Global patterns of population genetic differentiation in seed plants

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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 $\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$. 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.

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 $\mathrm{F_{ST}}$ (Wright,1951; see Holsinger & Weir, 2009; Meirmans & Hedrick, 2011 for a review of $\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$ 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-Perez, & 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, Gonzalez-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 ($G'_{ST}=G_{ST}\text{overall}/G_{ST}\text{max}$).

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 $\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$. 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). $\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$ 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.

$\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$ (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 $\mathrm{F_{ST}}$ 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 $\mathrm{F_{ST}}$ 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 $\mathbf{F_{ST}}$

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 (ΔAIC=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.

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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[?]0.005, thin underline: 0.005<P<0.05).

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.

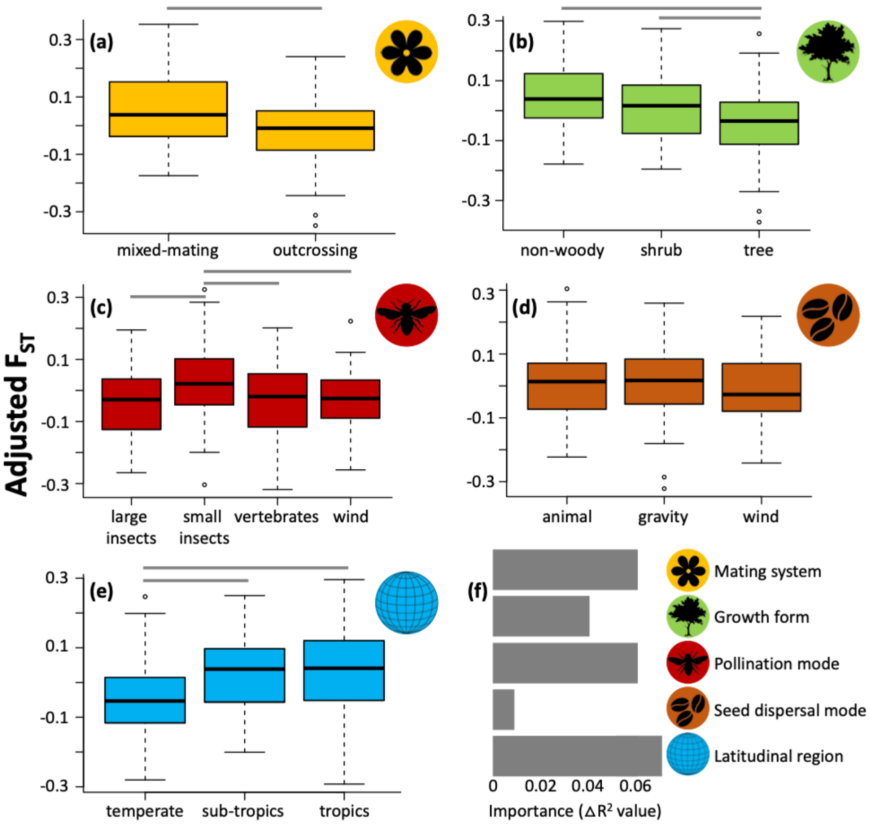
§ distance: natural logarithm of the maximum distance between populations.

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** Outcrossing	80 257	–0.07	0.01	–4.7	**<0.001**
Growth form **Tree** Non-woody Shrub	163 121 53	0.09 0.06	0.02 0.02	5.3 3	**<0.001 0.003**
Pollination mode **Small insects** Large insects Vertebrates Wind	176 48 44 69	–0.06 –0.05 –0.05	0.02 0.02 0.02	–3.4 –2.6 –3	**0.001 0.01 0.003**
Seed dispersal mode **Gravity** Animals Wind	82 147 108	–0.003 –0.02	0.02 0.02	–0.2 –1.4	0.8 0.1
Latitudinal region **Temperate** Sub-tropical Tropical	134 78 125	0.07 0.09	0.02 0.02	4.5 5.4	**<0.001 <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.



Supporting Information

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 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.