Revue Internationale de Statistique*, *51*(1), 59–72. doi: 10.2307/1402731

Quintero, I., González-Caro, S., Zalamea, P.-C., & Cadena, C. D. (2014). Asynchrony of seasons: Genetic differentiation associated with geographic variation in climatic seasonality and reproductive phenology. *The American Naturalist*, *184*(3), 352–363. doi: 10.1086/677261

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Revell, L. J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223. doi: 10.1111/j.2041-210X.2011.00169.x

Rhodes, M. K., Fant, J. B., & Skogen, K. A. (2017). Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Molecular Ecology*, *26*(16), 4296–4308. doi: 10.1111/mec.14115

Ricklefs, R. E. (1977). Environmental heterogeneity and plant species diversity: A hypothesis. *The American Naturalist*, *111*(978), 376–381. doi: 10.1086/283169

Ricklefs, R. E. (1987). Community diversity: Relative roles of local and regional processes. *Science*, *235*(4785), 167–171. doi: 10.1126/science.235.4785.167

Ricklefs, R. E. (2004). A comprehensive framework for global patterns in biodiversity. *Ecology Letters*, *7*(1), 1–15. doi: 10.1046/j.1461-0248.2003.00554.x

Ricklefs, R. E. (2006). Evolutionary diversification and the origin of the diversity–environment relationship. *Ecology*, *87*(sp7), S3–S13. doi: 10.1890/0012-9658(2006)87[3:EDATOO]2.0.CO;2

Riginos, C., Douglas, K. E., Jin, Y., Shanahan, D. F., & Treml, E. A. (2011). Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography*, *34*(4), 566–575. doi: 10.1111/j.1600-0587.2010.06511.x

Rolland, J., Condamine, F. L., Jiguet, F., & Morlon, H. (2014). Faster speciation and reduced extinction in the tropics contribute to the mammalian latitudinal diversity gradient. *PLoS Biology*, *12*(1), e1001775. doi: 10.1371/journal.pbio.1001775

Sarmiento, G. (1972). Ecological and floristic convergences between seasonal plant formations of tropical and subtropical South America. *Journal of Ecology*, *60*(2), 367–410. doi: 10.2307/2258353

Schemske, D. W., Mittelbach, G. G., Cornell, H. V., Sobel, J. M., & Roy, K. (2009). Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 245–269. doi: 10.1146/annurev.ecolsys.39.110707.173430

Singhal, S., Huang, H., Grundler, M. R., Marchán-Rivadeneira, M. R., Holmes, I., Title, P. O., … Rabosky, D. L. (2018). Does population structure predict the rate of speciation? A comparative test across Australia's most diverse vertebrate radiation. *The American Naturalist*, *192*(4), 432–447. doi: 10.1086/699515

Sitnikov I.G. (2009). Principal weather systems in subtropical and tropical zones. In Gruzaayloa, G.V (Ed.), *Environmental structure and function: Climate system* (pp. 120–139). Oxford, UK: EOLSS.

Skogen, K. A., Overson, R. P., Hilpman, E. T., & Fant, J. B. (2019). Hawkmoth pollination facilitates long-distance pollen dispersal and reduces isolation across a gradient of land-use change. *Annals of the Missouri Botanical Garden*, *104*(3), 495–511. doi: 10.3417/2019475

Smith, B. T., McCormack, J. E., Cuervo, A. M., Hickerson, M. J., Aleixo, A., Cadena, C. D., … Harvey, M. G. (2014). The drivers of tropical speciation. *Nature*, *515*(7527), 406. doi: 10.1038/nature13687

Smith, S. A., & Brown, J. W. (2018). Constructing a broadly inclusive seed plant phylogeny. *American Journal of Botany*, *105*(3), 302–314. doi: 10.1002/ajb2.1019

Sork, V. L., Nason, J., Campbell, D. R., & Fernandez, J. F. (1999). Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology & Evolution*, *14*(6), 219–224. doi: 10.1016/S0169-5347(98)01585-7

Stein, A., Gerstner, K., & Kreft, H. (2014). Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters*, *17*(7), 866–880. doi: 10.1111/ele.12277

Stevens, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 14, July 2017 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/.

Symonds, M. R. E., & Blomberg, S. P. (2014). A primer on Phylogenetic Generalized Least Squares. In L. Z. Garamszegi (Ed.), *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology* (pp. 105–130). doi: 10.1007/978-3-662-43550-2_5

Tello-Ramos, M. C., Hurly, T. A., & Healy, S. D. (2015). Traplining in hummingbirds: Flying short-distance sequences among several locations. *Behavioral Ecology*, *26*(3), 812–819. doi: 10.1093/beheco/arv014

Templeton, A. R. (1981). Mechanisms of speciation - A population genetic approach. *Annual Review of Ecology and Systematics*, *12*(1), 23–48. doi: 10.1146/annurev.es.12.110181.000323

ter Steege, H., Pitman, N. C. A., Sabatier, D., Baraloto, C., Salomão, R. P., Guevara, J. E., … Silman, M. R. (2013). Hyperdominance in the Amazonian tree flora. *Science*, *342*(6156), 1243092. doi: 10.1126/science.1243092

Theim, T. J., Shirk, R. Y., & Givnish, T. J. (2014). Spatial genetic structure in four understory *Psychotria* species (Rubiaceae) and implications for tropical forest diversity. *American Journal of Botany*, *101*(7), 1189–1199. doi: 10.3732/ajb.1300460

Tukey, J. W. (1970). *Exploratory Data Analysis: Limited Preliminary Ed*. Boston, MA: Addison-Wesley Publishing Company.

Vekemans, X., & Hardy, O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, *13*(4), 921–935. doi: 10.1046/j.1365-294X.2004.02076.x

Wallace, A. R. (1854). On the monkeys of the Amazon. *Annals and Magazine of Natural History*, *14*(84), 451–454. doi: 10.1080/037454809494374

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the analysis of population structure. *Evolution*, *38*(6), 1358. doi: 10.2307/2408641

Willing, E.-M., Dreyer, C., & van Oosterhout, C. (2012). Estimates of genetic differentiation measured by F_{ST} do not necessarily require large sample sizes when using many SNP markers. *PLoS ONE*, *7*(8), e42649. doi: 10.1371/journal.pone.0042649

Wright, S. (1943). Isolation by distance. *Genetics*, *28*(2), 114–138. PMID: 17247074, PMCID: PMC1209196

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics*, *15*(1), 323–354. doi: 10.1111/j.1469-1809.1949.tb02451.x

Wright, S. I., Ness, R. W., Foxe, J. P., & Barrett, S. C. H. (2008). Genomic consequences of outcrossing and selfing in plants. *International Journal of Plant Sciences*, *169*(1), 105–118. doi: 10.1086/523366

Data accessibility statement

Should the manuscript be accepted, the data and R scripts supporting the results will be archived in Dryad and their DOI will be included at the end of this article. No new data were used in this research because analyses were based on a literature review of published studies.

Author Contributions

DG and NM planned and designed the research. DG collected and analyzed the data. DG wrote the first draft of the manuscript. DG and NM contributed equally to substantial revisions of the manuscript.

Table 1 Phylogenetic multiple regressions explaining variation in F_{ST}. In each model only the main effect of factors is considered, i.e., no interactions. AIC and λ fit (scaling parameter to correct for phylogeny) were estimated using maximum likelihood. Underlined variables indicate that at least one of their terms was a significant factor in the corresponding model. (Thick underline: $P \leq 0.005$, thin underline: $0.005 < P < 0.05$) (next page).

MODEL	Variables †	R_2	AIC	λ fit
Null model	genetic marker mean sample size ‡ distance §	0.36	–437	0.57
Model 1	null model 	0.41	–463.5	0.48
Model 2	null model 	0.42	–466	0.46
Model 3	null model 	0.43	–480.1	0.37
Model 4	null model 	0.44	–482.3	0.35
Model 5	null model 	0.42	–488.6	<0.001
Model 6	null model 	0.45	–503.9	<0.001
Model 7	null model 	0.46	–502.9	<0.001

† yellow circle: mating system, green circle: growth form, brown circle: seed dispersal mode, red circle: pollination mode, blue circle: latitudinal region.

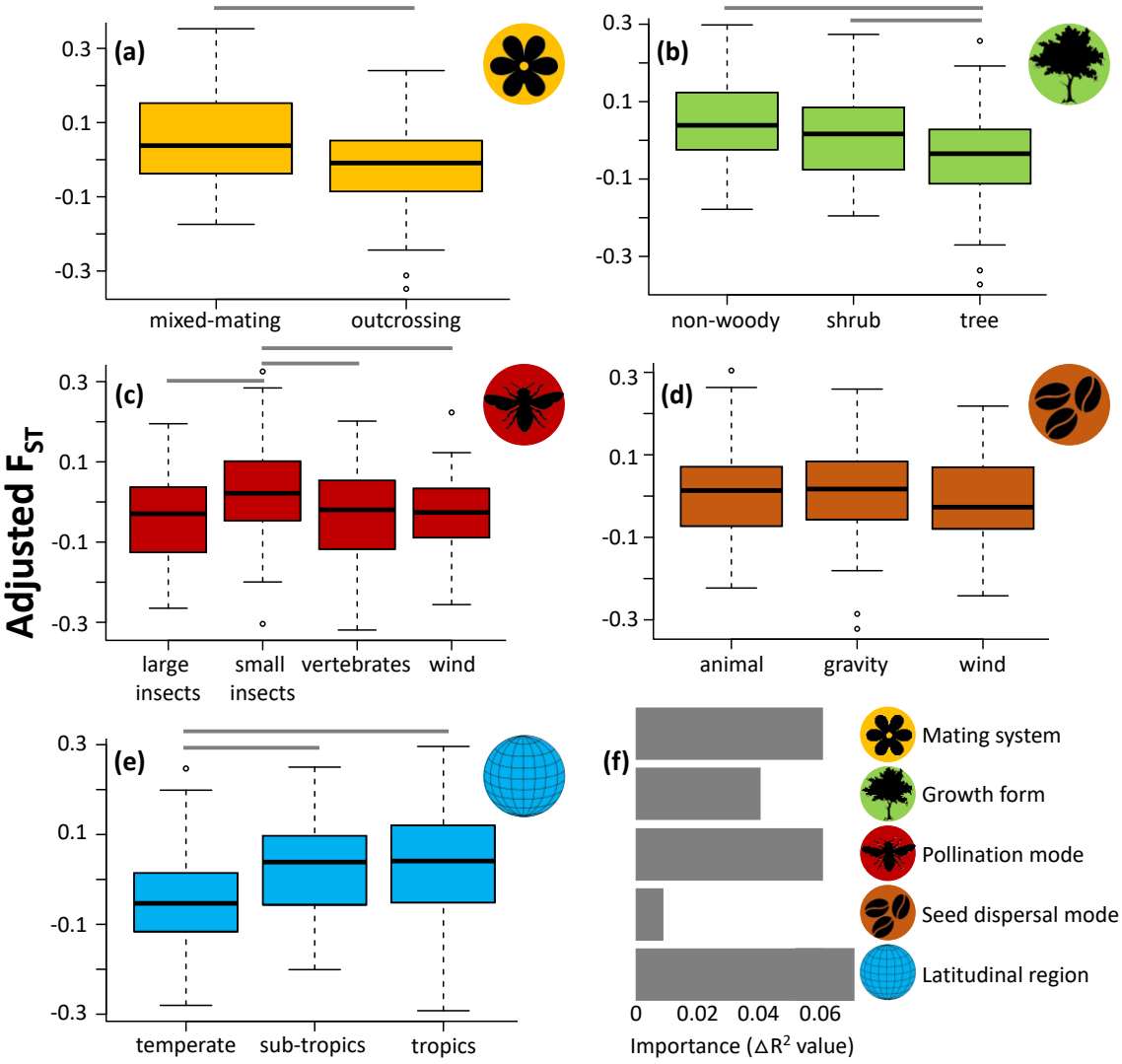
‡ mean sample size: natural logarithm of the mean sample size of individuals per population.

§ distance: natural logarithm of the maximum distance between populations.

Table 2 Details of model 7, the most inclusive phylogenetic model with factors of interest. Variables in bold indicate the reference level for each categorical factor. N indicates the sample size of each group without phylogenetic correction. Significant P values are in bold.

Variable	N	Estimate	Std. Error	T value	P value
Intercept		0.59	0.04	14.1	<0.001
Mating system					
Mixed-mating	80				
Outcrossing	257	–0.07	0.01	–4.7	**<0.001**
Growth form					
Tree	163				
Non-woody	121	0.09	0.02	5.3	**<0.001**
Shrub	53	0.06	0.02	3	**0.003**
Pollination mode					
Small insects	176				
Large insects	48	–0.06	0.02	–3.4	**0.001**
Vertebrates	44	–0.05	0.02	–2.6	**0.01**
Wind	69	–0.05	0.02	–3	**0.003**
Seed dispersal mode					
Gravity	82				
Animals	147	–0.003	0.02	–0.2	0.8
Wind	108	–0.02	0.02	–1.4	0.1
Latitudinal region					
Temperate	134				
Sub-tropical	78	0.07	0.02	4.5	**<0.001**
Tropical	125	0.09	0.02	5.4	**<0.001**

Fig. 1 Partial regression plots showing the effect of each factor on transformed F_{ST} values after accounting for the effect of other independent variables in model 7 (i.e., adjusted F_{ST}). Parallel boxplots of the partial residuals are drawn for the levels of each factor along with significant differences between groups depicted by the upper horizontal grey lines according to model 7 (Table 2): **(a)** mating system, **(b)** growth form, **(c)** pollination mode, **(d)** seed dispersal mode, and **(e)** latitudinal region. Thick horizontal black lines are median values, boxes indicate 25% and 75% quartiles, whiskers are maximum and minimum values, white circles are outliers. **(f)** Relative importance of each factor (ΔR_2 value); the change in R_2 after each individual factor is removed from model 7 (next page).


Adjusted F_{ST}
(a)
0.3
0.1
-0.1
-0.3
mixed-mating
outcrossing
(b)
non-woody
shrub
tree
(c)
large insects
small insects
vertebrates
wind
(d)
animal
gravity
wind
(e)
temperate
sub-tropics
tropics
(f)
0
0.02
0.04
0.06
Importance (ΔR^2 value)
Mating system
Growth form
Pollination mode
Seed dispersal mode
Latitudinal region

Additional supporting information that will appear in the expanded online version of this article:

Appendix S1. References of publications with data on F_{ST} and species traits used in this study.

Appendix S2. Data transformation.

Appendix S3. Tests of multicollinearity.

Appendix S4. Phylogeny.

Appendix S5. Phylogenetic signal.

Appendix S6. PhyloLM implementation.

Fig. S1. Phylogeny of studied species.

Fig. S2. Estimation of phylogenetic signal on model variables.

Table S1. Dataset used in this study (in Table S1.xlsx).

Table S2. Correlation tests between categorical variables.

Table S3. Estimates of the generalized variance inflation factor on predictors.

Table S4. Results from phylogenetic ANOVA on F_{ST}.

Table S5. Pairwise post-hoc tests between groups within each categorical variable, estimated after performing phylogenetic ANOVA.

Table S6. Details of model 7 including variables in the null model.

Appendix S1. References of publication with F_{ST} data and species traits used in this study.

de Abreu Moreira P, Brandão MM, de Araujo NH, de Oliveira DA, Fernandes GW. (2015). Genetic diversity and structure of the tree *Enterolobium contortisiliquum* (Fabaceae) associated with remnants of a seasonally dry tropical forest. *Flora - Morphology, Distribution, Functional Ecology of Plants* 210: 40–46.

Addisalem AB, Bongers F, Kassahun T, Smulders MJM. (2016). Genetic diversity and differentiation of the frankincense tree (*Boswellia papyrifera* (Del.) Hochst) across Ethiopia and implications for its conservation. *Forest Ecology and Management* 360: 253–260.

Affre L, Thompson JD. (1997). Population genetic structure and levels of inbreeding depression in the Mediterranean island endemic *Cyclamen creticum* (Primulaceae). *Biological Journal of the Linnean Society* 60: 527–549.

Afif M, Messaoud C, Boulila A, Chograni H, Bejaoui A, Rejeb MN, Boussaid M. (2008). Genetic structure of Tunisian natural carob tree (*Ceratonia siliqua* L.) populations inferred from RAPD markers. *Annals of Forest Science* 65: 710–710.

Alvarez-Buylla ER, Garay AA. (1994). Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. *Evolution* 48: 437–453.

Alves RM, Sebbenn AM, Artero AS, Clement C, Figueira A. (2007). High levels of genetic divergence and inbreeding in populations of cupuassu (*Theobroma grandiflorum*). *Tree Genetics & Genomes* 3: 289–298.

Amat ME, Silvertown J, Vargas P. (2013). Strong spatial genetic structure reduces reproductive success in the critically endangered plant genus *Pseudomisopates*. *Journal of Heredity* 104: 692–703.

Amico GC, Vidal-Russell R, Aizen MA, Nickrent D. (2014). Genetic diversity and population structure of the mistletoe *Tristerix corymbosus* (Loranthaceae). *Plant Systematics and Evolution* 300: 153–162.

Angelone S, Hilfiker K, Holderegger R, Bergamini A, Hoebee SE. (2007). Regional population dynamics define the local genetic structure in *Sorbus torminalis*. *Molecular Ecology* 16: 1291–1301.

Barbará T, Martinelli G, Fay MF, Mayo SJ, Lexer C. (2007). Population differentiation and species cohesion in two closely related plants adapted to neotropical high-altitude inselbergs, *Alcantarea imperialis* and *Alcantarea geniculata* (Bromeliaceae). *Molecular Ecology* 16: 1981–1992.

Barbará T, Martinelli G, Palma-Silva C, Fay MF, Mayo S, Lexer C. (2009). Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae). *Annals of Botany* 103: 65–77.

Baucom RS, Estill JC, Cruzan MB. (2005). The effect of deforestation on the genetic diversity and structure in *Acer saccharum* (Marsh): Evidence for the loss and restructuring of genetic variation in a natural system. *Conservation Genetics* 6: 39–50.

Beatty GE, Brown JA, Cassidy EM, Finlay CMV, McKendrick L, Montgomery WI, Reid N, Tosh DG, Provan J. (2015). Lack of genetic structure and evidence for long-distance dispersal in ash (*Fraxinus excelsior*) populations under threat from an emergent fungal pathogen: implications for restorative planting. *Tree Genetics and Genomes* 11: 53.

Beland JD, Krakowski J, Ritland CE, Ritland K, El-Kassaby YA. (2005). Genetic structure and mating system of northern *Arbutus menziesii* (Ericaceae) populations. *Canadian Journal of Botany* 83: 1581–1589.

Bessega C, Pometti CL, Ewens M, Saidman BO, Vilardi JC. (2016). Fine-scale spatial genetic structure analysis in two Argentine populations of *Prosopis alba* (Mimosoideae) with different levels of ecological disturbance. *European Journal of Forest Research* 135: 495–505.

Bizoux JP, Daïnou K, Bourland N, Hardy OJ, Heuertz M, Mahy G, Doucet JL. (2009). Spatial genetic structure in *Milicia excelsa* (Moraceae) indicates extensive gene dispersal in a low-density wind-pollinated tropical tree. *Molecular Ecology* 18: 4398–4408.

Bodare S, Ravikanth G, Ismail SA, Patel MK, Spanu I, Vasudeva R, Shaanker RU, Vendramin GG, Lascoux M, Tsuda Y. (2017). Fine- and local- scale genetic structure of *Dysoxylum malabaricum*, a late-successional canopy tree species in disturbed forest patches in the Western Ghats, India. *Conservation Genetics* 18: 1–15.

Boisselier-Dubayle M-C, Leblois R, Samadi S, Lambourdière J, Sarthou C. (2010). Genetic structure of the xerophilous bromeliad *Pitcairnia geyskesii* on inselbergs in French Guiana - a test of the forest refuge hypothesis. *Ecography* 33: 175–184.

Bottin L, Verhaegen D, Tassin J, Olivieri I, Vaillant A, Bouvet JM. (2005). Genetic diversity and population structure of an insular tree, *Santalum austrocaledonicum* in New Caledonian archipelago: sandalwood's genetic diversity and structure. *Molecular Ecology* 14: 1979–1989.

Brandão MM, Vieira F de A, Nazareno AG, Carvalho D de. (2015). Genetic diversity of neotropical tree *Myrcia splendens* (Myrtaceae) in a fragment–corridor system in the Atlantic rainforest. *Flora - Morphology, Distribution, Functional Ecology of Plants* 216: 35–41.

Broadhurst LM. (2012). Genetic diversity and population genetic structure in fragmented *Allocasuarina verticillata* (Allocasuarinaceae)–implications for restoration. *Australian Journal of Botany* 59: 770–780.

Broadhurst L, Coates D. (2002). Genetic diversity within and divergence between rare and geographically widespread taxa of the *Acacia acuminata* Benth. (Mimosaceae) complex. *Heredity* 88: 250–257.

Broadhurst LM, Coates DJ. (2004). Genetic divergence among and diversity within two rare *Banksia* species and their common close relative in the

subgenus *Isostylis* R.Br. (Proteaceae). *Conservation Genetics* 5: 837–846.

Broadhurst LM, Young AG, Murray BG. (2008). AFLPs reveal an absence of geographical genetic structure among remnant populations of pohutukawa (*Metrosideros excelsa*, Myrtaceae). *New Zealand Journal of Botany* 46: 13–21.

Brousseau L, Foll M, Scotti-Saintagne C, Scotti I. (2015). Neutral and adaptive drivers of microgeographic genetic divergence within continuous populations: the case of the neotropical tree *Eperua falcata* (Aubl.). *PLOS ONE* 10: e0121394.

Browne L, Ottewell K, Karubian J. (2015). Short-term genetic consequences of habitat loss and fragmentation for the neotropical palm *Oenocarpus bataua*. *Heredity* 115: 389–395.

Bustamante E, Búrquez A, Scheinvar E, Eguiarte LE. (2016). Population genetic structure of a widespread bat-pollinated columnar cactus. *PLOS ONE* 11: e0152329.

Butcher PA, McDonald MW, Bell JC. (2009). Congruence between environmental parameters, morphology and genetic structure in Australia's most widely distributed eucalypt, *Eucalyptus camaldulensis*. *Tree Genetics & Genomes* 5: 189–210.

Caetano S, Prado D, Pennington RT, Beck S, Oliveira-Filho A, Spichiger R, Naciri Y. (2008). The history of Seasonally Dry Tropical Forests in eastern South America: inferences from the genetic structure of the tree *Astronium urundeuva* (Anacardiaceae). *Molecular Ecology* 17: 3147–3159.

Caldiz MS, Premoli AC. (2005). Isozyme diversity in large and isolated populations of *Luma apiculata* (Myrtaceae) in north-western Patagonia, Argentina. *Australian Journal of Botany* 53: 781.

Campbell DR, Dooley JL. (1992). The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation by distance. *The American Naturalist* 139: 735–748.

Cavallari MM, Forzza RC, Veasey EA, Zucchi MI, Oliveira GCX. (2006). Genetic variation in three endangered species of *Encholirium* (Bromeliaceae) from Cadeia do Espinhaço, Brazil, detected using RAPD Markers. *Biodiversity and Conservation* 15: 4357–4373.

Cerón-Souza I, Bermingham E, McMillan W, Jones F. (2012). Comparative genetic structure of two mangrove species in Caribbean and Pacific estuaries of Panama. *BMC Evolutionary Biology* 12: 205.

Chase MR, Boshier DH, Bawa KS. (1995). Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree. 1. Genetic variation in natural populations. *American Journal of Botany* 82: 468–475.

Cheng Y-P, Hwang S-Y, Chiou W-L, Lin T-P. (2006). Allozyme variation of populations of *Castanopsis carlesii* (fagaceae) revealing the diversity centers and areas of the greatest divergence in Taiwan. *Annals of Botany* 98: 601–608.

Cheng J, Lyu L-S, Shen Y-B, Li K-X, Liu Z-H, Wang W-X, Xie L. (2016). Population structure and genetic diversity of *Lithocarpus litseifolius*

(Fagaceae) assessed using microsatellite markers. *Nordic Journal of Botany* 34: 752–760.

Chung MG. (2000). Spatial distribution of allozyme polymorphisms following clonal and sexual reproduction in populations of *Rhus javanica* (Anacardiaceae). *Heredity* 84: 178.

Chung MY, Kim K-J, Pak J-H, Park C-W, Sun B-Y, Myers ER, Chung MG. (2005). Inferring establishment histories in populations of *Quercus dentata* (Fagaceae) from the analysis of spatial genetic structure. *Plant Systematics and Evolution* 250: 231–242.

Chung MY, Nason J, Chung MG, Kim K-J, Park C-W, Sun B-Y, Pak J-H. (2002). Landscape-level spatial genetic structure in *Quercus acutissima* (Fagaceae). *American Journal of Botany* 89: 1229–1236.

Chybicki IJ, Oleska A, Burczyk J. (2011). Increased inbreeding and strong kinship structure in *Taxus baccata* estimated from both AFLP and SSR data. *Heredity* 107: 589–600.

Coart E, Vekemans X, Smulders MJM, Wagner I, Van Huylenbroeck J, Van Bockstaele E, Roldan-Ruiz I. (2003). Genetic variation in the endangered wild apple (*Malus sylvestris* (L.) Mill.) in Belgium as revealed by amplified fragment length polymorphism and microsatellite markers. *Molecular Ecology* 12: 845–857.

Cole CT, Biesboer DD. (1992). Monomorphism, reduced gene flow, and cleistogamy in rare and common species of *Lespedeza* (Fabaceae). *American Journal of Botany* 79: 567–575.

Collevatti RG, Grattapaglia D, Hay JD. (2001). Population genetic structure of the endangered tropical tree species *Caryocar brasiliense*, based on variability at microsatellite loci. *Molecular ecology* 10: 349–356.

Collevatti RG, Telles MPC, Lima JS, Gouveia FO, Soares TN. (2014). Contrasting spatial genetic structure in *Annona crassiflora* populations from fragmented and pristine savannas. *Plant Systematics and Evolution* 300: 1719–1727.

Colling G, Hemmer P, Bonniot A, Hermant S, Matthies D. (2010). Population genetic structure of wild daffodils (*Narcissus pseudonarcissus* L.) at different spatial scales. *Plant Systematics and Evolution* 287: 99–111.

Conson ARO, Ruas EA, Vieira BG, Rodrigues LA, Costa BF, Bianchini E, Prioli AJ, de Fátima Ruas C, Ruas PM. (2013). Genetic structure of the Atlantic Rainforest tree species *Luehea divaricata* (Malvaceae). *Genetica* 141: 205–215.

Conte R, Sedrez dos Reis M, Mantovani A, Vencovsky R. (2008). Genetic structure and mating system of *Euterpe edulis* Mart. populations: a comparative analysis using microsatellite and allozyme markers. *Journal of Heredity* 99: 476–482.

Costa J, Vaillancourt RE, Steane DA, Jones RC, Marques C. (2017). Microsatellite analysis of population structure in *Eucalyptus globulus* (AL Hipp, Ed.). *Genome* 60: 770–777.

Cota LG, Moreira PA, Brandão MM, Royo VA, Junior AFM, Menezes EV, Oliveira DA. (2017). Structure and genetic diversity of *Anacardium humile* (Anacardiaceae): a tropical shrub. *Genetics and Molecular Research* 16.

Culley TM, Wolfe AD. (2001). Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity* 86: 545–556.

Daïnou K, Mahy G, Duminil J, Dick CW, Doucet J-L, Donkpégan ASL, Pluijgers M, Sinsin B, Lejeune P, Hardy OJ. (2014). Speciation slowing down in widespread and long-living tree taxa: insights from the tropical timber tree genus *Milicia* (Moraceae). *Heredity* 113: 74–85.

Damasceno JO, Ruas EA, Rodrigues LA, Ruas CF, Bianchini E, Pimenta JA, Ruas PM. (2011). Genetic differentiation in *Aspidosperma polyneuron* (Apocynaceae) over a short geographic distance as assessed by AFLP markers. *Genetics and Molecular Research* 10: 1180–1187.

Dangasuk OG, Gudu S. (2000). Allozyme variation in 16 natural populations of *Faidherbia albida* (Del.) A. Chev. *Hereditas* 133: 133–145.

Debout GDG, Doucet J-L, Hardy OJ. (2011). Population history and gene dispersal inferred from spatial genetic structure of a Central African timber tree, *Distemonanthus benthamianus* (Caesalpinioideae). *Heredity* 106: 88–99.

De Carvalho MCCG, Da Silva DGG, Ruas PM, Medri ME, Ruas CF. Flooding tolerance and genetic diversity in populations of *Luehea divaricata*. *Biologia Plantarum* 52: 771–774.

De-Lucas AI, González-Martínez SC, Vendramin GG, Hidalgo E, Heuertz M. (2009). Spatial genetic structure in continuous and fragmented populations of *Pinus pinaster* Aiton. *Molecular Ecology* 18: 4564–4576.

de Melo Jr. AF, de Carvalho D, Vieira FA, Oliveira DA. (2012). Spatial genetic structure in natural populations of *Caryocar brasiliense* Camb. (Caryocareceae) in the North of Minas Gerais, Brazil. *Biochemical Systematics and Ecology* 43: 205–209.

Demenou BB, Doucet J-L, Hardy OJ. (2018). History of the fragmentation of the African rain forest in the Dahomey Gap: insight from the demographic history of *Terminalia superba*. *Heredity* 120: 547–561.

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.

Doligez A, Joly HI. (1997). Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, *Carapa procera* (Meliaceae), in French Guiana. *Heredity* 79:72–82.

Dry P, Burdon J. (1986). Genetic structure of natural populations of wild sunflowers (*Helianthus annuus* L.) in Australia. *Australian Journal of Biological Sciences* 39: 255.

Duminil J, Daïnou K, Kaviriri DK, Gillet P, Loo J, Doucet J-L, Hardy OJ. (2016). Relationships between population density, fine-scale genetic structure, mating system and pollen dispersal in a timber tree from African rainforests. *Heredity* 116: 295–303.

Dutech C, Joly HI, Jarne P. (2004). Gene flow, historical population dynamics and genetic diversity within French Guianan populations of a rainforest tree species, *Vouacapoua americana*. *Heredity* 92: 69–77.

El Mousadik A, Petit RJ. (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics* 92: 832–839.

England PR, Usher AV, Whelan RJ, Ayre DJ. (2002). Microsatellite diversity and genetic structure of fragmented populations of the rare, fire-dependent shrub *Grevillea macleayana*. *Molecular Ecology* 11: 967–977.

Fant JB, Havens K, Keller JM, Radosavljevic A, Yates ED. (2014). The influence of contemporary and historic landscape features on the genetic structure of the sand dune endemic, *Cirsium pitcheri* (Asteraceae). *Heredity* 112: 519–530.

Fenster CB, Vekemans X, Hardy OJ. (2003). Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculata* (Leguminosae). *Evolution* 57: 995–1007.

Fontaine C, Lovett PN, Sanou H, Maley J, Bouvet J-M. (2004). Genetic diversity of the shea tree (*Vitellaria paradoxa* C.F. Gaertn), detected by RAPD and chloroplast microsatellite markers. *Heredity* 93: 639–648.

Foster PF, Sork VL. (1997). Population and genetic structure of the West African rain forest liana *Ancistrocladus korupensis* (Ancistrocladaceae). *American Journal of Botany* 84: 1078–1091.

Franceschinelli EV, Kesseli R. (1999). Population structure and gene flow of the Brazilian shrub *Helicteres brevispira*. *Heredity* 82: 355–363.

Frascaria N, Santi F, Gouyon PH. (1993). Genetic differentiation within and among populations of chestnut (*Castanea sativa* Mill.) and wild cherry (*Prunus avium* L.). *Heredity* 70: 634–641.

Fuchs EJ, Hamrick JL. (2010). Genetic Diversity in the Endangered Tropical Tree, *Guaiacum sanctum* (Zygophyllaceae). *Journal of Heredity* 101: 284–291.

Ganzhorn SM, Thomas WW, Gaiotto FA, Lewis JD. (2015). Spatial genetic structure of *Manilkara maxima* (Sapotaceae), a tree species from the Brazilian Atlantic forest. *Journal of Tropical Ecology* 31: 437–447.

Gaudeul M, Till-Bottraud I, Barjon F, Manel S. (2004). Genetic diversity and differentiation in *Eryngium alpinum* L. (Apiaceae): comparison of AFLP and microsatellite markers. *Heredity* 92: 508–518.

Ge JP, Cai B, Ping W, Song G, Ling H, Lin P. (2005). Mating system and population genetic structure of *Bruguiera gymnorrhiza* (Rhizophoraceae), a viviparous mangrove species in China. *Journal of Experimental Marine Biology and Ecology* 326: 48–55.

Godt MJW, Hamrick JL. (1993). Genetic diversity and population structure in *Tradescantia hirsuticaulis* (Commelinaceae). *American Journal of Botany* 80: 959–966.

Godt MJW, Hamrick JL. (1999). Population genetic analysis of *Elliottia racemosa* (Ericaceae), a rare Georgia shrub. *Molecular Ecology* 8: 75–82.

Goetze M, Büttow MV, Zanella CM, Paggi GM, Bruxel M, Pinheiro FG, Sampaio JAT, Palma-Silva C, Cidade FW, Bered F. (2015). Genetic variation in *Aechmea winkleri*, a bromeliad from an inland Atlantic rainforest fragment in Southern Brazil. *Biochemical Systematics and Ecology* 58: 204–210.

Gonzalez-Astorga J. (2004). Diversity and genetic structure of the mexican endemic epiphyte *Tillandsia achyrostachys* E. Morr. ex Baker var. *achyrostachys* (Bromeliaceae). *Annals of Botany* 94: 545–551.

González-Martínez SC, Dubreuil M, Riba M, Vendramin GG, Sebastiani F, Mayol M. (2010). Spatial genetic structure of *Taxus baccata* L. in the western Mediterranean Basin: Past and present limits to gene movement over a broad geographic scale. *Molecular Phylogenetics and Evolution* 55: 805–815.

González-Pérez MA, Caujapé-Castells J, Sosa PA. (2004). Allozyme variation and structure of the Canarian endemic palm tree *Phoenix canariensis* (Arecaceae): implications for conservation. *Heredity* 93: 307–315.

Haase P. (1992). Isozyme variability and biogeography of *Nothofagus truncata* (Fagaceae). *New Zealand Journal of Botany* 30: 315–328.

Hahn T, Kettle CJ, Ghazoul J, Frei ER, Matter P, Pluess AR. (2012). Patterns of genetic variation across altitude in three plant species of semi-dry grasslands. *PLoS ONE* 7: e41608.

Hahn CZ, Michalski SG, Durka W. (2017). Gene flow in, and mating system of, *Rhododendron simsii* in a nature reserve in subtropical China. *Nordic Journal of Botany* 35: 1–7.

Hahn CZ, Michalski SG, Fischer M, Durka W. (2016). Genetic diversity and differentiation follow secondary succession in a multi-species study on woody plants from subtropical China. *Journal of Plant Ecology*: rtw054.

Hall P, Chase MR, Bawa KS. (1994). Low genetic variation but high population differentiation in a common tropical forest tree species. *Conservation Biology* 8: 471–482.

Hall P, Walker S, Bawa K. (1996). Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. *Conservation Biology* 10: 757–768.

Hansen OK, Changtragoon S, Ponoy B, Kjær ED, Minn Y, Finkeldey R, Nielsen KB, Graudal L. (2015). Genetic resources of teak (*Tectona grandis* Linn. f.)—strong genetic structure among natural populations. *Tree Genetics & Genomes* 11: 802.

Hardesty BD, Dick CW, Hamrick JL, Degen B, Hubbell SP, Bermingham E. (2010). Geographic influence on genetic structure in the widespread neotropical tree *Simarouba amara* (Simaroubaceae): landscape genetic diversity of *Simarouba amara*. *Tropical Plant Biology* 3: 28–39.

Hardy OJ, Vekemans X. (2001). Patterns of allozyme variation in diploid and tetraploid *Centaurea jacea* at different spatial scales. *Evolution* 55: 943–954.

Hardy OJ, Maggia L, Bandou E, Breyne P, Caron H, Chevallier M-E, Doligez A, Dutech C, Kremer A, Latouche-Hallé C, et al. (2006). Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species.

Molecular Ecology 15: 559–571.
He R, Wang J, Huang H. (2012). Long-distance gene dispersal inferred from spatial genetic structure in *Handeliodendron bodinieri*, an endangered tree from karst forest in southwest China. *Biochemical Systematics and Ecology* 44: 295–302.
Heer K, Kalko EKV, Albrecht L, García-Villacorta R, Staeps FC, Herre EA, Dick CW. (2015). Spatial scales of genetic structure in free-standing and strangler figs (*Ficus*, Moraceae) inhabiting neotropical forests. *PLOS ONE* 10: e0133581.
Helsen K, Jacquemyn H, Honnay O. (2015). Hidden founder effects: small-scale spatial genetic structure in recently established populations of the grassland specialist plant *Anthyllis vulneraria*. *Molecular Ecology* 24: 2715–2728.
Helsen K, Meekers T, Vranckx G, Roldán-Ruiz I, Vandepitte K, Honnay O. (2016). A direct assessment of realized seed and pollen flow within and between two isolated populations of the food-deceptive orchid *Orchis mascula* (N Vereecken, Ed.). *Plant Biology* 18: 139–146.
Heuertz M, Hausman J-F, Hardy OJ, Vendramin GG, Frascaria-Lacoste N, Vekemans X. (2004). Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L.). *Evolution* 58: 976–988.
Hipólito J, Viana BF, Selbach-Schnadelbach A, Galetto L, Kevan PG. (2012). Pollination biology and genetic variability of a giant perfumed flower (*Aristolochia gigantea* Mart. and Zucc., Aristolochiaceae) visited mainly by small Diptera. *Botany* 90: 815–829.
Hiraoka K, Tomaru N. (2009). Genetic divergence in nuclear genomes between populations of *Fagus crenata* along the Japan Sea and Pacific sides of Japan. *Journal of Plant Research* 122: 269–282.
Hmeljevski KV, Nazareno AG, Leandro Bueno M, dos Reis MS, Forzza RC. (2017). Do plant populations on distinct inselbergs talk to each other? A case study of genetic connectivity of a bromeliad species in an Ocbil landscape. *Ecology and Evolution* 7: 4704–4716.
Hoban SM, McCleary TS, Schlarbaum SE, Romero-Severson J. (2014). Spatial genetic structure in 21 populations of butternut, a temperate forest tree (*Juglans cinerea* L.), is correlated to spatial arrangement, habitat, and land-use history. *Forest Ecology and Management* 314: 50–58.
Hoey MT, Parks CR. (1994). Genetic Divergence in *Liquidambar styraciflua*, *L. formosana*, and *L. acalycina* (Hamamelidaceae). *Systematic Botany* 19: 308.
Hughes M. (2002). Population structure and speciation in *Begonia* L. PhD thesis, University of Glasgow, Scotland.
Hughes M, MacMaster G, Möller M, Bellstedt DU, Edwards TJ. (2006). Breeding system of a plesiomorphic floral type: an investigation of small flowered *Streptocarpus* (Gesneriaceae) species. *Plant Systematics and Evolution* 262: 13–24.

Hughes M, Moller M, Edwards TJ, Bellstedt DU, Villiers M d. (2007). The impact of pollination syndrome and habitat on gene flow: a comparative study of two *Streptocarpus* (Gesneriaceae) species. *American Journal of Botany* 94: 1688–1695.

Huh MK. (1999). Genetic diversity and population structure of Korean alder (*Alnus japonica*; Betulaceae). *Canadian journal of forest research* 29: 1311–1316.

Iddrisu MN, Ritland K. (2004). Genetic variation, population structure, and mating system in bigleaf maple (*Acer macrophyllum* Pursh). *Canadian Journal of Botany* 82: 1817–1825.

Izquierdo LY, Piñero D. (2000). High genetic diversity in the only known population of *Aechmea tuitensis* (Bromeliaceae). *Australian Journal of Botany* 48: 645.

Jacquemyn H, Brys R, Honnay O, Hermy M, Roldán-Ruiz I. (2005). Local forest environment largely affects below-ground growth, clonal diversity and fine-scale spatial genetic structure in the temperate deciduous forest herb *Paris quadrifolia*: effects of local environment on *Paris quadrifolia*. *Molecular Ecology* 14: 4479–4488.

Jacquemyn H, Honnay O, Galbusera P, Roldan-Ruiz I. (2004). Genetic structure of the forest herb *Primula elatior* in a changing landscape. *Molecular Ecology* 13: 211–219.

Jeong J-H, Park Y-J, Kim Z-S. (2007). Genetic diversity and spatial structure of *Symplocarpus renifolius* on Mt. Cheonma, Korea. 한국자원식물학회지 20: 530–539.

Jerome CA, Ford BA. (2002). The discovery of three genetic races of the dwarf mistletoe *Arceuthobium americanum* (Viscaceae) provides insight into the evolution of parasitic angiosperms. *Molecular Ecology* 11: 387–405.

Jia H, Jiao Y, Wang G, Li Y, Jia H, Wu H, Chai C, Dong X, Guo Y, Zhang L, *et al.* (2015). Genetic diversity of male and female Chinese bayberry (*Myrica rubra*) populations and identification of sex-associated markers. *BMC Genomics* 16: 394.

Jiménez P, Agundez D, Alia R, Gil L. (1999). Genetic variation in central and marginal populations of *Quercus suber* L. *Silvae Genetica* 48: 278–283.

Jolivet C, Höltken AM, Liesebach H, Steiner W, Degen B. (2011). Spatial genetic structure in wild cherry (*Prunus avium* L.): I. variation among natural populations of different density. *Tree Genetics and Genomes* 7: 271–283.

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

Jump AS, Rico L, Lloret F, Peñuelas J. (2009). Microspatial population genetic structure of the Mediterranean shrub *Fumana thymifolia*. *Plant Biology* 11: 152–160.

Kang M, Jiang M, Huang H. (2005). Genetic diversity in fragmented populations of *Berchemiella wilsonii* var. *pubipetiolata* (Rhamnaceae). *Annals of Botany* 95: 1145–1151.

Kassa A, Konrad H, Geburek T. (2017). Landscape genetic structure of *Olea europaea* subsp. *cuspidata* in Ethiopian highland forest fragments. *Conservation Genetics* 18: 1463–1474.

Kim SH, Jang YS, Han JG, Chung HG, Lee SW, Cho KJ. (2006). Genetic variation and population structure of *Dendropanax morbifera* Lev. (Araliaceae) in Korea. *Silvae Genetica* 55: 7–13.

Kitamoto N, Honjo M, Ueno S, Takenaka A, Tsumura Y, Washitani I, Ohsawa R. (2005). Spatial genetic structure among and within populations of *Primula sieboldii* growing beside separate streams: spatial genetic structure of *P. sieboldii*. *Molecular Ecology* 14: 149–157.

Kitamura K, Kawano S. (2001). Regional differentiation in genetic components for the American beech, *Fagus grandifolia* Ehrh., in relation to geological history and mode of reproduction. *Journal of Plant Research* 114: 353–368.

Kloss L, Fischer M, Durka W. (2011). Land-use effects on genetic structure of a common grassland herb: A matter of scale. *Basic and Applied Ecology* 12: 440–448.

Knight SE, Waller DM. (1987). Genetic Consequences of outcrossing in the cleistogamous annual, *Impatiens capensis*. I. Population-Genetic Structure. *Evolution* 41: 969.

Kramer AT, Fant JB, Ashley MV. (2011). Influences of landscape and pollinators on population genetic structure: Examples from three *Penstemon* (Plantaginaceae) species in the Great Basin. *American Journal of Botany* 98: 109–121.

Kreivi M, Aspi J, Leskinen E. (2011). Regional and local spatial genetic structure of Siberian primrose populations in Northern Europe. *Conservation Genetics* 12: 1551–1563.

Kudoh H, Whigham DF. (1997). Microgeographic genetic structure and gene flow in *Hibiscus moscheutos* (Malvaceae) populations. *American Journal of Botany* 84: 1285–1293.

Kuss P, Pluess AR, Aegisdottir HH, Stocklin J. (2008). Spatial isolation and genetic differentiation in naturally fragmented plant populations of the Swiss Alps. *Journal of Plant Ecology* 1: 149–159.

Kyndt T, Assogbadjo AE, Hardy OJ, Glele Kakaï R, Sinsin B, Van Damme P, Gheysen G. (2009). Spatial genetic structuring of baobab (*Adansonia digitata*, Malvaceae) in the traditional agroforestry systems of West Africa. *American Journal of Botany* 96: 950–957.

de Lafontaine G, Ducousso A, Lefèvre S, Magnanou E, Petit RJ. (2013). Stronger spatial genetic structure in recolonized areas than in refugia in the European beech. *Molecular Ecology* 22: 4397–4412.

Lamont RW, Conroy GC, Reddell P, Ogbourne SM. (2016). Population genetic analysis of a medicinally significant Australian rainforest tree, *Fontainea picrosperma* C.T. White (Euphorbiaceae): biogeographic patterns and implications for species domestication and plantation establishment. *BMC Plant Biology* 16: 57.

Lasso E, Dalling JW, Bermingham E. (2011). Strong spatial genetic structure in five tropical *Piper* species: should the Baker-Fedorov hypothesis be revived for tropical shrubs?. *Ecology and Evolution* 1: 502–516.

Latouche-Hallé C, Ramboer A, Bandou E, Caron H, Kremer A. (2003). Nuclear and chloroplast genetic structure indicate fine-scale spatial dynamics in a neotropical tree population. *Heredity* 91: 181–190.

Lau CPY, Saunders RMK, Ramsden L. (2009). Floral biology, breeding systems and population genetic structure of three climbing *Bauhinia* species (Leguminosae: Caesalpinioideae) in Hong Kong, China. *Journal of Tropical Ecology* 25: 147–159.

Le Corre V, Dumolin-Lapègue S, Kremer A. (1997). Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. *Molecular Ecology* 6: 519–529.

Ledig FT. (2000). Founder effects and the genetic structure of Coulter pine. *Journal of Heredity* 91: 307–315.

Lee Y-J, Hwang S-Y, Ho K-C, Lin T-P. (2006). Source populations of *Quercus glauca* in the last glacial age in taiwan revealed by nuclear microsatellite markers. *Journal of Heredity* 97: 261–269.

Lee CT, Lee SL, Ng KKS, Salwana HS, Norwati M, Saw LG. (2007). Allozyme diversity of *Koompassia malaccensis* (Leguminosae) in peninsular Malaysia. *Journal of Tropical Forest Science* 19(2): 73–78.

Lee S-L, Ng KK-S, Saw L-G, Norwati A, Salwana MHS, Lee C-T, Norwati M. (2002). Population genetics of *Intsia palembanica* (Leguminosae) and genetic conservation of Virgin Jungle Reserves in Peninsular Malaysia. *American Journal of Botany* 89: 447–459.

Lee SL, Wickneswari R, Mahani MC, Zakri AH. (2000). Genetic diversity of a tropical tree species, *Shorea leprosula* Miq. (Dipterocarpaceae), in Malaysia: implications for conservation of genetic resources and tree improvement. *Biotropica* 32: 213–224.

Lemos RPM, D'Oliveira CB, Stefenon VM. (2015). Genetic structure and internal gene flow in populations of *Schinus molle* (Anacardiaceae) in the Brazilian Pampa. *Tree Genetics & Genomes* 11: 75.

Leonarduzzi C, Piotti A, Spanu I, Vendramin GG. (2016). Effective gene flow in a historically fragmented area at the southern edge of silver fir (*Abies alba* Mill.) distribution. *Tree Genetics & Genomes* 12: 95.

Ley AC, Hardy OJ. (2016). Spatially limited clonality and pollen and seed dispersal in a characteristic climber of Central African rain forests: *Haumania danckelmaniana* (Marantaceae). *Biotropica* 48: 618–627.

Leys M, Petit EJ, El-Bahloul Y, Liso C, Fournet S, Arnaud J-F. (2014). Spatial genetic structure in *Beta vulgaris* subsp. *maritima* and *Beta macrocarpa* reveals the effect of contrasting mating system, influence of marine currents, and footprints of postglacial recolonization routes. *Ecology and Evolution* 4: 1828–1852.

Lhuillier E, Butaud J-F, Bouvet J-M. (2006). Extensive clonality and strong differentiation in the insular pacific tree *Santalum insulare*: Implications for its conservation. *Annals of Botany* 98: 1061–1072.

Li C. (2000). RAPD analysis of genetic variation in *Eucalyptus microtheca* F. Muell. populations. *Hereditas* 132: 151–156.

Liengsiri C, Yeh FrancisC, Boyle TJB. (1995). Isozyme analysis of a tropical forest tree, *Pterocarpus macrocarpus* Kurz. in Thailand. *Forest Ecology and Management* 74: 13–22.

Lin T-P. (2001). Allozyme variations in *Michelia formosana* (Kanehira) Masamune (Magnoliaceae), and the inference of a glacial refugium in Taiwan: *Theoretical and Applied Genetics* 102: 450–457.

Listl D, Reisch C. (2012). spatial genetic structure of the sedge *Carex Nigra* reflects hydrological conditions in an Alpine fen. *Arctic, Antarctic, and Alpine Research* 44: 350–358.

Liu M, Compton SG, Peng F-E, Zhang J, Chen X-Y. (2015). Movements of genes between populations: are pollinators more effective at transferring their own or plant genetic markers? *Proceedings of the Royal Society B: Biological Sciences* 282: (2015)0290–(2015)0290.

Loiselle BA, Sork VL, Nason J, Graham C. (1995). Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82: 1420–1425.

Lopez L, Barreiro R. (2013). Genetic guidelines for the conservation of the endangered polyploid *Centaurea borjae* (Asteraceae). *Journal of Plant Research* 126: 81–93.

Lowe AJ, Jourde B, Breyne P, Colpaert N, Navarro C, Wilson J, Cavers S. (2003). Fine-scale genetic structure and gene flow within Costa Rican populations of mahogany (*Swietenia macrophylla*). *Heredity* 90: 268–275.

Magalhaes IS, Gleiser G, Labouche A-M, Bernasconi G. (2011). Comparative population genetic structure in a plant-pollinator/seed predator system: genetic structure in a plant-pollinator system. *Molecular Ecology* 20: 4618–4630.

Maguire TL, Saenger P, Baverstock P, Henry R. (2000). Microsatellite analysis of genetic structure in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae). *Molecular Ecology* 9: 1853–1862.

Mahy G, Vekemans X, Jacquemart A-L. (1999). Patterns of allozymic variation within *Calluna vulgaris* populations at seed bank and adult stages. *Heredity* 82: 432–440.

Marchelli P, Gallo LA. (2001). Genetic diversity and differentiation in a southern beech subjected to introgressive hybridization. *Heredity* 87: 284–293.

Mariette S, Cottrell J, Csaikl UM, Goikoechea P, Konig A, Lowe AJ, Van Dam BC, Barreneche T, Bodénès C, Streiff R. (2002). Comparison of levels of genetic diversity detected with AFLP and microsatellite markers within and among mixed *Q. petraea* (Matt.) Liebl. and *Q. robur* L. stands. *Silvae Genetica* 51: 72–79.

Mariot A. (2002). Genetic diversity in natural populations of *Piper cernuum*. *Journal of Heredity* 93: 365–369.

Marquardt PE, Epperson BK. (2004). Spatial and population genetic structure of microsatellites in white pine: genetic variation in white pine. *Molecular Ecology* 13: 3305–3315.

Marsico TD, Hellmann JJ, Romero-Severson J. (2009). Patterns of seed dispersal and pollen flow in *Quercus garryana* (Fagaceae) following post-glacial climatic changes. *Journal of Biogeography* 36: 929–941.

Martín MA, Mattioni C, Molina JR, Alvarez JB, Cherubini M, Herrera MA, Villani F, Martín LM. (2012). Landscape genetic structure of chestnut (*Castanea sativa* Mill.) in Spain. *Tree Genetics & Genomes* 8: 127–136.

Matolweni LO, Balkwill K, McLellan T. (2000). Genetic diversity and gene flow in the morphologically variable, rare endemics *Begonia dregei* and *Begonia homonyma* (Begoniaceae). *American Journal of Botany* 87: 431–439.

Matter P, Kettle CJ, Ghazoul J, Pluess AR. (2013). Extensive contemporary pollen-mediated gene flow in two herb species, *Ranunculus bulbosus* and *Trifolium montanum*, along an altitudinal gradient in a meadow landscape. *Annals of Botany* 111: 611–621.

Mayol M, Palau C, Rosselló JA, González-Martínez SC, Molins A, Riba M. (2012). Patterns of genetic variability and habitat occupancy in *Crepis triasii* (Asteraceae) at different spatial scales: insights on evolutionary processes leading to diversification in continental islands. *Annals of Botany* 109: 429–441.

McDonald MW, Rawlings M, Butcher PA, Bell JC. (2003). Regional divergence and inbreeding in *Eucalyptus cladocalyx* (Myrtaceae). *Australian Journal of Botany* 51: 393.

McGranahan M, Slee M, Bell JC, Moran GF. (1997). High genetic divergence between geographic regions in the highly outcrossing species *Acacia aulacocarpa* (Cunn. ex Benth.). *International Journal of Forest Genetics* 4: 1–13.

Medina-Macedo L, Sebbenn AM, Lacerda AEB, Ribeiro JZ, Soccol CR, Bittencourt JVM. (2015). High levels of genetic diversity through pollen flow of the coniferous *Araucaria angustifolia*: a landscape level study in Southern Brazil. Tree Genetics & Genomes 11: 814(1–14).

Meirmans PG, Goudet J, IntraBioDiv Consortium, Gaggiotti OE. (2011). Ecology and life history affect different aspects of the population structure of 27 high-alpine plants: life history, ecology and genetic structure. *Molecular Ecology* 20: 3144–3155.

Melo AT de O, Franceschinelli EV. (2016). Gene flow and fine-scale spatial genetic structure in *Cabralea canjerana* (Meliaceae), a common tree species from the Brazilian Atlantic forest. *Journal of Tropical Ecology* 32: 135–145.

Michaud H, Lumaret R, Romane F. (1992). Variation in the genetic structure and reproductive biology of holm oak populations. In: *Quercus ilex* L. ecosystems: function, dynamics and management. Springer, 107–113.

Montalvo AM, Conard SG, Conkle MT, Hodgskiss PD. (1997). Population structure, genetic diversity, and clone formation in *Quercus chrysolepis* (Fagaceae). *American Journal of Botany* 84: 1553–1564.

Moran G, Bell J, Turnbull J. (1989a). A Cline in Genetic Diversity in River She-Oak *Casuarina cunninghamiana*. *Australian Journal of Botany* 37: 169.

Moran GF, Muona O, Bell JC. (1989b). *Acacia mangium*: a tropical forest tree of the coastal lowlands with low genetic diversity. *Evolution* 43: 231–235.
Moreira PA, Fernandes GW, Collevatti RG. (2009). Fragmentation and spatial genetic structure in *Tabebuia ochracea* (Bignoniaceae) a seasonally dry Neotropical tree. *Forest Ecology and Management* 258: 2690–2695.
Moreira RG, McCauley RA, Cortés-Palomec AC, Fernandes GW, Oyama K. (2010). Spatial genetic structure of *Coccoloba cereifera* (Polygonaceae), a critically endangered microendemic species of Brazilian rupestrian fields. *Conservation Genetics* 11: 1247–1255.
Muloko-Ntoutoume N. (2000). Chloroplast DNA variation in a rainforest tree (*Aucoumea klaineana*, Burseraceae) in Gabon. *Molecular Ecology* 9: 359.
Murawski DA, Bawa KS. (1994). Genetic structure and mating system of *Stemonoporus oblongifolius* (Dipterocarpaceae) in Sri Lanka. *American Journal of Botany* 81: 155–160.
Murawski DA, Hamrick JL. (1990). Local genetic and clonal structure in the tropical terrestrial bromeliad, *Aechmea magdalenae*. *American Journal of Botany* 77: 1201–1208.
Nakagawa M. (2004). Genetic diversity of fragmented populations of *Polygala reinii* (Polygalaceae), a perennial herb endemic to Japan. *Journal of Plant Research* 117: 355–361.
Navarro C, Cavers S, Pappinen A, Tigerstedt P, Lowe A, Merilä J. (2005). Contrasting quantitative Traits and Neutral Genetic Markers for Genetic Resource Assessment of Mesoamerican *Cedrela odorata*. *Silvae Genetica* 54: 281–292.
Nettel A, Dodd RS, Afzal-Rafii Z. (2009). Genetic diversity, structure, and demographic change in tanoak, *Lithocarpus densiflorus* (Fagaceae), the most susceptible species to sudden oak death in California. *American Journal of Botany* 96: 2224–2233.
Nguyen TPT, Tran TH, Nguyen MD, Sierens T, Triest L. (2014). Genetic population of threatened *Hopea odorata* Roxb. in the protected areas of Vietnam. *Journal of Vietnamese Environment* 6: 69–76.
Nicoletti F, De Benedetti L, Airò M, Ruffoni B, Mercuri A, Minuto L, Casazza G. (2012). Spatial genetic structure of *Campanula sabatia*, a threatened narrow endemic species of the Mediterranean Basin. *Folia Geobotanica* 47: 249–262.
Noreen AME, Webb EL. (2013). High genetic diversity in a potentially vulnerable tropical tree species despite extreme habitat loss. *PLoS ONE* 8: e82632.
Oddou-Muratorio, Klein EK. (2008). Comparing direct vs. indirect estimates of gene flow within a population of a scattered tree species. *Molecular Ecology* 17: 2743–2754.
Ohsako T, Hirai M, Yamabuki M. (2010). Spatial structure of microsatellite variability within and among populations of wild radish *Raphanus sativus* L. var. *hortensis* Backer f. *raphanistroides* Makino (Brassicaceae) in Japan. *Breeding Science* 60: 195–202.

Ojeda-Camacho M, Kjær ED, Philipp M. (2013). Population genetics of *Guibourtia chodatiana* (Hassl.) J. Leonard, in a dry Chiquitano forest of Bolivia. *Forest Ecology and Management* 289: 525–534.

Oleas NH, von Wettberg EJB, Negrón-Ortiz V. (2014). Population genetics of the federally threatened miccosukee gooseberry (*Ribes echinellum*), an endemic North American species. *Conservation Genetics*.

Omondi SF, Kireger E, Dangasuk OG, Chikamai B, Odee DW, Cavers S, Khasa DP. (2010). Genetic diversity and population structure of *Acacia senegal* (L) Willd. in Kenya. *Tropical Plant Biology* 3: 59–70.

Pakkad G, James C, Torre F, Elliott S, Blakesley D. (2003). Genetic variation of *Prunus cerasoides* D. Don, a framework tree species in northern Thailand. *New forests* 27: 189–200.

Palma-Silva C, Lexer C, Paggi GM, Barbará T, Bered F, Bodanese-Zanettini MH. (2009). Range-wide patterns of nuclear and chloroplast DNA diversity in *Vriesea gigantea* (Bromeliaceae), a neotropical forest species. *Heredity* 103: 503–512.

Pandey M, Rajora OP. (2012). Genetic diversity and differentiation of core vs. peripheral populations of eastern white cedar, *Thuja occidentalis* (Cupressaceae). *American Journal of Botany* 99: 690–699.

Pandey M, Sharma J. (2015). Disjunct populations of a locally common North American orchid exhibit high genetic variation and restricted gene flow. *Open Journal of Genetics* 05: 159–175.

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

Parks CR, Wendel JF, Sewell MM, Qiu Y-L. (1994). The significance of allozyme variation and introgression in the *Liriodendron tulipifera* complex (Magnoliaceae). *American Journal of Botany* 81: 878–889.

Payn KG, Dvorak WS, Janse BJH, Myburg AA. (2008). Microsatellite diversity and genetic structure of the commercially important tropical tree species *Eucalyptus urophylla*, endemic to seven islands in eastern Indonesia. *Tree Genetics & Genomes* 4: 519–530.

Peakall R, Beattie AJ. (1995). Does ant dispersal of seeds in *Sclerolaena diacantha* (Chenopodiaceae) generate local spatial genetic structure? *Heredity* 75: 351–361.

Peakall R, Beattie AJ. (1996). Ecological and genetic consequences of pollination by sexual deception in the orchid *Caladenia tentactulata*. *Evolution* 50: 2207.

Pither R, Shore JS, Kellman M. (2003). Genetic diversity of the tropical tree *Terminalia amazonia* (Combretaceae) in naturally fragmented populations. *Heredity* 91: 307–313.

Playford J, Bell J, Moran G. (1993). A major disjunction in genetic diversity over the geographic range of *Acacia melanoxylon* R.Br. *Australian Journal of Botany* 41: 355.

Pluess AR. (2011). Pursuing glacier retreat: genetic structure of a rapidly expanding *Larix decidua* population: genetic structure of an expanding population. *Molecular Ecology* 20: 473–485.

Pometti C, Bessega C, Cialdella A, Ewens M, Saidman B, Vilardi J. (2018). Spatial genetic structure within populations and management implications of the South American species *Acacia aroma* (Fabaceae). *PLOS ONE* 13: e0192107.

Premoli AC. (1997). Genetic variation in a geographically restricted and two widespread species of South American *Nothofagus*. *Journal of Biogeography* 24: 883–892.

Prober SM, Brown AHD. (1994). Conservation of the grassy white box woodlands: population genetics and fragmentation of *Eucalyptus albens*. *Conservation Biology* 8: 1003–1013.

Qiu Y-L, Parks CR. (1994). Disparity of allozyme variation levels in three *Magnolia* (Magnoliaceae) species from the southeastern United States. *American Journal of Botany* 81: 1300–1308.

Quevedo AA, Schleuning M, Hensen I, Saavedra F, Durka W. (2013). Forest fragmentation and edge effects on the genetic structure of *Clusia sphaerocarpa* and *C. lechleri* (Clusiaceae) in tropical montane forests. *Journal of Tropical Ecology* 29: 321–329.

Raspé O, Jacquemart A-L. (1998). Allozyme diversity and genetic structure of European populations of *Sorbus aucuparia* L. (Rosaceae: Maloideae). *Heredity* 81: 537.

Reif BP, Mathiasen RL, Kenaley SC, Allan GJ. (2015). Genetic Structure and Morphological Differentiation of Three Western North American Dwarf Mistletoes (*Arceuthobium*: Viscaceae). *Systematic Botany* 40: 191–207.

Ribeiro FE, Baudouin L, Lebrun P, Chaves LJ, Brondani C, Zucchi MI, Vencovsky R. (2010). Population structures of Brazilian tall coconut (*Cocos nucifera* L.) by microsatellite markers. *Genetics and Molecular Biology* 33: 696–702.

Ritchie AL, Nevill PG, Sinclair EA, Krauss SL. (2017). Does restored plant diversity play a role in the reproductive functionality of *Banksia* populations?: Reproductive functionality of restored keystone species. *Restoration Ecology* 25: 414–423.

Robertson A, Newton AC, Ennos RA. (2004). Multiple hybrid origins, genetic diversity and population genetic structure of two endemic Sorbus taxa on the Isle of Arran, Scotland. *Molecular Ecology* 13: 123–134.

Rocha OJ, Lobo JA. (1996). Genetic variation and differentiation among five populations of the guanacaste tree (*Enterolobium cyclocarpum* Jacq.) in Costa Rica. *International Journal of Plant Sciences* 157: 234–239.

Ross-Davis A, Ostry M, Woeste KE. (2008). Genetic diversity of butternut (*Juglans cinerea*) and implications for conservation. *Canadian Journal of Forest Research* 38: 899–907.

Rossetto M, Jones R, Hunter J. (2004). Genetic effects of rainforest fragmentation in an early successional tree (*Elaeocarpus grandis*). *Heredity* 93: 610–618.

Rusanen M, Vakkari P, Blom A. (2003). Genetic structure of *Acer platanoides* and *Betula pendula* in northern Europe. *Canadian Journal of Forest Research* 33: 1110–1115.

Rüter B, Hamrick JL, Wood BW. (1999). Genetic diversity within provenance and cultivar germplasm collections versus natural populations of pecan (*Carya illinoinensis*). *Journal of Heredity* 90: 521–528.

Saenz-Romero C, Guries RP, Monk AI. (2001). Landscape genetic structure of *Pinus banksiana*: allozyme variation. *Canadian Journal of Botany* 79: 871–878.

Santos AS, Cazetta E, Dodonov P, Faria D, Gaiotto FA. (2016). Landscape-scale deforestation decreases gene flow distance of a keystone tropical palm, *Euterpe edulis* Mart (Arecaceae). *Ecology and Evolution* 6: 6586–6598.

Sato T, Isagi Y, Sakio H, Osumi K, Goto S. (2006). Effect of gene flow on spatial genetic structure in the riparian canopy tree *Cercidiphyllum japonicum* revealed by microsatellite analysis. *Heredity* 96: 79–84.

Schnabel A, Hamrick JL. (1990a). Comparative analysis of population genetic structure in *Quercus macrocarpa* and *Q. gambelii* (Fagaceae). *Systematic Botany* 15: 240.

Schnabel A, Hamrick JL. (1990b). Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). *American Journal of Botany* 77: 1060–1069.

Searle SD, Bell JC, Moran GF. (2000). Genetic diversity in natural populations of *Acacia mearnsii*. *Australian Journal of Botany* 48: 279.

Sgorbati S, Labra M, Grugni E, Barcaccia G, Galasso G, Boni U, Mucciarelli M, Citterio S, Benavides Iramátegui A, Venero Gonzales L, *et al.* (2004). A survey of genetic diversity and reproductive biology of *Puya raimondii* (Bromeliaceae), the endangered queen of the Andes. *Plant Biology* 6: 222–230.

Shapcott A. (1994). Genetic and ecological variation in *Atherosperma moschatum* and the Implications for Conservation of Its Biodiversity. *Australian Journal of Botany* 42: 663.

Shapcott. (1998). The patterns of genetic diversity in *Carpentaria acuminata* (Arecaceae), and rainforest history in northern Australia. *Molecular Ecology* 7: 833–847.

Shapcott A. (1999). Vagility and the monsoon rain forest archipelago of northern Australia: patterns of genetic diversity in *Syzygium nervosum* (Myrtaceae). *Biotropica* 31: 579–590.

Sheely DL, Meagher TR. (1996). Genetic diversity in Micronesian island populations of the tropical tree *Campnosperma brevipetiolata* (Anacardiaceae). *American Journal of Botany* 83: 1571–1579.

Sherman-Broyles SL, Broyles SB, Hamrick JL. (1992). Geographic distribution of allozyme variation in *Ulmus crassifolia*. *Systematic Botany* 17: 33.

Slavov GT, Leonardi S, Adams WT, Strauss SH, DiFazio SP. (2010). Population substructure in continuous and fragmented stands of *Populus trichocarpa*. *Heredity* 105: 348–357.

Sochor M, Vašut RJ, Bártová E, Majeský Ľ, Mráček J. (2013). Can gene flow among populations counteract the habitat loss of extremely fragile biotopes? An example from the population genetic structure in *Salix daphnoides*. *Tree Genetics & Genomes* 9: 1193–1205.

Soltis DE, Gilmartin AJ, Rieseberg L, Gardner S. (1987). Genetic variation in the epiphytes *Tillandsia ionantha* and *T. recurvata* (Bromeliaceae). *American Journal of Botany* 74: 531–537.

Song Z, Zhang M, Li F, Weng Q, Zhou C, Li M, Li J, Huang H, Mo X, Gan S. (2016). Genome scans for divergent selection in natural populations of the widespread hardwood species *Eucalyptus grandis* (Myrtaceae) using microsatellites. *Scientific Reports* 6: 34941.

Sork V, Huang S, Wiener E. (1993). Macrogeographic and fine-scale genetic structure in a North American oak species, *Quercus rubra* L. *Annales des Sciences Forestières* 50: 261s–270s.

Stanton S, Honnay O, Jacquemyn H, Roldán-Ruiz I. (2009). A comparison of the population genetic structure of parasitic *Viscum album* from two landscapes differing in degree of fragmentation. *Plant Systematics and Evolution* 281: 161–169.

Starr TN, Gadek KE, Yoder JB, Flatz R, Smith CI. (2013). Asymmetric hybridization and gene flow between Joshua trees (Agavaceae: *Yucca*) reflect differences in pollinator host specificity. *Molecular Ecology* 22: 437–449.

Stefenon VM, Gailing O, Finkeldey R. (2007). Genetic structure of *Araucaria angustifolia* (Araucariaceae) populations in Brazil: Implications for the in situ conservation of genetic resources. *Plant Biology* 9: 516–525.

Stein K, Rosche C, Hirsch H, Kindermann A, Köhler J, Hensen I. (2014). The influence of forest fragmentation on clonal diversity and genetic structure in *Heliconia angusta*, an endemic understory herb of the Brazilian Atlantic rain forest. *Journal of Tropical Ecology* 30: 199–208.

Suarez-Gonzalez A, Good SV. (2014). Pollen limitation and reduced reproductive success are associated with local genetic effects in Prunus virginiana, a widely distributed self-incompatible shrub. *Annals of Botany* 113: 595–605.

Sujii PS, Martins K, Wadt LH de O, Azevedo VCR, Solferini VN. (2015). Genetic structure of *Bertholletia excelsa* populations from the Amazon at different spatial scales. *Conservation Genetics* 16: 955–964.

Suma TB, Balasundaran M. (2003). Isozyme variation in five provenances of *Santalum album* in India. *Australian Journal of Botany* 51: 243.

Sun M. (1999). Cleistogamy in *Scutellaria indica* (Labiatae): effective mating system and population genetic structure. *Molecular Ecology* 8: 1285–1295.

Sun R, Lin F, Huang P, Zheng Y. (2016). Moderate genetic diversity and genetic differentiation in the relict tree *Liquidambar formosana* Hance revealed by genic simple sequence repeat markers. *Frontiers in plant science* 7: 1411.

Surget-Groba Y, Kay KM. (2013). Restricted gene flow within and between rapidly diverging Neotropical plant species. *Molecular Ecology* 22: 4931–4942.

Swift JF, Smith SA, Menges ES, Bassüner B, Edwards CE. (2016). Analysis of mating system and genetic structure in the endangered, amphicarpic plant, Lewton's polygala (*Polygala lewtonii*). *Conservation Genetics* 17: 1269–1284.

Tambarussi EV, Sebbenn AM, Alves-Pereira A, Vencovsky R, Cambuim J, Da Silva A, Moraes M, De Moraes MLT. (2017). *Dipteryx alata* Vogel (Fabaceae) a neotropical tree with high level of selfing: implication for conservation and breeding programs. *Annals of Forest Research* 2: 1–19.

Tarazi R, Moreno MA, Gandara FB, Martins-Ferraz E, Moraes MLT, Vinson CC, Ciampi, AY, Vencovsky R, Kageyama PY. (2010). High levels of genetic differentiation and selfing in the Brazilian cerrado fruit tree *Dipteryx alata* Vog. (Fabaceae). *Genetics and Molecular Biology* 33: 78–85.

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.

Tinio CE, Finkeldey R, Prinz KA, Fernando ES. (2014). Genetic variation in natural and planted populations of *Shorea guiso* (Dipterocarpaceae) in the Philippines revealed by microsatellite DNA markers. *Asia Life Sciences* 23: 75–91.

Tomimatsu H, Ohara M. (2003). Genetic diversity and local population structure of fragmented populations of *Trillium camschatcense* (Trilliaceae). *Biological Conservation* 109: 249–258.

Tsuda Y, Ide Y. (2005). Wide-range analysis of genetic structure of Betula maximowicziana, a long-lived pioneer tree species and noble hardwood in the cool temperate zone of Japan: genetic structure of Betula maximowicziana. *Molecular Ecology* 14: 3929–3941.

Turchetto C, Lima JS, Rodrigues DM, Bonatto SL, Freitas LB. (2015). Pollen dispersal and breeding structure in a hawkmoth-pollinated Pampa grasslands species *Petunia axillaris* (Solanaceae). *Annals of Botany* 115: 939–948.

Twyford AD, Kidner CA, Ennos RA. (2014). Genetic differentiation and species cohesion in two widespread Central American *Begonia* species. *Heredity* 112: 382–390.

Ueno S, Setsuko S, Kawahara T, Yoshimaru H. (2006). Genetic diversity and differentiation of the endangered Japanese endemic tree *Magnolia stellata* using nuclear and chloroplast microsatellite markers. *Conservation Genetics* 6: 563–574.

Van Rossum F, Campos De Sousa S, Triest L. (2004). Genetic consequences of habitat fragmentation in an agricultural landscape on the common *Primula veris*, and comparison with its rare congener, *P. vulgaris*. *Conservation Genetics* 5: 231–245.

Vergara R, Gitzendanner MA, Soltis DE, Soltis PS. (2014). Population genetic structure, genetic diversity, and natural history of the South American

species of *Nothofagus* subgenus *Lophozonia* (Nothofagaceae) inferred from nuclear microsatellite data. *Ecology and Evolution* 4: 2450–2471.
Victory ER, Glaubitz JC, Rhodes OE, Woeste KE. (2006). Genetic homogeneity in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. *American Journal of Botany* 93: 118–126.
Vieira FDA. (2009). Genetic differentiation and temporal aspects of the fine-scale genetic structure in fragments-vegetation corridors: inferences from a dioecious-dominant neotropical tree. PhD Thesis, Universidade Federal de Lavras, Minas Gerais, Brazil.
Wadt LH de O, Kageyama PY. (2004). Estrutura genética e sistema de acasalamento de *Piper hispidinervum*. *Pesquisa Agropecuária Brasileira* 39: 151–157.
Walisch TJ, Matthies D, Hermant S, Colling G. (2015). Genetic structure of *Saxifraga rosacea* subsp. *sponhemica*, a rare endemic rock plant of Central Europe. *Plant Systematics and Evolution* 301: 251–263.
Wang R, Compton SG, Shi Y-S, Chen X-Y. (2012). Fragmentation reduces regional-scale spatial genetic structure in a wind-pollinated tree because genetic barriers are removed. *Ecology and Evolution* 2: 2250–2261.
Wang H, Pei D, Gu R, Wang B. (2008). Genetic diversity and structure of walnut populations in central and southwestern china revealed by microsatellite markers. *Journal of the American Society for Horticultural Science* 133: 197–203.
Wickneswari R, Norwati M. (1993). Genetic diversity of natural-populations of *Acacia auriculiformis*. *Australian Journal of Botany* 41: 65.
Williams CF, Guries RP. (1994). Genetic consequences of seed dispersal in three sympatric forest herbs. i. Hierarchical population-genetic structure. *Evolution* 48: 791.
Williams, Jr., JH, Arnold ML. (2001). Sources of genetic structure in the woody perennial *Betula occidentalis*. *International Journal of Plant Sciences* 162: 1097–1109.
Xie C-Y, El-Kassaby YA, Ying CC. (2002). Genetics of red alder (*Alnus rubra* Bong.) populations in British Columbia and its implications for gene resources management. *New forests* 24: 97–112.
Y. Fu. (2003). Allozyme Variation in Endangered *Castanea pumila* var. *pumila*. *Annals of Botany* 92: 223–230.
Yan J, Chu H-J, Wang H-C, Li J-Q, Sang T. (2009). Population genetic structure of two *Medicago* species shaped by distinct life form, mating system and seed dispersal. *Annals of Botany* 103: 825–834.
Yan T-F, Zu Y-G, Yan X-F, Zhou F-J. (2003). Genetic structure of endangered *Rhodiola sachalinensis*. *Conservation Genetics* 4: 213–218.
Yang A, Dick CW, Yao X, Huang H. (2016). Impacts of biogeographic history and marginal population genetics on species range limits: a case study of *Liriodendron chinense*. *Scientific Reports* 6: 25632.
Zárate S, Pérez-Nasser N, Casas A. (2005). Genetics of wild and managed populations of *Leucaena esculenta* subsp. *esculenta* (Fabaceae;

Mimosoideae) in La Montaña of Guerrero, Mexico. *Genetic Resources and Crop Evolution* 52: 941–957.
Zeng X, Michalski SG, Fischer M, Durka W. (2012). Species diversity and population density affect genetic structure and gene dispersal in a subtropical understory shrub. *Journal of Plant Ecology* 5: 270–278.
Zucchi MI, Pinheiro JB, Chaves LJ, Coelho ASG, Couto MA, Morais LK de, Vencovsky R. (2005). Genetic structure and gene flow of *Eugenia dysenterica* natural populations. *Pesquisa Agropecuária Brasileira* 40: 975–980.

Appendix S2. Data transformation.

We applied transformations to continuous variables in order to improve normality. F_{ST} was transformed using Tukey's ladder of powers transformation (Tukey, 1970) with the function transformTukey from the R package rcompanion (Mangiafico, 2018). This function finds the power that makes a variable as normally distributed as possible based on the Shapiro-Wilk test (Shapiro & Wilk, 1965). Transformed F_{ST} resulted in F_{ST}^0.275 (Shapiro-Wilk statistic=0.27, P=0.7). For continuous predictors, the best transformation to improve normality was the natural logarithm of the maximum distance between populations and the mean sample size per population.

Appendix S3. Tests of multicollinearity.

Because multicollinearity can complicate the identification of an optimal set of explanatory variables for a statistical model, we assessed the correlation between species traits. We calculated the Pearson Chi-Square test of independence (Plackett, 1983), which is appropriate for categorical data, between all pairs of variables. We then calculated Cramer V values, which gives

a measure of the strength of the association, using the the R functions chisq.test and cramerV. Cramer V values less than 0.3 represent a moderately low association and excluding associations higher than 0.3 helps prevent multicollinearity issues (Acock & Stavig, 1979). We also estimated the variance inflation factor generalized to account for degrees of freedom of each factor (GVIF, Fox & Monette, 1992) with the R function VIF. GVIF values smaller than 5 are generally considered to not cause collinearity problems in model inferences. All Cramer V values were ≤0.3 and GVIF values were <2 (Table S2 and S3). Thus, multicollinearity did not affect our model inference.

Appendix S4. Phylogeny.

A species-level phylogeny was produced with the R package V.PhyloMaker (Jin & Qian, 2019). This program uses as the backbone tree the latest seed plant mega-phylogeny (Smith & Brown, 2018), which is inferred from seven nuclear regions retrieved from GenBank and fossil calibrated to include branch lengths. Species are pruned from this backbone tree based on a custom species list. Species not present in the backbone tree were added as polytomies within their respective clade using the same method as Phylomatic (Webb & Donoghue, 2005), with a branch length calculation as implemented with the branch length adjuster algorithm (Webb et al., 2008). Qian & Jin (2016) showed that such approach results in phylogenies very similar to empirical species-level phylogenies. Of the 337 species in our dataset, 239 were already in the backbone tree and 98 were newly added. After these additions, V.PhyloMaker

pruned our custom phylogenetic tree to remove tips not in our dataset. Because V.PhyloMaker assigns age divergences to particular nodes in the target topology, and then places the remaining nodes evenly between them, the resulting time-calibrated tree is actually a pseudo-chronogram. Pseudo-chronograms show lower variability in branch length than well-calibrated phylogenies that use molecular clocks, yet they remain appropriate for phylogenetic comparative methods (Molina-Venegas & Rodríguez, 2017).

Appendix S5. Phylogenetic signal.

For categorical traits, we performed Abouheif's method of serial independence (Abouheif, 1999), which is equivalent to Moran's *I* when computed with a specific matrix of phylogenetic weights based on branch lengths and trait distance between tips in the phylogeny (Pavoine *et al.*, 2008). Moran's *I* and its significance were estimated with 1000 permutations of the dataset using the function abouheif.moran from the package adephylo (Jombart *et al.*, 2010). For continuous variables, we estimated Pagel's λ (Pagel, 1999) and its significance with 1000 simulations with the function phylosig from phytools (Revell, 2012). We chose Pagel's λ over Blomberg's K (Blomberg *et al.*, (2003)) because simulations demonstrate that Blomberg's K estimates can be highly inflated in both type I and II error when calculated using pseudo-chronograms rather than fully time-calibrated phylogenies, while Pagel's λ is strongly robust to branch-length biases (Molina-Venegas & Rodríguez, 2017).

Appendix S6. Phylolm implementation.

We performed phylogenetic multiple regression models with the function and package phylolm (Ho & Ané, 2014). We implemented the lambda phylogenetic model for the correction of the error term. The lambda parameter in this model is used to transform the error associated to the autocorrelation in the variance–covariance matrix assuming a Brownian motion model of evolution. We chose this model because it consistently had the lowest AIC value when compared to the other six methods available in phylolm. Lambda is useful for improving the fit of the phylogenetic regression, but the actual evolutionary process resulting in lambda is hard to interpret (Revell *et al.*, 2008).

References (Appendix S2 – Appendix S6).

Abouheif, E. (1999). A method for testing the assumption of phylogenetic independence in comparative data. Evol. *Evolutionary Ecology Research*, 1, 895–909.

Acock, A.C. & Stavig, G.R. (1979). A Measure of Association for Nonparametric Statistics. *Social Forces*, 57, 1381–1386.

Blomberg, S.P., Garland, T. & Ives, A.R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57, 717–745.

Fox, J. & Monette, G. (1992). Generalized Collinearity Diagnostics. *Journal of the American Statistical Association*, 87, 178–183.

Jin, Y. & Qian, H. (2019). V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography*, ecog.04434.

Jombart, T., Balloux, F. & Dray, S. (2010). Adephylo: new tools for investigating the phylogenetic signal in biological traits. *Bioinformatics*, 26, 1907–1909.

Mangiafico, S. (2018). rcompanion: functions to support extension education program evaluation. Available at [https://rdrr.io/cran/rcompanion/#vignettes]. Last accessed 1 February 2019.

Molina-Venegas, R. & Rodríguez, M.Á. (2017). Revisiting phylogenetic signal; strong or negligible impacts of polytomies and branch length information? *BMC Evolutionary Biology*, 17, 53.

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*,

401, 877–884.
Pavoine, S., Ollier, S., Pontier, D. & Chessel, D. (2008). Testing for phylogenetic signal in phenotypic traits: New matrices of phylogenetic proximities. *Theoretical Population Biology*, 73, 79–91.
Plackett, R.L. (1983). Karl Pearson and the Chi-Squared Test. *International Statistical Review / Revue Internationale de Statistique*, 51, 59–72.
Qian, H. & Jin, Y. (2016). An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *Journal of Plant Ecology*, 9, 233–239.
Revell, L.J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223.
Revell, L.J., Harmon, L.J. & Collar, D.C. (2008). Phylogenetic signal, evolutionary process, and rate. *Systematic Biology*, 57, 591–601.
Shapiro, S.S. & Wilk, M.B. (1965). An Analysis of Variance Test for Normality (Complete Samples). *Biometrika*, 52, 591–611.
Smith, S.A. & Brown, J.W. (2018). Constructing a broadly inclusive seed plant phylogeny. *American Journal of Botany*, 105, 302–314.
Tukey, J.W. (1970). Exploratory Data Analysis: Limited Preliminary Ed. Addison-Wesley Publishing Company, Boston, MA, USA.
Ho, T.L. & Ané, C. (2014). A Linear-Time Algorithm for Gaussian and Non-Gaussian Trait Evolution Models. *Systematic Biology*, 63, 397–408.
Webb, C.O., Ackerly, D.D. & Kembel, S.W. (2008). Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, 24, 2098–2100.
Webb, C.O. & Donoghue, M.J. (2005). Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes*, 5, 181–183.

Fig. S1. Phylogeny produced with the R package V.PhyloMaker (See Appendix S4 for details).

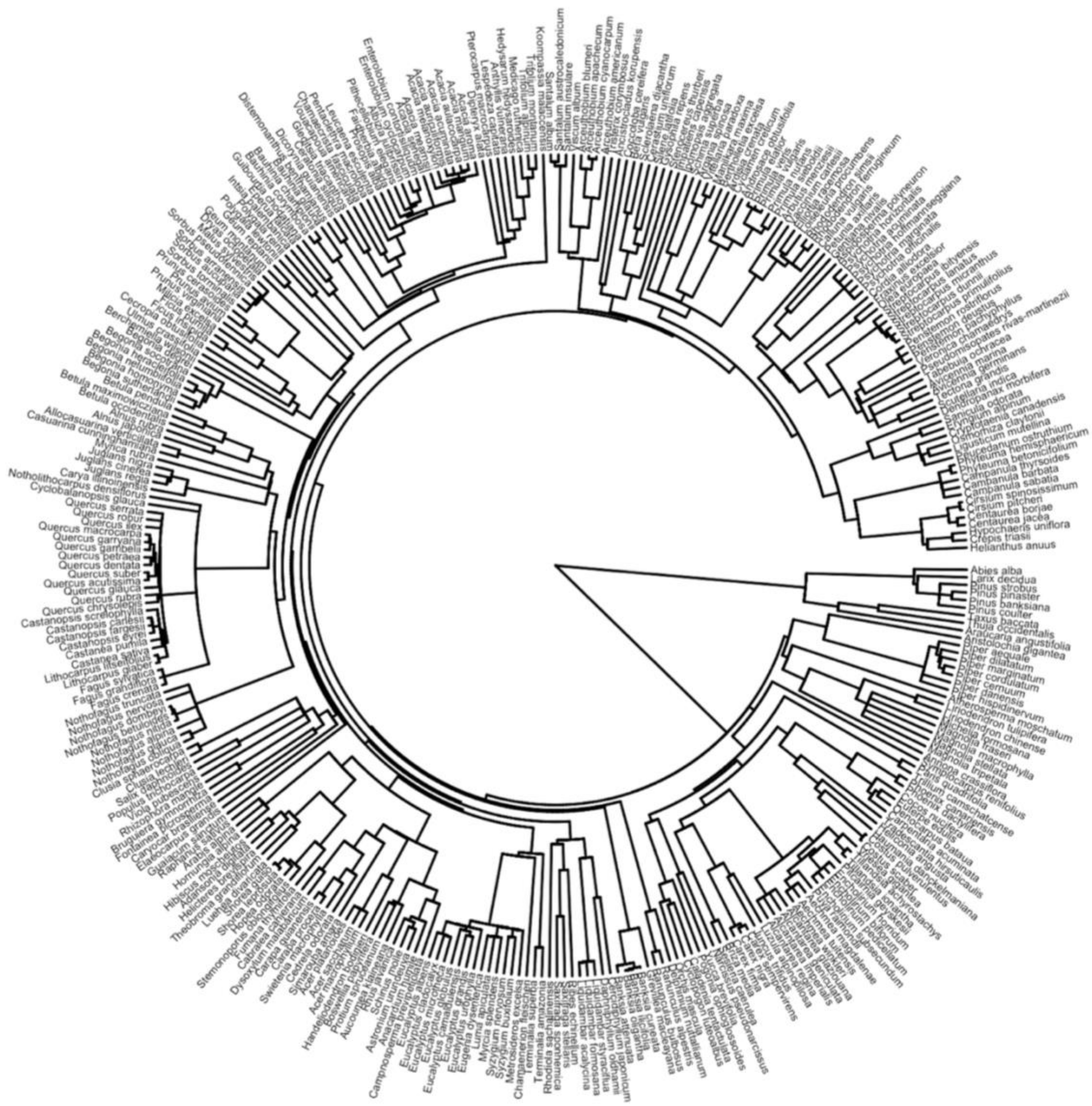


Fig. S2. Phylogenetic signal and its significance with Moran's *I* obtained with Abouheif's method for categorical species traits in the dataset. Asterisks denote statistical significance based on 1000 permutations: P=0.001 (See Appendix S5 for details).

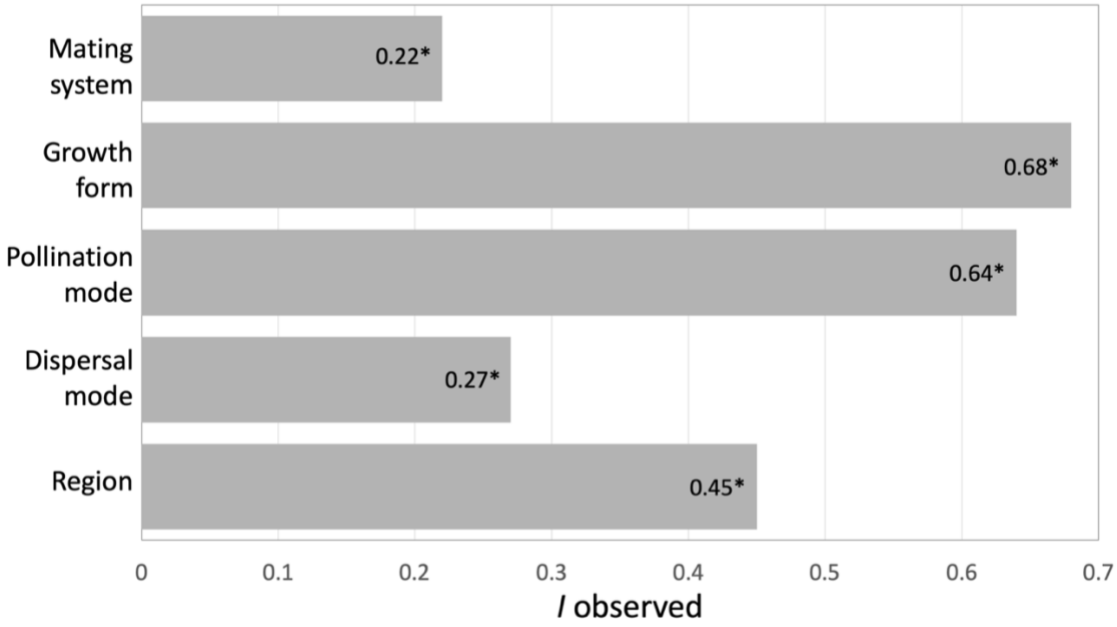


Table S1. Dataset used in this study (in file Table S1.xlsx). Abbreviations are as follow: MS, mating system; GF, growth form; PM, pollination mode; DM, dispersal mode; MaxDP, maximum distance between populations in km; MSS, mean sample size of individuals per population. When more than one publication is included per species, the first one reports the F_{ST} value used in this study.

Table S2. Pearson Chi-squared test for correlation between categorical variables and Cramer's V degree of association between variables. Significant P values are in bold † (next page).

† Refer to Appendix S3 for details. The strongest association was between mating system and life form; most trees are outcrossing species, while most mixed-mating species are non-woody plants. Pollination mode was significantly associated with growth form, as well as with region; wind pollinated plants are almost entirely trees from temperate regions, while vertebrate pollination is more common in non-woody tropical plants. Growth form and seed dispersal were also correlated; most gravity-dispersed plants are non-woody, most animal dispersed plants are trees, and shrubs are rarely wind dispersed. Mating system and seed dispersal were also correlated; most outcrossing plants have seeds dispersed by animals. Growth form and region were also significantly associated; most tropical and subtropical plants are trees, while most non-woody plants are from temperate regions. Lastly, seed dispersal and region significantly correlated; wind dispersal is more common in the temperate zones, while animal dispersal is more common in the tropics.

Tested correlations	**Chi_2**	**DF**	**P value**	**Cramer's V**
Seed dispersal – Growth form	51.8	4	**<0.001**	0.28
Seed dispersal – Mating system	8.7	2	**0.01**	0.16
Seed dispersal – Pollination mode	11.8	6	0.06	0.13
Seed dispersal – Region	14.5	4	**0.006**	0.15
Growth form – Mating system	30.4	2	**<0.001**	0.30
Growth form – Pollination mode	41.1	6	**<0.001**	0.25
Growth form – Region	15.8	4	**0.003**	0.15
Mating system – Pollination mode	7.2	3	0.07	0.14
Mating system – Region	1.3	2	0.52	0.06
Pollination mode – Region	54.1	6	**<0.001**	0.28

Table S3. Estimates of the generalized variance inflation factor (GVIF), and its adjusted value accounting for the degrees of freedom (GVIF^(1/(2*Df))) for each variable in tested models.

Variable	GVIF	Df	GVIF^(1/(2*Df))
ln(distance †)	1.28	1	1.13
ln(MSS ‡)	1.50	1	1.22
Marker	1.71	4	1.07
Mating system	1.13	1	1.06
Growth form	1.79	2	1.16
Pollination mode	1.69	3	1.09
Seed dispersal mode	1.30	2	1.07
Region	1.56	2	1.12

† distance: maximum distance between populations in km.

‡ MSS: mean sample size of individuals per population.

Table S4. Results from phylogenetic ANOVA on each categorical variable (predictor) and F_{ST} as the response variable. P values are based on 1000 simulations. Significant P values are in bold.

Predictor	Sum Sq	Mean Sq	F value	P value
Mating system	0.5	0.5	24.38	**0.002**
Residuals	6.83	0.02		
Growth form	0.95	0.48	25.06	**0.014**
Residuals	6.37	0.02		
Pollination mode	1.17	0.39	21.03	**0.025**
Residuals	6.16	0.02		
Dispersal mode	0.2	0.1	4.77	0.21
Residuals	7.12	0.02		
Region	0.2	0.1	4.78	0.24
Residuals	7.12	0.02		

Table S5. Pairwise post-hoc tests between groups within each categorical variable, estimated after performing the phylogenetic ANOVA. P value corrections were done with the Holm-Bonferroni method. Significant P values are in bold (next page).

Pairwise comparisons	Pairwise T values	Pairwise P values
Mating system		
Mixed-mating – Outcrossing	4.93	**0.002**
Growth form		
Tree – Non-woody	6.9	**0.045**
Tree – Shrub	3.71	0.136
Non-woody – Shrub	1.46	0.497
Pollination mode		
Small insects – Large insects	5.01	**0.006**
Small insects – Vertebrates	1.7	1
Small insects – Wind	7.18	0.09
Large insects – Vertebrates	2.54	0.68
Large insects – Wind	1.08	1
Vertebrates – Wind	3.8	0.68
Seed dispersal mode		
Gravity – Wind	2.93	0.24
Gravity – Biotic	2.54	0.35
Wind – Biotic	0.62	0.72
Region		
Temperate – Subtropical	2.41	0.24
Temperate – Tropical	2.77	0.35
Subtropical – Tropical	0.01	0.99

Table S6. Details of model 7 including variables in the null model. Variables in bold indicate the reference level for each categorical factor; the intercept of all other levels is compared to the intercept of this reference. N indicates the sample size of each group without phylogenetic correction. The R_2 relates to the importance of each factor to the explained variance in F_{ST} after accounting for the other variables in the model. Significant P values are in bold.

Variable †	**N**	**Estimate**	**Std. Error**	**T value**	**P value**	**R_2**
Intercept		0.59	0.04	14.1	2.2E-16	
Marker						0.04
SSR	14					
AFLP	60	0.06	0.02	2.8	**0.005**	
Allozymes	114	0.01	0.02	0.1	0.3	
ISSR	7	0.03	0.04	0.6	0.53	
RAPD	16	0.08	0.03	2.7	**0.007**	
ln(distance)	337	0.01	0.002	5.5	**<0.001**	0.08
ln(mean sample size)	337	–0.03	0.007	–3.7	**<0.001**	0.04

† SSR: simple sequence repeat (microsatellites), AFLP: amplified fragment length polymorphism, ISSR: inter-simple sequence repeat, RAPD: random amplification of polymorphic DNA. Distance: maximum distance between populations. Mean sample size: mean sample size of individuals per population.

Chapter II: Flowering asynchrony contributes to genetic divergence in tropical plants

Running title: Flowering asynchrony drives differentiation

Diana Gamba, Department of Biology, University of Missouri at Saint Louis, One University Blvd., 223R Research Hall, St. Louis, MO 63121, USA; dlgtk5@mail.umsl.edu

Alexander Linan, Center for Conservation and Sustainable Development, Missouri Botanical Garden, 4344 Shaw Blvd., St. Louis, MO 63110, USA; alinan@mobot.org

Nathan Muchhala, Department of Biology, University of Missouri at Saint Louis, One University Blvd., 223R Research Hall, St. Louis, MO 63121, USA; muchhalan@umsl.edu

Author for correspondence: Diana Gamba; One University Boulevard, 223R Research Hall, Saint Louis, MO 63121, USA; dlgtk5@mail.umsl.edu; +1 (314) 702-0326.

In review: New Phytologist

Abstract: 200 words

Total word count (main text): 4932

Abstract

Speciation rates are frequently higher in tropical clades relative to temperate counterparts, yet the underlying mechanisms behind regional differences remain poorly understood. One compelling but relatively untested idea is the ‘asynchrony of seasons hypothesis’ (ASH). It posits that, while seasons are relatively synchronized over large areas in temperate regions, there can be seasonal asynchrony over short distances in tropical regions due to differences in the onset of rainfall between nearby sites. Climatic seasonal asynchrony leads to reproductive seasonal asynchrony, imposing a temporal barrier to gene flow and thus promoting population genetic divergence among subpopulations, which in turn may promote speciation. Here, we focused on understory angiosperms in two cloud forest sites in northwestern Ecuador that diverge in rainfall seasonality. We tested a central prediction of the ASH: that species with higher flowering asynchrony between sites will have genetically more divergent populations. We documented flowering phenology for nine species at both sites over one year and inferred population genetic parameters with a genome-wide genotyping approach. We found a strong positive cross-species association between flowering asynchrony and population differentiation. Our results suggest that seasonal asynchrony between sites can contribute significantly to population genetic divergence, and thus potentially to speciation, in tropical angiosperms.

Key words: Andes, angiosperms, cloud forest, flowering asynchrony, population genetic differentiation, 2b-RAD sequencing.

Introduction

Understanding the spatial and temporal processes that shape the patterns of angiosperm diversity is of central interest in biology (Fedorov 1966; Davies et al. 2004; Soltis et al. 2019). One prominent pattern exhibited by many clades is that of higher diversification rates in the tropics than in the temperate zones (Mittelbach et al. 2007; Brown 2014). Phylogenetic evidence from fossil and extant species suggest that this is due to higher speciation rates –rather than to lower extinction rates– in the tropics, which are predicted to coincide with higher population genetic divergence (reviewed in Mittelbach et al. 2007). However, the underlying mechanisms responsible for higher population genetic divergence and speciation in the tropics remain largely unknown. Several explanations suggest that dispersal, and thus gene flow, is more restricted in the tropics than in the temperate zones (Salisbury et al. 2012; Schluter and Pennell 2017). Limited gene flow between populations promotes population genetic divergence, resulting ultimately in reproductive isolation and allopatric speciation (Haffer 1997; Claramunt et al. 2012).

Several factors may contribute to gene flow being more restricted in the tropics. For example, the complex topography and environmental heterogeneity of the region can limit the movement of organisms and thus gene flow, resulting in isolated subpopulations (Wallace 1854; Benham and Witt 2016). Furthermore, the low temperature seasonality in the tropics can result in subpopulations that evolve relatively narrow niches that adapt them to local conditions. If local conditions vary widely over short distances, local adaptation would further restrict

gene flow among subpopulations, and increase isolation (Janzen 1967; Ghalambor et al. 2006). Moreover, local adaptation of subpopulations might result in mismatched timing of their reproductive cycles over short distances, disrupting gene flow between subpopulations. This temporal disruption to gene flow is central to the ‘asynchrony of seasons’ hypothesis (Martin et al. 2009), a compelling but relatively untested explanation for higher rates of population genetic divergence and speciation in the tropics.

The ‘asynchrony of seasons hypothesis’ (ASH) is based on the observation that seasons in temperate zones are determined by relatively constant temperature regimes over large geographical distances, while seasons in the tropics are determined primarily by rainfall patterns, which can vary greatly over short distances. This results in a geographical mosaic of climatic seasonality in the tropics, i.e. high climatic asynchrony between nearby sites. Because organisms usually time their reproductive cycles to seasons, such climatic asynchrony could result in reproductive asynchrony, which in turn would disrupt gene flow among subpopulations and promote population genetic divergence and speciation. Thus, a central prediction of the ASH is that tropical species with higher reproductive asynchrony will have more highly genetically divergent populations. One study found support for this prediction among new world birds: seasonal asynchrony was a strong predictor of genetic distance across intraspecific pairs of individuals, after accounting for potential geographic barriers to dispersal (Quintero et al. 2014). While compelling, this study only examined seasonal asynchrony across sites, and did not document whether this in fact

corresponds with reproductive asynchrony, a task which would be somewhat daunting for birds. Reproductive cycles are relatively easy to document for angiosperms, on the other hand, by simply observing when plants are in flower. Thus, angiosperms represent a logical group for an additional test of the ASH which can more directly examine the association between reproductive asynchrony and genetic divergence.

The impact of differences in flowering time on gene flow has been evaluated among sympatric individuals of the same species (Taylor and Friesen 2017), but little is known about how differences across a species range can impact gene flow among subpopulations. A model of incipient sympatric speciation showed that asynchronic flowering time among individuals quickly lead to reproductive isolation and speciation (Devaux and Lande 2008) because it results in assortative mating among individuals with overlapping flowering (also see (Hendry and Day 2005; Gaudinier and Blackman 2019)). In an allopatric scenario, flowering time should shift between sites with different seasonality as plants adapt to local conditions to maximize their reproductive success (Blackman 2017; Gaudinier and Blackman 2019). If shifts in flowering time can cause speciation in sympatry (Hendry and Day 2005), we expect they would be even more likely to cause speciation in allopatry, in line with the ASH.

Here, we examine the ASH for the first time, to our knowledge, in tropical angiosperms. We test the central prediction that species with higher reproductive asynchrony between sites should have greater population genetic divergence. We focus on two sites in northern Ecuador, located in the western slope of the

Andes. These sites are close enough to share many species, but differ in the onset of the rainy season, which we expected would promote divergent flowering time between subpopulations. For nine understory species, we documented flowering phenology at both study sites for one year. To infer population genetic divergence between sites, we used a genome-wide genotyping approach using single nucleotide polymorphisms. We then tested whether flowering asynchrony between sites explained differences in population genetic divergence across species.

Materials and Methods

Study sites

This study was performed in Golondrinas and Santa Lucía reserves, two cloud forests located in the northwestern slope of the Andean cordillera of Ecuador, in the provinces of Carchi and Pichincha, respectively (Fig. 1A). Sites are ~100 km apart from each other and range from 1500–2500 m in elevation. Rainfall seasonality was inferred from monthly precipitation data extracted from the WorldClim database at a projected resolution of 30 arcseconds (Hijmans et al. 2005). We delimited two polygons using the coordinates of our focal plants at each site (Golondrinas: 0.80–0.84 N, 78.07–78.15 W; Santa Lucía; 0.10–0.13 N, 78.59–78.64 W). Based on the area of these polygons we extracted mean monthly precipitation and calculated standard errors (Fig. 1B). The rainy season in Golondrinas extends from October to May, peaking in April, while the rainy season in Santa Lucía extends from December to May, peaking in March.

Moreover, Santa Lucía receives twice as much rainfall as Golondrinas each year. We expected that these differences in precipitation should affect the flowering phenology of some portion of our focal species, leading to asynchrony between sites.

Study species

To select our focal species, we began by compiling a list of species occurring at both sites using the Tropicos.org database of the Missouri Botanical Garden. Through fieldwork, we further narrowed this list to nine perennial understory angiosperms, based on sufficient abundance in both study sites for flowering phenology surveys and population genetic work. These included *Begonia tiliifolia* C. DC. (Begoniaceae), *Besleria solanoides* Kunth (Gesneriaceae), *Burmeistera multiflora* Zahlbr. (Campanulaceae), *Centropogon solanifolius* Benth. (Campanulaceae), *Drymonia tenuis* (Benth.) J.L. Clark (Gesneriaceae), *Fuchsia macrostigma* Benth. (Onagraceae), *Gasteranthus quitensis* Benth. (Gesneriaceae), *Kohleria affinis* (Fritsch) Roalson & Boggan (Gesneriaceae), and *Meriania tomentosa* (Cogn.) Wurdack (Melastomataceae).

Based on our observations in the field, all focal species have dichogamous hermaphrodite flowers with male parts developing before female parts, except for *B. tiliifolia*, which is monoecious with male flowers developing before female flowers. Dichogamy likely reduces self-fertilization for all species, although some of them produce multiple flowers at the same time, which might result in geitonogamy. Pollination of most species is achieved by hummingbirds,

while two species are bat pollinated, and one species is insect pollinated (Muchhala 2006; Weinstein and Graham 2017; Dellinger et al. 2019) (Table 1). Seed dispersal in focal species remains largely unknown, and we were unable to detect seed dispersers from field observations. Those species with berries and fleshy capsules (Table 1) are hypothesized to be animal dispersed (Kvist and Skog 1992; Loiselle and Blake 1993), while other types of capsules are hypothesized to be gravity dispersed (Gamba et al. 2017).

Estimation of flowering phenology

To assess phenological patterns, we marked 10–25 individuals per species located along trails in the reserves. We selected individuals that were at least 5 m apart from each other to limit spatial autocorrelation. We recorded the number of flowers during twice-per-month surveys over one year (July 2017 through June 2018; Table S1). For each species at each site, the date with the highest number of flowers was taken as the 100% flowering peak and used to calculate the percentage of flowers for the rest of survey dates (Table S2).

Evaluation of flowering seasonality and asynchrony

We evaluated flowering seasonality from the twice-per-month flowering percentages with a Fourier spectral analysis using the function ‘spec.pgram’ in the stats R package in RStudio V 1.2.5019 (R Core Team 2018). Such analysis decomposes the flowering time series into sinusoidal curves representing different periodicities (Platt and Denman 1975; Zalamea et al. 2011; Quintero et

al. 2014). For each species, we evaluated the fit of the flowering data to periodicities corresponding to one peak of flowering per year with a 12-month period between peaks (i.e., annual pattern), two peaks of flowering per year with a 6-month period (biannual), and three and four peaks of flowering per year, with 4- and 3-month periods, respectively (sub-annual patterns). To evaluate whether the fit of the selected pattern for each species was greater than would be expected by chance, we constructed a null distribution of flowering times for each species by randomly resampling the flowering data 10,000 times (as in Zalamea et al. 2011). All species exhibited statistically significant phenological seasonality, exceeding the 95% quantiles of the corresponding null distributions, and this pattern was consistent between sites.

After establishing the periodicity of phenological patterns at each site, we then performed Fourier cospectral analyses to estimate the magnitude of intraspecific flowering asynchrony between sites. This analysis gives a value in radians corresponding to an angle positioning that represents the lag between flowering peaks between sites (Quintero et al. 2014). We transformed this value to degrees and subsequently to percent asynchrony, where 0º corresponds 0% asynchrony (both peaks occurring at the same time), and 180º corresponds to 100% asynchrony (the peak of flowering in one site coinciding with the valley of flowering on the other site). We also used similar Fourier analyses as outlined above to estimate rainfall seasonality and percent asynchrony between study sites, using the WorldClim data described previously (Table S3).

Genomic library preparation and sequencing

We began molecular work by extracting whole genomic DNA from silica-dried leaf tissue from 20 individuals per species from each study site. We followed the CTAB protocol (Doyle and Doyle 1987), modified slightly by incorporating additional ethanol washes of the DNA pellet. We quantified DNA with a Qubit 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific), using the manufacturer's protocol. For each of our samples with sufficient DNA, we obtained single nucleotide polymorphisms (SNPs) to use as genetic markers for population divergence inferences with the restriction site-associated DNA sequencing technique called 2b-RAD (Wang et al. 2012). We constructed 2b-RAD libraries for each individual following the protocol of (Wang et al. 2012). 500 ng of total genomic DNA were digested with a type IIb endonuclease, BcgI (New England Biolabs), which cuts DNA on both sides of a recognition site to obtain uniform 36-bp fragments scattered across the genome. Oligonucleotide Illumina sequences were ligated to these fragments with 12 double-stranded barcoded adapters, one per each column of a 96-sample plate. In order to increase sequence coverage per locus, we utilized reduced representation barcoded adapters which reduce the total number of loci sequenced. Samples with different barcoded adapters were pooled into 8 groups of 12 samples. Following initial pooling, Illumina RAD PCR primers (1–8) were incorporated into the fragments of each pool via 14 cycles of PCR amplification. Amplified pools were then purified via gel electrophoresis, and fragments of 75bp were size-selected by excising target bands from the agarose gel. We then used a Min Elute Gel

extraction kit (Qiagen) to purify target bands. Purified samples were quantified and pooled into a single library in equimolar concentrations. We generated three libraries, which together included ~15 individuals per species per study site. Libraries were sequenced on Illumina HiSeq 2500 (Brigham Young University, UT) and HiSeq 4000 (Duke University, NC) machines, to generate single-end 50 bp reads.

Building loci and genotyping individuals

Reads were demultiplexed using a custom script (trim2bRAD) generated by the Matz lab at the University of Austin, TX (https://github.com/z0on/2bRAD_denovo). This script trims 2b-RAD fragments from barcodes to produce one fastq file per sample. The resulting files were quality filtered with FastQC (Babraham Bioinformatics) and the FASTX-toolkit (Gordon and Hannon 2010). We discarded low quality reads and obtained sequences that were 36 bp in length, with a minimum of 90% bases having a Phred quality score of at least 20 and an input quality offset of 33. We then used the Stacks v2.3e pipeline to genotype individuals and produce a catalog of loci for each species (Catchen et al. 2013). We ran Stacks using the default parameter settings for building loci, which we considered to be appropriate for the short size of the 2b-RAD fragments. These parameter settings included a maximum distance of 2 nucleotide differences allowed between reads, a minimum depth of coverage of 3 reads required to create a stack, and a maximum distance of 4 nucleotide differences allowed to align secondary reads

to primary stacks. We also allowed one gap between stacks before merging into putative loci. We filtered loci with the program ‘populations’ on the same pipeline. We excluded loci that were genotyped in <40% of individuals in each population. To avoid effects of linkage disequilibrium in our analyses, we only used one random SNP per locus. To prevent potential low-frequency SNP miscalls, we discarded alleles that had a frequency <5% in any locus across all individuals. To avoid repetitive or paralogous loci, the maximum number of heterozygous individuals that may be present in any locus was set to 75%. Lastly, we used the program VCFtools v0.1.16 (Danecek et al. 2011) to identify individuals with >50% missing data relative to variant sites, which we removed from subsequent analyses.

Inference of population genetic divergence

We used the program GenoDive v3.0 (Meirmans and Van Tienderen 2004) to calculate genetic diversity statistics. We assessed population genetic divergence between study sites for each species using the pairwise fixation index, F_{ST} (Wright 1965; Nei 1977), and the allelic differentiation statistic, Jost’s *D* (Jost 2008; Jost et al. 2018). The statistical significance of diversity statistics was assessed using 1000 random permutations of the data, while standard deviations of diversity statistics were obtained by jackknifing over loci and 95% confidence intervals were obtained by bootstrapping over loci.

To further visualize genetic divergence, we inspected genetic clustering in focal species. We conducted assignment tests using the program STRUCTURE

v.2.3.4 (Pritchard et al. 2000) as implemented in the ipyrad analysis toolkit (https://ipyrad.readthedocs.io/en/latest/API-analysis/cookbook-structure.html). We examined whether the data fit to K = 1–4 genetic clusters using 20 replicates per K with 300,000 generations used as burn in followed by 500,000 generations to achieve convergence. Optimal K values were inferred using the Evanno method (Evanno et al. 2005). Results were summarized with the program CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007).

Testing the relationship between flowering asynchrony and population genetic divergence

We used linear regressions to test if flowering asynchrony predicts population genetic divergence between sites across our focal species. To evaluate whether this relationship was robust to different measures of population genetic divergence, we repeated analyses with either pairwise F_{ST} or Jost's *D* as response variables. We also performed phylogenetic regressions to account for potential autocorrelation in the data due to evolutionary relationships. To this end, we extracted a species-level phylogeny containing the focal taxa (Fig. S1) from an angiosperm mega-tree (Smith and Brown 2018) in the R package V.PhyloMaker (Jin and Qian 2019). Branch lengths were inferred using the branch length adjuster algorithm in the same package (Qian and Jin 2016). We performed linear regressions of population genetic divergence on flowering asynchrony with the R function 'lm', and phylogenetic regressions with function 'phylolm' from the phylolm R package (Ho and Ané 2014). To assess the fit of

our phylolm models to the data, the likelihood of parameters was calculated under a Brownian motion model of trait evolution (Symonds and Blomberg 2014). We compared the fit of the models using AIC scores (Akaike 1974; Burnham and Anderson 2004). To provide a more thorough evaluation of model fit, we also measured phylogenetic signal in the error term of each linear regression (as in Revell 2010) using Pagel's λ (Pagel 1999).

Results

Flowering seasonality and asynchrony

Fourier spectral analyses found an annual flowering periodicity to be the most common pattern in both study sites (Table 2). Most species flowered earlier in Golondrinas than in Santa Lucía (Fig. 2), as might be expected given the earlier onset of the rainy season in Golondrinas. The only species with patterns different from annual were *B. multiflora*, in which the production of flowers was steady with three peaks in the year, and *B. solanoides*, in which we recorded two clear peaks in the year separated by periods of 0% production.

Among the 7 annually-flowering species, there was variation in the extent to which they were also flowering in other parts of the year, which can be summarized as three general patterns: 1) constant flower production at >30% of highest flower count throughout the year (*B. tiliifolia*), 2) constant flower production at >10% of highest flower count throughout the year (*D. tenuis* and *G. quitensis*), and 3) discrete flower production, with periods of 0% production lasting 1–4 months (*C. solanifolius*, *F. macrostigma*, *K. affinis*, *M. tomentosa*).

Fourier cospectral analyses identified a range of flowering asynchrony values across the species, from 2.7–87.0% (Table 2, mean = 26.1 ± 29.5 SD). *Burmeistera multiflora* and *F. macrostigma* showed the lowest asynchrony, while *K. affinis*, *M. tomentosa* and *C. solanifolius* showed the highest. The remaining species presented asynchronies between 9.3–16.8%. Similar Fourier cospectral analyses of rainfall patterns from WorldClim data across the two study sites detected a significant annual pattern in precipitation for both sites and a precipitation asynchrony of 19% between them (Table S3).

Filtered genetic datasets

After SNP calling and quality control using different filtering procedures, we obtained a mean of 2,174,885 SNP loci per species (± 834,061 SD; range: 1,071,520–3,370,979), with a mean coverage ranging from 12.6–22.7 read depth per loci across species (Table S4). After removing individuals with >50% missing data, final sample sizes of individuals per species per study site ranged from 7–12 (mean = 9 ± 1.5 SD), and the number of variant loci ranged from 1,082–7,624 (mean = 3,840 ± 2,199 SD) across species, with missing data across species ranging from 35–40% (mean = 38 ± 2.3 SD) (Table S5 and S6).

Gene diversity was similar across species, with He (expected heterozygosity) within sites ranging from 0.19–0.26 (mean = 0.24 ± 0.02). Additionally, all species showed statistically significant levels of inbreeding, as indicated by significant G_{IS} values, when these values are pooled across sites for each species (mean = 0.51 ± 0.2 SD; Table S5) as well as when they are

analyzed separately by site for each species (mean = 0.51 ± 0.2 SD, Table S6).

Population genetic divergence

Population genetic divergence between sites was significant for all species (Fig. 3). Pairwise F_{ST} values ranged from 0.09–0.30 (mean = 0.16 ± 0.09 SD), and Jost's D values from 0.03–0.13 (mean = 0.06 ± 0.04 SD). Further inspection of genetic divergence based on clustering STRUCTURE analyses showed that K = 2 was the most common supported number of clusters within species for all of the species, with the exception of *D. tenuis* for which K = 3 was the most likely number (Fig. 3 and Fig. S2). These genetic clusters most frequently followed geography, with one genetic cluster assigned to each of the two study sites. For *B. tiliifolia* and *B. multiflora*, there was one admixed individual identified at each site based on STRUCTURE Q values, while *F. macrostigma* and *M. tomentosa* showed no evidence of admixture between clusters. *Centropogon solanifolius*, *G. quitensis* and *K. affinis* exhibited a directional pattern of admixture, with varying amounts of alleles from Santa Lucía in Golondrinas but not vice-versa. For *D. tenuis*, Santa Lucía was almost homogeneous in cluster assignment except for one admixed individual, while all three genetic clusters were present in Golondrinas. Lastly, *B. solanoides* was composed of two genetic clusters present in both study sites (Fig. 3). This unexpected result might indicate that *B. solanoides* is composed of two cryptic species which are present at both sites.

Flowering asynchrony and genetic divergence

We performed linear and phylogenetic regressions to evaluate the relationship across species between flowering asynchrony and genetic divergence (in terms of pairwise F_{ST} and Jost's *D* values). Because genetic clustering results suggest that individuals of *B. solanoides* may potentially represent two species, we repeated regressions either including or excluding *B. solanoides* (Table 3).

Results demonstrate that flowering asynchrony is a significant predictor of pairwise F_{ST} $F(1, 7) = 39.1$, adjusted-$R^2 = 0.83$, $p = 0.0004$) and Jost's *D* ($F(1, 7) = 33.5$, adjusted-$R^2 = 0.80$, $p = 0.0007$) (Table 3). The same analyses without *B. solanoides* yielded similar positive associations between flowering asynchrony and pairwise F_{ST} ($F(1, 6) = 36.3$, adjusted-$R^2 = 0.83$, $p = 0.0009$) and Jost's *D* ($F(1, 6) = 29.2$, adjusted-$R^2 = 0.80$, $p = 0.002$) (Table 3 and Fig. 4A, B). Phylogenetic regressions did not improve model fit and produced identical results. Similarly, Pagel's λ tests of phylogenetic signal on the error term of all linear regressions were non-significant (Table 3), consistent with a lack of phylogenetic autocorrelation in the data.

Discussion

Our results reveal a robust positive association between flowering asynchrony and population genetic divergence across our nine focal species of Andean angiosperms (Table 3, Fig. 4). Those species with greater shifts in flowering patterns across our two study sites had greater levels of genetic

divergence between their two subpopulations. Given that precipitation patterns were significantly different across these sites, these results support the idea that spatial variation in climatic seasonality may drive increased levels of genetic divergence, which in turn might be an important mechanism for the origin of new species of angiosperms.

Our study design controlled for many other factors that might impact population genetic divergence, increasing the probability that the association we found is in fact due directly to flowering asynchrony rather than a confounding variable. For instance, by choosing the same two study sites for all species, geographic distance could not influence differences in F_{ST} values across species. Similarly, study species are likely all exposed to the same geographic barriers. They all occur in the understory of cloud forests on the same slope of the Andes, and both sites belong to the southern end of the Choco Andean corridor (Mordecai et al. 2009) and are presumably well-connected by a continuous corridor of forests due to the presence of the Cotacachi-Cayapas national park between them. Finally, differences in inbreeding levels do not seem to underlie the differences in population genetic divergence. Inbreeding can affect population genetic structure (Duminil et al. 2007), however we do not find such association in our dataset: the inbreeding coefficient (G_{IS} in Table S5) does not predict F_{ST} ($F_{(1, 7)} = 0.19$, adjusted $R^2 = -0.11$, $p = 0.7$).

We note that six of our study species presented relatively high inbredding coeffiecients (i.e., F_{IS} values were > 0.5 in *B. tiliifolia*, *B. solanoides*, *C. solanaoides*, *D. tenuis*, *G. quitensis*, and *K. affinis*), which is generally associated

with selfing. This is stricking given that five of the species are largely visited by hummingbirds (Weinstein and Graham 2017), while only one (*B*. *tiliifolia*) is presumably insect pollinated (*pers. obs.*). Studies of the pollination biology of *B*. *tiliifolia* are lacking, but it is possible that this monoecious herb is self-compatible, as are many other *Begonia* (Agren and Schemske 1993; Matolweni et al. 2000; Waytt & Sazima 2011). Self-compatibility is also common among other species related to our focal taxa, as has been shown in *Besleria* (Martin-Gajardo 1999), *Drymonia* (Steiner 1985), and other neotropical species (Schatz 1990). However, spontaneous self-pollination is unlikely due to monoecy in *B. tiliifolia*, and protandry in the hummingbird pollinated species. It is likely that pollinators promote geitonogamy and thus increase inbreeding within subpopulations, especially for hummingbird pollinated species that produce multiple flowers simultaneously (i.e., *G. quitensis* and *K. affinis*).

We also note that species with lower genetic divergence (e.g., *B. multiflora*) showed a more constant production of flowers throughout the year, while species with greater genetic divergence showed markedly interrupted production of flowers, with periods of 0% production ranging from 1–4 months. Specifically, in *M. tomentosa* zero-flowering periods were long and extended (~ 4 months, one valley per year, figure 2), while in *C. solanifolius* zero-flowering periods were short and intermittent (~ 2 months or shorter, multiple valleys per year, Fig. 2). Thus, some zero-flowering periods at a given site may be an important contributor to cutting off gene flow between nearby sites.

The mode of gene dispersal between subpopulations could also affect

the importance of flowering asynchrony in population genetic divergence. If gene flow between nearby sites is mainly achieved via pollen dispersal, flowering asynchrony would be the primary mechanism for genetic divergence. However, if gene flow is also achieved via seed dispersal, flowering asynchrony might not be as important to promote genetic divergence. In the presence of seed dispersal, the association between flowering asynchrony and genetic divergence will largely depend on the fate of migrant seeds in a new site in combination with the underlying drivers of flowering time. If flowering time is a phenotypically plastic response to rainfall patterns (Levin 2009), adult migrants would flower at the same time as the local population, while if it is an evolved response to some other cue (Hall and Willis 2006), these migrants may remain out-of-synch with conspecifics in the new site. Common garden experiments (as in Fudickar et al. 2016), or reciprocal transplants (as in Hall and Willis 2006), would help to evaluate the role of phenotypic plasticity and environmental cues in determining flowering phenology.

If migrants remain out of synch with conspecifics in the new site, flowering asynchrony could arise within a site and prevent gene flow between sympatric individuals. Asynchrony in flowering time among sympatric individuals is often termed allochrony (Gaudinier and Blackman 2019) and has been proposed as a possible mechanism for reproductive isolation in sympatry (Hendry and Day 2005; Taylor and Friesen 2017). A model of speciation in sympatry proposes that reproductive isolation can quickly evolve within small populations exhibiting long population-level periods of flowering, but short

individual-level periods of flowering, as this will cluster individuals genetically according to their flowering time (Devaux and Lande 2008). However, whether or how frequently this occurs in nature remains unclear. Allochrony has also been proposed as a mechanism that strengthens boundaries between incipient species when ranges rejoin in secondary contact, with prominent empirical examples in nature (Briscoe Runquist et al. 2014; Hipperson et al. 2016; Spriggs et al. 2019). This evidence suggests that flowering asynchrony likely evolves in allopatry, in line with the 'asynchrony of seasons hypothesis' (ASH), and its persistence after secondary contact helps to reduce gene flow and maintain species boundaries.

Among our focal species, *B. solanoides* was the only taxon for which we detected two genetic clusters that did not correspond to the two study sites, but rather both occurred at both study sites. Interestingly, we note that one genetic cluster (in blue in figure 3) corresponds to early bloomers in both study sites, while the other (in orange) is composed of late bloomers in both study sites. Thus, these clusters might represent cryptic species separated by flowering time. This pattern suggests empirical support for the scenario discussed above, where shifts in flowering time evolved in allopatry (as per the ASH) and now maintain boundaries of these hypothetical cryptic species after one or both expanded their range into sympatry. Remarkably, the pairwise F_{ST} between genetic clusters was 0.23 ($p<0.001$), greater than the pairwise F_{ST} between sites (0.09, Table 3). A thorough taxonomic and demographic study including individuals across *B. solanoides*' range would help to evaluate this hypothesized scenario of cryptic

speciation after secondary contact driven by flowering asynchrony.

One important caveat to our study is that the relationship between flowering asynchrony and population genetic divergence between sites only establishes a correlation, not a causation. Greater asynchrony may drive increased genetic divergence, as we have argued above. However, it could also be that subpopulations in each study site first became genetically differentiated due to other factors, and this divergence then led to differences in flowering phenologies. In such a case, flowering asynchrony would further strengthen the existing genetic divergence between subpopulations. Nonetheless, whether shifts in flowering time cause or strengthen genetic divergence, our main finding supports flowering asynchrony as an important mechanism that limits gene flow between subpopulations.

Our study provides the first test to date of the ‘asynchrony of seasons hypothesis’ (Martin et al. 2009) in flowering plants. We found evidence for a central prediction of the ASH, namely that reproductive asynchrony between tropical sites with different seasonality is associated with increased population genetic divergence. Thus, reproductive asynchrony may accelerate rates of population differentiation, and ultimately speciation in tropical plants. Before our study, ASH had only been tested in birds (Moore et al. 2005; Quintero et al. 2014). We thus encourage more phenological studies, in flowering plants and other organisms, to broadly document patterns of reproductive asynchrony and how these relate to ‘isolation by time’ in allopatry. Future work should also examine whether reproductive asynchrony is more prevalent in tropical than in

temperate systems, as predicted by their increased seasonal asynchrony. If so, flowering asynchrony could represent a key explanation for the latitudinal diversity gradient observed in flowering plants.

Acknowledgements

Ben Weinstein and Holger Beck were instrumental for locating plants in Santa Lucía and provided useful preliminary phenological data for a number of species. Thanks to Nora Oleas and Paola Peña for help with the research permit in Ecuador (MAE-DNB-CM-2015-017). Robert Ricklefs, Christine Edwards, and Carmen Ulloa provided great advice in this study. Thanks to field assistants Hugo Quintanchala, Paola Peña, Nelly Muñoz, Justin Zweck, An Nguyen, and Carlos Imery, and to families at Santa Lucía cloud forest reserve and at Bosque Protector Golondrinas for their hospitality. Joel Swift offered useful guidance for 2b-RAD, and Isabel Loza for Fourier analyses. Discussions with members of the Muchhala lab at the University of Missouri-Saint Louis (UMSL) greatly improved a previous version of this manuscript. This study was funded with graduate student research grants from the Whitney R. Harris World Ecology Center at the University of Missouri at St. Louis, the Botanical Society of America, the Society of Systematic Biologists, and the American Philosophical Society to DG, and a grant from the Office of Research Administration at the University of Missouri at St. Louis to NM.

References

Agren, J., and D. W. Schemske. 1993. Outcrossing rate and the inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. Evolution. 47:125–135.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19:716–723.

Benham, P. M., and C. C. Witt. 2016. The dual role of Andean topography in primary divergence: functional and neutral variation among populations of the hummingbird, *Metallura tyrianthina*. BMC Evol. Biol. 16:22–22.

Blackman, B. K. 2017. Changing responses to changing seasons: natural variation in the plasticity of flowering time. Plant Physiol. 173:16–26.

Briscoe Runquist, R. D., E. Chu, J. L. Iverson, J. C. Kopp, and D. A. Moeller. 2014. Rapid evolution of reproductive isolation between incipient outcrossing and selfing Clarkia species. Evolution 68:2885–2900.

Brown, J. H. 2014. Why are there so many species in the tropics? J. Biogeogr. 41:8–22.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociol. Methods Res. 33:261–304. Sage Publications Sage CA: Thousand Oaks, CA.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22:3124–3140.

Claramunt, S., E. P. Derryberry, J. V. Remsen, and R. T. Brumfield. 2012. High dispersal ability inhibits speciation in a continental radiation of passerine birds. Proc. R. Soc. B Biol. Sci. 279:1567–1574.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin, and 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Davies, T. J., T. G. Barraclough, M. W. Chase, P. S. Soltis, D. E. Soltis, and V. Savolainen. 2004. Darwin's abominable mystery: Insights from a supertree of the angiosperms. Proc. Natl. Acad. Sci. 101:1904–1909.

Dellinger, A. S., L. M. Scheer, S. Artuso, D. Fernández-Fernández, F. Sornoza, D. S. Penneys, R. Tenhaken, S. Dötterl, and J. Schönenberger. 2019. Bimodal pollination systems in Andean Melastomataceae involving birds, bats, and rodents. Am. Nat. 194:104–116.

Devaux, C., and R. Lande. 2008. Incipient allochronic speciation due to non-selective assortative mating by flowering time, mutation and genetic drift. Proc. R. Soc. B Biol. Sci. 275:2723–2732.

Doyle, J., and J. L. Doyle. 1987. Genomic plant DNA preparation from fresh tissue-CTAB method. Phytochem Bull 19:11–15.

Duminil, J., S. Fineschi, A. Hampe, P. Jordano, D. Salvini, G. G. Vendramin, and R. J. Petit. 2007. Can Population Genetic Structure Be Predicted from Life-History Traits? Am. Nat. 169:662–672.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters

of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611–2620.
Fedorov, A. 1966. The structure of the tropical rain forest and speciation in the humid tropics. J. Ecol. 54:1–11.
Fudickar, A. M., T. J. Greives, J. W. Atwell, C. A. Stricker, and E. D. Ketterson. 2016. Reproductive allochrony in seasonally sympatric populations maintained by differential response to photoperiod: implications for population divergence and response to climate change. Am. Nat. 187:436–446.
Gamba, D., N. R. Maguiña, C. A. Calderón-Acevedo, K. Torres, and N. C. Muchhala. 2017. Seed dispersal for the unusual inflated berries of Burmeistera (Campanulaceae). Neotropical Biodivers. 3:10–17.
Gaudinier, A., and B. K. Blackman. 2019. Evolutionary processes from the perspective of flowering time diversity. New Phytol. nph.16205.
Ghalambor, C. K., R. B. Huey, P. R. Martin, J. J. Tewksbury, and G. Wang. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. Integr. Comp. Biol. 46:5–17.
Gordon, A., and G. Hannon. 2010. Fastx-toolkit. FASTQ/A short-reads pre-processing tools. Unpubl. Httphannonlab Cshl Edufastxtoolkit 5.
Haffer, J. 1997. Alternative models of vertebrate speciation in Amazonia: an overview. Biodivers. Conserv. 6:451–476.
Hall, M. C., and J. H. Willis. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. Evolution 60:2466–2477.
Hendry, A. P., and T. Day. 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. Mol. Ecol. 14:901–916.
Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high-resolution interpolated climate surfaces for global land areas. Int. J. Climatol. J. R. Meteorol. Soc. 25:1965–1978.
Hipperson, H., L. T. Dunning, W. J. Baker, R. K. Butlin, I. Hutton, A. S. T. Papadopulos, C. M. Smadja, T. C. Wilson, C. Devaux, and V. Savolainen. 2016. Ecological speciation in sympatric palms: 2. Pre- and post-zygotic isolation. J. Evol. Biol. 29:2143–2156.
Ho, L. si T., and C. Ané. 2014. A Linear-time algorithm for gaussian and non-gaussian trait evolution models. Syst. Biol. 63:397–408.
Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.
Janzen, D. H. 1967. Why mountain passes are higher in the tropics. Am. Nat. 101:233–249.
Jin, Y., and H. Qian. 2019. V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. Ecography ecog.04434.
Jost, L., F. Archer, S. Flanagan, O. Gaggiotti, S. Hoban, and E. Latch. 2018. Differentiation measures for conservation genetics. Evol. Appl. 11:1139–1148.
Jost, L. O. U. 2008. G_{ST} and its relatives do not measure differentiation. Mol.

Ecol. 17:4015–4026.
Kvist, L. P., and L. E. Skog. 1992. Revision of Kohleria (Gesneriaceae). Smithson. Contrib. Bot.
Levin, D. A. 2009. Flowering-time plasticity facilitates niche shifts in adjacent populations. New Phytol. 183:661–666.
Loiselle, B. A., and J. G. Blake. 1993. Spatial distribution of understory fruit-eating birds and fruiting plants in a neotropical lowland wet forest. Pp. 177–189 in T. H. Fleming and A. Estrada, eds. Frugivory and seed dispersal: ecological and evolutionary aspects. Springer Netherlands, Dordrecht.
Martin, P., F. Bonier, I. Moore, and J. J. Tewksbury. 2009. Latitudinal variation in the asynchrony of seasons: implications for higher rates of population differentiation and speciation in the tropics. Ideas Ecol. Evol. 2.
Meirmans, P. G., and P. H. Van Tienderen. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4:792–794.
Mittelbach, G. G., D. W. Schemske, H. V. Cornell, A. P. Allen, J. M. Brown, M. B. Bush, S. P. Harrison, A. H. Hurlbert, N. Knowlton, H. A. Lessios, C. M. McCain, A. R. McCune, L. A. McDade, M. A. McPeek, T. J. Near, T. D. Price, R. E. Ricklefs, K. Roy, D. F. Sax, D. Schluter, J. M. Sobel, and M. Turelli. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecol. Lett. 10:315–331.
Moore, I. T., F. Bonier, and J. C. Wingfield. 2005. Reproductive asynchrony and population divergence between two tropical bird populations. Behav. Ecol. 16:755–762.
Mordecai, R. S., R. J. Cooper, and R. Justicia. 2009. A threshold response to habitat disturbance by forest birds in the Choco Andean corridor, Northwest Ecuador. Biodivers. Conserv. 18:2421–2431.
Matolweni, L. O., K. Balkwill, and T. McLellan. 2000. Genetic diversity and gene flow in the morphologically variable, rare endemics *Begonia degrei* and *Begonia homonyma* (Begoniaceae). Am. J. Bot. 87:431–439.
Muchhala, N. 2006. The pollination biology of *Burmeistera* (Campanulaceae): specialization and syndromes. Am. J. Bot. 93:1081–1089.
Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. 41:225–233.
Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.
Platt, T., and K. L. Denman. 1975. Spectral analysis in ecology. Annu. Rev. Ecol. Syst. 6:189–210.
Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.
Qian, H., and Y. Jin. 2016. An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. J. Plant Ecol. 9:233–239.
Quintero, I., S. González-Caro, P.-C. Zalamea, and C. D. Cadena. 2014. Asynchrony of seasons: genetic differentiation associated with geographic

variation in climatic seasonality and reproductive phenology. Am. Nat. 184:352–363.
R Core Team. 2018. R Foundation for Statistical Computing; Vienna, Austria: 2015. R Lang. Environ. Stat. Comput. 2013.
Revell, L. J. 2010. Phylogenetic signal and linear regression on species data: Phylogenetic regression. Methods Ecol. Evol. 1:319–329.
Salisbury, C. L., N. Seddon, C. R. Cooney, and J. A. Tobias. 2012. The latitudinal gradient in dispersal constraints: ecological specialisation drives diversification in tropical birds. Ecol. Lett. 15:847–855.
San Martin-Gajardo, I., and L. Freitas.1999. Hummingbird pollination in Besleria longimucronata Hoehne (Gesneriaceae) in South-Eastern Brazil. Biociências 7:13–24.
Schatz, G. E. 1990. Some aspects of pollination biology in Central American forests. Pp. 69–84 in K. S. Bawa, and M. Hadley, eds. Reproductive ecology of tropical forest plants. Unesco.
Schluter, D., and M. W. Pennell. 2017. Speciation gradients and the distribution of biodiversity. Nature 546:48–55.
Smith, S. A., and J. W. Brown. 2018. Constructing a broadly inclusive seed plant phylogeny. Am. J. Bot. 105:302–314.
Soltis, P. S., R. A. Folk, and D. E. Soltis. 2019. Darwin review: angiosperm phylogeny and evolutionary radiations. Proc. R. Soc. B Biol. Sci. 286:20190099.
Spriggs, E. L., C. Schlutius, D. A. Eaton, B. Park, P. W. Sweeney, E. J. Edwards, and M. J. Donoghue. 2019. Differences in flowering time maintain species boundaries in a continental radiation of Viburnum. Am. J. Bot.
Steiner, K. E. 1985. The role of nectar and oil in the pollination of *Drymonia serrulata* (Gesneriaceae) by Epicharis bees (Anthophoridae) in Panama. Biotropica 17:217–229.
Symonds, M. R. E., and S. P. Blomberg. 2014. A Primer on Phylogenetic Generalised Least Squares. Pp. 105–130 in L. Z. Garamszegi, ed. Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology. Springer Berlin Heidelberg, Berlin, Heidelberg.
Taylor, R. S., and V. L. Friesen. 2017. The role of allochrony in speciation. Mol. Ecol. 26:3330–3342.
Wallace, A. R. 1854. On the monkeys of the Amazon. Ann. Mag. Nat. Hist. 14:451–454.
Wang, S., E. Meyer, J. K. McKay, and M. V. Matz. 2012. 2b-RAD: a simple and flexible method for genome-wide genotyping. Nat. Methods 9:808–810.
Weinstein, B. G., and C. H. Graham. 2017. Persistent bill and corolla matching despite shifting temporal resources in tropical hummingbird-plant interactions. Ecol. Lett. 20:326–335.
Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420.
Waytt, G. E., and M. Sazima. 2011. Pollination and reproductive biology of thirteen species of Begonia in the Serra do Mar State Park, São Paulo, Brazil. J. Poll. Ecol. 6(14):95–107.

Zalamea, P.-C., F. Munoz, P. R. Stevenson, C. E. T. Paine, C. Sarmiento, D. Sabatier, and P. Heuret. 2011. Continental-scale patterns of *Cecropia* reproductive phenology: evidence from herbarium specimens. Proc. R. Soc. B Biol. Sci. 278:2437–2445.

Data accessibility statement

Should the manuscript be accepted, the data and R scripts supporting the results will be archived in Dryad and their DOI will be included at the end of this article.

Author contributions

DG and NM planned and designed the research. DG collected and analyzed the data. AL performed STRUCTURE analyses. DG wrote the first draft of the manuscript. DG, AL, and NM contributed equally to substantial revisions of the manuscript.

Fig. 1 (a) Location of study sites in northwestern Ecuador, South America, with map color representing elevation over sea level in m. The grey circle is Bosque Protector Golondrinas and the black circle is Santa Lucía Cloud Forest Reserve. **(b)** Rainfall seasonality at study sites: the y-axis is the amount of monthly rainfall in mm. Boxplots show the distribution of rainfall data across the geographic extent of each reserve; black circles are monthly means, horizontal grey lines are medians, and the boxes' lower and upper limits are 25th and 75th percentiles. Elevation and monthly rainfall data come from WorldClim raster layers at a projected resolution of 1 km^2.

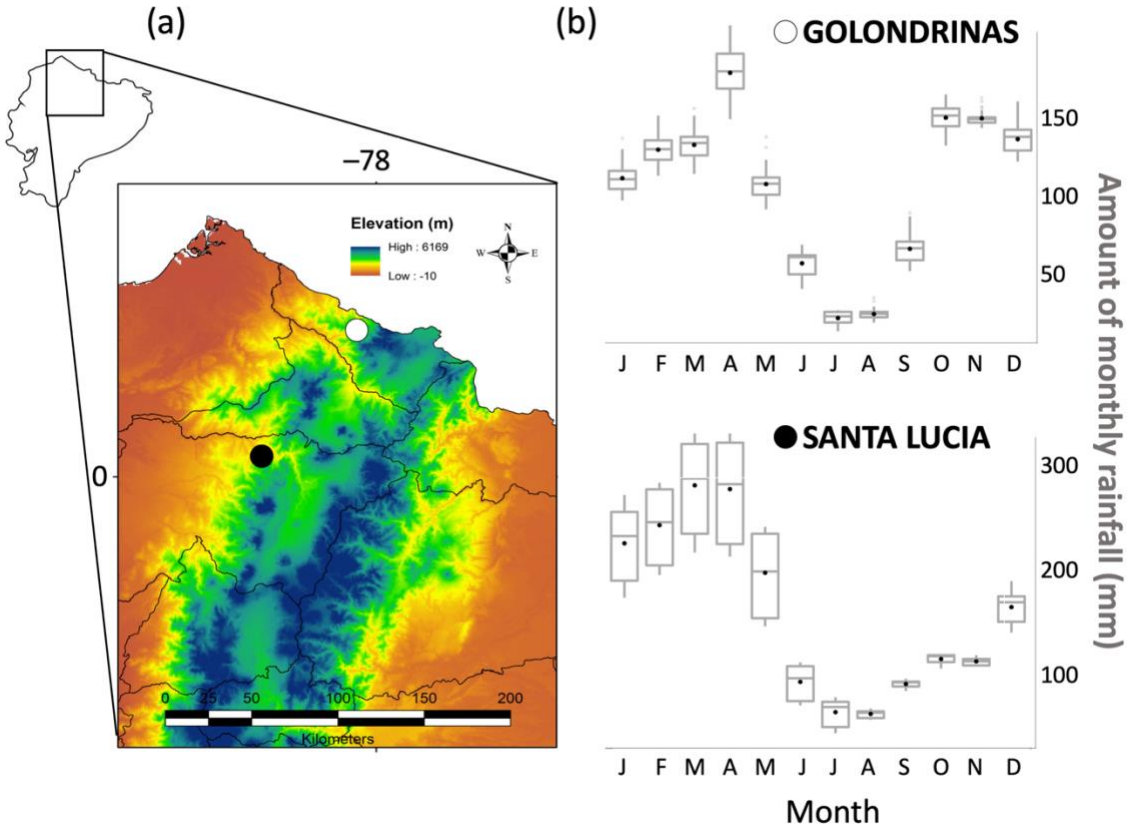


Fig. 2 Flowering phenology of the nine studied species recorded for one year (July 2017 – June 2018). Flowering data is depicted in the y-axis as a monthly percent of peak flowering in the year. Grey lines correspond to flowering in Golondrinas, and black lines in Santa Lucía.

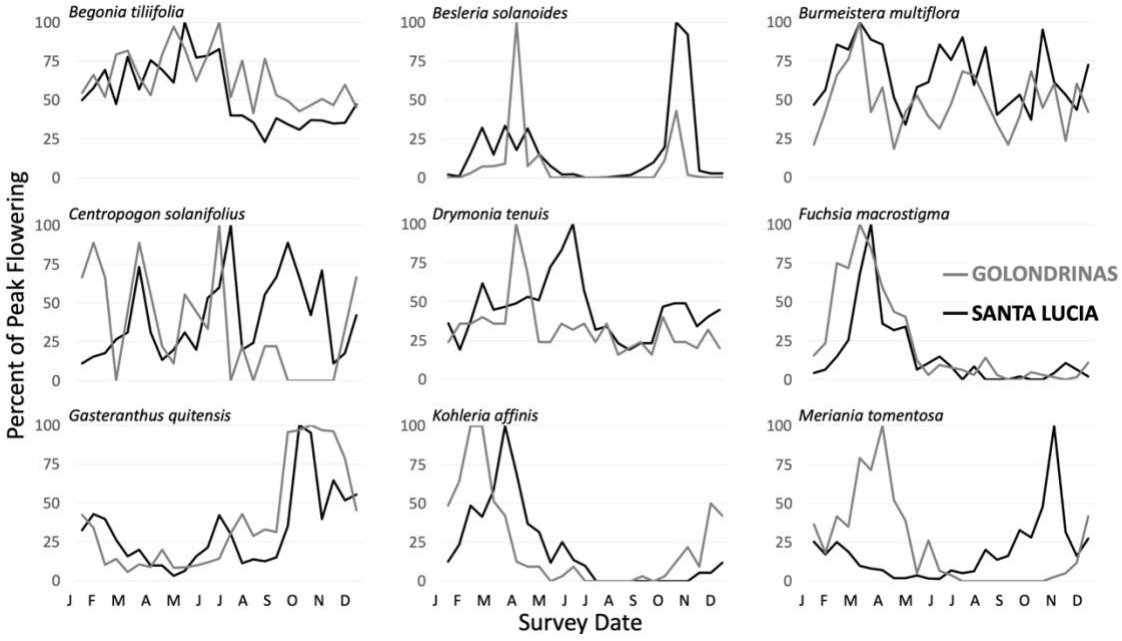


Fig. 3 Identified genetic clusters and Bayesian admixture proportions depicted for individual plants of each species. For most species K = 2 was the best K-fit to the data, except for *D. tenuis* which best K = 3. The black vertical bar on each structure plot separates individuals from Santa Lucía to the left and Golondrinas to the right (clusters between species are independent). Measures of genetic divergence between sites are indicated with pairwise F_{ST} values (fixation index) and Jost's *D* values (allelic differentiation). All statistics were significant ($p<0.005$) based on 1000 permutations.

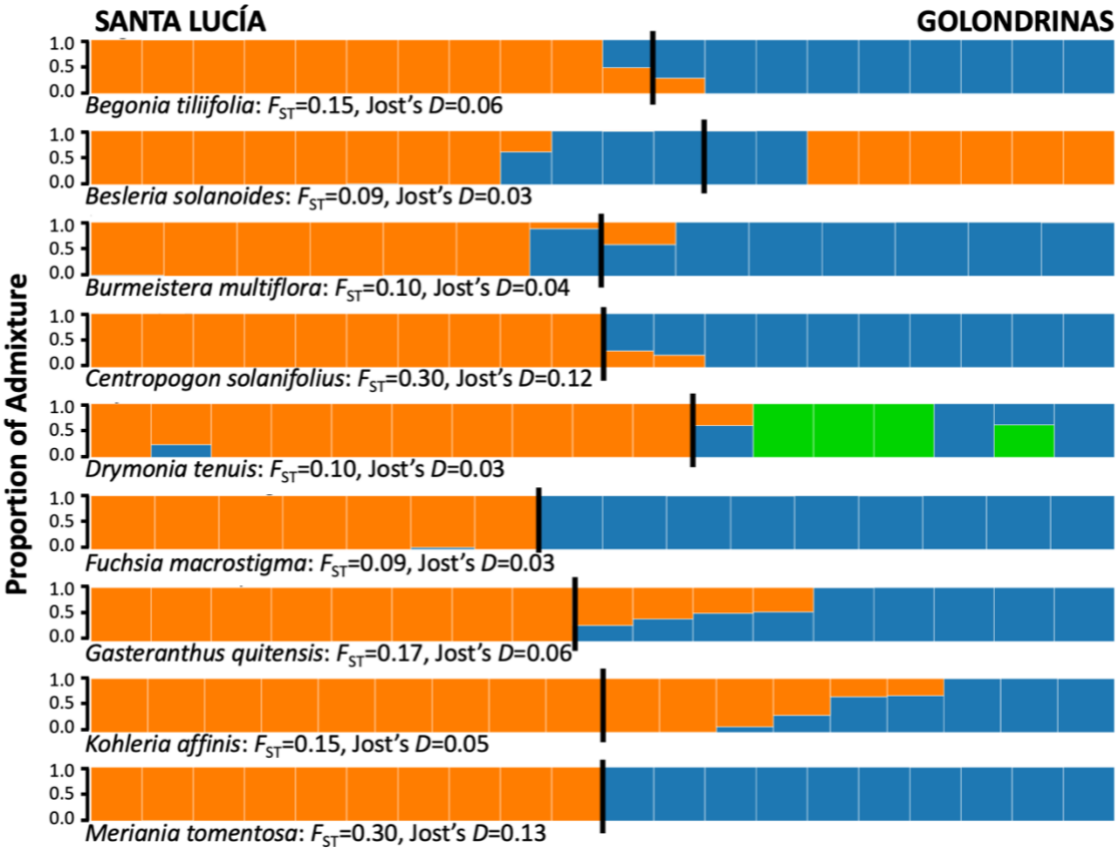


Fig. 4 The positive and significant ($p < 0.005$) association between flowering asynchrony and population genetic divergence across eight species of tropical angiosperms (excluding *B. solanoides*): **A** with pairwise F_{ST} in the y-axis, and **B** with Jost's *D* in the y-axis. The blue line represents the prediction based on linear models with associated error in grey shading.

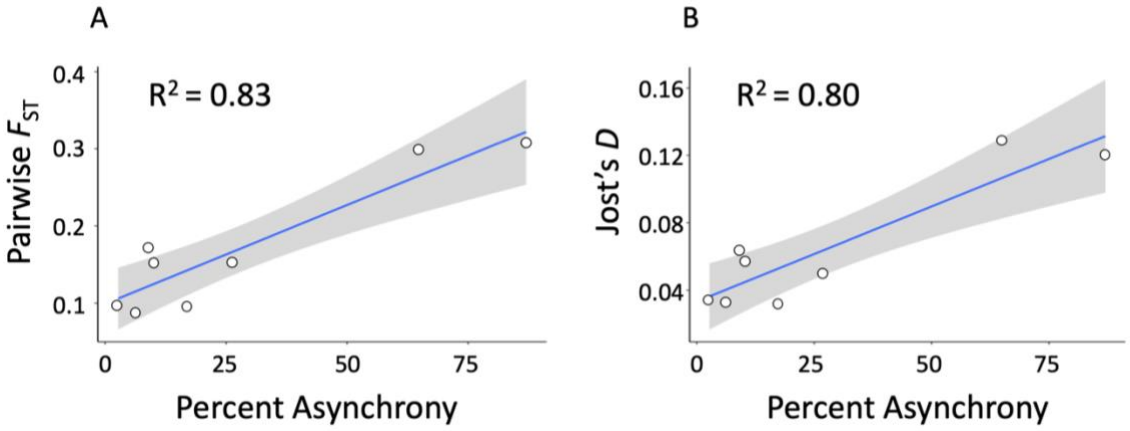


Table 1 Characteristics of studied species.

Species	Growth form	Pollinators	Fruit type
Begonia tiliifolia	Herbaceous	Insects (pers. obs.)	Indehiscent capsule
Besleria solanoides	Shrub	Hummingbirds (1)	Berry
Burmeistera multiflora	Herbaceous	Bats (2)	Inflated berry
Centropogon solanifolius	Herbaceous	Hummingbirds (1)	Indehiscent capsule
Drymonia tenuis	Sub-shrub	Hummingbirds (1)	Berry
Fuchsia macrostigma	Herbaceous	Hummingbirds (1)	Indehiscent capsule
Gasteranthus quitensis	Sub-shrub	Hummingbirds (1)	Fleshy capsule
Kohleria affinis	Epiphyte	Hummingbirds (1)	Fleshy capsule
Meriania tomentosa	Shrub	Bats and hummingbirds (3)	Indehiscent capsule

(1) Weinstein and Graham 2017

(2) Muchhala 2006

(3) Dellinger et al. 2019

Table 2 Flowering seasonality and asynchrony of studied species. A significance test of Fourier spectral analyses indicated that periodicity (i.e. seasonality) was significant for all studied species and consistent between sites ($p < 0.05$). A Fourier cospectral analysis was used to quantify flowering asynchrony (% async) between sites.

	Seasonality (sample size)		
Species	**Santa Lucía**	**Golondrinas**	**% async**
Begonia tiliifolia	annual (25)	annual (10)	10.5
Besleria solanoides	bi-annual (25)	bi-annual (25)	11.1
Burmeistera multiflora	sub-annual (25)	sub-annual (10)	2.7
Centropogon solanifolius	annual (25)	annual (25)	87.0
Drymonia tenuis	annual (25)	annual (10)	16.8
Fuchsia macrostigma	annual (25)	annual (25)	6.4
Gasteranthus quitensis	annual (25)	annual (15)	9.3
Kohleria affinis	annual (25)	annual (15)	26.3
Meriania tomentosa	annual (20)	annual (15)	64.6

Table 3 Results of linear regressions of population genetic divergence as predicted by flowering asynchrony for four tests. Tests (1) and (2) include *B. solanoides*. Tests (3) and (4) exclude *B. solanoides*. Significance of linear models is denoted in bold. Pagel's λ measures phylogenetic signal in the error term of each linear model. Phylogenetic regressions produced identical results.

Test	Response	Estimate	Std. E	t-value	*p*-value	adj-R^2	AIC	λ
1	intercept	0.092	0.016	5.7	0.0007			
	F_{ST}	0.003	0.0004	6.3	**0.0004**	0.83	-30.9	<0.001
2	intercept	0.031	0.008	4.0	0.005			
	Jost's *D*	0.001	0.0002	5.7	**0.0007**	0.80	-44.3	<0.001
3	intercept	0.100	0.02	5.8	0.001			
	F_{ST}	0.003	0.0004	6.0	**0.0009**	0.83	-27.3	<0.001
4	intercept	0.033	0.008	3.9	0.008			
	Jost's *D*	0.001	0.0002	5.4	**0.002**	0.80	-38.7	<0.001

Additional supporting information that will appear in the expanded online version of this article:

Fig. S1 Phylogeny of studied species extracted with V.PhyloMaker.

Fig. S2 Summary of Delta K results for each species.

Table S1 Total flower count per species at each survey date (Table S1.xlsx).

Table S2 Percent of flowering peak data per species (TableS2.xlsx).

Table S3 Results from Fourier spectral and cospectral analyses (TableS3.xlsx).

Table S4 Unfiltered catalog of loci for studied species.

Table S5 Genetic diversity of studied species across loci.

Table S6 Genetic diversity of studied species within sites.

Fig. S1 Phylogeny of studied species extracted from a backbone tree in V.PhyloMaker.

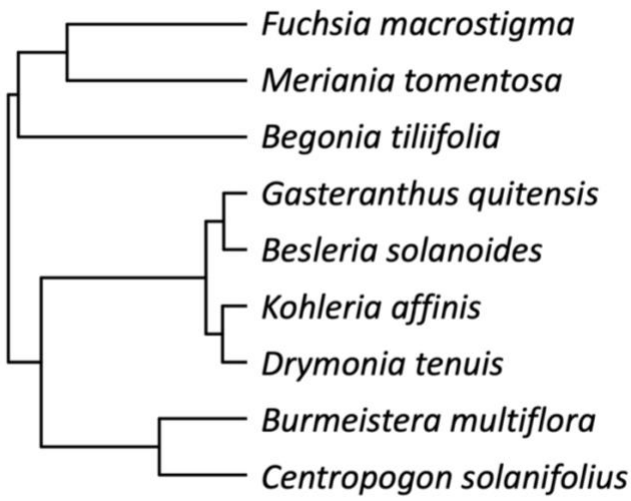


Fig. S2 Summary of Delta K results for each species based on the Evanno et al. (2005) method. K = 2 was the best-fit to the data for most species, except for *D. tenuis* where the best K = 3.

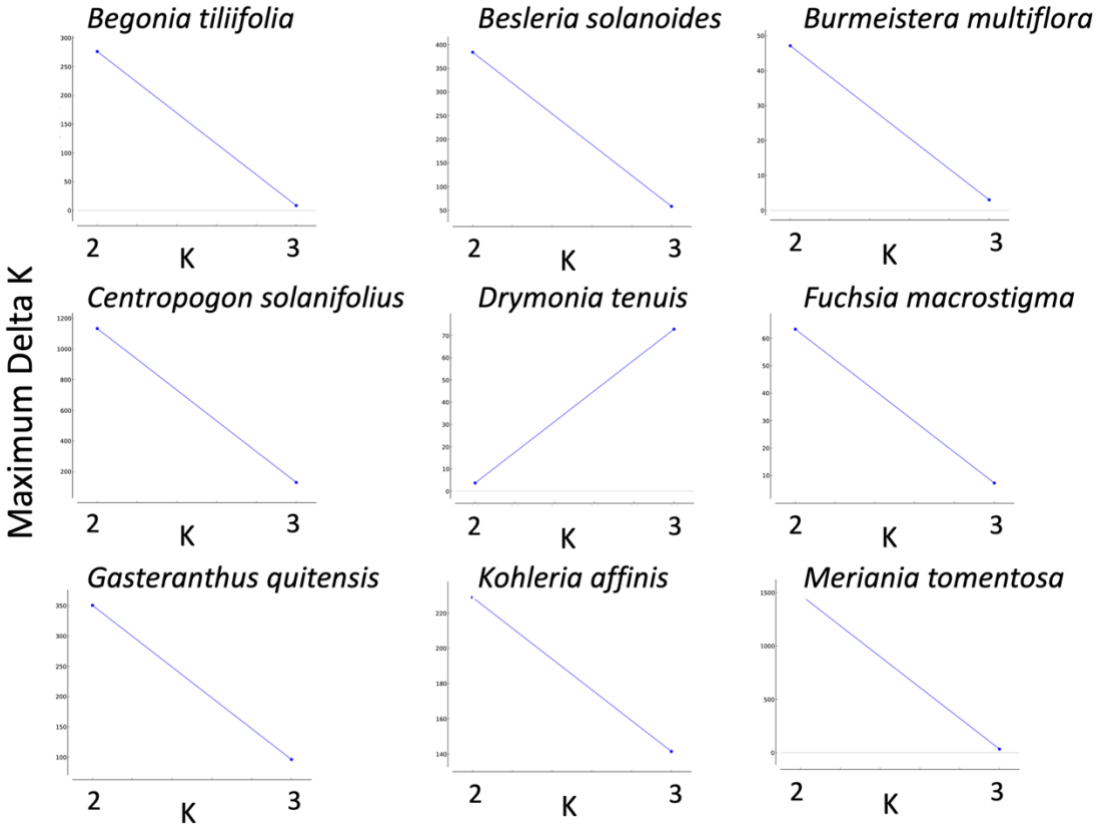


Table S1 Total flower count per species at each survey date (in file TableS1.TotalFlowers.xlsx).

Table S2 Percent of flowering peak data per species per site used for Fourier spectral and cospectral analyses (in file TableS2.FlowersRdata.xlsx)

TableS3 Results of Fourier spectral and cospectral analyses, significance of seasonality tests and estimated flowering asynchrony (in TableS3.SpectralCospectralResults.xlsx)

Table S4 Unfiltered catalog of loci recovered with the STACKS v2.3e pipeline for non-model organisms. N is the number of individuals. Coverage refers to the mean depth of reads used to build loci.

			Effective depth of coverage (reads/loci)			
Species	**total N genotyped**	**total loci genotyped**	**mean**	**stdev**	**min**	**max**
Begonia tiliifolia	25	2196507	15.6	4	8.4	26
Besleria solanoides	26	1751170	12.6	3.8	6.1	19.8
Burmeistera multiflora	21	3370979	17.7	7.6	9.1	33.5
Centropogon solanifolius	23	1469286	20.8	5	11.7	31.3
Drymonia tenuis	26	3266205	16	5.7	6.4	31.4
Fuchsia macrostigma	21	2439480	16.1	5.3	6.6	26.5
Gasteranthus quitensis	23	1326095	16.2	3.8	11.3	23.9
Kohleria affinis	22	1071520	15.2	3.9	10.7	30
Meriania tomentosa	27	2682725	22.7	13.1	7.1	51.8

Table S5 Genetic diversity of studied species estimated across filtered loci. N var loci: number of variant loci, N total a: total number of alleles, %md: percent missing data, N a: mean number of alleles per locus, Ne a: mean effective number of alleles per locus, Ho: observed heterozygosity, Hs: mean expected heterozygosity across subpopulations, Ht: total expected heterozygosity over all subpopulations, G_{IS}: inbreeding coefficient. Standard deviations of statistics (in parentheses) were obtained through jackknifing over loci and significance ($p < 0.005$) through 1000 permutations (denoted in bold).

Species	**N var loci**	**N total a**	**% md**	**N a**	**Ne a**	**Ho**	**Hs**	**Ht**	**G_{IS}**
				1.96	1.29	0.09	0.24	0.26	**0.62**
Begonia tiliifolia	4608	9035	40	(0.003)	(0.003)	(0.002)	(0.002)	(0.002)	(0.01)
				1.98	1.31	0.11	0.25	0.26	**0.55**
Besleria solanoides	1082	2144	35	(0.004)	(0.007)	(0.005)	(0.004)	(0.004)	(0.019)
				1.86	1.30	0.19	0.24	0.25	**0.22**
Burmeistera multiflora	7624	14175	36	(0.004)	(0.002)	(0.002)	(0.002)	(0.002)	(0.008)
				1.97	1.25	0.08	0.22	0.26	**0.62**
Centropogon solanifolius	3182	6281	40	(0.003)	(0.003)	(0.003)	(0.002)	(0.003)	(0.011)
				1.88	1.28	0.11	0.24	0.25	**0.53**
Drymonia tenuis	2389	4708	39	(0.006)	(0.005)	(0.003)	(0.003)	(0.003)	(0.011)
				1.95	1.31	0.17	0.25	0.27	**0.32**
Fuchsia macrostigma	6634	12908	37	(0.003)	(0.003)	(0.002)	(0.002)	(0.002)	(0.007)
				1.90	1.27	0.06	0.24	0.27	**0.77**
Gasteranthus quitensis	3251	6179	41	(0.005)	(0.004)	(0.002)	(0.003)	(0.003)	(0.009)
				1.92	1.26	0.08	0.22	0.24	**0.66**
Kohleria affinis	1457	2716	36	(0.007)	(0.005)	(0.004)	(0.003)	(0.004)	(0.016)
				1.95	1.29	0.16	0.23	0.28	**0.33**
Meriania tomentosa	4224	8236	36	(0.003)	(0.003)	(0.003)	(0.002)	(0.002)	(0.010)

Table S6 Genetic diversity of studied species within sites estimated from filtered loci. S: Santa Lucía, G: Golondrinas, N: number of individuals in the final genetic dataset, Ne: effective number of individuals, P a: number of private alleles, % P a: proportion of private to total alleles, N a: mean number of alleles per locus, Ne a: mean effective number of alleles per locus, Ho: observed heterozygosity, Hs: mean expected heterozygosity within site, G_{IS}: inbreeding coefficient. Significance ($p < 0.005$) was obtained through 1000 permutations and is denoted in bold.

Species	Site	N	Ne	P a	% P a	N a	Ne a	Ho	Hs	G_{IS}
Begonia tiliifolia	S	11	7.4	1809	0.20	1.74	1.36	0.09	0.26	**0.67**
	G	9	6.3	945	0.10	1.54	1.29	0.09	0.22	**0.57**
Besleria solanoides	S	12	8.2	295	0.14	1.73	1.32	0.11	0.23	**0.54**
	G	8	5.6	269	0.13	1.70	1.37	0.12	0.27	**0.56**
Burmeistera multiflora	S	7	5.9	2563	0.18	1.62	1.34	0.20	0.24	**0.17**
	G	7	4.9	1721	0.12	1.60	1.33	0.18	0.24	**0.27**
Centropogon solanifolius	S	10	6.7	1086	0.17	1.58	1.29	0.09	0.21	**0.60**
	G	10	6.2	1090	0.17	1.60	1.30	0.08	0.23	**0.63**
Drymonia tenuis	S	10	5.7	535	0.11	1.59	1.34	0.12	0.26	**0.54**
	G	7	7.0	668	0.14	1.62	1.30	0.10	0.22	**0.54**
Fuchsia macrostigma	S	7	5.8	1954	0.15	1.68	1.35	0.18	0.25	**0.30**
	G	9	5.9	1701	0.13	1.66	1.35	0.17	0.25	**0.33**
Gasteranthus quitensis	S	8	6.0	1081	0.17	1.54	1.32	0.06	0.25	**0.77**
	G	9	6.4	1127	0.18	1.59	1.32	0.06	0.24	**0.78**
Kohleria affinis	S	9	6.4	388	0.14	1.50	1.25	0.07	0.19	**0.63**
	G	9	6.2	628	0.23	1.65	1.35	0.08	0.26	**0.69**
Meriania tomentosa	S	10	7.9	1507	0.18	1.65	1.35	0.16	0.24	**0.33**
	G	10	7.0	1082	0.13	1.60	1.33	0.15	0.23	**0.32**

Chapter III: Pollination by hummingbirds strongly decreases genetic structure between and within plant populations relative to pollination by insects

Running title: Effects of animal pollination on plant gene flow in tropical plants

Diana Gamba, Department of Biology, University of Missouri at Saint Louis
dlgtk5@mail.umsl.edu
Nathan Muchhala, Department of Biology, University of Missouri at Saint Louis
muchhalan@umsl.edu

Author for correspondence: Diana Gamba; One University Boulevard, 223R Research Hall, Saint Louis, MO 63121, USA; dlgtk5@mail.umsl.edu; +1 (314) 702-0326.

Target journal: Annals of Botany

Abstract: 205

Total word count (main text): 4302

Abstract

Animal pollinators have a direct effect on plant gene flow because they carry the pollen grains. Pollinators with restricted mobility are predicted to limit gene flow within and among populations, while pollinators that fly longer distances likely promote genetic cohesion. Such predictions, however, remain surprisingly poorly tested. Here, we examined population genetic structure and fine-scale spatial genetic structure (SGS) in six perennial understory angiosperms in Andean cloud forests of northwestern Ecuador. Species belong to three families and within each family we selected one insect-pollinated species and one hummingbird-pollinated species. Based on differences in foraging behavior and flying ability, we tested the predictions that species pollinated by insects should have greater population genetic differentiation among study sites (as quantified with the F_{ST} statistic), and stronger SGS (as quantified with the S_P statistic), than species pollinated by hummingbirds. We confirmed putative pollinators through a literature review and fieldwork, and inferred population genetic parameters with a genome-wide genotyping approach. Generalized linear mixed-effects models showed that insect pollination is significantly associated with both greater population genetic differentiation and stronger SGS than hummingbird pollination. Our results clearly show for the first time that pollination by insects significantly restricts the spatial scale of intraspecific gene flow relative to pollination by hummingbirds

Key words: 2b-RAD sequencing, Andean cloud forest understory, fine-scale spatial genetic structure, animal pollination, population genetic structure.

Introduction

Understanding how plant mutualists influence spatial patterns of genetic diversity is central to plant biology, especially in the present scenario of biodiversity decline due to human-accelerated environmental change (Hardy *et al.* 2006; Dick *et al.* 2008; Aguilar *et al.* 2008, 2019). Animal pollinators directly affect gene flow within and among flowering plant populations because they are the carriers of pollen grains (Loveless and Hamrick 1984; Hamrick *et al.* 1992). Previous broad-scale studies on patterns of genetic structure in plants have lumped together all animals, and compared them to wind, thus overlooking the effect of different animals on gene flow dynamics within and among plant population (Hamrick and Godt 1996; Duminil *et al.* 2007). Findings from such studies reveal that wind tends to homogenize plant gene pools, while animal pollination is associated with higher population genetic differentiation as well as stronger fine-scale spatial genetic structure (i.e., the non-random spatial distribution of closely related individuals) (Dick *et al.* 2008; Gelmi-Candusso *et al.* 2017). Thus, in general, animal pollination may significantly disrupt gene flow relative to wind pollination within and among populations. Such patterns, however, should vary depending on the pollen dispersal ability of the pollinator, which will depend on foraging behavior and pollen carry-over capacity (Levin 1979). Pollinators with large foraging areas can carry pollen long distances, potentially enhancing gene flow within and among plant populations. In contrast, pollinators with local foraging behavior potentially reduce pollen dispersal, likely disrupting gene flow within and among plant populations. This potential trend has

been suggested in seminal reviews (Levin 1981; Loveless and Hamrick 1984), and in some empirical studies (Linhart *et al.* 1987; Linhart and Grant 1996; Kramer *et al.* 2011; Amico *et al.* 2014). However, no study to date has formally tested the prediction that pollinators with limited mobility should lead to stronger patterns of isolation by distance across individuals, potentially increasing population genetic differentiation across subpopulations, relative to pollinators that fly longer distances.

Vertebrate pollinators, such as nectarivorous bats and birds, generally fly longer distances during foraging bouts than insects, likely enhancing pollen flow among distantly spaced individuals and subpopulations, even across fragmented habitats (Levin 1979; Machado *et al.* 1998; Sahley 2001; Southerton *et al.* 2004; Byrne *et al.* 2007; Dick *et al.* 2008; Hadley and Betts 2009; McCulloch *et al.* 2013; Breed *et al.* 2015; Krauss *et al.* 2017; Solís-Hernández and Fuchs 2019). Thus, pollination by volant vertebrates potentially results in larger genetic plant neighborhoods (sensu Wright 1946; Webb 1984) than pollination by insects (Karron *et al.* 1995; Krauss 2000; Krauss *et al.* 2009; Bezemer *et al.* 2016). Although studies on the contrasting effects of pollination by volant vertebrates vs. insects on plant gene flow are remarkably lacking, this idea is supported by pollination studies on focal species. For example, studies in entomophilous plants show that small insects such as flies, solitary bees, and small beetles generally visit most flowers in a single plant, and then move to nearby plants restricting foraging to relatively small areas (Campbell 1985; Escaravage and Wagner 2004; Hasegawa *et al.* 2015). Furthermore, large insects such as large

bees and lepidoptera have larger foraging areas, frequently associated with traplining behavior (i.e., repeated sequence of floral visits over several locations) (Levin 1979; Schmitt 1980; Murawski and Gilbert 1986; Rhodes *et al.* 2017). Similarly, vertebrate pollinators such as non-territorial hummingbirds and bats also follow a traplining foraging behavior (Fleming 1982; Lemke 1984, 1985; Tello-Ramos *et al.* 2015), and potentially cover even larger areas than large insects (Linhart 1973; Webb and Bawa 1983; Melampy 1987; Campbell and Dooley 1992; Sahley 2001; Castellanos *et al.* 2003; Serrano-Serrano *et al.* 2017). Taken together, pollination by volant vertebrates should increase the spatial scale of intraspecific plant gene flow relative to pollination by insects.

In this study we aimed to test two predictions: (1) insect pollination is associated with greater genetic differentiation between plant populations than hummingbird pollination, and (2) insect pollination is associated with stronger fine-scale spatial genetic structure (SGS) within plant populations than hummingbird pollination. We focused on six perennial understory angiosperms in the Andean cloud forest of northwestern Ecuador, a highly diverse but threatened ecosystem. Species belong to three families and within each family we selected one insect-pollinated species (euglossine bees, or small buzzing bees, or hoverflies and wasps), and one hummingbird-pollinated species (traplining hummingbirds) (Renner 1989; Gamba and Almeda 2014; Weinstein and Graham 2017; Dellinger *et al.* 2019) (Table 1). All six focal species are likely very limited in their seed dispersal, as they are dispersed by gravity or by understory birds with sedentary lifestyles (Renner 1989; Loiselle and Blake 1993, 1999; Kessler-

Ríos and Kattan 2012; Theim *et al.* 2014). Thus, we expect that any trend of variation in population genetic differentiation and SGS across species will be due primarily to pollination mode. We confirmed putative pollinators through field work, and we used a genome-wide genotyping approach to obtain genetic data. We then tested whether animal pollination mode explained differences in population genetic differentiation, as well as in strength of SGS, across species.

Materials and Methods

Study sites

We performed this study in Santa Lucía (0.12 N, 78.6 W), El Pahuma (0.02 N, 78.6 W), Bellavista (0.01 S, 78.7 W), and Las Tángaras (0.08 S, 78.8 W), four private reserves located on the northwestern slope of the Andean cordillera of Ecuador, in the province of Pichincha around 40 km northwest of Quito. Sites are 5–23 km apart from each other and are composed of secondary and primary cloud forest ranging from 1800–2500 m in elevation. Because they are nearby and similar in elevation, they share many species, yet the distance between them potentially imposes a physical barrier for movement of pollinators, making them ideal for testing our predictions.

Study species and pollinators

To select our focal species, we began by compiling a list of species occurring at all sites using the Tropicos.org database of the Missouri Botanical Garden. Through fieldwork we further narrowed this list to six perennial

understory angiosperms from three families, with one insect-pollinated and one hummingbird-pollinated species per family, including *Drymonia brochidodroma* Wiehler and *Drymonia tenuis* (Benth.) J.L. Clark (Gesneriaceae), *Miconia rubescens* (Triana) Gamba & Almeda and *Meriania tomentosa* (Cogn.) Wurdack (Melastomataceae), and *Notopleura longipedunculoides* (C.M. Taylor) C.M. Taylor and *Palicourea demissa* Standl. (Rubiaceae; with the hummingbird-pollinated species listed second in each case). Among study species, *M. tomentosa* is also pollinated by nectarivorous bats (Muchhala and Jarrín-V 2002). Pairing by family allowed us to control for phylogenetic autocorrelation in subsequent tests. Based on our observations in the field, the spatial distribution of all species appeared widespread and consistent within sites, with occasional clusters of individuals. Additionally, seed dispersal in selected species is mostly achieved by understory birds with sedentary lifestyles such as tanagers and manakins, as has been shown for fleshy berries in Rubiaceae (Loiselle and Blake 1993, Loiselle et al. 1995; Theim *et al.* 2014) and Melastomataceae (Renner 1989; Loiselle and Blake 1999; Kessler-Ríos and Kattan 2012), and for fleshy capsules (often referred as display-capsules) in understory Gesneriaceae (Clark et al. 2012). The dry indehiscent capsules of *M. tomentosa* are likely gravity dispersed, as are many understory Melastomataceae with the same type of fruit (Renner 1989).

We obtained information on pollination mode from peer-reviewed literature of studied species (Renner 1989; Muchhala and Jarrín-V 2002; Gamba and Almeda 2014; Weinstein and Graham 2017; Dellinger *et al.* 2019), and by

videotaping plants in the field (Table 1). Specifically, for species with little information on pollination mode (*D. brochidodroma* and *N. longipedunculoides*), we confirmed putative pollinators by videotaping flowers with four high definition Sony digital camcorders for four days at each site. Cameras simultaneously videotaped four individuals per day (one species per day, eight individuals per species per site). Flowers were videotaped in the morning (0630 to 1130) and in the afternoon (1330 to 1830) (Additional file 1).

Genomic sampling, library preparation and sequencing

For molecular work, we collected leaf tissue in silica gel from 20 individuals per species from each of the three study sites (see Table 1 for sampled sites per species). We largely followed available trails in the reserves, making sure sampled individuals were at least 20 m apart from each other, and taking geographic coordinates in decimal degrees for each of them (Additional file 2).

We extracted total genomic DNA from silica-dried leaf tissue following the CTAB protocol (Doyle and Doyle 1987), but incorporating two additional ethanol washes of the DNA pellet. We quantified DNA with a Qubit 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific), using the manufacturer's protocol. For each of our samples with sufficient DNA, we obtained single nucleotide polymorphisms (SNPs) using 2b-RAD, a restriction site-associated DNA sequencing technique (Wang *et al.* 2012). We constructed 2b-RAD libraries for each individual following the available protocol (Wang *et al.* 2012). Five hundred

ng of total genomic DNA were digested with a type IIb endonuclease, *Bcg*I (New England Biolabs), which cuts DNA on both sides of a recognition site to obtain uniform 36-bp fragments distributed across the genome. Oligonucleotide Illumina sequences were ligated to these fragments with 12 double-stranded barcoded adapters, one per each column of a 96-sample plate. In order to increase sequence coverage per locus, we utilized reduced representation barcoded adapters which reduce the total number of loci sequenced. Samples with different barcoded adapters were pooled into 8 groups of 12 samples. Following initial pooling, Illumina RAD PCR primers (1–8) were incorporated into the fragments of each pool via 14 cycles of PCR amplification. Amplified pools were then purified via gel electrophoresis. Fragments of 75bp were size selected by excising target bands from the agarose gel. We then used a Min Elute Gel extraction kit (Qiagen) to purify target bands. Purified samples were quantified and pooled into a single library in equimolar concentrations. We generated three libraries, which together included ~ 15–20 individuals per species per study site. Libraries were sequenced on Illumina HiSeq 4000 (Duke University, NC) machines, to generate single end 50 bp reads.

Building loci and genotyping individuals

Reads were demultiplexed using a custom script (trim2bRAD) generated by the Matz lab at the University of Austin, TX (https://github.com/z0on/2bRAD_denovo). This script trims 2b-RAD fragments from barcodes to produce one fastq file per sample. The resulting files were

quality filtered with FastQC (Babraham Bioinformatics) and the FASTX-toolkit (Gordon and Hannon 2010). We discarded low quality reads and obtained sequences that were 36 bp in length, with a minimum of 90% bases having a Phred quality score of at least 20 and an input quality offset of 33 (fastq files will be available in the Dryad repository). We then used the Stacks v2.3e pipeline to genotype individuals and produce a catalog of loci for each species (Catchen *et al.* 2013). We ran Stacks using the default parameter settings for building loci, which we considered to be appropriate for the short size of the 2b-RAD fragments, including a maximum distance of 2 nucleotide differences allowed between reads, a minimum depth of coverage of 3 reads required to create a stack, and a maximum distance of 4 nucleotide differences allowed to align secondary reads to primary stacks. We also allowed one gap between stacks before merging into putative loci. We filtered loci with the program 'populations' on the same pipeline. We excluded loci that were genotyped in <40% of individuals within each species. To avoid using SNPs in high linkage disequilibrium, we used one random SNP per locus. To prevent potential low-frequency SNP miscalls, we discarded alleles that had a frequency <5% in any locus across all individuals per species. To avoid repetitive or paralogous loci, the maximum number of heterozygous individuals that may be present in any locus was set to 75%. Lastly, we used the program VCFtools v0.1.16 (Danecek *et al.* 2011) to identify individuals with >50% missing data relative to variant sites and removed these individuals from subsequent analyses. We removed a total of 51 individuals across all species, with an average of 9 individuals/species (± 4

SD, range = 2–17 individuals/species).

Inference of population genetic parameters

We used the program GenoDive v3.0 (Meirmans and Van Tienderen 2004) to calculate genetic diversity statistics for each species. We assessed population genetic structure using the F-statistics derived from an Analysis of Molecular Variance or AMOVA (Excoffier *et al.* 1992). AMOVA determines the proportion of genetic variance partitioned within individuals, among individuals within subpopulations, and among subpopulations. Related F-statistics were obtained with an infinite allele model; thus, they are equivalent to G-statistics (Nei 1973; Nei and Chesser 1983). These include F_{IT} (the mean reduction in heterozygosity of an individual relative to the total population), F_{IS} (the inbreeding coefficient among individuals within sites), and F_{ST} (the global genetic differentiation among sampled sites). The statistical significance of diversity statistics was assessed using 1000 random permutations of the data, while their standard deviations were obtained by jackknifing over loci.

Inference of fine-scale spatial genetic structure (SGS)

We evaluated SGS for each species via spatial autocorrelation analyses at the individual level (Vekemans and Hardy 2004) using the program SPAGeDi v. 1.3a (Hardy and Vekemans 2002). We first transformed individuals' decimal degrees coordinates into the Universal Transverse Mercator coordinate system, which is compatible with the SPAGeDi version we used. We then assessed

genetic relatedness between all pairs of individuals i and j with Nason's kinship coefficient, F_{ij} (Loiselle *et al.* 1995). We specified 5 distance intervals for each species and allowed the program to define their maximal distance such that the number of pairwise comparisons within each interval was kept approximately constant. F_{ij} values were regressed on the natural logarithm of the spatial distance separating pairs of individuals, $\ln(d_{ij})$, in order to quantify regression slopes, *b*. To test for SGS, spatial positions of individuals were permuted 1000 times to obtain a frequency distribution of *b* under the null hypothesis that F_{ij} and $\ln(d_{ij})$ are not correlated. We quantified the strength of SGS with the S_P statistic (Vekemans and Hardy 2004), which is calculated as $-b/(1 - F_1)$, where F_1 is the mean F_{ij} between all pairs of individuals in the first distance interval containing nearest neighbors (< ~1 km for all species). The S_P statistic mainly depends on the slope of the kinship-distance curve, allowing direct comparisons of SGS among species (Vekemans and Hardy 2004). Standard errors of all SGS statistics were obtained by jackknifing over loci. To visualize SGS, we plotted the mean F_{ij} at each distance interval over the five distance intervals for each species.

Testing for the effect of animal pollinators on plant F_{ST} and SGS

We used generalized linear mixed-effects models in RStudio V 1.2.5019 (R Core Team 2018) to examine if insect pollination is associated with both higher genetic differentiation across subpopulations (i.e., higher F_{ST} values) and stronger SGS across individuals (i.e., higher S_p values) than hummingbird

pollination, across our study species. Given that the natural logarithm of F_{ST} and S_P values are normally distributed, we fitted models with the R function glmer() and the 'lognormal' distribution (family=gaussian, link='log') for the structure of the residuals, specifying taxonomic family as a random effect.

Results

Pollinators

We recorded a total of 10 individuals and 30 hours (i.e., ~3 hours/individual) for *Drymonia brochidodroma*, and 12 individuals and 35 hours (i.e., ~2.9 hours/individual) for *Notopleura longipedunculoides*. From these videos, we observed that *D. brochidodroma* was exclusively visited by Euglossine bees, with 5 bee visits lasting ~10 seconds each, while *N. longipeduncoloides* was visited by wasps, hoverflies, and small bees. We recorded 18 wasp visits lasting ~60 seconds each, 10 hoverfly visits ~ 30 seconds each, and 5 bees visits ~15 seconds each.

Filtered genetic datasets

After SNP calling and quality control using different filtering procedures, we obtained a mean of 2,797,308 SNP loci per species (± 1,091,949 SD; range: 879,138–4,151,836), with a mean coverage ranging from 14–95.1 read depth per loci across species (Table S1). After removing individuals with >50% missing data, final sample sizes of individuals per species per study site ranged from 8–18 (mean = 13 ± 3 SD), and the number of variant loci ranged from 1,044–4,907

(mean = 2,699 ± 1,427 SD) across species, with missing data across species ranging from 24–38% (mean = 33% ± 5 SD) (Table S2 and S3).

Gene diversity was similar across species; total expected heterozygosity (H_T) ranged from 0.21–0.25 (mean = 0.23 ± 0.02) across species (Table S2) and mean expected heterozygosity within sites (H_S) ranged from 0.17–0.26 (mean = 0.22 ± 0.02). Additionally, all species showed statistically significant levels of inbreeding, as indicated by significant G_{IS} values whether these are pooled across sites (mean = 0.30 ± 0.14 SD; Table S2) or analyzed separately by site (mean = 0.32 ± 0.16 SD, Table S3).

Population genetic structure

AMOVA results revealed that in all species most of the genetic diversity resides within individuals and among individuals within sites, while less genetic diversity resides among sites (Table S4). AMOVA F_{IT} showed that for most species a large proportion of individuals across study sites were out of Hardy-Weinberg equilibrium, likely due to inbreeding among individuals. In fact, AMOVA F_{IS} was significant for all species, congruent with our G_{IS} estimates above, and confirming that there is substantial genetic inbreeding within sites across studied species. Furthermore, AMOVA F_{ST} was variable (range = 0.03–0.21, mean = 0.10 ± 0.06) but significant for all species, hence there is considerable genetic differentiation among study sites (Table 2).

Fine-scale spatial genetic structure (SGS)

SGS was significant for all studied species; regression slopes *b* of pairwise kinship coefficients on the natural logarithm of spatial distance were significantly negative in all species (Table 3). Additionally, the extent of SGS as quantified with the S_P statistic was quite variable across species, ranging from 0.009–0.089 (mean = 0.04 ± 0.03 SD). Such variation is evident in our SGS visualizations (Fig. 1, Tables S5–S10), which show that species pollinated by insects tend to have steeper average kinship-distance slopes (Fig. 1 a, c, e) than species pollinated by vertebrates (Fig. 1 b, d, f). Given that standard errors associated with each average F_{ij} are vanishingly small (Tables S5–S10), they are not observable in Fig. 1.

Effect of insect vs. vertebrate pollination modes on plant F_{ST} and SGS

We hypothesized that insect pollination results in both stronger SGS and higher population genetic differentiation than hummingbird pollination. On average, plants pollinated by insects had greater F_{ST} values (0.14 ± 0.07 SD) than plants pollinated by hummingbirds (0.06 ± 0.04 SD) (Table 2). We observed a similar trend for S_P values; 0.054 ± 0.03 SD for plants pollinated by insects vs. 0.017 ± 0.01 SD for plants pollinated by hummingbirds (Table 3). Results from a generalized linear mixed-effects model (GLMM), specifying taxonomic family as a grouping factor, supported our predictions: insect pollination is associated with both significantly higher F_{ST} and significantly higher S_P values than vertebrate pollination (Fig. 2, Table 4).

Discussion

The contrasting effect of different animal pollinators on plant gene flow has remained largely unexplored across plant species. Our study provides an important advance in this matter and our results supported our predictions: species pollinated by insects had significantly greater levels of population genetic differentiation and stronger fine-scale spatial genetic structure than species pollinated by hummingbirds (Table 4, Fig.1 and 2). Our findings support the idea that pollinator movement during foraging has strong effects on the spatial scale of intraspecific plant gene flow. The limited movement of insects restricts gene flow within and among populations, while the traplining behavior of hummingbirds promotes genetic cohesion.

Our chosen study species allowed us to control for other factors that might impact plant population genetic structure and SGS, increasing the probability that the association we found is in fact due directly to animal pollination mode rather than a confounding variable. For example, choosing species pairs with distinct animal pollination modes (insect vs. vertebrate), each pair in one plant family, allowed us to control for evolutionary relationships that could have resulted in phylogenetic autocorrelation in our dataset. Furthermore, all species belong to cloud forest understory sites inside the southern end of the Choco Andean corridor (Mordecai *et al.* 2009) that are relatively well-connected by a continuous corridor of forests. Thus, pollinator movement between sites for all species should be constrained by the same type of geographic barriers inherent to the landscape heterogeneity of the Andes. Likewise, seed dispersal across species

is likely limited; seeds either fall under mother plants or are dispersed by sedentary understory birds like tanagers and manakins (Loiselle and Blake 1993, 1999; Smith 2001; Gamba and Almeda 2014). Additionally, most species pairs have the same type of fruit: x and x of gesner havex, x and x of x have x. The exception are the Melastomataceae pair, in which *Miconia rubescens* has fleshy berries and *Meriania tomentosa* has indehiscent capsules. We would expect indehiscent capsules to be more dispersed limited that fleshy berries, resulting in higher F_{ST} and S_P values. Our data instead found that *M. tomentosa* has smaller F_{ST} and S_P values than *M. rubescens*, suggesting vertebrate pollination in the former may override any dispersal limitation imposed by the indehiscent capsules. Overall, we expect that seed dispersal likely contributes little to gene flow. Finally, differences in inbreeding levels do not seem to underlie the differences in population genetic differentiation or strength of SGS. Inbreeding can affect population genetic structure and SGS (Vekemans and Hardy 2004; Duminil *et al.* 2007), however we do not find such association in our dataset: the inbreeding coefficient (AMOVA F_{IS} in Table 2) does not predict F_{ST} (GLMM, $p=0.9$) or S_P values (GLMM, $p=0.5$).

We note that differences in F_{ST} and S_P values were more pronounced between the Rubiaceae species pairs (7 and 10-fold, respectively), followed by the Melastomataceae pairs (2.2 and 2.5-fold, respectively), and lastly by the Gesneriaceae pairs (almost equivalent values) (Table 2 and 3). *Notopleura longipedunculoides* is largely pollinated by tiny wasps and hoverflies that probe most flowers in the same individual and stay among nearby plants (pers. obs),

consistent with the greatest observed F_{ST} and S_P values. *Miconia rubescens* is pollinated by *Melipona* and *Trigona*, which are relatively small pollen collecting bees (Renner 1989), consistent with the intermediate F_{ST} and S_P values. Finally, *Drymonia brochidodroma* is pollinated by euglossine bees (pers. obs.), which are larger and have been reported to flight long distances (Janzen 1971; López-Uribe *et al.* 2008), which is in line with *D. brochidodroma* having the smallest F_{ST} and S_P values among our insect pollinated plants. Thus, differences between insect pollinators may explain this pattern. Among vertebrate pollinated plants, *Palicourea demissa* is visited by ~15 hummingbird species, *Meriania tomentosa* is visited by ~8 hummingbird species and by nectarivorous bats (Muchhala and Jarrín-V 2002), and *Drymonia tenuis* is visited by ~7 hummingbird species (Weinstein and Graham 2017), consistent with lower F_{ST} and S_P values. The fact that the two *Drymonia* species had such similar F_{ST} and S_P values suggests that euglossine bees and hummingbirds are similar in their pollen dispersal ability. Direct measures of pollen dispersal based on paternity analyses are in line with the patterns of genetic structure we found, in that bats and hummingbirds can transport pollen for several kilometers, large insects such as large bees (including euglossine bees) for over 600 meters, while most small insects (smaller than a honeybee) rarely transfer pollen more than 300 meters (Webb and Bawa 1983; Dick *et al.* 2008).

One important consideration of our study is that we categorized pollination systems fairly broadly as insects vs. vertebrates. But in the same way that insects can vary in pollen dispersal ability, as described above, different

vertebrates may also differ in pollen dispersal. For instance, traplining vs. territorial behavior among hummingbirds might strongly impact plant gene flow (Murawski and Gilbert 1986; Cuevas *et al.* 2018; Schmidt-Lebuhn *et al.* 2019), since territorial hummingbirds have been shown to move pollen much shorter distances than traplining hummingbirds (Ohashi and Thomson 2009; Wolowski *et al.* 2013; Betts *et al.* 2015). There also might be differences between hummingbirds and bats, as the latter have been found to carry pollen more efficiently (Muchhala and Thomson 2010) and to longer distances than hummingbirds (Lemke 1984, 1985; Tello-Ramos *et al.* 2015). Future work should look more in depth at how plant gene flow is affected by differences within pollinator guilds, including large vs. small insects, territorial vs. traplining hummingbirds, and nectarivorous bats vs. hummingbirds.

Our study provides new evidence on the contrasting effect that different animal pollinators can have on the spatial scale of intraspecific plant gene flow. We found that insect-pollinated plants have significantly higher population genetic differentiation and stronger fine-scale spatial genetic structure than hummingbird pollinated plants. Thus, the effect of animal pollinators on plant gene flow is significant at local (within populations) and regional (among populations) scales. Our results support the idea that plants pollinated by insects are likely very susceptible to habitat fragmentation (more so than vertebrate pollinated plants; e.g. Côrtes *et al.* 2013), because it can further isolate populations and result in loss of genetic variability due to increased genetic drift (Aguilar *et al.* 2008, 2019). Nevertheless, focal studies reveal that hummingbird

and bat pollinated plants can also experience detrimental effects due to habitat fragmentation (Wanderley *et al.* 2020). Increased deforestation results in significant declines of hummingbird species richness and thus of pollinator availability (Hadley and Betts 2009; Hadley *et al.* 2018). Furthermore, habitat destruction due to urbanization likely decreases areas of cross-pollination mediated by nectarivorous bats, because their habitat becomes restricted to few forest fragments inside large tropical cities (Nunes *et al.* 2017). Future studies should seek to compare how animal foraging behavior and its related effect on plant gene flow might be altered due to anthropogenic disturbance. In general, the current scenario of human-accelerated change should push conservation efforts to maintain connectivity between fragments that harbor many understory tropical species.

Acknowledgements

Thanks to Nora Oleas and Paola Peña for help with the research permit in Ecuador (MAE-DNB-CM-2015-017). Robert Ricklefs, Christine Edwards, and Carmen Ulloa provided advice in this study. We also thank field assistants An Nguyen, Carlos Imery, and Alexander Lascher-Posner for their valuable help. Thanks to families at Santa Lucía, Bellavista, El Pahuma, and Las Tángaras cloud forest reserves for their conservation efforts and hospitality. Finally, we thank Amanda Grusz for help with SPAGeDi analyses. This research was supported by two graduate research grants from the Whitney R. Harris World Ecology Center at UMSL and one research grant from the American Society of

Plant Taxonomists to DG.

References

Aguilar R, Cristóbal-Pérez EJ, Balvino-Olvera FJ, *et al.* 2019. Habitat fragmentation reduces plant progeny quality: a global synthesis (JM Gomez, Ed.). *Ecology Letters* **22**: 1163–1173.

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J. **2008**. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology* **17**: 5177–5188.

Amico GC, Vidal-Russell R, Aizen MA, Nickrent D. **2014**. Genetic diversity and population structure of the mistletoe Tristerix corymbosus (Loranthaceae). *Plant Systematics and Evolution* **300**: 153–162.

Betts MG, Hadley AS, Kress WJ. **2015**. Pollinator recognition by a keystone tropical plant. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 3433–3438.

Bezemer N, Krauss SL, Phillips RD, Roberts DG, Hopper SD. **2016**. Paternity analysis reveals wide pollen dispersal and high multiple paternity in a small isolated population of the bird-pollinated Eucalyptus caesia (Myrtaceae). *Heredity* **117**: 460–471.

Breed M F, Ottewell KM, Gardner MG, Marklund MHK, Stead MG, Harris JBC, Lowe AJ. **2015**. Mating system and early viability resistance to habitat fragmentation in a bird-pollinated eucalypt. *Heredity* **115**: 100–107.

Byrne M, Elliott CP, Yates CJ, Coates D J. **2007**. Extensive pollen dispersal in a bird-pollinated shrub, Calothamnus quadrifidus, in a fragmented landscape. *Molecular Ecology* **16**: 1303–1314.

Campbell DR. **1985**. Pollen and gene dispersal: the influences of competition for pollinators. *Evolution* **39**: 418–431.

Campbell DR, Dooley JL. **1992**. The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation by distance. *The American Naturalist* **139**: 735–748.

Castellanos MC, Wilson P, Thomson JD. **2003**. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in Penstemon. *Evolution* **57**: 2742–2752.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. **2013**. Stacks: an analysis tool set for population genomics. *Molecular ecology* **22**: 3124–3140.

Clark JL, Funke MM, Duffy AM, Smith JF. **2012**. Phylogeny of a neotropical clade in the Gesneriaceae: more tales of convergent evolution. *International Journal of Plant Sciences* **173**: 894–916.

Côrtes MC, Uriarte M, Lemes MR, *et al.* 2013. Low plant density enhances gene dispersal in the Amazonian understory herb Heliconia acuminata. *Molecular Ecology* **22**: 5716–5729.

Cuevas E, Espino J, Marques I. **2018**. Reproductive isolation between Salvia elegans and S. fulgens, two hummingbird-pollinated sympatric sages. *Plant Biology* **20**: 1075–1082.

Danecek P, Auton A, Abecasis G, *et al.* 2011. The variant call format and VCFtools. *Bioinformatics* **27**: 2156–2158.

Dellinger AS, Scheer LM, Artuso S, *et al.* 2019. Bimodal Pollination Systems in Andean Melastomataceae Involving Birds, Bats, and Rodents. *The American Naturalist* **194**: 104–116.

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.

Doyle J, Doyle JL. **1987**. Genomic plant DNA preparation from fresh tissue-CTAB method. *Phytochem Bull* **19**: 11–15.

Duminil J, Fineschi S, Hampe A, *et al.* 2007. Can Population Genetic Structure Be Predicted from Life-History Traits? *The American Naturalist* **169**: 662–672.

Escaravage N, Wagner J. **2004**. Pollination effectiveness and pollen dispersal in a Rhododendron ferrugineum (Ericaceae) population. *Plant Biology* **6**: 606–615.

Excoffier L, Smouse PE, Quattro JM. **1992**. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.

Fleming TH. **1982**. Foraging strategies of plant-visiting bats In: *Ecology of bats*. Springer, 287–325.

Gamba D, Almeda F. **2014**. Systematics of the Octopleura clade of Miconia (Melastomataceae: Miconieae) in tropical America. *Phytotaxa* **179**: 1–174.

Gelmi-Candusso TA, Heymann EW, Heer K. **2017**. Effects of zoochory on the spatial genetic structure of plant populations. *Molecular Ecology* **26**: 5896–5910.

Gordon A, Hannon G. **2010**. Fastx-toolkit. FASTQ/A short-reads pre-processing tools. *Unpublished http://hannonlab. cshl. edu/fastx_toolkit* **5**.

Hadley AS, Betts MG. **2009**. Tropical deforestation alters hummingbird movement patterns. *Biology letters* **5**: 207–210.

Hadley AS, Frey SJ, Robinson WD, Betts MG. **2018**. Forest fragmentation and loss reduce richness, availability, and specialization in tropical hummingbird communities. *Biotropica* **50**: 74–83.

Hamrick JL, Godt MJW. **1996**. Effects of Life History Traits on Genetic Diversity in Plant Species. *Philosophical Transactions: Biological Sciences* **351**: 1291–1298.

Hamrick JL, Godt MJW, Sherman-Broyles SL. **1992**. Factors influencing levels of genetic diversity in woody plant species. *New forests* **6**: 95–124.

Hardy OJ, Maggia L, Bandou E, *et al.* 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular ecology* **15**: 559–571.

Hardy OJ, Vekemans X. **2002**. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular ecology notes* **2**: 618–620.

Hasegawa Y, Suyama Y, Seiwa K. **2015**. Variation in pollen-donor composition among pollinators in an entomophilous tree species, Castanea crenata, revealed by single-pollen genotyping. *PLOS ONE* **10**: e0120393.

Janzen DH. **1971**. Euglossine bees as long-distance pollinators of tropical plants. *Science, New Series* **171**: 203–205.

Karron JD, Tucker R, Thumser NN, Reinartz JA. **1995**. Comparison of pollinator flight movements and gene dispersal patterns in Mimulus ringens. *Heredity* **75**: 612–617.

Kessler-Rios MM, Kattan GH. **2012**. Fruits of Melastomataceae: phenology in Andean forest and role as a food resource for birds. *Journal of Tropical Ecology* **28**: 11–21.

Kramer AT, Fant JB, Ashley MV. **2011**. Influences of landscape and pollinators on population genetic structure: Examples from three *Penstemon* (Plantaginaceae) species in the Great Basin. *American Journal of Botany* **98**: 109–121.

Krauss SL. **2000**. Patterns of mating in *Persoonia mollis* (Proteaceae) revealed by an analysis of paternity using AFLP: Implications for conservation. *Australian Journal of Botany* **48**: 349–356.

Krauss SL, He T, Barrett LG, Lamont BB, Enright NJ, Miller BP, Hanley ME. **2009**. Contrasting impacts of pollen and seed dispersal on spatial genetic structure in the bird-pollinated Banksia hookeriana. *Heredity* **102**: 274–285.

Krauss SL, Phillips RD, Karron JD, Johnson SD, Roberts DG, Hopper SD. **2017**. Novel consequences of bird pollination for plant mating. *Trends in Plant Science* **22**: 395–410.

Lemke TO. **1984**. Foraging ecology of the long-nosed bat, *Glossophaga soricina*, with respect to resource availability. *Ecology* **65**: 538–548.

Lemke TO. **1985**. Pollen carrying by the nectar-feeding bat *Glossophaga soricina* in a suburban environment. *Biotropica* **17**: 107–111.

Levin DA. **1979**. Pollinator foraging behavior: genetic implications for plants In: *Topics in plant population biology*. Springer, 131–153.

Levin DA. **1981**. Dispersal versus gene flow in plants. *Annals of the Missouri Botanical Garden* **68**: 233.

Linhart YB. **1973**. Ecological and behavioral determinants of pollen dispersal in hummingbird-pollinated Heliconia. *The American Naturalist* **107**: 511–523.

Linhart YB, Busby WH, Beach JH, Feinsinger P. **1987**. Forager behavior, pollen dispersal, and inbreeding in two species of hummingbird-pollinated plants. *Evolution* **41**: 679–682.

Linhart YB, Grant MC. **1996**. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* **2**: 237–277.

Loiselle BA, Blake JG. **1993**. Spatial distribution of understory fruit-eating birds and fruiting plants in a neotropical lowland wet forest In: Fleming TH,

Estrada A, eds. *Frugivory and seed dispersal: ecological and evolutionary aspects*. Dordrecht: Springer Netherlands, 177–189.

Loiselle BA, Blake JG. **1999**. Dispersal of melastome seeds by fruit-eating birds of tropical forest understory. *Ecology* **80**: 330–336.

Loiselle BA, Sork VL, Nason J, Graham C. **1995**. Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). *American Journal of Botany* **82**: 1420–1425.

López-Uribe MM, Oi CA, Del Lama MA. **2008**. Nectar-foraging behavior of Euglossine bees (Hymenoptera: Apidae) in urban areas. *Apidologie* **39**: 410–418.

Loveless MD, Hamrick JL. **1984**. Ecological Determinants of Genetic Structure in Plant Populations. *Annual Review of Ecology and Systematics* **1**: 65–95.

Machado ICS, Sazima I, Sazima M. **1998**. Bat pollination of the terrestrial herbIrlbachia alata (Gentianaceae) in northeastern Brazil. *Plant Systematics and Evolution* **209**: 231–237.

McCulloch ES, Sebastián Tello J, Whitehead A, Rolón-Mendoza CM, Maldonado-Rodríguez MC, Stevens RD. **2013**. Fragmentation of Atlantic Forest has not affected gene flow of a widespread seed-dispersing bat. *Molecular ecology* **22**: 4619–4633.

Meirmans PG, Van Tienderen PH. **2004**. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* **4**: 792–794.

Melampy MN. **1987**. Flowering phenology, pollen flow and fruit production in the Andean shrub Befaria resinosa. *Oecologia* **73**: 293–300.

Mordecai RS, Cooper RJ, Justicia R. **2009**. A threshold response to habitat disturbance by forest birds in the Choco Andean corridor, Northwest Ecuador. *Biodiversity and Conservation* **18**: 2421–2431.

Muchhala N, Thomson JD. **2010**. Fur versus feathers: pollen delivery by bats and hummingbirds and consequences for pollen production. *The American Naturalist* **175**: 717–726.

Murawski DA, Gilbert LE. **1986**. Pollen flow in Psiguria warscewiczii: a comparison of Heliconius butterflies and hummingbirds. *Oecologia* **68**: 161–167.

Nei M. **1973**. Analysis of Gene Diversity in Subdivided Populations. *Proceedings of the National Academy of Sciences* **70**: 3321.

Nei M, Chesser RK. **1983**. Estimation of fixation indices and gene diversities. *Annals of human genetics* **47**: 253–259.

Nunes H, Rocha FL, Cordeiro-Estrela P. **2017**. Bats in urban areas of Brazil: roosts, food resources and parasites in disturbed environments. *Urban Ecosystems* **20**: 953–969.

Ohashi K, Thomson JD. **2009**. Trapline foraging by pollinators: its ontogeny, economics and possible consequences for plants. *Annals of botany* **103**: 1365–1378.

R Core Team. **2018**. R Foundation for Statistical Computing; Vienna, Austria: 2015. *R: A language and environment for statistical computing*: 2013.

Renner SS. **1989**. A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. *Annals of the Missouri Botanical Garden*: 496–518.

Rhodes MK, Fant JB, Skogen KA. **2017**. Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Molecular ecology* **26**: 4296–4308.

Sahley CT. **2001**. Vertebrate pollination, fruit production, and pollen dispersal of Stenocereus thurberi (Cactaceae). *The Southwestern Naturalist*: 261–271.

Schmidt-Lebuhn AN, Müller M, Pozo Inofuentes P, Encinas Viso F, Kessler M. **2019**. Pollen analogues are transported across greater distances in bee-pollinated than in hummingbird-pollinated species of Justicia (Acanthaceae). *Biotropica* **51**: 99–103.

Schmitt J. **1980**. Pollinator foraging behavior and gene dispersal in Senecio (Compositae). *Evolution* **34**: 934–943.

Serrano-Serrano ML, Rolland J, Clark JL, Salamin N, Perret M. **2017**. Hummingbird pollination and the diversification of angiosperms: an old and successful association in Gesneriaceae. *Proceedings of the Royal Society B: Biological Sciences* **284**: 20162816.

Smith JF. **2001**. High species diversity in fleshy-fruited tropical understory plants. *The American Naturalist* **157**: 646–653.

Solís-Hernández W, Fuchs E-J. **2019**. Effective gene flow patterns across a fragmented landscape in southern Costa Rica for Symphonia globulifera (Clusiaceae); a species with mobile seed and pollen dispersers. *Revista de Biología Tropical* **67**: S95–S111.

Southerton SG, Birt P, Porter J, Ford HA. **2004**. Review of gene movement by bats and birds and its potential significance for eucalypt plantation forestry. *Australian Forestry* **67**: 44–53.

Tello-Ramos MC, Hurly TA, Healy SD. **2015**. Traplining in hummingbirds: flying short-distance sequences among several locations. *Behavioral ecology* **26**: 812–819.

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.

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

Wanderley AM, dos Santos EKR, Galetto L, Benko-Iseppon AM, Machado ICS. **2020**. Pollen flow within and among isolated populations of two rare, self-compatible plant species from inselbergs of Northeast Brazil. *Plant Ecology*: 1–12.

Wang S, Meyer E, McKay JK, Matz MV. **2012**. 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nature Methods* **9**: 808–810.

Webb CJ. **1984**. Hummingbird pollination of Malvaviscus arboreus in Costa Rica. *New Zealand journal of botany* **22**: 575–581.

Webb CJ, Bawa KS. **1983**. Pollen dispersal by hummingbirds and butterflies: a comparative study of two lowland tropical plants. *Evolution*: 1258–1270.

Weinstein BG, Graham CH. **2017**. Persistent bill and corolla matching despite shifting temporal resources in tropical hummingbird-plant interactions (R Irwin, Ed.). *Ecology Letters* **20**: 326–335.
Wolowski M, Saad CF, Ashman T-L, Freitas L. **2013**. Predominance of self-compatibility in hummingbird-pollinated plants in the Neotropics. *Naturwissenschaften* **100**: 69–79.
Wright S. **1946**. Isolation by distance under diverse systems of mating. *Genetics* **31**: 39.

Data accessibility statement

Should the manuscript be accepted, the data and R scripts supporting the results will be archived in Dryad and their DOI will be included at the end of this article.

Author Contributions

DG and NM planned and designed the research. DG collected and analyzed the data. DG wrote the initial draft of the manuscript. DG and NM contributed equally to substantial revisions of the manuscript.

Table 1 Characteristics of studied species and sites where they were sampled.

Species	**Growth form**	**Pollinators (source)**	**Fruit type**	**Sites †**
Drymonia brochidodroma	Herbaceous	Euglossine bees (pers. obs.)	Fleshy capsule	SL, T
Drymonia tenuis	Sub-shrub	Traplining hummingbirds (1)	Fleshy capsule	SL, P, B
Miconia rubescens	Shrub	Small-buzzing bees (2)	Berry	SL, P, B
Meriania tomentosa	Shrub	Traplining hummingbirds/bats (1, 3)	Dry capsule	SL, P, B
Notopleura longipedunculoides	Sub-shrub	Wasps/flies/bees (pers. obs.)	Berry	SL, P, B
Palicourea demissa	Shrub	Traplining hummingbirds (1)	Berry	SL, B

(1) Weinstein and Graham 2017

(2) Gamba and Almeda 2004

(3) Dellinger *et al.* 2019

† SL: Santa Lucía, T: Las Tángaras, P: El Pahuma, B: Bellavista.

Table 2 Estimates of population genetic structure for each studied species. N total, number of genotyped individuals in the final genetic dataset; N loci, number of variant loci in the final genetic dataset; AMOVA F_{IT} represents the deviation from Hardy-Weinberg Equilibrium within individuals relative to the expected heterozygosity in the total population; AMOVA F_{IS} represents the inbreeding coefficient among individuals within sites; AMOVA F_{ST} represents the global genetic differentiation among sampled sites. Population genetic parameters (F_{IS} and F_{ST}) were all statistically significant (p = 0.001 in bold) based on 1000 permutations of the data.

Species	**N total**	**N loci**	**AMOVA F_{IT} (SE)**	**AMOVA F_{IS} (SE)**	**AMOVA F_{ST} (SE)**
Drymonia brochidodroma	35	4907	0.42 (0.007)	**0.37** (0.007)	**0.08** (0.004)
Drymonia tenuis	29	1044	0.56 (0.014)	**0.51** (0.015)	**0.10** (0.010)
Miconia rubescens	34	2171	0.50 (0.009)	**0.43** (0.010)	**0.13** (0.005)
Meriania tomentosa	32	3883	0.29 (0.008)	**0.24** (0.008)	**0.06** (0.003)
Notopleura longipedunculoides	41	1815	0.35 (0.013)	**0.17** (0.014)	**0.21** (0.008)
Palicourea demissa	30	2376	0.22 (0.012)	**0.19** (0.012)	**0.03** (0.003)

Table 3 Estimates of SGS parameters for each studied species. N pairs, number of comparisons between all pairs of conspecific individuals; F_1, kinship coefficient between individuals in the first distance interval (separated by <1 km); *b* ln(distance), slope of the regression of kinship coefficients on the natural logarithm of spatial distance; S_P, intensity of SGS for each species. Standard errors (SE) were obtained through jackknifing over loci. SGS parameters (F_1 and *b*) were all statistically significant ($p < 0.01$ in bold) based on 1000 permutations of individual locations.

Species	**N pairs**	F_1 **(SE)**	***b*** **ln(distance)**	S_P **(SE)**
Drymonia brochidodroma	595	**0.053** (0.003)	**−0.024**	0.025 (0.001)
Drymonia tenuis	406	**0.044** (0.007)	**−0.021**	0.022 (0.002)
Miconia rubescens	561	**0.105** (0.005)	**−0.043**	0.048 (0.002)
Meriania tomentosa	496	**0.051** (0.002)	**−0.018**	0.019 (0.001)
Notopleura longipedunculoides	820	**0.180** (0.006)	**−0.073**	0.089 (0.003)
Palicourea demissa	435	**0.018** (0.002)	**−0.009**	0.009 (0.001)

Table 4 Results from generalized linear mixed-effects models with taxonomic family specified as a grouping factor and pollination mode as a fixed effect on (1) F_{ST} values and (2) S_P values across six species of cloud forest understory angiosperms. Significant p-values (<0.05) are denoted in bold.

TEST	Response	Estimate	Std. Error	t-value	p-value
1	Intercept	−2.88	0.46	−6.19	<0.0001
	F_{ST}	0.91	0.44	2.07	**0.04**
			Groups	**Variance**	**Std. Dev.**
			Family	0.03	0.18
			Residual	0.002	0.04
2	Intercept	−4.63	0.61	−7.65	<0.0001
	S_P	1.49	0.43	3.45	0.0006
			Groups	**Variance**	**Std. Dev.**
			Family	0.14	0.39
			Residual	0.0002	**0.014**

Figure 1 Average kinship-distance curves of each studied species. Filled symbols represent significant ($p < 0.05$) average kinship coefficient values based on 1000 permutations of individual spatial locations among all individuals. For associated standard errors of average F_{ij} at each distance interval refer to tables S5–S10. (a) *Drymonia brochidodroma*. (b) *Drymonia tenuis*. (c) *Miconia rubescens*. (d) *Meriania tomentosa*. (e) *Notopleura longipedunculoides*. (f) *Palicourea demissa*.

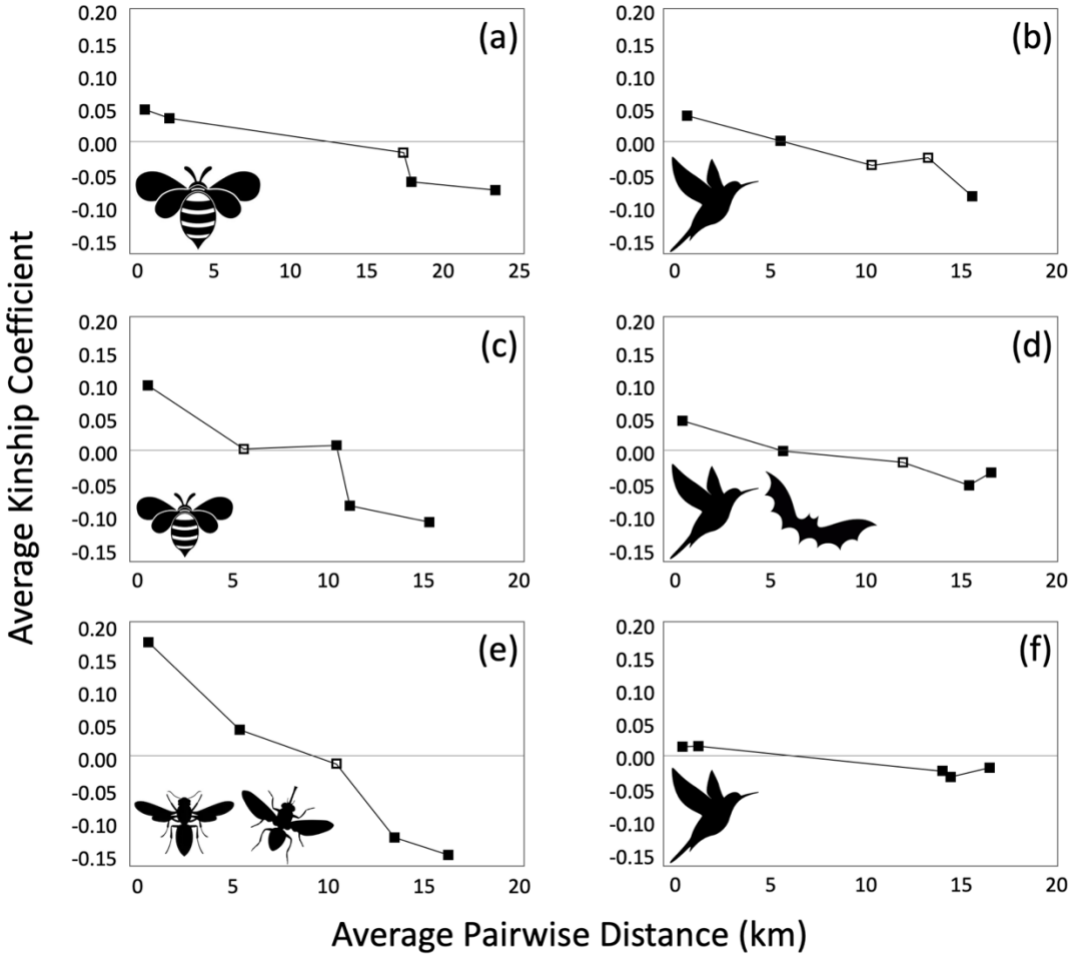
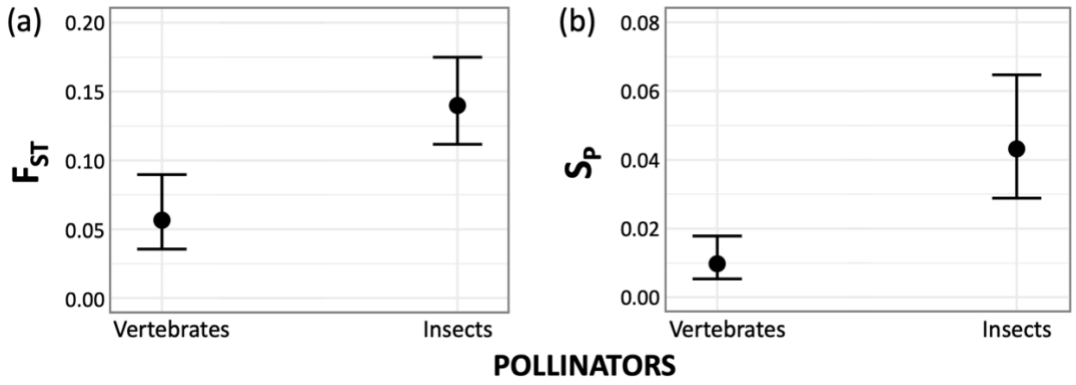


Figure 2 Marginal effect of animal pollination mode on predicted (a) F_{ST} values and (b) S_P values in the GLMMs with taxonomic family specified as a random effect. Black dots are predicted means for each category and surrounded black bars correspond to ± one standard deviation. Vertebrate and insect pollination modes were significantly different on both models (p<0.05).



Additional supporting information that will appear in the expanded online version of this article:

Table S1 Unfiltered catalog of loci recovered with STACKS v2.3e

Table S2 Genetic diversity of studied species across filtered loci.

Table S3 Genetic diversity of studied species within sites.

Table S4 AMOVA results showing the percent of genetic variation partitioning.

Tables S5–S10 Results of the spatial genetic structure (SGS) analysis.

Table S1 Unfiltered catalog of loci recovered with the STACKS v2.3e pipeline for non-model organisms. N is the number of individuals. Coverage refers to the mean depth of reads used to build loci.

Species	total N genotyped	total loci genotyped	Effective depth of coverage (reads/loci)			
			mean	stdev	min	max
Drymonia brochidodroma	37	4151836	14	3.8	8.7	31
Drymonia tenuis	42	3215520	16	5.3	6.6	32.4
Miconia rubesens	41	2923808	22.5	10.6	9.8	51.3
Meriania tomentosa	44	3164936	23.3	13.4	7	56.8
Notopleura longipedunculoides	50	879138	95.1	36.5	9.5	170.3
Palicourea demissa	38	2448608	18.9	6.2	9.2	42

Table S2 Genetic diversity of studied species estimated across filtered loci. N ind: number of genotyped individuals in the final genetic dataset, N var loci: number of variant loci, N total a: total number of alleles, %md: percent missing data, N a: mean number of alleles per locus, Ne a: mean effective number of alleles per locus, H_O: observed heterozygosity, H_S: mean expected heterozygosity across subpopulations, H_T: total expected heterozygosity over all subpopulations, G_{IS}: inbreeding coefficient. Standard deviations of statistics (in parentheses) were obtained through jackknifing over loci and significance of G_{IS} ($p < 0.005$) through 1000 permutations (all were statistically significant).

Species	**N ind**	**N var loci**	**N total a**	**% md**	**N a**	**Ne a**	**H_O**	**H_S**	**H_T**	**G_{IS}**
Drymonia brochidodroma	35	4907	9800	37	2.00 (0.001)	1.29 (0.003)	0.14 (0.002)	0.22 (0.002)	0.23 (0.002)	**0.37** (0.01)
Drymonia tenuis	29	1044	2018	38	1.93 (0.008)	1.22 (0.006)	0.09 (0.003)	0.19 (0.004)	0.21 (0.004)	**0.51** (0.02)
Miconia rubescens	34	2171	4314	34	1.99 (0.002)	1.29 (0.004)	1.13 (0.003)	0.23 (0.003)	0.25 (0.003)	**0.45** (0.01)
Meriania tomentosa	32	3883	7727	30	1.99 (0.001)	1.31 (0.004)	0.18 (0.003)	0.23 (0.002)	0.25 (0.002)	**0.25** (0.01)
Notopleura longipedunculoides	41	1815	3621	24	2.00 (0.002)	1.28 (0.005)	0.18 (0.004)	0.21 (0.003)	0.25 (0.003)	**0.17** (0.01)
Palicourea demissa	30	2376	4691	32	1.97 (0.003)	1.29 (0.005)	0.18 (0.003)	0.22 (0.003)	0.22 (0.003)	**0.19** (0.01)

Table S3 Genetic diversity of studied species within sites estimated from filtered loci. B: Bellavista, T: Las Tángaras, P: Pahuma, S: Santa Lucía. N: number of individuals in the final genetic dataset, Ne: effective number of individuals, P a: number of private alleles, % P a: proportion of private to total alleles, N a: mean number of alleles per locus, Ne a: mean effective number of alleles per locus, H_O: observed heterozygosity, H_S: mean expected heterozygosity within site, G_{IS}: inbreeding coefficient. Significance ($p < 0.005$) was obtained through 1000 permutations (all were statistically significant).

Species	**Site**	**N**	**Ne**	**P a**	**% P a**	**N a**	**Ne a**	**H_O**	**H_S**	**G_{IS}**
Drymonia brochidodroma	T	18	12	930	0.19	1.83	1.31	0.14	0.22	**0.37**
	S	17	11	737	0.15	1.81	1.30	0.14	0.22	**0.37**
Drymonia tenuis	B	9	7	74	0.07	1.51	1.24	0.10	0.17	**0.44**
	P	11	8	80	0.08	1.65	1.28	0.09	0.21	**0.55**
	S	9	8	114	0.11	1.52	1.26	0.09	0.19	**0.54**
Miconia rubescens	B	8	7	183	0.08	1.57	1.34	0.10	0.24	**0.58**
	P	13	9	80	0.04	1.71	1.33	0.15	0.23	**0.35**
	S	13	9	164	0.08	1.73	1.33	0.14	0.23	**0.40**
Meriania tomentosa	B	11	9	136	0.04	1.73	1.34	0.18	0.23	**0.21**
	P	11	9	162	0.04	1.65	1.33	0.17	0.22	**0.20**
	S	10	8	385	0.10	1.73	1.37	0.17	0.26	**0.32**
Notopleura longipedunculoides	B	14	11	57	0.03	1.56	1.29	0.18	0.18	**0.02**
	P	14	11	339	0.19	1.77	1.38	0.17	0.25	**0.32**
	S	13	11	202	0.11	1.59	1.32	0.18	0.20	**0.12**
Palicourea demissa	B	14	10	235	0.10	1.77	1.31	0.18	0.22	**0.18**
	S	16	13	479	0.20	1.84	1.31	0.18	0.22	**0.20**

Table S4. AMOVA results showing the percent of genetic variation partitioned within individuals, among individuals within sites, and among sites for all studied species.

Species	Source of Variation		
	Within individuals	Among individuals within sites	Among sites
Drymonia brochidodroma	0.58	0.34	0.08
Drymonia tenuis	0.44	0.46	0.10
Miconia rubescens	0.50	0.37	0.13
Meriania tomentosa	0.71	0.23	0.06
Notopleura longipedunculoides	0.65	0.14	0.21
Palicourea demissa	0.78	0.19	0.03

Legend for Tables S5–S10: Results of the spatial genetic structure (SGS) analysis based on all pairs of individuals within six studied species. Maximum distance: the upper limit of each distance interval. Mean distance: the average distance separating pairs of individuals within each interval. Mean ln(distance): the average natural logarithm of the distance separating pairs of individuals within each interval. Number of pairs: the number of pairs of individuals separated by the given distance interval. % partic: the percentage of individuals participating at least once in a pairwise comparison within each interval. CV partic: the coefficient of variation (i.e. the ratio of the standard deviation over the average) of the number of times each individual participates in pairwise comparisons within each interval. Kinship coefficients (F_{ij}) were calculated according to Loiselle et al. (1995). Respective standard errors (SE) were obtained through jackknifing over loci. Significance tests ($p < 0.05$ is denoted in bold) are based on the comparison of the observed F_{ij} values with the corresponding frequency distributions of 1000 random permutations of individual spatial locations among all individuals (next 2 pages).

Table S5 SGS analysis for *Drymonia brochidodroma* based on 35 individuals and 4907 loci.

Distance interval	**1**	**2**	**3**	**4**	**5**
Maximum distance (km)	0.47	2.06	17.40	17.93	23.43
Mean distance (km)	0.18	1.08	10.80	17.68	19.05
Mean ln(distance)	-2.04	0.01	2.14	2.87	2.94
Number of pairs	119	119	119	119	119
% partic	94	94	100	86	97
CV partic	0.43	0.45	0.95	0.47	0.76
F_{ij} (Loiselle)	**0.053**	**0.040**	-0.013	**-0.057**	**-0.069**
SE	0.003	0.003	0.002	0.003	0.003

Table S6 SGS analysis for *Drymonia tenuis* based on 29 individuals and 1044 loci.

Distance interval	**1**	**2**	**3**	**4**	**5**
Maximum distance (km)	0.62	5.50	10.32	13.26	15.57
Mean distance (km)	0.18	3.03	6.68	11.00	13.89
Mean ln(distance)	-2.26	0.87	1.87	2.40	2.63
Number of pairs	81	81	81	81	82
% partic	93	100	76	90	66
CV partic	0.58	0.66	0.74	0.68	0.78
F_{ij}	**0.044**	**0.005**	-0.032	**-0.021**	**-0.079**
SE	0.007	0.006	0.005	0.006	0.008

Table S7 SGS analysis for *Miconia rubescens* based on 34 individuals and 2171 loci.

Distance interval	**1**	**2**	**3**	**4**	**5**
Maximum distance (km)	0.51	5.56	10.42	11.12	15.29
Mean distance (km)	0.26	2.47	7.74	10.78	13.83
Mean ln(distance)	-1.59	0.45	2.01	2.38	2.62
Number of pairs	112	112	112	112	113
% partic	97	100	97	74	85
CV partic	0.52	0.54	0.76	0.72	0.79
F_{ij}	**0.105**	0.007	**0.013**	**-0.079**	**-0.104**
SE	0.005	0.005	0.003	0.005	0.006

Table S8 SGS analysis for *Meriania tomentosa* based on 32 individuals and 3883 loci.

Distance interval	**1**	**2**	**3**	**4**	**5**
Maximum distance (km)	0.36	5.65	11.94	15.40	16.56
Mean distance (km)	0.20	3.19	8.31	12.80	15.82
Mean ln(distance)	-1.88	0.65	2.06	2.55	2.76
Number of pairs	99	99	99	99	100
% partic	91	91	94	88	100
CV partic	0.55	0.81	0.63	0.63	0.77
F_{ij}	**0.051**	0.005	**-0.013**	**-0.048**	**-0.028**
SE	0.002	0.002	0.002	0.002	0.002

Table S9 SGS analysis for *Notopleura longipedunculoides* based on 41 individuals and 1815 loci.

Distance interval	**1**	**2**	**3**	**4**	**5**
Maximum distance (km)	0.55	5.33	10.42	13.46	16.28
Mean distance (km)	0.30	2.90	6.68	11.60	14.45
Mean ln(distance)	-1.43	0.78	1.87	2.45	2.67
Number of pairs	164	164	164	164	164
% partic	100	100	81	83	68
CV partic	0.41	0.62	0.75	0.66	0.75
F_{ij}	**0.180**	0.046	**-0.006**	**-0.118**	**-0.144**
SE	0.006	0.007	0.003	0.006	0.009

Table S10 SGS analysis for *Palicourea demissa* based on 30 individuals and 2376 loci.

Distance interval	**1**	**2**	**3**	**4**	**5**
Maximum distance (km)	0.36	1.20	14.02	14.44	16.47
Mean distance (km)	0.20	0.74	8.61	14.22	15.24
Mean ln(distance)	-1.82	-0.38	1.71	2.65	2.72
Number of pairs	87	87	87	87	87
% partic	87	100	100	80	90
CV partic	0.67	0.49	0.83	0.66	0.81
F_{ij}	**0.018**	**0.018**	**-0.019**	**-0.028**	**-0.014**
SE	0.002	0.002	0.002	0.002	0.002

Chapter IV: Impact of animal pollinators and latitudinal regions on the spatial genetic structure of plants: a global test

Running title: Drivers of spatial genetic structure in plants

Diana Gamba, Department of Biology, University of Missouri at Saint Louis
dlgtk5@mail.umsl.edu
Nathan Muchhala, Department of Biology, University of Missouri at Saint Louis
muchhalan@umsl.edu

Author for correspondence: Diana Gamba; One University Boulevard, 223R Research Hall, Saint Louis, MO 63121, USA; dlgtk5@mail.umsl.edu; +1 (314) 702-0326.

Target journal: Proceedings of the Royal Society B: Biological Sciences

Abstract: 200 words

Total word count (main text): 5178

Abstract

Spatial genetic structure (SGS) in plants results from the nonrandom distribution of genotypes within populations, which is influenced by life-history traits including mating system, growth form, and seed dispersal mode. However, the effect of animal pollination and latitudinal region remain largely unknown. Based on their lower flying ability compared to other animals, we predict that SGS should be stronger in plants pollinated by small insects relative to plants pollinated by large insects and vertebrates. Likewise, we predict that plant SGS should be stronger in the tropics than in temperate zones, because higher spatial heterogeneity at local scales, lower population densities and higher species richness in the tropics may restrict plant gene flow. To test our predictions, we performed a literature review and assembled a 147-species global dataset of animal-pollinated plants with data on SGS intensity, as quantified with the S_P statistic. Generalized linear models demonstrated that pollination mode, latitudinal region, and growth form were all significant predictors of S_P values, while mating system and seed dispersal mode were not significant. Our findings strongly supported our predictions, particularly in non-woody plants and shrubs, highlighting differences among latitudinal regions, and the importance of animal pollination mode in shaping patterns of plant SGS.

Key words: animal pollination, flowering plants, fine-scale spatial genetic structure, latitudinal region, SGS, S_P statistic.

Introduction

Fine-scale spatial genetic structure (SGS) in plants results from the non-random distribution of closely related individuals in space and represents the spatial scale of intraspecific gene flow within populations [1]. Understanding the factors that affect plant SGS is critical for analyzing demographic patterns such as the extent of genetic cohesion, or 'neighborhood size' [2,3], within natural and fragmented populations. Likewise, factors that influence plant SGS can strongly affect evolutionary processes within populations, such as local adaptation [4], and the maintenance of genetic diversity [5]. Plant life-history traits such as mating system, growth form, pollination mode and seed dispersal mode can influence patterns of SGS because they are directly involved in gene dispersal. In general, selfing herbs have significantly greater SGS than outcrossing trees [6], and animal-pollinated plants have greater SGS than wind-pollinated ones [5]. Additionally, SGS is greater in species with short-distance dispersers, lower in species dispersed by birds, and highly variable in species dispersed by active or passive seed accumulators [4,5], suggesting that dispersal limitation leads to high SGS. In fact, seed dispersal is often assumed to be the main determinant of SGS [7]. However, this relationship will ultimately depend on how successfully seeds establish and become adult plants. If most seeds fall under a mother plant —a common sign of dispersal limitation— but do not survive, then other factors that affect plant gene flow, such as pollination mode and landscape heterogeneity in a given region, should become important determinants of plant SGS. The effect of different animal pollinators on broad-scale patterns of plant

SGS, however, remains largely understudied.

Different pollinators can differ substantially in their flying ability and pollen carry-over capacity. Volant vertebrates and large insects, for example, generally fly longer distances during foraging bouts than small insects [5,8–12]. Studies on pollen carry-over in entomophilous plants reveal that small insects such as flies, solitary bees, and small beetles generally visit most flowers in a single plant, and then usually stay among nearby plants in the same patch [10–13]. In contrast to this, bumblebees are generally associated with significantly greater pollen carry-over and pollen dispersal distances [15]. For example, enclosed experiments and studies in natural populations show that although bumblebees deposit most pollen in nearby plants, significant amounts of pollen are transported to more distant flowers even after grooming [14,16,17]. Similarly, honeybees deposit pollen across distances three times larger than predicted by common exponential functions that evaluate pollen deposition, fitting a leptokurtic distribution comparable to that of bumblebees [18,19]. Furthermore, bumblebees and butterflies are highly directional in their flight while foraging, suggesting they can increase pollen flow distances when pollen carry-over is successful [8,20]. Studies of pollinator movement show that euglossine bees, hawkmoths, birds and bats can all travel quite far, even across fragmented habitats, potentially connecting individual plants across large distances [21–29]. In support of this, direct measures of pollen dispersal reveal that bats can transport pollen for several kilometers, large insects such as honeybees can transport pollen for >600 meters, while pollen transfer by most small insects (smaller than a

honeybee) rarely reaches 300 meters (reviewed in [5]). Based on these differences in the extent of pollen dispersal among animal pollinators, we predict that plants pollinated by small insects (smaller than a honeybee) should have stronger SGS than plants pollinated by large insects (honeybee or larger) or volant vertebrates (nectar-feeding birds and bats).

Furthermore, the influence of different latitudinal regions (i.e., temperate, tropical, subtropical), which differ substantially in landscape heterogeneity, is poorly understood. Across broader latitudinal scales, there are important environmental differences that may result in distinct patterns of SGS between plants in different latitudinal regions. For example, tropical regions have substantial habitat heterogeneity at a local scale, resulting in contrasting microclimates that could restrict plant demographic-range expansion at a given site [30–32]. Such restriction could limit gene flow within plant populations, and in turn potentially increase plant SGS in tropical plants relative to temperate ones. Subtropical forests similarly show considerable heterogeneity at a local scale compared to temperate ones [33], which could also result in higher plant SGS in subtropical than in temperate regions. Moreover, population densities tend to be significantly lower in tropical regions than temperate zones, which is usually associated with higher species diversity [5]. For instance, in a study of *Ardisia crenata* populations in subtropical China, sites with low population density and high species diversity were associated with greater SGS, relative to sites with high population density and low species diversity [34]. Given all of the above, we predict that species in tropical and subtropical regions should associate with

stronger SGS than species in temperate regions.

The strength of SGS can be quantified with the S_P statistic [6], which is based on a model of isolation by distance at migration–drift equilibrium [2,3]. This model describes the degree to which genetic relatedness between individuals, as quantified with the kinship coefficient F_{ij} [1], decreases with increasing geographic distance. S_P is defined as $-b/(1 - F_1)$, where b is the regression slope of genetic relatedness (F_{ij}) on geographic distance (d_{ij}) between individuals i and j, and F_1 is the mean F_{ij} [1] between all pairs of individuals in the first distance interval containing nearest neighbors. Because S_P mainly depends on the regression slope b, it is not affected by an arbitrary choice of distance intervals defined in a given study, making it comparable across species and thus ideal for investigating the factors that affect the strength of plant SGS globally. Additionally, studies that use the S_P statistic to characterize plant SGS frequently work at intermediate spatial scales (typically tens to hundreds of kilometers) at which both pollen and seed dispersal patterns have important effects on genetic diversity and population structure [5]. This is because the majority of seed dispersal often occurs at a small scale (i.e, <0.1 km), at which its effect is expected to determine plant SGS. At larger scales, i.e., beyond the bulk of seed dispersal, pollen dispersal can become equally or more important [5,35]. Thus, studies that report S_P values allow investigation of the effects of pollen dispersal mode across zoophilous species.

While the effects of animal pollination mode and latitudinal region have been largely overlooked in previous reviews on plant SGS variation [4–6,35],

they were evaluated in a recent review on global patterns of population genetic differentiation in seed plants based on F_{ST} values (D. Gamba and N. Muchhala, *in review*). Results of that study showed that tropical and subtropical mixed-mating non-woody plants pollinated by small insects were associated with higher F_{ST} values relative to temperate outcrossing trees and to plants pollinated by large insects and vertebrates. F_{ST} represents the proportion of genetic diversity partitioned among subpopulations, relative to the total population, and is usually taken at larger geographic scales than SGS studies (typically hundreds to thousands of kilometers). Thus, the S_P statistic describes isolation by distance among conspecific individuals, while the F_{ST} statistic may be used to examine isolation by distance among conspecific subpopulations [2,36,37]. Although S_P and F_{ST} values describe the arrangement of genetic diversity at different spatial scales, i.e., within (fine-scale) and among (large-scale) populations, respectively, the same processes, namely genetic drift, gene flow, and selection, underlie their patterns of variation. Thus, we expect that the same factors that affect F_{ST} also affect S_P, in line with our predictions. To our knowledge, however, no study to date has tried to connect patterns of S_P and F_{ST} variation. Furthermore, because seed dispersal is generally considered to be more important locally [4,5], it likely affects plant S_P values more than plant F_{ST} values. On the other hand, because pollen dispersal can generally reach longer distances [5,35], it likely affects plant S_P values as much as plant F_{ST} values.

Here, we took advantage of the wealth of publications that report S_P values and assembled a 147-species dataset of animal-pollinated plants at a

global scale. To the best of our knowledge, ours is the largest plant SGS dataset to be analyzed to date. We aimed to evaluate the effect of animal pollination mode and latitudinal region on S_P values, while also accounting for other factors that have been shown to affect S_P, namely mating system, growth form, seed dispersal mode, and genetic marker choice. Using multiple regressions, we tested two predictions: (1) that species pollinated by small insects (smaller than a honeybee) have on average greater S_P values that species pollinated by large insects (honeybees or larger) and vertebrates (hummingbirds and bats), and (2) that species from regions at tropical and subtropical latitudes have on average greater S_P values that species from regions at temperate latitudes. We also examined the relative contributions of factors to explaining variation in S_P values, in order to identify the most important factor affecting plant SGS.

Materials and Methods

Dataset compilation

We constructed an S_P dataset by conducting a systematic literature search in Google Scholar (key words: “fine-scale spatial genetic structure” OR “SGS” OR “spatial genetic structure” OR “S_P statistic”) focused on articles published through June 2018. This search yielded 254 peer-reviewed publications on seed plants for which S_P values based on nuclear markers were available. We also included 6 more species from a recent unpublished study (D. Gamba & N. Muchhala, *in prep.*). Because we were mainly interested in animal-pollinated plants, we did not include wind-pollinated or selfing species in the

database. Furthermore, we only considered studies of adult plants, rather than on seedlings or saplings, given that adults should better represent the long-term effects of animal pollinators on SGS. Based on these criteria, our final dataset included mean S_P values and metadata for 147 species (Table S1, Appendix S1). When a single study reported S_P values for multiple populations of the same species, we calculated the mean S_P value for all populations surveyed. When multiple studies reported S_P values for the same species, we calculated the mean S_P value for all populations across studies. For clonal species (*Asclepias syriaca* and *Piper* sp.), we used the published S_P value based on genets (excluding clones).

Previous studies suggest that the S_P statistic can be unduly influenced by the genetic marker chosen to infer SGS parameters [4,38,39]. Thus, we also scored the genotyping technique used for each species (microsatellites; allozymes; AFLP: amplified fragment length polymorphism; SNP: single-nucleotide polymorphisms). When a single species was analyzed with multiple markers, we used the marker with the greatest sample size of individuals per population. We did not include studies based on RAPD (randomly amplified polymorphic DNA) markers, because these were scarce (N = 3) and we wanted to minimize potential bias on S_P estimates due to marker type.

Species traits

We extracted information on species traits directly from the source publications, including pollination mode (small insects; large insects;

vertebrates), latitudinal region (tropics; subtropics; temperate), growth form (non-woody; shrub; tree), mating system (mixed-mating; outcrossing), and seed dispersal mode (animals; gravity; wind). Below, we explain how we coded factors in more detail.

Pollination mode— Small insect pollinators of species in our dataset included small Hymenoptera (*Trigona* and *Melipona* bees and wasps), Diptera (hoverflies and gnats), Coleoptera (small curculionids), Hemiptera (Anthocoridae and Miridae), and Thysanoptera (i.e., thrips). Large insects included large bees (honeybees, bumblebees, carpenter bees, euglossine bees) and Lepidoptera (hawk moths and yucca moths, monarch butterflies). Vertebrates included bats, hummingbirds, and other nectarivorous birds such as honeyeaters and sunbirds.

Latitudinal region— Tropical regions included sites between the Tropic of Cancer and Tropic of Capricorn (23.5° north and south of the equator, respectively), sub-tropical regions included latitudes from 23.5° to 35° (north and south of the equator), and temperate regions included latitudes greater than 35° (north and south of the equator).

Growth form— Trees included woody plants >10 m tall, typically with a single trunk coming from the base. Shrubs included upright woody plants <10 m tall, typically with one or several trunks coming from the base. Hemi-epiphytes (*Ficus citrifolia* and *F. obtusifolia*) and woody climbers (*Ancistrocladus korupensis*) were included in the shrub category, while epiphytes (*Aechmea nudicaulis*) and non-woody climbers (*Borderea pyrenaica*, *Dioscorea japonica*, and *Haumania danckelmaniana*) were included in the non-woody category.

Mating system— Mixed-mating species included those that undergo both outcrossing and selfing to some extent, through either autogamy or geitonogamy. Outcrossing species included plants that are self-incompatible, unisexual (i.e. monoecious or dioecious), or dichogamous hermaphrodites—i.e. either having the male reproductive organs come to maturity before the female organs (protandry), or vice versa (protogyny).

Seed dispersal mode— Plants that presented fruits or seeds that were particularly light and/or winged were coded as wind dispersed. Plants with no adaptations for vector-mediated seed dispersal were coded as gravity dispersed. Publications often did not include disperser identities for animal-dispersed species, and some species were dispersed by many taxonomic groups, making animal dispersal difficult to characterize. Thus, we maintained a broad animal dispersal category including all zoochorous plants (effects of zoochory on plant SGS are reviewed in [4]).

Statistical analyses

We used multiple regression models to examine the influence of different animal pollinators and latitudinal regions on plant SGS intensity, while accounting for other potentially significant predictors (growth form, mating system, seed dispersal mode, and genetic marker). Given that natural logarithm-transformed S_P values are normally distributed, we fitted generalized linear models (GLMs) with the 'glm' function in RStudio V 1.2.5019 [40] under a lognormal distribution structure for the residuals (family = 'Gaussian', link = 'log'). First, we built a GLM

that included all variables to estimate multicollinearity between predictors with the generalized variance inflation factor (GVIF) [41] calculated using the 'vif' R function. All GVIF values were >1 and <3.05 (Table S2), indicating the presence of some correlations among predictors, but that these were not sufficiently problematic to create multicollinearity issues negatively influencing a multiple regression [42]. Then, we examined our most inclusive model and sequentially removed factors that did not significantly contribute to the explained variation in S_P values in order to find the best-fit model to the data. We compared the fit of GLMs using model selection based on the Akaike Information Criterion (AIC) [43,44]. Finally, we tested for two-way interactions of pollination mode and latitudinal region with other factors in the best-fit model.

In order to measure and account for potential autocorrelations among the data due to evolutionary relationships, we calculated phylogenetic signal in the residual error of all models simultaneously with the regression parameters, following recommendations by Revell [45]. We extracted a species-level phylogeny containing our focal taxa (Fig. 1) from the angiosperm mega-tree [46] available in the V.PhyloMaker R package [47]. Branch lengths were inferred using the branch length adjuster algorithm in V.PhyloMaker [48]. Phylogenetic signal was measured with Pagel's λ [49] as implemented in the 'phylosig' R function in phytools [50]. We consistently obtained $\lambda < 0.001$ ($p = 1$), indicating a lack of phylogenetic autocorrelation in the residuals of our GLMs; thus, we only present and interpret results from non-phylogenetic GLMs.

After finding the best-fit model, we used the rr2 R package [51] and the

'R2.lik' function to obtain the unique contribution of each factor, in terms of the amount of S_P variance explained, by comparing the best-fit model with a reduced model not including the factor of interest. We also obtained the partial R^2 for each interaction term found to be significant. We visualized the marginal effect of each factor on S_P values in the best-fit model using the R packages sjPlot and ggplot2 [52,53] and the function 'plot_model' (with type = 'eff'). For conditional effects among factors (i.e., interactions), we set the plot_model type to 'int'.

Results

Taxonomic scope and phylogeny

The 147 animal-pollinated species were distributed in 113 genera, representing 54 families in 28 orders. The majority of species (118) belonged to the Eudicots, followed by 20 Monocots, 8 Magnoliids, and one Gymnosperm (*Zamia fairchildiana*). The families Fabaceae and Moraceae (mostly *Ficus*; 9 species) were the most well represented in the dataset, with 16 and 10 species, respectively (Table S1). The resulting phylogeny had 147 tips and 138 internal nodes (Fig. 1), indicating that 94% of the phylogeny was resolved, and only 9 tips (6%) belonged to polytomies. These polytomies were located within clades for which phylogenetic information remains scarce or unclear [54]: *Alcantarea* (Bromeliaceae) and *Psychotria* (Rubiaceae).

Best-fit model explaining variation in SGS intensity

Among the predictors we tested, pollination mode, latitudinal region and

life form had significant effects on S_P values, while the effect of mating system was only marginally significant (Table 1). Seed dispersal mode and genetic marker did not enter the best-fit model. Although animal-dispersed plants, and plants for which S_P was obtained with AFLP markers, tended to have slightly higher mean S_P values than the other groups (Fig. S1), these differences were not statistically significant ($p > 0.05$). In fact, removing these factors from the most-inclusive model (Table S3) greatly increased model fit to the data ($\Delta AIC = 5.95$).

Our estimation of the relative contribution of each factor to the explained variance of S_P values showed that growth form was the most important predictor in the best-fit model, with a partial R^2 of 0.20. Latitudinal region was second in importance with a partial R^2 of 0.13, followed by pollination mode (partial $R^2 = 0.05$), and lastly by mating system (partial $R^2 = 0.02$).

Patterns of S_P variation

Our results reveal that species pollinated by small insects are associated with significantly greater S_P values than species pollinated by vertebrates and large insects, while the latter two animal pollination modes did not differ from each other (Fig. 2a). We also found that species in tropical regions have significantly greater S_P values than species in subtropical and temperate regions, while the latter two regions did not differ from each other (Fig. 2b). Consistent with initial expectations, we confirm that trees have significantly lower S_P values relative to non-woody plants and shrubs. The three types of growth form were

also significantly different from each other, with mean S_P values increasing from trees to shrubs to non-woody plants (Fig. 2c). Lastly, mixed-mating plant species were associated with marginally higher S_P values than outcrossing species (Fig. 2d).

Because we were mostly interested in examining the effect of different animal pollinators and latitudinal regions on S_P values, we tested for interactions between pollination mode and latitudinal region with the other factors in our best-fit model, respectively. First, we found that differences between animal pollinators were significantly conditional on growth form ($p = 0.03$). Pollination by small insects is associated with higher mean S_P values relative to vertebrate and large insect pollination in non-woody plants and shrubs, but not in trees. Rather, vertebrate pollination tends to increase mean S_P in trees relative to large insects (Fig. 3a). The amount of variance explained by the model with this interaction was $R^2 = 0.26$, and this interaction had a partial $R^2 = 0.04$. Including it in the best-fit model, however, decreased model fit to the data (model with interaction AIC = −721.57, ΔAIC = 2.58). Second, we found that differences between latitudinal regions are marginally conditional on growth form ($p = 0.08$). Tropical regions tend to be associated with higher S_P values relative to subtropical and temperate zones in non-woody plants, but not in shrubs and trees. In shrubs, tropical regions seem related with higher S_P values relative to subtropical regions, while values from temperate regions were highly variable and appeared not different from other regions. Trees, on the other hand, did not seem to differ in S_P values among latitudinal regions (Fig. 3b). The amount of variance explained by the

model with this interaction was $R_2 = 0.26$, and this interaction had a partial $R_2 = 0.03$. Including this interaction in the best model, however, decreased model fit to the data (model with interaction AIC = −720.11, ΔAIC = 4.04).

Discussion

Here, we analyzed for the first time the effects of animal pollination mode and latitudinal region on plant SGS using a comprehensive global dataset of S_P values. Our results revealed a number of interesting patterns. Strikingly, we found that small insect pollination significantly increases S_P values relative to large insect and vertebrate pollination, particularly in non-woody plants and shrubs (Fig. 2a, 3a). Likewise, species from tropical regions are associated with higher S_P values relative to those from subtropical and temperate regions, especially for non-woody plants (Fig. 2b, 3b). Growth form was the most important predictor of S_P values relative to the other factors, followed by latitudinal region and pollination mode, while mating system was the least important and only marginally significant. Seed dispersal mode and genetic marker were not significant predictors of S_P. Before discussing the roles of these different factors in influencing SGS in more detail, below we compare our results to those from a review on global patterns of population genetic differentiation (as quantified with the F_{ST} statistic) in seed plants (D. Gamba & N. Muchhala, in review).

Our results are largely concordant with general patterns of variation in F_{ST} values, particularly with our predictions in respect to animal pollination mode and

latitudinal region. In general, small insect pollination is associated with higher F_{ST} and S_P values compared to both large insect and vertebrate pollination. Similarly, species from tropical regions have significantly higher F_{ST} and S_P values compared to species from temperate regions. Additionally, trees have significantly lower F_{ST} and S_P values relative to non-woody plants. These patterns of variation suggest that the same factors affect the arrangement of genetic diversity at different spatial scales: from fine-scale spatial structure within populations to broad-scale spatial structure among populations. Although this is expected given that any structuring of genetic diversity ultimately depends on the fundamental processes of gene flow, genetic drift and selection, ours is the first study we are aware of to link patterns of F_{ST} and S_P variation at a broad scale. Furthermore, seed dispersal mode was also not significant for explaining variation in F_{ST} or S_P values. Because seed dispersal is generally considered to be more important at local scales [1,4–7,60], we expected that it would have an effect on S_P values, particularly when comparing gravity vs. other modes of seed dispersal. We think that unrecorded secondary movement of seeds that fall under mother plants potentially precluded us from finding such difference. Finally, one difference between patterns of variation of F_{ST} and S_P values was the effect of mating system. It was a significant predictor for F_{ST} values, but only marginally significant for S_P values, with mixed-mating species generally associated with higher values. This was somewhat unexpected, given that mating system affects inbreeding, which lowers within-population variation, inflating between-population differentiation. Thus mixed-mating should increase both F_{ST} and S_P values due to

increased local genetic drift. Our result could simply be due to considerable amounts of outcrossing among the mixed-mating species in our S_P dataset, counteracting local genetic drift.

Influence of pollination mode on S_P

The strength of SGS was higher in species pollinated by small insects than in species pollinated by large insects and vertebrates (Fig. 2a). This is in line with differences in foraging behavior, pollen carry-over capacity, and flying ability among animal pollinators, which indicate that pollen dispersal by small insects is more limited compared to large insects and vertebrates [5,8,15]. Direct measures of pollen dispersal based on paternity analyses also support the limited distance covered by small insects in trees, as they reach maximum 300 meters [5]. This idea is also supported by indirect measures of pollen dispersal—i.e., obtained from observed SGS values derived from an isolation-by-distance process at equilibrium combined with estimates of the effective population density— which suggest they rarely surpass 20 meters in non-woody plants and shrubs [6,11,34], and 265 m in trees [5]. A remarkable exception is the pollen dispersal of fig trees by tiny agaonid wasps, which with the help of wind can achieve cross-pollination between trees separated by several kilometers [55]. Our dataset included 5 *Ficus* trees classified as pollinated by small insects. The mean S_P value for such *Ficus* was 0.017 (± 0.015 SD), which was not lower than expected compared to the mean S_P value of other tree species pollinated by small insects (0.013 ± 0.01 SD). However, the mean S_P value for all trees

pollinated by small insects (0.014 ± 0.01 SD) was considerably lower than that of non-woody plants and shrubs pollinated by small insects (0.032 ± 0.03 SD). This difference between trees vs. non-tree species in our dataset suggests that small insect pollination does not result in larger S_P values in trees. In fact, we also found that differences between animal pollinators in their effect on plant S_P values are rather restricted to non-woody plants and shrubs (Fig. 3a). Although it is not clear why this is the case, we propose that, as in agaonid wasps, other small insects that pollinate trees in our dataset could also be transported by wind when they reach the canopy. This would result in large breeding areas for many small insect pollinated trees, corresponding to their observed small S_P values.

Influence of latitudinal region on S_P

We predicted that species from tropical and subtropical regions should have stronger SGS than species from temperate regions. We did in fact find that tropical species had greater S_P values than temperate species, however subtropical and temperate species did not differ from each other (Fig. 2b). In general, tropical regions have greater species richness and higher habitat heterogeneity at local scales [30,56], and this combination could be underlying the pattern of S_P variation we found. This is because such combination likely makes gene dispersal less effective at local scales, decreasing the spatial scale of intraspecific gene flow and thus increasing S_P values. For example, high species richness implies that conspecific individuals are potentially separated by interspecific ones [57], making cross-pollination and thus intraspecific gene flow

harder to achieve across long distances in the tropics. Furthermore, high habitat heterogeneity at local scales in the tropics may result in tropical species and their mutualists to be highly restricted to certain microclimates due to local adaptation [58]. Such fine-scale narrow niches suggest that conspecific individuals should become rapidly genetically isolated with increasing geographic distance, associating with high S_P values.

Differences among latitudinal regions, however, tend to be restricted to non-woody plants, to a lesser extent to shrubs, and not apparent in trees (Fig. 3b). A similar pattern was reported in Dick et al. [5], where S_P values were not different between temperate and tropical trees. This result is in line with findings showing that trees worldwide can have extensive breeding areas, thus high gene flow among distant individuals, even in tropical regions where inbreeding has been hypothesized to be prevalent [5,55,59]. Even if trees are very good at dispersing their genes, either via pollen or seed, it is not clear why differences between latitudinal regions affect other types of growth forms but not trees. The mode of zoochory might be a more important determinant of SGS strength in trees (see [4,5,60]), which we were not able to analyze in our dataset, precluding us from finding a pattern of S_P variation among trees.

Influence of growth form on S_P

Growth form in animal-pollinated plants was by far the most important predictor of S_P variation in our best-fit model, with S_P values increasing from trees to shrubs to non-woody plants (Fig. 2c). A similar pattern was reported by

Vekemans and Hardy [6], although they did not provide an explanation for it. This pattern may reflect the fact that larger plants will be higher in the canopy and thus better at dispersing genes, whether via pollen or seeds. The pattern may also simply reflect scale: smaller plants show more fine-grained dispersal and thus will have more fine-grained genetic structure. Furthermore, growth form is frequently tightly linked to habitat, in that non-woody plants and shrubs live in the understory while many trees reach the canopy. The understory may restrict gene flow more than the canopy, due to the lower dispersal propensity and the sedentary lifestyle of animal mutualists in the understory [61–63].

Factors that did not influence S_P

We did not find a significant effect of mating system on S_P values in the animal-pollinated plant species included in our study. Mixed-mating plants tend to have higher S_P values than outcrossing plants (Fig. S1d, 2d), but the difference between them was only marginally significant (Table 1). Selfing increases local genetic drift by reducing the effective number of reproductive individuals, which associates with higher S_P values than outcrossing [6]. Moreover, gene dispersal in outcrossing plants occurs via pollen and seed dispersal, whereas gene dispersal in selfing plants is solely determined by seed dispersal, increasing S_P values in selfing plants. We note that we did not include solely-selfing species in our analysis, thus the amounts of outcrossing in the mixed-mating species may have led to the only marginally significant effects of mating system that we detected.

We also failed to find an effect of seed dispersal mode on S_P values either (Table 1, Fig S1e). However, we note that our classification of dispersal mode was somewhat coarse, in that we lumped together all zoochorous plants. Indeed, differences in foraging behavior among seed dispersing animals have previously been found to affect plant S_P: species with short-distance dispersers have greater Sp values than those dispersed by birds, while Sp values are highly variable in species dispersed by scatter-hoarding animals [4,60]. Our dataset included gravity dispersed plants, which should be the most dispersal limited, but surprisingly they were not associated with higher S_P values. This is probably due to some animals (like ants and rodents) creating equally restricted seed dispersal patterns, and because some gravity-dispersed species might have unrecorded secondary seed vectors. Similarly, S_P values for wind dispersal were highly variable in our study. Previous studies suggest that wind dispersal is often restricted [5,60], but our results suggest that wind does not have a predictable effect on gene dispersal.

Conclusions

Our results have important implications for understanding the origin and maintenance of biodiversity and can inform conservation strategies. For example, we found a general pattern in which genetic relatedness rapidly decreases with increasing geographic distance (i.e., high S_P values) among tropical non-woody plants and shrubs pollinated by small insects. This suggests that such plants likely have more genetically isolated subpopulations than other animal-pollinated

plants. A recent review on global patterns of population genetic differentiation in seed plants supports this idea. Non-woody tropical species pollinated by small insects were associated with greater F_{ST} values than other plants (D. Gamba & N. Muchhala, *in review*). Such genetic isolation at small to large spatial scales (i.e., within and among populations) could result in nearby subpopulations that harbor unique genetic diversity. This in turn, could increase the probability for local adaptation and reproductive isolation if divergent selection between close-by sites is strong and seed-mediated gene flow is ineffective. Non-woody/shrubby tropical species pollinated by small insects, nonetheless, are likely very susceptible to non-random habitat fragmentation (more so than vertebrate pollinated plants; e.g. [64]), which can further isolate populations and result in loss of genetic variability due to increased genetic drift [65,66]. The current scenario of human-accelerated change should thus push conservation efforts to maintain connectivity between fragments that harbor many understory tropical species pollinated by small insects.

Acknowledgements

We thank the researchers whose published data we used in this paper. We also thank Robert Ricklefs, Christine Edwards, and Carmen Ulloa for advice on study design. Many thanks to members of the Muchhala lab at the University of Missouri at Saint Louis for constructive discussions on a previous version of this manuscript. This research was supported by funds from the Whitney Harris World Ecology Center at the University of Missouri–Saint Louis.

References

1. Loiselle BA, Sork VL, Nason J, Graham C. 1995 Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). *Am. J. Bot.* **82**, 1420–1425. (doi:10.1002/j.1537-2197.1995.tb12679.x)
2. Wright S. 1943 Isolation by Distance. *Genetics* **28**, 114–138.
3. Wright S. 1946 Isolation by distance under diverse systems of mating. *Genetics* **31**, 39.
4. Gelmi-Candusso TA, Heymann EW, Heer K. 2017 Effects of zoochory on the spatial genetic structure of plant populations. *Mol. Ecol.* **26**, 5896–5910. (doi:10.1111/mec.14351)
5. Dick CW, Hardy OJ, Jones FA, Petit RJ. 2008 Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Trop. Plant Biol.* **1**, 20–33. (doi:10.1007/s12042-007-9006-6)
6. Vekemans X, Hardy OJ. 2004 New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol. Ecol.* **13**, 921–935. (doi:10.1046/j.1365-294X.2004.02076.x)
7. Hamrick JL, Trapnell DW. 2011 Using population genetic analyses to understand seed dispersal patterns. *Acta Oecologica* **37**, 641–649. (doi:10.1016/j.actao.2011.05.008)
8. Levin DA. 1979 Pollinator foraging behavior: genetic implications for plants. In *Topics in plant population biology*, pp. 131–153. Springer.
9. Rhodes MK, Fant JB, Skogen KA. 2017 Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Mol. Ecol.* **26**, 4296–4308.
10. Schmitt J. 1983 Density-dependent pollinator foraging, flowering phenology, and temporal pollen dispersal patterns in Linanthus bicolor. *Evolution* **37**, 1247–1257.
11. Fenster CB. 1991 Gene flow in Chamaecrista fasciculata (Leguminosae) I. Gene dispersal. *Evolution* **45**, 398–409.
12. Fenster CB, Vekemans X, Hardy OJ. 2003 Quantifying gene flow from spatial genetic structure data in a metapopulation of Chamaecrista fasciculata (Leguminosae). *Evolution* **57**, 995–1007.
13. Campbell DR. 1985 Pollen and gene dispersal: the influences of competition for pollinators. *Evolution* **39**, 418–431. (doi:10.1111/j.1558-5646.1985.tb05678.x)
14. Escaravage N, Wagner J. 2004 Pollination effectiveness and pollen dispersal in a Rhododendron ferrugineum (Ericaceae) population. *Plant Biol.* **6**, 606–615.
15. Hasegawa Y, Suyama Y, Seiwa K. 2015 Variation in pollen-donor composition among pollinators in an entomophilous tree species, Castanea crenata, revealed by single-pollen genotyping. *PLOS ONE* **10**, e0120393. (doi:10.1371/journal.pone.0120393)
16. Thomson JD, Plowright RC. 1980 Pollen carryover, nectar rewards, and pollinator behavior with special reference to Diervilla lonicera. *Oecologia* **46**, 68–74. (doi:10.1007/BF00346968)

17. Thomson JD. 1986 Pollen transport and deposition by bumblebees in Erythronium: influences of floral nectar and bee grooming. *J. Ecol.* **74**, 329–341. (doi:10.2307/2260258)
18. Cresswell JE, Bassom AP, Bell SA, Collins SJ, Kelly TB. 1995 Predicted pollen dispersal by honeybees and three species of bumblebees foraging on oil-seed rape: a comparison of three models. *Funct. Ecol.* **9**, 829–841.
19. Morris WF, Mangel M, Adler FR. 1995 Mechanisms of pollen deposition by insect pollinators. *Evol. Ecol.* **9**, 304–317. (doi:10.1007/BF01237776)
20. Schmitt J. 1980 Pollinator foraging behavior and gene dispersal in Senecio (Compositae). *Evolution* **34**, 934–943.
21. Janzen DH. 1971 Euglossine bees as long-distance pollinators of tropical plants. *Sci. New Ser.* **171**, 203–205.
22. Machado ICS, Sazima I, Sazima M. 1998 Bat pollination of the terrestrial herbIrlbachia alata (Gentianaceae) in northeastern Brazil. *Plant Syst. Evol.* **209**, 231–237. (doi:10.1007/BF00985230)
23. Sahley CT. 2001 Vertebrate pollination, fruit production, and pollen dispersal of Stenocereus thurberi (Cactaceae). *Southwest. Nat.* **46**, 261–271.
24. López-Uribe MM, Oi CA, Del Lama MA. 2008 Nectar-foraging behavior of Euglossine bees (Hymenoptera: Apidae) in urban areas. *Apidologie* **39**, 410–418.
25. Brunet J, Larson-Rabin Z, Stewart CM. 2012 The distribution of genetic diversity within and among populations of the Rocky Mountain Columbine: the impact of gene flow, pollinators, and mating system. *Int. J. Plant Sci.* **173**, 484–494. (doi:10.1086/665263)
26. McCulloch ES, Sebastián Tello J, Whitehead A, Rolón-Mendoza CM, Maldonado-Rodríguez MC, Stevens RD. 2013 Fragmentation of Atlantic Forest has not affected gene flow of a widespread seed-dispersing bat. *Mol. Ecol.* **22**, 4619–4633.
27. Finger A, Kaiser-Bunbury CN, Kettle CJ, Valentin T, Ghazoul J. 2014 Genetic connectivity of the moth pollinated tree Glionnetia sericea in a highly fragmented habitat. *PloS One* **9**, e111111.
28. Krauss SL, Phillips RD, Karron JD, Johnson SD, Roberts DG, Hopper SD. 2017 Novel consequences of bird pollination for plant mating. *Trends Plant Sci.* **22**, 395–410.
29. Skogen KA, Overson RP, Hilpman ET, Fant JB. 2019 Hawkmoth pollination facilitates long-distance pollen dispersal and reduces isolation across a gradient of land-use change. *Ann. Mo. Bot. Gard.* **104**, 495–511. (doi:10.3417/2019475)
30. Ricklefs RE. 1977 Environmental heterogeneity and plant species diversity: a hypothesis. *Am. Nat.* **111**, 376–381.
31. Baraloto C, Goldberg DE, Bonal D. 2005 Performance trade-offs among tropical tree seedlings in contrasting microhabitats. *Ecology* **86**, 2461–2472.
32. Baraloto C, Couteron P. 2010 Fine-scale microhabitat heterogeneity in a French Guianan forest. *Biotropica* **42**, 420–428.

33. Iacopetti G, Bussotti F, Selvi F, Maggino F, Pollastrini M. 2019 Forest ecological heterogeneity determines contrasting relationships between crown defoliation and tree diversity. *For. Ecol. Manag.* **448**, 321–329.
34. Zeng X, Michalski SG, Fischer M, Durka W. 2012 Species diversity and population density affect genetic structure and gene dispersal in a subtropical understory shrub. *J. Plant Ecol.* **5**, 270–278. (doi:10.1093/jpe/rtr029)
35. Hardy OJ *et al.* 2006 Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Mol. Ecol.* **15**, 559–571.
36. Wright S. 1951 The genetical structure of populations. *Ann. Eugen.* **15**, 323–354. (doi:10.1111/j.1469-1809.1949.tb02451.x)
37. Wright S. 1965 The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**, 395–420.
38. Jump AS, Peñuelas J. 2006 Extensive spatial genetic structure revealed by AFLP but not SSR molecular markers in the wind-pollinated tree, Fagus sylvatica. *Mol. Ecol.* **16**, 925–936. (doi:10.1111/j.1365-294X.2006.03203.x)
39. Jump AS, Rico L, Coll M, Peñuelas J. 2012 Wide variation in spatial genetic structure between natural populations of the European beech (Fagus sylvatica) and its implications for SGS comparability. *Heredity* **108**, 633–639. (doi:10.1038/hdy.2012.1)
40. R Core Team. 2018 R Foundation for Statistical Computing; Vienna, Austria: 2015. *R Lang. Environ. Stat. Comput.* , 2013.
41. Fox J, Monette G. 1992 Generalized Collinearity Diagnostics. *J. Am. Stat. Assoc.* **87**, 178–183. (doi:10.1080/01621459.1992.10475190)
42. Mansfield ER, Helms BP. 1982 Detecting multicollinearity. *Am. Stat.* **36**, 158–160.
43. Akaike H. 1974 A new look at the statistical model identification. *IEEE Trans. Autom. Control* **19**, 716–723. (doi:10.1109/TAC.1974.1100705)
44. Burnham KP, Anderson DR. 2004 Multimodel inference: understanding AIC and BIC in model selection. *Sociol. Methods Res.* **33**, 261–304.
45. Revell LJ. 2010 Phylogenetic signal and linear regression on species data: Phylogenetic regression. *Methods Ecol. Evol.* **1**, 319–329. (doi:10.1111/j.2041-210X.2010.00044.x)
46. Smith SA, Brown JW. 2018 Constructing a broadly inclusive seed plant phylogeny. *Am. J. Bot.* **105**, 302–314. (doi:10.1002/ajb2.1019)
47. Jin Y, Qian H. 2019 V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography* , ecog.04434. (doi:10.1111/ecog.04434)
48. Qian H, Jin Y. 2016 An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *J. Plant Ecol.* **9**, 233–239. (doi:10.1093/jpe/rtv047)
49. Pagel M. 1999 Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884. (doi:10.1038/44766)
50. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223.

51. Ives AR. 2018 R s for Correlated Data: Phylogenetic Models, LMMs, and GLMMs. *Syst. Biol.* **68**, 234–251.
52. Wickham H. 2011 ggplot2. *Wiley Interdiscip. Rev. Comput. Stat.* **3**, 180–185.
53. Lüdecke D. 2018 sjPlot: Data visualization for statistics in social science. R package version 2.4. 0. *Compr. R Arch. Netw. HttpsCRAN R-Proj. Orgpackage SjPlot*
54. Stevens PF. 2001 Angiosperm Phylogeny Website.
55. Nason JD, Herre EA, Hamrick JL. 1996 Paternity analysis of the breeding structure of strangler fig populations: evidence for substantial long-distance wasp dispersal. *J. Biogeogr.* **23**, 501–512.
56. ter Steege H *et al.* 2013 Hyperdominance in the Amazonian Tree Flora. *Science* **342**, 1243092. (doi:10.1126/science.1243092)
57. Comita LS, Queenborough SA, Murphy SJ, Eck JL, Xu K, Krishnadas M, Beckman N, Zhu Y. 2014 Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *J. Ecol.* **102**, 845–856. (doi:10.1111/1365-2745.12232)
58. Torroba-Balmori P, Budde KB, Heer K, González-Martínez SC, Olsson S, Scott-Saintagne C, Casalis M, Sonké B, Dick CW, Heuertz. 2017 Altitudinal gradients, biogeographic history and microhabitat adaptation affect fine-scale spatial genetic structure in African and Neotropical populations of an ancient tropical tree species. *PLOS ONE* **12**, e0182515. (doi:10.1371/journal.pone.0182515)
59. Fedorov A. 1966 The structure of the tropical rain forest and speciation in the humid tropics. *J. Ecol.* **54**, 1–11. (doi:10.2307/2257656)
60. Hamrick JL, Murawski DA, Nason JD. 1993 The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. In *Frugivory and seed dispersal: ecological and evolutionary aspects* (eds TH Fleming, A Estrada), pp. 281–297. Dordrecht: Springer Netherlands. (doi:10.1007/978-94-011-1749-4_20)
61. Givnish TJ. 2010 Ecology of plant speciation. *TAXON* **59**, 1326–1366. (doi:10.1002/tax.595003)
62. Theim TJ, Shirk RY, Givnish TJ. 2014 Spatial genetic structure in four understory Psychotria species (Rubiaceae) and implications for tropical forest diversity. *Am. J. Bot.* **101**, 1189–1199. (doi:10.3732/ajb.1300460)
63. Burney CW, Brumfield RT. 2009. Ecology predicts levels of genetic differentiation in neotropical birds. *Am. Nat.* **174**, 358–368. (doi:10.1086/603613)
64. Côrtes MC, Uriarte M, Lemes MR, Gribel R, John Kress W, Smouse PE, Bruna EM. 2013 Low plant density enhances gene dispersal in the Amazonian understory herb Heliconia acuminata. *Mol. Ecol.* **22**, 5716–5729. (doi:10.1111/mec.12495)
65. Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J. 2008 Genetic consequences of habitat fragmentation in plant populations:

susceptible signals in plant traits and methodological approaches. *Mol. Ecol.* **17**, 5177–5188. (doi:10.1111/j.1365-294X.2008.03971.x)
66. Aguilar R *et al.* 2019 Habitat fragmentation reduces plant progeny quality: a global synthesis. *Ecol. Lett.* **22**, 1163–1173. (doi:10.1111/ele.13272)

Data accessibility statement

Should the manuscript be accepted, the data and R scripts supporting the results will be archived in Dryad and their DOI will be included at the end of this article.

Author Contributions

DG and NM planned and designed the research. DG collected and analyzed the data. DG wrote the initial draft of the manuscript. DG and NM contributed equally to substantial revisions of the manuscript.

Table 1 Details of the best-fit model explaining variation in S_P values. Variables in bold indicate the reference level for each categorical factor. N indicates the sample size of each group. Significant *p*-values are in bold. Model R_2 = 0.24, Model AIC = −724.15.

Variable	N	Estimate	Std. Error	*t*-value	*p*-value
Intercept		−2.58	0.21	−12.302	<0.001
Pollination mode					
Small insects	82				
Large insects	38	−0.38	0.19	−1.97	**0.05**
Vertebrates	27	−0.50	0.21	−2.42	**0.02**
Latitudinal region					
Tropics	97				
Subtropics	17	−0.70	0.27	−2.60	**0.01**
Temperate	33	−1.01	0.25	−4.12	**<0.001**
Growth form					
Non-woody	43				
Shrub	37	−0.45	0.19	−2.39	**0.02**
Tree	67	−1.26	0.23	−5.48	**<0.001**
Mating system					
Mixed-mating	34				
Outcrossing	113	−0.26	0.21	−1.89	0.06

Figure 1 Phylogeny of studied species showing the taxonomic extent of this study with plotted S_P values in a logathmic scale, revealing their general lability across the phylogenetic tree. Plotting of S_P values was achieved with the R package ‘phytools’ and the function ‘contMap’.

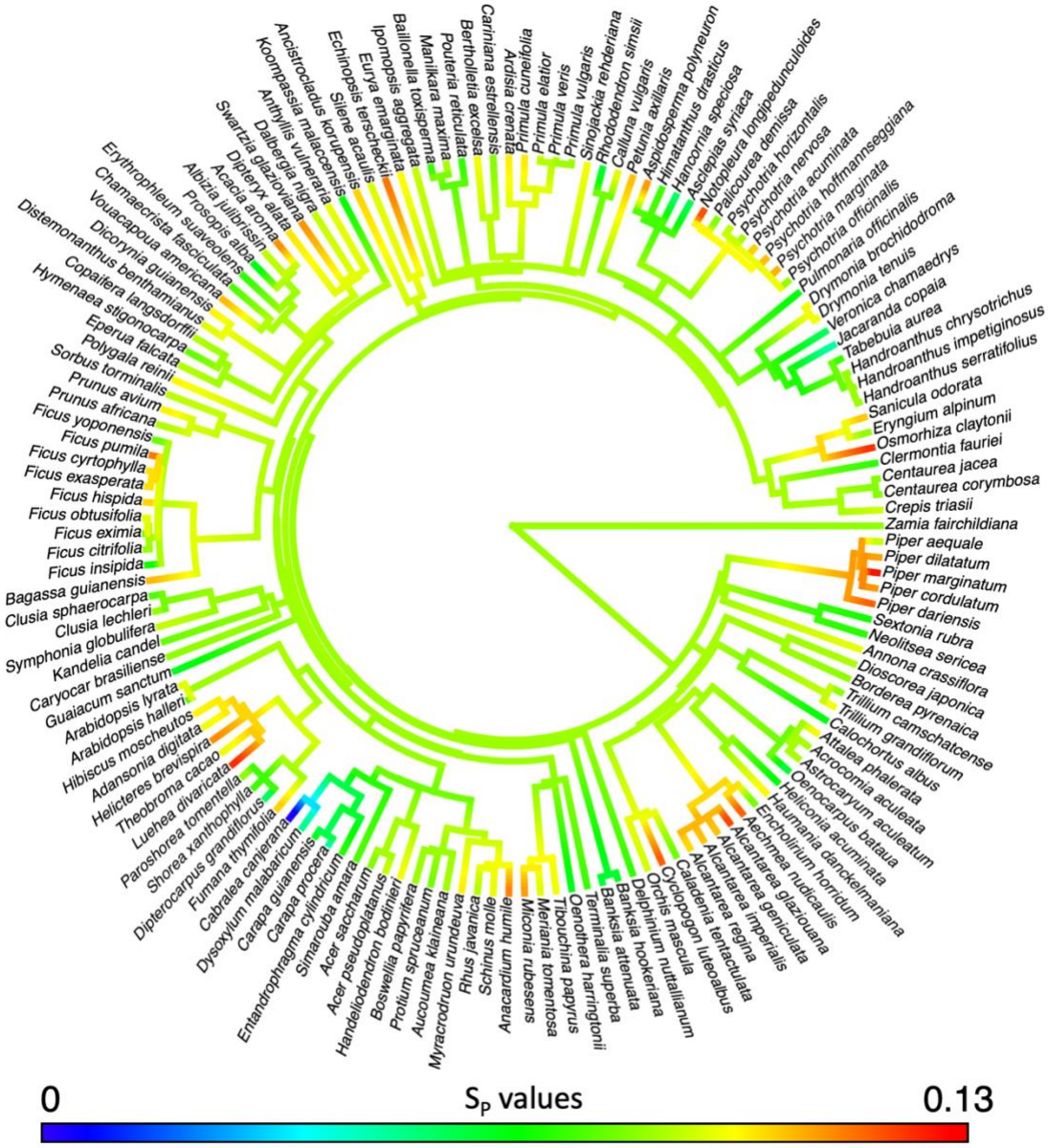


Figure 2 Marginal effects of factors on predicted S_P values in the best-fit model: (a) pollination mode, (b) latitudinal region, (c) growth form, (d) mating system. Black dots are predicted S_P means and surrounding bars correspond to ± one standard deviation. Significant differences between groups are depicted by letters on top of bar.

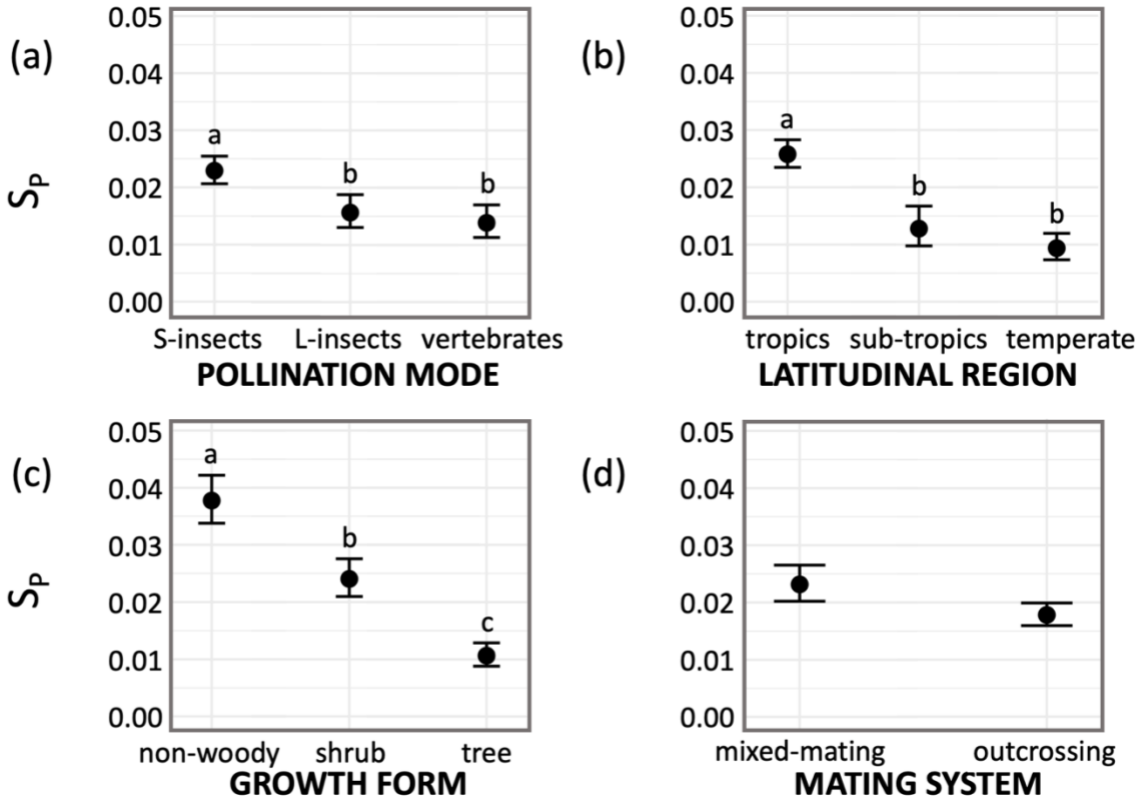
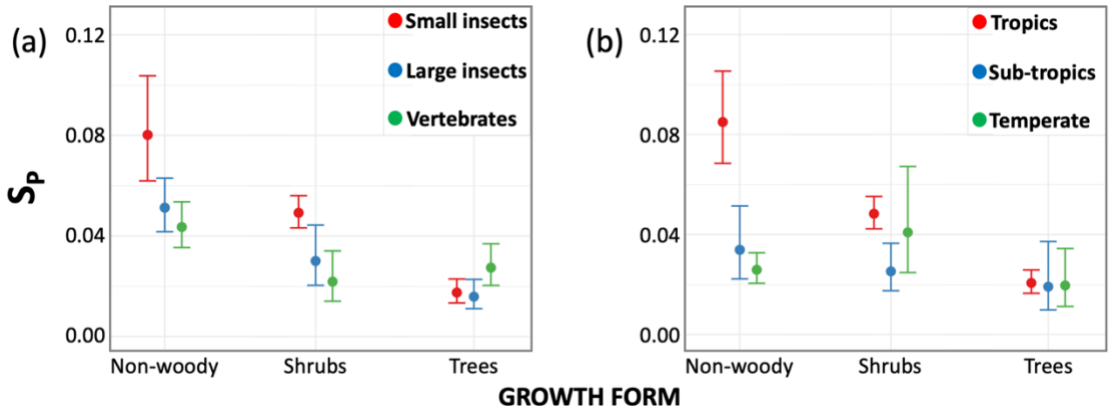


Figure 3 Marginal effects conditional on growth form of predicted S_P values for (a) animal pollination mode and (b) latitudinal region. Colors correspond to grouping categories (animal pollination modes or latitudinal regions). Each interaction was estimated as an additional term in the best-fit model. Dots in the plot are predicted S_P means and surrounding bars correspond to ± one standard deviation.



Additional supporting information that will appear in the expanded online version of this article:

Appendix S1. References of publication with S_P data and species traits used in this study.

Fig. S1 Violin plots of S_P values as a function of factors tested in this study.

Table S1 Dataset used in this study (in file Table S1.xlsx).

Table S2 Estimates of the generalized variance inflation factor on predictors.

Table S3 Details of the most-inclusive model explaining variation in S_P values.

Appendix S1. References of publication with S_P data and species traits used in this study.

Addisalem, A. B., J. Duminil, D. Wouters, F. Bongers, and M. J. M. Smulders. 2016. Fine-scale spatial genetic structure in the frankincense tree Boswellia papyrifera (Del.) Hochst. and implications for conservation. Tree Genetics & Genomes 12:86.

Araújo, M. R. G., A. F. de Melo Júnior, E. V. Menezes, M. M. Brandão, L. G. Cota, D. A. de Oliveira, V. de A. Royo, et al. 2017. Fine-scale spatial genetic structure and gene flow in Acrocomia aculeata (Arecaceae): Analysis in an overlapping generation. Biochemical Systematics and Ecology 71:147–154.

Baldauf, C., M. Ciampi-Guillardi, T. J. Aguirra, C. E. Corrêa, F. A. M. dos Santos, A. P. de Souza, and A. M. Sebbenn. 2014. Genetic diversity, spatial genetic structure and realised seed and pollen dispersal of Himatanthus drasticus (Apocynaceae) in the Brazilian savanna. Conservation Genetics 15:1073–1083.

Baldoni, A. B., L. H. O. Wadt, T. Campos, V. S. Silva, V. C. R. Azevedo, L. R. Mata, A. A. Botin, et al. 2017. Contemporary pollen and seed dispersal in natural populations of Bertholletia excelsa (Bonpl.). Genetics and Molecular Research 16.

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

Batista Leite, F. A., R. L. Brandão, R. S. de O. Buzatti, J. P. de Lemos-Filho, and M. B. Lovato. 2014. Fine-scale genetic structure of the threatened rosewood Dalbergia nigra from the Atlantic Forest: comparing saplings versus adults and small fragment versus continuous forest. Tree Genetics & Genomes 10:307–316.

Berens, D. G., C. Braun, S. C. González-Martínez, E. M. Griebeler, R. Nathan, and K. Böhning-Gaese. 2014. Fine-scale spatial genetic dynamics over the life cycle of the tropical tree Prunus africana. Heredity 113:401–407.

Bessega, C., C. L. Pometti, M. Ewens, B. O. Saidman, and J. C. Vilardi. 2016. Fine-scale spatial genetic structure analysis in two Argentine populations of Prosopis alba (Mimosoideae) with different levels of ecological disturbance. European Journal of Forest Research 135:495–505.

Bodare, S., G. Ravikanth, S. A. Ismail, M. K. Patel, I. Spanu, R. Vasudeva, R. U. Shaanker, et al. 2017. Fine- and local- scale genetic structure of Dysoxylum malabaricum, a late-successional canopy tree species in disturbed forest patches in the Western Ghats, India. Conservation Genetics 18:1–15.

Born, C., O. J. Hardy, M.-H. Chevallier, S. Ossari, C. Attéké, E. J. Wickings, and M. Hossaert-Mckey. 2008. Small-scale spatial genetic structure in the

Central African rainforest tree species Aucoumea klaineana: a stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. Molecular Ecology 17:2041–2050.

Brousseau, L., M. Foll, C. Scotti-Saintagne, and I. Scotti. 2015. Neutral and Adaptive Drivers of Microgeographic Genetic Divergence within Continuous Populations: The Case of the Neotropical Tree Eperua falcata (Aubl.). (F. A. Aravanopoulos, ed.)PLOS ONE 10:e0121394.

Browne, L., K. Ottewell, and J. Karubian. 2015. Short-term genetic consequences of habitat loss and fragmentation for the neotropical palm Oenocarpus bataua. Heredity 115:389–395.

Buzatti, R. S. de O., R. A. Ribeiro, J. P. de Lemos Filho, and M. B. Lovato. 2012. Fine-scale spatial genetic structure of Dalbergia nigra (Fabaceae), a threatened and endemic tree of the Brazilian Atlantic Forest. Genetics and Molecular Biology 35:838–846.

Campbell, D. R., and J. L. Dooley. 1992. The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation by distance. The American Naturalist 139:735–748.

Chaves, C. L., A. M. Sebbenn, A. Baranoski, B. D. Goez, A. P. S. C. Gaino, C. F. Ruas, E. Ruas, et al. 2016. Gene dispersal via seeds and pollen and their effects on genetic structure in the facultative-apomictic Neotropical tree Aspidosperma polyneuron. Silvae Genetica 65:46–57.

Choo, J., T. E. Juenger, and B. B. Simpson. 2012. Consequences of frugivore-mediated seed dispersal for the spatial and genetic structures of a neotropical palm. Molecular Ecology 21:1019–1031.

Chung, M. G. 2000*a*. Clonal and spatial genetic structure in Eurya emarginata (Theaceae). Heredity 84:170.

———. 2000*b*. Spatial distribution of allozyme polymorphisms following clonal and sexual reproduction in populations of Rhus javanica (Anacardiaceae). Heredity 84:178.

Collevatti, R. G., R. Estolano, M. L. Ribeiro, S. G. Rabelo, E. J. Lima, and C. B. R. Munhoz. 2014*a*. High genetic diversity and contrasting fine-scale spatial genetic structure in four seasonally dry tropical forest tree species. Plant Systematics and Evolution 300:1671–1681.

Collevatti, R. G., J. S. Lima, T. N. Soares, and M. P. de C. Telles. 2010. Spatial Genetic Structure and Life History Traits in Cerrado Tree Species: Inferences for Conservation. Natureza & Conservação 08:54–59.

Collevatti, R. G., M. P. C. Telles, J. S. Lima, F. O. Gouveia, and T. N. Soares. 2014*b*. Contrasting spatial genetic structure in Annona crassiflora populations from fragmented and pristine savannas. Plant Systematics and Evolution 300:1719–1727.

Côrtes, M. C., M. Uriarte, M. R. Lemes, R. Gribel, W. John Kress, P. E. Smouse, and E. M. Bruna. 2013. Low plant density enhances gene dispersal in the Amazonian understory herb Heliconia acuminata. Molecular Ecology 22:5716–5729.

Costa, C. F., R. G. Collevatti, L. J. Chaves, J. de S. Lima, T. N. Soares, and M. P. de C. Telles. 2017. Genetic diversity and fine-scale genetic structure in

Hancornia speciosa Gomes (Apocynaceae). Biochemical Systematics and Ecology 72:63–67.

Cota, L. G., P. A. Moreira, M. M. Brandão, V. A. Royo, A. F. M. Junior, E. V. Menezes, and D. A. Oliveira. 2017. Structure and genetic diversity of Anacardium humile (Anacardiaceae): a tropical shrub. Genetics and Molecular Research 16.

de Almeida Vieira, F., C. G. Fajardo, A. M. de Souza, and D. de Carvalho. 2010. Landscape-level and fine-scale genetic structure of the neotropical tree Protium spruceanum (Burseraceae). International Journal of Forestry Research 2010.

de Almeida Vieira, F., C. G. Fajardo, A. M. de Souza, C. A. F. Reis, and D. de Carvalho. 2012. Fine-scale genetic dynamics of a dominant neotropical tree in the threatened Brazilian Atlantic Rainforest. Tree Genetics & Genomes 8:1191–1201.

de Souza Lima, J., R. G. Collevatti, T. N. Soares, L. J. Chaves, and M. P. de Campos Telles. 2015. Fine-scale genetic structure in Tibouchina papyrus (Pohl) Toledo (Melastomataceae), an endemic and habitat-restricted species from Central Brazil. Plant Systematics and Evolution 301:1207–1213.

Debout, G. D. G., J.-L. Doucet, and O. J. Hardy. 2011. Population history and gene dispersal inferred from spatial genetic structure of a Central African timber tree, Distemonanthus benthamianus (Caesalpinioideae). Heredity 106:88–99.

Dev, S. A., F. Kjellberg, M. Hossaert-McKey, and R. M. Borges. 2011. Fine-scale Population Genetic Structure of Two Dioecious Indian Keystone Species, Ficus hispida and Ficus exasperata (Moraceae). Biotropica 43:309–316.

Dick, C. W., O. J. Hardy, F. A. Jones, and R. J. Petit. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. Tropical Plant Biology 1:20–33.

Duminil, J., K. Daïnou, D. K. Kaviriri, P. Gillet, J. Loo, J.-L. Doucet, and O. J. Hardy. 2016*a*. Relationships between population density, fine-scale genetic structure, mating system and pollen dispersal in a timber tree from African rainforests. Heredity 116:295–303.

Duminil, J., D. T. Mendene Abessolo, D. Ndiade Bourobou, J.-L. Doucet, J. Loo, and O. J. Hardy. 2016*b*. High selfing rate, limited pollen dispersal and inbreeding depression in the emblematic African rain forest tree Baillonella toxisperma – Management implications. Forest Ecology and Management 379:20–29.

Fenster, C. B., X. Vekemans, and O. J. Hardy. 2003. Quantifying gene flow from spatial genetic structure data in a metapopulation of Chamaecrista fasciculata (Leguminosae). Evolution 57:995–1007.

Foster, P. F., and V. L. Sork. 1997. Population and genetic structure of the West African rain forest liana *Ancistrocladus korupensis* (Ancistrocladaceae). American Journal of Botany 84:1078–1091.

Franceschinelli, E. V., and R. Kesseli. 1999. Population structure and gene flow of the Brazilian shrub Helicteres brevispira. Heredity 82:355–363.

Fuchs, E. J., and James. L. Hamrick. 2010. Spatial genetic structure within size classes of the endangered tropical tree Guaiacum sanctum (Zygophyllaceae). American Journal of Botany 97:1200–1207.

Gaino, A. P. S. C., A. M. Silva, M. A. Moraes, P. F. Alves, M. L. T. Moraes, M. L. M. Freitas, and A. M. Sebbenn. 2010. Understanding the effects of isolation on seed and pollen flow, spatial genetic structure and effective population size of the dioecious tropical tree species Myracrodruon urundeuva. Conservation Genetics 11:1631–1643.

Ganzhorn, S. M., W. W. Thomas, F. A. Gaiotto, and J. D. Lewis. 2015. Spatial genetic structure of Manilkara maxima (Sapotaceae), a tree species from the Brazilian Atlantic forest. Journal of Tropical Ecology 31:437–447.

Gaudeul, M., I. Till-Bottraud, F. Barjon, and S. Manel. 2004. Genetic diversity and differentiation in Eryngium alpinum L. (Apiaceae): comparison of AFLP and microsatellite markers. Heredity 92:508–518.

Gelmi-Candusso, T. A., E. W. Heymann, and K. Heer. 2017. Effects of zoochory on the spatial genetic structure of plant populations. Molecular Ecology 26:5896–5910.

Geng, Q., C. Lian, S. Goto, J. Tao, M. Kimura, M. S. Islam, and T. Hogetsu. 2008. Mating system, pollen and propagule dispersal, and spatial genetic structure in a high-density population of the mangrove tree Kandelia candel. Molecular Ecology 17:4724–4739.

Hahn, C. Z., S. G. Michalski, and W. Durka. 2017. Gene flow in, and mating system of, Rhododendron simsii in a nature reserve in subtropical China. Nordic Journal of Botany 35:1–7.

Hardy, O. J., S. C. Gonzalez-Martinez, H. Freville, G. Boquien, A. Mignot, B. Colas, and I. Olivieri. 2004. Fine-scale genetic structure and gene dispersal in Centaurea corymbosa (Asteraceae) I. Pattern of pollen dispersal. Journal of Evolutionary Biology 17:795–806.

Hardy, O. J., L. Maggia, E. Bandou, P. Breyne, H. Caron, M. H. Chevallier, A. Doligez, et al. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. Molecular ecology 15:559–571.

Hardy, O. J., and X. Vekemans. 2001. Patterns of Allozyme Variation in Diploid and Tetraploid Centaurea jacea at Different Spatial Scales. Evolution 55:943–954.

He, R., J. Wang, and H. Huang. 2012. Long-distance gene dispersal inferred from spatial genetic structure in Handeliodendron bodinieri, an endangered tree from karst forest in southwest China. Biochemical Systematics and Ecology 44:295–302.

Heer, K., E. K. V. Kalko, L. Albrecht, R. García-Villacorta, F. C. Staeps, E. A. Herre, and C. W. Dick. 2015. Spatial Scales of Genetic Structure in Free-Standing and Strangler Figs (Ficus, Moraceae) Inhabiting Neotropical Forests. (W. Arthofer, ed.)PLOS ONE 10:e0133581.

Helsen, K., H. Jacquemyn, and O. Honnay. 2015. Hidden founder effects: small-scale spatial genetic structure in recently established populations of the grassland specialist plant Anthyllis vulneraria. Molecular Ecology 24:2715–2728.

Helsen, K., T. Meekers, G. Vranckx, I. Roldán-Ruiz, K. Vandepitte, and O. Honnay. 2016. A direct assessment of realized seed and pollen flow within and between two isolated populations of the food-deceptive orchid Orchis mascula. (N. Vereecken, ed.)Plant Biology 18:139–146.

Henss, J. M., J. R. Moeller, T. J. Theim, and T. J. Givnish. 2013. Spatial scales of genetic structure and gene flow in Calochortus albus (Liliaceae). Ecology and Evolution 3:1461–1470.

Hirao, A. S., and G. Kudo. 2008. The effect of segregation of flowering time on fine-scale spatial genetic structure in an alpine-snowbed herb Primula cuneifolia. Heredity 100:424–430.

Hmeljevski, K. V., M. S. dos Reis, and R. C. Forzza. 2015. Patterns of Gene Flow in Encholirium horridum L.B.Sm., a Monocarpic Species of Bromeliaceae From Brazil. Journal of Heredity 106:93–101.

Jacquemyn, H., O. Honnay, P. Galbusera, and I. Roldan-Ruiz. 2004. Genetic structure of the forest herb Primula elatior in a changing landscape. Molecular Ecology 13:211–219.

Jennings, H., K. Wallin, J. Brennan, A. D. Valle, A. Guzman, D. Hein, S. Hunter, et al. 2016. Inbreeding, low genetic diversity, and spatial genetic structure in the endemic Hawaiian lobeliads Clermontia fauriei and Cyanea pilosa ssp. longipedunculata. Conservation Genetics 17:497–502.

Jolivet, C., A. M. Höltken, H. Liesebach, W. Steiner, and B. Degen. 2011. Spatial genetic structure in wild cherry (Prunus avium L.): I. variation among natural populations of different density. Tree Genetics & Genomes 7:271–283.

Jones, F. A., and S. P. Hubbell. 2006. Demographic spatial genetic structure of the Neotropical tree, Jacaranda copaia. Molecular Ecology 15:3205–3217.

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

Jump, A. S., L. Rico, F. Lloret, and J. Peñuelas. 2009. Microspatial population genetic structure of the Mediterranean shrub Fumana thymifolia. Plant Biology 11:152–160.

Kabat, S. M. 2010. Genetic Structure of the Common Milkweed, Asclepias syriaca L. 60.

Kalisz, S., J. D. Nason, F. M. Hanzawa, and S. J. Tonsor. 2001. Spatial Population Genetic Structure in Trillium grandiflorum: The Roles of Dispersal, Mating, History, and Selection. Evolution 55:1560–1568.

Kettle, C. J., P. M. Hollingsworth, D. F. R. P. Burslem, C. R. Maycock, E. Khoo, and J. Ghazoul. 2011. Determinants of fine-scale spatial genetic structure in three co-occurring rain forest canopy trees in Borneo. Perspectives in Plant Ecology, Evolution and Systematics 13:47–56.

Kloss, L., M. Fischer, and W. Durka. 2011. Land-use effects on genetic structure of a common grassland herb: A matter of scale. Basic and Applied Ecology 12:440–448.

Krauss, S. L., T. He, L. G. Barrett, B. B. Lamont, N. J. Enright, B. P. Miller, and M. E. Hanley. 2009. Contrasting impacts of pollen and seed dispersal on

spatial genetic structure in the bird-pollinated Banksia hookeriana. Heredity 102:274–285.
Kudoh, H., and D. F. Whigham. 1997. Microgeographic genetic structure and gene flow in *Hibiscus moscheutos* (Malvaceae) populations. American Journal of Botany 84:1285–1293.
Kyndt, T., A. E. Assogbadjo, O. J. Hardy, R. Glele Kakaï, B. Sinsin, P. Van Damme, and G. Gheysen. 2009. Spatial genetic structuring of baobab (Adansonia digitata, Malvaceae) in the traditional agroforestry systems of West Africa. American Journal of Botany 96:950–957.
Lasso, E., J. W. Dalling, and E. Bermingham. 2011. Strong spatial genetic structure in five tropical Piper species: should the Baker-Fedorov hypothesis be revived for tropical shrubs? Ecology and Evolution 1:502–516.
Lemos, R. P. M., C. B. D'Oliveira, and V. M. Stefenon. 2015. Genetic structure and internal gene flow in populations of Schinus molle (Anacardiaceae) in the Brazilian Pampa. Tree Genetics & Genomes 11:75.
Ley, A. C., and O. J. Hardy. 2016. Spatially limited clonality and pollen and seed dispersal in a characteristic climber of Central African rain forests: Haumania danckelmaniana (Marantaceae). Biotropica 48:618–627.
Llaurens, V., V. Castric, F. Austerlitz, and X. Vekemans. 2008. High paternal diversity in the self-incompatible herb Arabidopsis halleri despite clonal reproduction and spatially restricted pollen dispersal. Molecular Ecology 17:1577–1588.
Loh, R., F. R. Scarano, M. Alves-Ferreira, and F. Salgueiro. 2015. Clonality strongly affects the spatial genetic structure of the nurse species Aechmea nudicaulis (L.) Griseb. (Bromeliaceae). Botanical Journal of the Linnean Society 178:329–341.
Loiselle, B. A., V. L. Sork, J. Nason, and C. Graham. 1995. Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). American Journal of Botany 82:1420–1425.
Lopez-Gallego, C., and P. O'Neil. 2010. Life-history variation following habitat degradation associated with differing fine-scale spatial genetic structure in a rainforest cycad. Population Ecology 52:191–201.
Mahy, G., X. Vekemans, and A.-L. Jacquemart. 1999. Patterns of allozymic variation within Calluna vulgaris populations at seed bank and adult stages. Heredity 82:432–440.
Mayol, M., C. Palau, J. A. Rosselló, S. C. González-Martínez, A. Molins, and M. Riba. 2012. Patterns of genetic variability and habitat occupancy in Crepis triasii (Asteraceae) at different spatial scales: insights on evolutionary processes leading to diversification in continental islands. Annals of Botany 109:429–441.
Meeus, S., O. Honnay, and H. Jacquemyn. 2013. Differences in fine-scale spatial genetic structure across the distribution range of the distylous forest herb Pulmonaria officinalis (Boraginaceae). BMC Genetics 14:101.
Melo, A. T. de O., and E. V. Franceschinelli. 2016. Gene flow and fine-scale spatial genetic structure in Cabralea canjerana (Meliaceae), a common

tree species from the Brazilian Atlantic forest. Journal of Tropical Ecology 32:135–145.

Mizuki, I., K. Ishida, N. Tani, and Y. Tsumura. 2010. Fine-scale spatial structure of genets and sexes in the dioecious plant Dioscorea japonica, which disperses by both bulbils and seeds. Evolutionary Ecology 24:1399–1415.

Monthe, F. K., O. J. Hardy, J.-L. Doucet, J. Loo, and J. Duminil. 2017. Extensive seed and pollen dispersal and assortative mating in the rain forest tree Entandrophragma cylindricum (Meliaceae) inferred from indirect and direct analyses. Molecular Ecology 26:5279–5291.

Moreira, P. A., G. W. Fernandes, and R. G. Collevatti. 2009. Fragmentation and spatial genetic structure in Tabebuia ochracea (Bignoniaceae) a seasonally dry Neotropical tree. Forest Ecology and Management 258:2690–2695.

Nagel, J. C., D. E. Ceconi, I. Poletto, and V. M. Stefenon. 2015. Historical gene flow within and among populations of Luehea divaricata in the Brazilian Pampa. Genetica 143:317–329.

Nakagawa, M. 2010. Fine-scale genetic structure within plots of Polygala reinii (Polygalaceae) having an ant-dispersal seed. Journal of Plant Research 123:355–362.

Nazareno, A. G., A. L. Alzate-Marin, and R. A. S. Pereira. 2013. Dioecy, more than monoecy, affects plant spatial genetic structure: the case study of Ficus. Ecology and Evolution n/a-n/a.

Ndiade-Bourobou, D., O. J. Hardy, B. Favreau, H. Moussavou, E. Nzengue, A. Mignot, and J.-M. Bouvet. 2010. Long-distance seed and pollen dispersal inferred from spatial genetic structure in the very low-density rainforest tree, Baillonella toxisperma Pierre, in Central Africa: LONG-DISTANCE SEED AND POLLEN DISPERSAL. Molecular Ecology 19:4949–4962.

Noreen, A. M. E., and E. L. Webb. 2013. High Genetic Diversity in a Potentially Vulnerable Tropical Tree Species Despite Extreme Habitat Loss. (T. Brown, ed.)PLoS ONE 8:e82632.

Pandey, M., O. Gailing, H. H. Hattemer, and R. Finkeldey. 2012. Fine-scale spatial genetic structure of sycamore maple (Acer pseudoplatanus L.). European Journal of Forest Research 131:739–746.

Pardini, E. A., and J. L. Hamrick. 2008. Inferring recruitment history from spatial genetic structure within populations of the colonizing tree Albizia julibrissin (Fabaceae). Molecular Ecology 17:2865–2879.

Peakall, R., and A. J. Beattie. 1996. Ecological and Genetic Consequences of Pollination by Sexual Deception in the Orchid Caladenia tentactulata. Evolution 50:2207.

Pérez-Collazos, E., J. G. Segarra-Moragues, L. Villar, and P. Catalán. 2015. Ant pollination promotes spatial genetic structure in the long-lived plant Borderea pyrenaica (Dioscoreaceae). Biological Journal of the Linnean Society 116:144–155.

Perry, D. J., and P. Knowles. 1991. Spatial genetic structure within three sugar maple (Acer saccharum Marsh.) stands. Heredity 66:137–142.

Pither, R., J. S. Shore, and M. Kellman. 2003. Genetic diversity of the tropical tree Terminalia amazonia (Combretaceae) in naturally fragmented populations. Heredity 91:307–313.

Pometti, C., C. Bessega, A. Cialdella, M. Ewens, B. Saidman, and J. Vilardi. 2018. Spatial genetic structure within populations and management implications of the South American species Acacia aroma (Fabaceae). (G. G. Vendramin, ed.)PLOS ONE 13:e0192107.

Quevedo, A. A., M. Schleuning, I. Hensen, F. Saavedra, and W. Durka. 2013. Forest fragmentation and edge effects on the genetic structure of Clusia sphaerocarpa and C. lechleri (Clusiaceae) in tropical montane forests. Journal of Tropical Ecology 29:321–329.

Quipildor, V. B., P. Mathiasen, and A. C. Premoli. 2017. Population Genetic Structure of the Giant Cactus Echinopsis terscheckii in Northwestern Argentina Is Shaped by Patterns of Vegetation Cover. Journal of Heredity 108:469–478.

Ramos, S. L. F., G. Dequigiovanni, A. M. Sebbenn, M. T. G. Lopes, P. Y. Kageyama, J. L. V. de Macêdo, M. Kirst, et al. 2016. Spatial genetic structure, genetic diversity and pollen dispersal in a harvested population of Astrocaryum aculeatum in the Brazilian Amazon. BMC Genetics 17:63.

Rhodes, M. K., J. B. Fant, and K. A. Skogen. 2014. Local topography shapes fine-scale spatial genetic structure in the Arkansas valley Evening Primrose, Oenothera harringtonii (Onagraceae). Journal of Heredity 105:900–909.

Ritchie, A. L., and S. L. Krauss. 2012. A Genetic Assessment of Ecological Restoration Success in Banksia attenuata. Restoration Ecology 20:441–449.

Ritchie, A. L., P. G. Nevill, E. A. Sinclair, and S. L. Krauss. 2017. Does restored plant diversity play a role in the reproductive functionality of Banksia populations?: Reproductive functionality of restored keystone species. Restoration Ecology 25:414–423.

Schroeder, J. W., H. T. Tran, and C. W. Dick. 2014. Fine scale spatial genetic structure in Pouteria reticulata (Engl.) Eyma (Sapotaceae), a dioecious, vertebrate dispersed tropical rain forest tree species. Global Ecology and Conservation 1:43–49.

Sebbenn, A. M., A. C. M. Carvalho, M. L. M. Freitas, S. M. B. Moraes, A. P. S. C. Gaino, J. M. da Silva, C. Jolivet, et al. 2011. Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree Copaifera langsdorffii Desf. Heredity 106:134–145.

Silva, C. R. S., P. S. B. Albuquerque, F. R. Ervedosa, J. W. S. Mota, A. Figueira, and A. M. Sebbenn. 2011. Understanding the genetic diversity, spatial genetic structure and mating system at the hierarchical levels of fruits and individuals of a continuous Theobroma cacao population from the Brazilian Amazon. Heredity 106:973–985.

Silva, M. B., M. Kanashiro, A. Y. Ciampi, I. Thompson, and A. M. Sebbenn. 2008. Genetic effects of selective logging and pollen gene flow in a low-density

population of the dioecious tropical tree Bagassa guianensis in the Brazilian Amazon. Forest Ecology and Management 255:1548–1558.
Spoladore, J., V. F. Mansano, M. R. Lemes, L. C. D. de Freitas, and A. M. Sebbenn. 2017. Genetic conservation of small populations of the endemic tree Swartzia glazioviana (Taub.) Glaz. (Leguminosae) in the Atlantic Forest. Conservation Genetics 18:1105–1117.
Tambarussi, E. V., A. M. Sebbenn, A. Alves-Pereira, R. Vencovsky, J. Cambuim, A. Da Silva, M. Moraes, et al. 2017. Dipteryx alata Vogel (Fabaceae) a neotropical tree with high level of selfing: implication for conservation and breeding programs. Annals of Forest Research 0.
Tarazi, R., A. M. Sebbenn, P. Y. Kageyama, and R. Vencovsky. 2013. Long-distance dispersal in a fire- and livestock-protected savanna. Ecology and Evolution 3:1003–1015.
Theim, T. J., R. Y. Shirk, and T. J. Givnish. 2014. Spatial genetic structure in four understory Psychotria species (Rubiaceae) and implications for tropical forest diversity. American journal of botany 101:1189–1199.
Torroba-Balmori, P., K. B. Budde, K. Heer, S. C. González-Martínez, S. Olsson, C. Scotti-Saintagne, M. Casalis, et al. 2017. Altitudinal gradients, biogeographic history and microhabitat adaptation affect fine-scale spatial genetic structure in African and Neotropical populations of an ancient tropical tree species. (Z. Wang, ed.)PLOS ONE 12:e0182515.
Turchetto, C., J. S. Lima, D. M. Rodrigues, S. L. Bonatto, and L. B. Freitas. 2015. Pollen dispersal and breeding structure in a hawkmoth-pollinated Pampa grasslands species Petunia axillaris (Solanaceae). Annals of Botany 115:939–948.
Van Rossum, F., and L. Triest. 2003. Spatial genetic structure and reproductive success in fragmented and continuous populations of Primula vulgaris. Folia Geobotanica 38:239–254.
Van Rossum, F., and L. Triest. 2007. Fine-scale spatial genetic structure of the distylous Primula veris in fragmented habitats. Plant Biology 9:374–382.
Vaughan, S. P., J. E. Cottrell, D. J. Moodley, T. Connolly, and K. Russell. 2007. Distribution and fine-scale spatial-genetic structure in British wild cherry (Prunus avium L.). Heredity 98:274–283.
Vekemans, X., and O. J. Hardy. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Molecular Ecology 13:921–935.
Wang, R., B. Ai, B.-Q. Gao, S. Yu, Y.-Y. Li, and X.-Y. Chen. 2009. Spatial genetic structure and restricted gene flow in a functionally dioecious fig, Ficus pumila L. var. pumila (Moraceae). Population Ecology 51:307–315.
Williams, C. F. 1994. Genetic Consequences of Seed Dispersal in Three Sympatric Forest Herbs. II. Microspatial Genetic Structure within Populations. Evolution 48:1959.
Yamagishi, H., H. Tomimatsu, and M. Ohara. 2007. Fine-Scale Spatial Genetic Structure within Continuous and Fragmented Populations of Trillium camschatcense. Journal of Heredity 98:367–372.

Yao, X., J. Zhang, Q. Ye, and H. Huang. 2011. Fine-scale spatial genetic structure and gene flow in a small, fragmented population of Sinojackia rehderiana (Styracaceae), an endangered tree species endemic to China. Plant Biology 13:401–410.

Zeng, X., S. G. Michalski, M. Fischer, and W. Durka. 2012. Species diversity and population density affect genetic structure and gene dispersal in a subtropical understory shrub. Journal of Plant Ecology 5:270–278.

Zhou, H.-P., and J. Chen. 2010. Spatial genetic structure in an understory dioecious fig species: the roles of seed rain, seed and pollen-mediated gene flow, and local selection. Journal of Ecology 98:1168–1177.

Figure S1 Violin plots of S_P values as a function of (a) pollination mode, (b) latitudinal region, (c) growth form, (d) mating system, (e) seed dispersal mode, and (f) genetic marker. Central black dots indicate the mean S_P for each group, surrounding black dots are all observations. Thick horizontal grey lines are median values, boxes indicate 25% and 75% quartiles, and grey bars are minimum and maximum values. (Abbreviations: S-ins: small insects, L-ins: large insects, verts: vertebrates, mixed-m: mixed-mating, allo: allozymes, SSR: microsatellites) (next page).

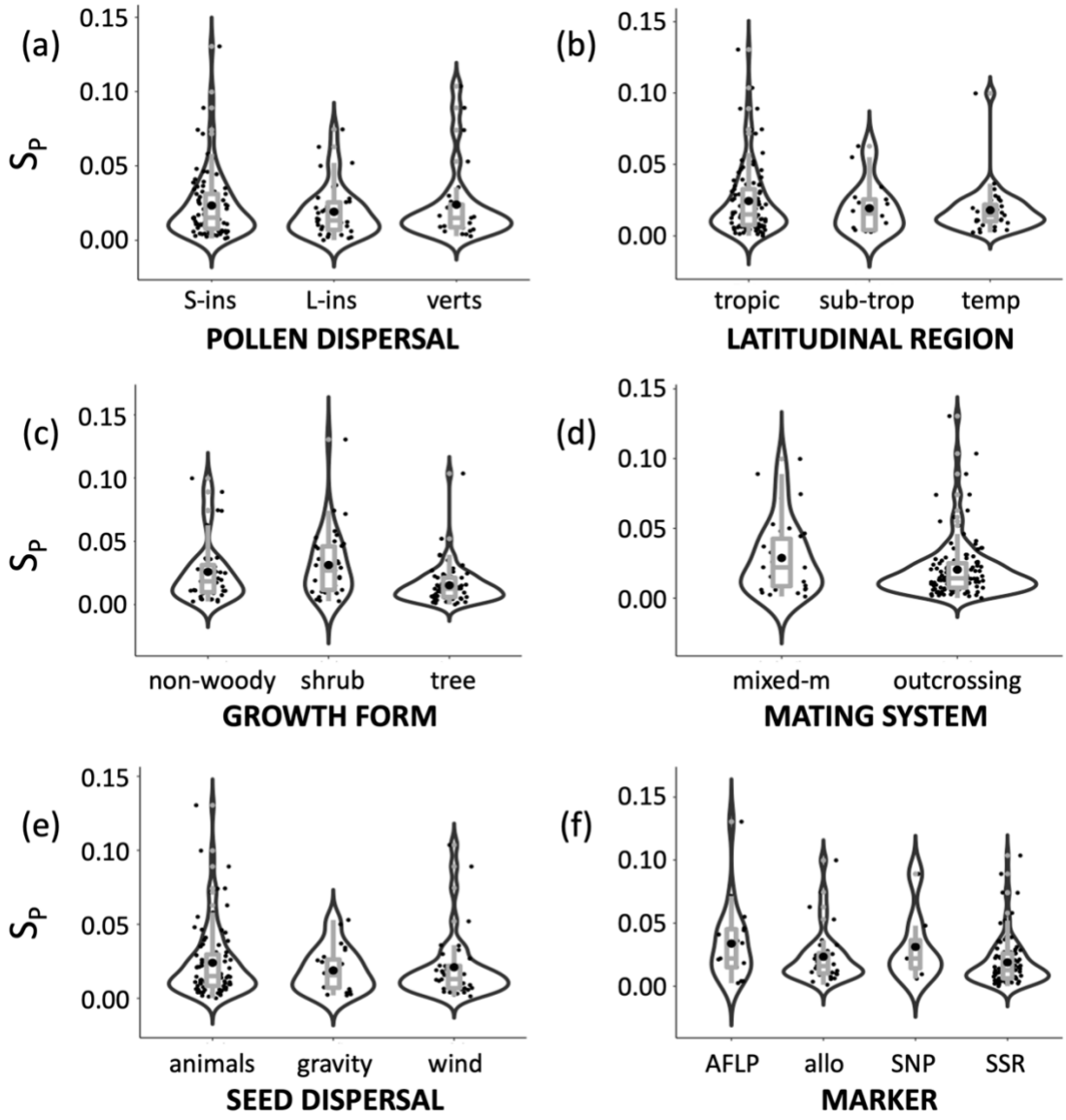

(a)
S_P
0.15
0.10
0.05
0.00
S-ins
L-ins
verts
POLLEN DISPERSAL
(b)
tropic
sub-trop
temp
LATITUDINAL REGION
(c)
non-woody
shrub
tree
GROWTH FORM
(d)
mixed-m
outcrossing
MATING SYSTEM
(e)
animals
gravity
wind
SEED DISPERSAL
(f)
AFLP
allo
SNP
SSR
MARKER

Table S1 Dataset used in this study (in file Table S1.xlsx).

Table S2 Estimates of the generalized variance inflation factor (GVIF), and its adjusted value accounting for the degrees of freedom (GVIF^(1/(2*Df))) for each factor in the most-inclusive model explaining variation in S_P values.

Variable	GVIF	Df	GVIF^(1/(2*Df))
Marker	3.05	3	1.20
Mating system	1.79	2	1.16
Growth form	1.27	1	1.13
Animal pollination mode	2.88	2	1.30
Seed dispersal mode	2.28	2	1.23
Latitudinal region	1.94	2	1.18

Table S3 Details of the most-inclusive model explaining variation in S_P values. Variables in bold indicate the reference level for each categorical factor. N indicates the sample size of each group. Significant *p*-values are in bold. Model R_2 = 0.26, Model AIC = −718.2.

Variable	N	Estimate	Std. Error	*t*-value	*p*-value
Intercept		−2.39	0.27	−8.99	<0.001
Genetic marker					
AFLP	19				
Allozymes	32	−0.02	0.23	−0.08	0.94
Microsatellites	89	−0.29	0.21	−1.37	0.17
SNPs	7	−0.33	0.28	−1.21	0.23
Seed dispersal mode					
Animals	84				
Gravity	24	−0.15	0.26	−0.57	0.57
Wind	39	−0.08	0.19	−0.41	0.68
Pollination mode					
Small insects	82				
Large insects	38	−0.34	0.21	−1.66	0.10
Vertebrates	27	−0.40	0.22	−1.82	0.07
Latitudinal region					
Tropics	97				
Subtropics	17	−0.72	0.28	−2.54	**0.01**
Temperate	33	−1.08	0.29	−3.76	**<0.001**
Growth form					
Non-woody	43				
Shrub	37	−0.54	0.22	−2.50	**0.01**
Tree	67	−1.25	0.25	−5.01	**<0.001**
Mating system					
Mixed-mating	34				
Outcrossing	113	−0.19	0.15	−1.27	0.21