8-24-2023

Assessing Threats to Plant Populations: Linking Pollinator Differences to Patterns of Plant Fitness and Population Genetics

Rieka Yu
University of Missouri-St. Louis, rymcf@umsystem.edu

Recommended Citation

Yu, Rieka, "Assessing Threats to Plant Populations: Linking Pollinator Differences to Patterns of Plant Fitness and Population Genetics" (2023). *Dissertations*. 1392.
https://irl.umsl.edu/dissertation/1392

Assessing Threats to Plant Populations: Linking Pollinator Differences to Patterns of Plant Fitness and Population Genetics

by

Rieka Yu

M.S., Biology, University of Missouri – St. Louis, 2021
B.A., Biology, Boston University, 2018

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

December
2023

Advisory Committee

Nathan Muchhala, Ph.D., Chairperson

Aimee S. Dunlap, Ph.D.

Robert J. Marquis, Ph.D.

Matthew Betts, Ph.D.

Sara Miller, Ph.D.

Abstract

Land use change is a major driver of biodiversity loss and consequently has led to the loss of genetic diversity in many plant populations due to declines in population sizes and an increase in genetic isolation. However, not all plant populations respond similarly to land use change, suggesting additional mechanisms mediating plant population genetic patterns. Here, I examine the role of pollinators as a mediating factor, investigating how pollinator tolerance to habitat degradation and pollination services differ, and how these can influence plant fitness. In Chapter 1 I conducted a meta-analysis on population differentiation (F_{ST}), which measure historic gene flow using adult plants, and pollen pool differentiation (Φ_{FT}), which measure current gene flow from maternal plants and seedlings, to investigate how different types of pollinators drive changes in gene flow for plant populations in disturbed habitats. Using phylogenetic linear mixed-effects models, I found that different types of pollinators mediate different levels of gene flow in plant populations, with large bees specifically facilitating smaller values of Φ_{FT} than non-bee insects. I also found that large bees are seemingly tolerant to habitat disturbance, maintaining equal levels of genetic connectivity in disturbed and undisturbed habitats. This shows that the relationship between disturbance and pollinators is indeed an important component of plant population genetics. In analyzing both F_{ST} and Φ_{FT}, we found that Φ_{FT} captures differences in disturbed habitats that F_{ST} did not. The ability to detect differences in disturbed habitats with Φ_{FT} and not F_{ST} is likely due to Φ_{FT} measuring gene flow in the most recent generation, making Φ_{FT} an important statistic for identifying threats to plant populations. In Chapter 2 I studied the relationship between landscape and habitat characteristics, pollinator community, and plant mating quality

within a single pollination system. *Campanula americana* has a generalist pollination system and is pollinated by bumblebees, *Megachile campanulae*, and small bees. With structural equation modeling, I found that different types of pollinators respond to different aspects of the environment and differentially affect mating quality. Bumblebees responded positively to floral resource availability and habitats with nesting areas while *M. campanulae* and small bees were tolerant to habitat loss and different land use types. *M. campanulae* visits led to greater seed set and haplotype diversity in *C. americana* while small bee visits decreased haplotype diversity. These results show pollinator-based differences in responses to habitat and plant mating patterns, further establishing that pollinators are important determinants of plant population genetic change in the face of disturbance. In Chapter 3, I investigated the consequences of differences between pollinators in their pollen deposition patterns by focusing on fitness effects in offspring. I tested the pollen competition hypothesis which states that competition between pollen grains will select for more vigorous offspring. I specifically tested if pollination by multiple donors selects for offspring with higher fitness in *Allium stellatum*. I found that pollen loads with a greater number of donors increased female reproductive success at the expense of slower growing seedlings. However, this trade-off appears to be due to a trade-off in seed number and size, with seeds originating from larger seed sets growing more slowly. This can be attributed to the perennial life history of *A. stellatum* and the need to conserve resources for future reproduction. Because plants are resource limited, they cannot allocate resources for both large seeds and large seed sets. Ultimately, I found that the diversity of deposited pollen loads has important effects on population dynamics as seen by the effects on female reproductive success. As habitats change and plant populations become smaller and more isolated due to anthropogenic disturbance, assessing the mechanisms behind plant population genetic patterns is an important contribution for conservation. With this dissertation, I have contributed to this assessment, illuminating the intricate role that pollinators play in influencing fitness and mating dynamics in plant populations.

Table of contents

Chapter 1: A meta-analysis shows pollinators differentially mediate changes in gene flow in disturbed habitats

Rieka Yu[1] and Nathan Muchhala[1]

[1]University of Missouri – St. Louis

One University Blvd. 223R Research Hall, Biology Department, St. Louis, MO 63121

Abstract

Land use change is a major threat to biodiversity, decreasing plant population genetic diversity through habitat loss and disturbance. However, emerging evidence suggests not all plant populations suffer the expected losses of genetic diversity, due to differences in how pollinators facilitate gene flow across disturbed landscapes. We performed phylogenetically independent meta-analyses to determine if and how pollen vectors, including bats, passerine birds, hummingbirds, insects, large bees, small bees, and wind, mediate changes in pollen pool differentiation (Φ_{FT}) and population differentiation (F_{ST}) in the face of habitat disturbance. When only considering pollen vector and not disturbance, we found that wind led to the lowest Φ_{FT} and F_{ST} values, with large bees, birds, and bats having intermediate values, and small bees, insects, and hummingbirds having the highest values. Habitat disturbance had no effect on F_{ST}, which reflects historical patterns in adult plants, but led to higher Φ_{FT}, which reflects current patterns of pollen movement. These effects of disturbance on Φ_{FT} depended on pollinator type, with hummingbird- and large bee- pollinated plants showing no difference. Overall, our results

provide evidence that pollinators drive plant population genetic patterns, as well as a clearer understanding of how particular pollinators differ in mediating gene flow.

Introduction

One of the greatest threats to biodiversity this century is land use change (Sala *et al.* 2000). Land use change impacts plant populations through modification of landscape structure in the form of habitat loss and disturbance (Fahrig 2003). These physical changes to populations are expected to cause decreased genetic diversity, which has implications for population persistence as it can determine population viability and response to selection pressures (Young *et al.* 1996). Habitat loss leads to small, fragmented populations that are predicted to suffer from genetic bottlenecks as well as genetic drift and inbreeding in subsequent generations due to limited gene flow (Templeton *et al.* 1990; Young *et al.* 1996; Hadley & Betts 2012).

The increased focus on effects of disturbance on plant population genetics in the past decade has produced mixed results, with many but not all showing the predicted negative impact on genetic diversity (Young *et al.* 1996; Breed *et al.* 2015). These different results could be explained by differences in pollinator behavior, as pollinators can mediate the degree of plant genetic change in disturbed habitats (Hadley & Betts 2012). One empirical study supports this idea, finding that habitat loss leads to decreased outcrossing rates in insect-pollinated *Eucalyptus* but not bird-pollinated *Eucalyptus* (Breed *et al.* 2015). Such a pattern is likely due to vertebrate pollinators being better able to move between isolated habitat patches (Aizen & Feinsinger 1994; Quesada *et al.* 2001, 2004; Byrne *et al.* 2007).

Although few studies directly compared how pollinators influence plant population genetics, meta-analyses of genetic variables also support the idea that there are large differences among pollinators (Aguilar *et al.* 2019; Gamba & Muchhala 2020).

A global analysis of F_{ST} values, a measure of population genetic differentiation, found differences in population genetics for plants pollinated by small insects, large insects, vertebrates, and wind (Gamba & Muchhala 2020). Similarly, in a meta-analysis of genetic diversity in continuous and disturbed habitats, Aguilar *et al.* (2019) found that habitat disturbance decreases genetic diversity and outcrossing rates and increases inbreeding for invertebrate-pollinated but not for vertebrate-pollinated species.

Despite compelling evidence that pollinators mediate plant population genetic patterns, there is little empirical evidence for the expected underlying differences in actual pollen movement patterns between pollinator types. Oftentimes, vertebrates are considered highly mobile and are expected to maintain genetic connectivity in disturbed habitats (Aguilar *et al.* 2008, 2019; Breed *et al.* 2015; Krauss *et al.* 2017), however, there is evidence that this is not always the case. For example, nectarivorous bats have mixed responses to habitat disturbance depending on the species; while some are not found in forest fragments, others are able to travel between fragments and continuous forest, and even visit flowers in fragments more frequently (Quesada *et al.* 2004). For hummingbirds, forest specialists are unable to reach forest fragments and will avoid flying through open canopy (Hadley & Betts 2009; Kormann *et al.* 2016; Volpe *et al.* 2016). On the other hand, although invertebrates are not expected to maintain genetic connectivity in disturbed habitats (Aguilar *et al.* 2008, 2019; Breed *et al.* 2015), studies show a wide range in their mobility and tolerance of anthropogenic disturbance. For instance, large bees such as bumblebees can travel distances up to 3-km, while some small solitary bees forage less than 1-km from their nests, and euglossine bees have been found to fly more than 40-km (Janzen 1971; Dressler 1982; Gathmann & Tscharntke

2002; Chapman *et al.* 2003; Zurbuchen *et al.* 2010). Along with being able to fly long distances, bees are apparently tolerant of disturbance, as they are common in anthropogenically altered habitats (Tonhasca *et al.* 2002; Kells & Goulson 2003; Winfree *et al.* 2009).

The F_{ST} statistic provides a useful general approach to quantifying genetic structure across populations, however, new methods allow researchers to more accurately compare genetic structure of pollen carried by pollinators. The intraclass correlation coefficient, Φ_{FT}, developed by (Smouse *et al.* 2001), measures the structure of the male gametes that contributed to offspring from a single bout of pollination. Φ_{FT} is analogous to the F_{ST}-statistic; while F_{ST} measures population differentiation as the amount of genetic variation among individuals within a population relative to between populations, Φ_{FT} measures pollen pool differentiation, or the genetic variation of male gametes among seeds within maternal plants relative to among maternal plants (Smouse *et al.* 2001). Higher Φ_{FT} values show divergence among male gametes and indicate different females are sampling from different pollen pools. Lower Φ_{FT} values show less divergence among male gametes and indicate different females are sampling from the same pollen pool due to high pollen movement across the population.

Because Φ_{FT} is calculated from the genetic material of seeds, it is highly representative of current mating patterns. This timescale is helpful for observing recent pollinator responses to habitat disturbance. By contrast, F_{ST} is typically calculated from the genetic material of adults, and thus represents historical patterns of gene flow. While genetic patterns in adults do show differences based on pollination mode, they will not show recent changes in mating patterns, e.g. if the habitat disturbance occurred after adult establishment (Gamba & Muchhala 2020).

In the present study, we perform a meta-analysis on data collected from published literature to assess how disturbance differentially affects Φ_{FT} and F_{ST} values for species with different pollen vectors. We review both Φ_{FT} and F_{ST} values because they potentially provide information about gene flow at different spatial and time scales. Our goal is to provide a comprehensive assessment on how differences between pollinators drive changes in gene flow among plant populations in disturbed landscapes.

Methods

Data collection

We compiled a dataset of published Φ_{FT} values by conducting a systematic literature search in Google Scholar (key words: "Φ_{FT}" AND "pollen"). This produced 133 results, which we reduced to 70 peer-reviewed publications that recorded Φ_{FT} values in disturbed and/or continuous populations for 70 different species. We additionally compiled a dataset of F_{ST} values using the dataset originally collected by (Gamba & Muchhala 2020) supplemented by a Google Scholar search (key words: "F_{ST}" AND "pollen). This resulted in 257 peer-reviewed publications that recorded F_{ST} values in disturbed and/or continuous populations for 346 species. See Appendix S1 and S2 for the list of publications.

For each recorded Φ_{FT} and F_{ST} value, we coded habitat disturbance as a binary variable (continuous or disturbed). Values were categorized as disturbed when they were calculated from populations that suffered any type of habitat degradation. This included degradation of focal populations from logging resulting in lower plant densities as well as agricultural or urban disturbances that led to fragmentation of populations into smaller and isolated patches.

For each recorded Φ_{FT} and F_{ST} value we additionally recorded the primary pollen dispersal vector as one of seven types: bat, passerine bird, hummingbird, large bee, small bee, insect, and wind. Pollen dispersal vectors were recorded as large bee if they were the size of honeybees or larger (e.g. *Bombus*, Euglossini); bees smaller than honeybees (e.g. Meliponini) were recorded as small bees. Pollen dispersal vectors were recorded as insects if they were non-bee insects or if the study only specified pollinators as 'insects.'

Because life history traits as well as sampling methods have been shown to influence F_{ST}, we additionally recorded seed dispersal mode (bat, bird, non-volant mammal, gravity, wind, and water), mating system (outcrossing, mixed-mating, selfing), growth form (tree, shrub, non-woody), and latitudinal region (temperate, subtropical, tropical) as defined by Gamba & Muchhala (2020). We also collected variables related to sampling methods; for Φ_{FT} this was mean offspring per maternal plant, maximum distance between maternal plants, and genetic marker, while for F_{ST} this was mean sample size per population, maximum distance between populations, and genetic marker. The collected data and list of publications can be found in the supporting information.

Phylogenetic meta-analysis

Before analyses, we normalized Φ_{FT} and F_{ST} with Tukey's ladder of powers transformation for the full dataset and subsets of data. Transformed values from the full data sets resulted in $\Phi_{FT}^{0.350}$ and $F_{ST}^{0.300}$. Transformed values from the subsets of data are reported with the results. We additionally created a species-level phylogeny with the R package *V.PhyloMaker* (Jin & Qian 2019) in order to run models corrected for phylogenetic signal. The package starts with the mega-tree of vascular trees (Smith & Brown 2018) and prunes it to include only those species in our analyses.

Prior to testing how pollinator type and habitat disturbance affect pollen and population differentiation, we ran two phylogenetic multiple linear regressions with all variables to separately determine significant predictors of Φ_{FT} and F_{ST}. For Φ_{FT}, significant predictor variables included pollination mode, disturbance, maximum distance between maternal plants, and genetic marker. For F_{ST}, significant predictor variables included pollination mode, mating system, growth form, latitudinal region, maximum distance between populations, genetic marker, and mean sample size per population. These variables were then included as random effects in the final phylogenetic models with pollination mode included as a random effect when not included as a fixed-effect.

In our final phylogenetic linear regressions, we tested whether Φ_{FT} and F_{ST} differed for plant species with different pollinators, as well as whether they differed between populations that occurred in disturbed and undisturbed habitats. We included the previously determined significant variables and each published study as random variables, with a phylogenetic correction. Statistical differences between pollinator type were determined by sequentially changing the reference category level and using z-values and their given p-values that are provided with model coefficients. Due to an increase in the risk of type I error from multiple comparisons, we used the graphically sharpened method of a false discovery rate (FDR) procedure to calculate adjusted p-values (Pike 2011).

We additionally tested whether Φ_{FT} and F_{ST} differed between disturbed and undisturbed habitats for each subgroup of pollination modes. To do this we performed phylogenetic linear regressions to test the effect of habitat disturbance on subgroups of data, each with plant species with different pollination modes. As with the full datasets we included the significant predictor variables and study as random factors with a phylogenetic correction.

Finally, we tested whether Φ_{FT} and F_{ST} differed between disturbed and undisturbed habitats for different subgroups of growth forms. We tested this relationship because growth form is a significant predictor of F_{ST} and not Φ_{FT}, and these two values measure gene flow at different time scales which may show up as differences between different growth forms.

Results

For both Φ_{FT} and $F_{ST,}$ pollination mode and disturbance were significant predictors of genetic variation. Although Φ_{FT} and F_{ST} values do not differ between plant species with different vertebrate pollinators, there are significant differences in Φ_{FT} between different invertebrate pollinators (Figure 1; Tables S1 and S2). For Φ_{FT}, wind-pollinated species have lower pollen pool differentiation compared to small bee-, insect-, and hummingbird- pollinated species, while large bee-pollinated species have less pollen pool differentiation than insect-pollinated species do (Table S1). For F_{ST}, insect-pollinated species have greater population differentiation than wind pollinated species (Table S2). Φ_{FT} and F_{ST} did not otherwise differ between species with other pollen vectors.

For disturbance, we found that disturbed habitats are associated overall with significantly higher pollen pool differentiation ($z = 3.065$, $p = 0.002$) but no difference in population differentiation ($z = 1.848$, $p = 0.065$; Figure 2) compared to continuous habitats. When analyzing effects of disturbance separately for plant species with different pollinators, disturbance was found to significantly increase pollen pool differentiation (Φ_{FT}) for all plants except those pollinated by hummingbirds and large bees (Figure 2a; Table 1). On the other hand, disturbance was only found to increases population differentiation (F_{ST}) for bat-pollinated species, with no difference for plants with other pollinators (Figure 2b; Table 1).

Regarding growth form, Φ_{FT} was higher in disturbed populations for trees and shrubs, but no difference was found for non-woody species (Figure S1, Table S3). For F_{ST} there was no effect of disturbance for any of the growth forms.

Discussion

Here, we provide the first analysis we are aware of to date that generalizes plant mating patterns based on a fine-scale partitioning of pollinators. The results of our meta-analysis support the hypothesis that pollinators mediate effects of habitat disturbance on plant population genetics. We show differences in Φ_{FT} but not F_{ST} between continuous and disturbed plant populations, indicating habitat disturbance has an effect on pollen pool differentiation and not on population differentiation. Additionally, we found that not only do patterns of gene flow differ for species with different pollinators overall, but that habitat disturbance differentially affects species with different pollinators.

As expected, disturbance is associated with increased pollen pool differentiation (Φ_{FT} values; Figure 1a), in support of the idea that habitat loss and fragmentation limits plant mating (Young *et al.* 1996; Fahrig 2003; Ghazoul 2005; Hadley & Betts 2012). These results agree with findings from previous meta-analyses that show habitat fragmentation leads to decreased genetic diversity and outcrossing rates as well as increased correlated paternity and inbreeding (Aguilar *et al.* 2008, 2019). As plant populations become more isolated after disturbance, the surrounding matrix serves as a barrier to pollinators (Fahrig 2003). With limited pollinator movement and fewer pollinators visiting isolated populations, pollen may be moving shorter distances, leading to subsampling by females and high pollen pool differentiation within a population. This pattern of pollen pool differentiation is also expected from increased inbreeding in isolated plant populations (Young *et al.* 1996).

Interestingly, we did not find a similar pattern of greater population differentiation (Fst values) in disturbed habitats (Figure 1b). This could be due to a time lag, given that population differentiation is calculated for adult individuals, and thus may represent genetic patterns pre-disturbance. This idea is further supported by the fact that there was no effect of disturbance on F_{ST} for different growth forms, but there was a positive effect on Φ_{FT} for trees and shrubs (Figure S1 and Table S3). For example, trees may have been present before disturbance occurred, leading to similar F_{ST} values in disturbed and undisturbed populations. However, after disturbance there would be changes in mating patterns, resulting in higher Φ_{FT} values in disturbed populations.

In our assessment of pollinator differences, we found that body size appears to mediate the degree of gene flow by invertebrate pollinators (Figure 1), in that large bees facilitate lower population and pollen pool differentiation than small bees or other insects. This concurs with a previous meta-analysis of F_{ST} values, which found greater gene flow by honey-bee-sized or larger invertebrates relative to smaller ones (Gamba & Muchhala 2020). This pattern may simply be due to larger bees traveling farther in foraging bouts. However, interestingly, (Castilla *et al.* 2017) actually found that large bees do not in fact move pollen longer distance but do facilitate greater seed set per flower. The ability to deposit larger pollen loads (also see Földesi et al. (2020)) can lead to greater gene flow between populations and among maternal plants, and thus may be the primary driver for the observed decrease in genetic differentiation among plants pollinated by large bees.

We found that all vertebrate pollinators facilitate similar degrees of gene flow within and between populations (Figure 1). However, we note that for Φ_{FT} the lack of differences among vertebrates may be due to low sample sizes, with only five species each of hummingbird- and bat-pollinated species. Nevertheless, no study we are aware of has compared differences between vertebrate pollinators, and our results lends credence to other studies that have treated vertebrate pollinators as a single group.

Along with differences between the pollinator types, and the differences in disturbed versus continuous habitats, we also found an interaction between these factors in that species with different pollinators have different responses to disturbance (Table 1). While most pollination modes show increased pollen pool differentiation in disturbed sites, hummingbirds and large bees may buffer plant populations, as species pollinated by those vectors did not suffer negative genetic effects from habitat degradation.

One limitation of this study is the lack of detailed pollinator identification for some of the plant species in our dataset. For example, certain species were coded as insect-pollinated because studies failed to specify the type of insect pollinator. There were also limitations in identifying pollinators to species level, which would have given information on whether pollinators were habitat generalists or specialists. Such information is necessary to more accurately determine differences in how pollinators facilitate pollen movement, and we encourage future plant population genetic studies to include specific details on their pollinators.

Although our study focused on how disturbance affects the movement of pollinators between plant individuals, it is also important to consider more direct impacts of disturbance on pollinator populations. For instance, the type of habitat and habitat quality of plant populations and their surrounding matrices can influence nesting and roosting sites of pollinators (Cosson *et al.* 1999; Mola *et al.* 2021). A landscape may have sufficient foraging habitat with little disturbance, but have few nesting and roosting habitats, leading to low attraction of pollinators and lack of pollination services. It is therefore important for more studies to examine how different aspects of land use change affect pollinators and their services.

Overall, this meta-analysis highlights the need to consider pollinators as major contributors to plant population dynamics in degraded habitats. Results demonstrate that different pollinators mediate the degree of gene flow within and between plant populations and the degree to which plant populations suffer from habitat disturbance. We also find that pollen pool differentiation (Φ_{FT}) detects an effect of disturbance while population differentiation does not (F_{ST}), emphasizing the need for the careful selection of measures of gene flow to effectively assess changes in plant population genetics. With the looming threat of continued habitat loss and fragmentation due to human activities, accurate assessments of environmental change are necessary to combat habitat degradation. These assessments should include detailed pollinator identification, ideally to species level so that analyses can include information on pollination performance, foraging ranges, and nesting habitats if available. Including these details could uncover fine-grain differences and lead to better generalizations that are better attributed to behavior, rather than just the relative size or type of pollinator. Understanding how pollinators differentially influence plant mating dynamics can help identify threats to both pollinators and plant populations and can inform restoration ecologists of the best practices to improve and conserve pollinator communities and plant population genetic diversity.

Author contributions

All authors contributed to study design. R.Y. collected data, performed analyses, and wrote the manuscript. All authors contributed substantially to revisions.

Literature cited

Aguilar, R., Cristóbal-Pérez, E.J., Balvino-Olvera, F.J., de Jesús Aguilar-Aguilar, M., Aguirre-Acosta, N., Ashworth, L., *et al.* (2019). Habitat fragmentation reduces plant progeny quality: a global synthesis. *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.

Aizen, M.A. & Feinsinger, P. (1994). Habitat Fragmentation , Native Insect Pollinators , and Feral Honey Bees in Argentine “Chaco Serrano.” *Ecological Applications*, 4, 378–392.

Bezemer, N., Krauss, S.L., Phillips, R.D., Roberts, D.G. & Hopper, S.D. (2016). Paternity analysis reveals wide pollen dispersal and high multiple paternity in a small isolated population of the bird-pollinated Eucalyptus caesia (Myrtaceae), 117, 460–471.

Breed, M.F., Ottewell, K.M., Gardner, M.G., Marklund, M.H.K., Dormontt, E.E. & Lowe, A.J. (2015). Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. *Heredity*, 115, 108–114.

Byrne, M., Elliott, C.P., Yates, C. & Coates, D.J. (2007). Extensive pollen dispersal in a bird-pollinated shrub, Calothamnus quadrifidus, in a fragmented landscape. *Molecular Ecology,* 16, 1303–1314.

Castilla, A.R., Pope, N.S., O’Connell, M., Rodriguez, M.F., Treviño, L., Santos, A., *et al.* (2017). Adding landscape genetics and individual traits to the ecosystem function paradigm reveals the importance of species functional breadth. *Proceedings of the National Academy of Sciences of the United States of America*, 114, 12761–12766.

Chapman, R.E., Wang, J. & Bourke, A.F.G. (2003). Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators. *Molecular Ecology*, 12, 2801–2808.

Cosson, J.F., Pons, J.M. & Masson, D. (1999). Effects of forest fragmentation on frugivorous and nectarivorous bats in French Guiana. *Journal of Tropical Ecology*, 15, 515–534.

Dressler, R.L. (1982). Biology of the Orchid Bees (Euglossini), 13, 373–394.

Fahrig, L. (2003). Effects of Habitat Fragmentation on Biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, 34, 487–515.

Gamba, D. & Muchhala, N. (2020). Global patterns of population genetic differentiation in seed plants. *Molecular Ecology*, 29, 3413–3428.

Gathmann, A. & Tscharntke, T. (2002). Foraging ranges of solitary bees. *Journal of Animal Ecology*, 71, 757–764.

Ghazoul, J. (2005). Pollen and seed dispersal among dispersed plants. *Biological Reviews of the Cambridge Philosophical Society*, 80, 413–443.

Hadley, A.S. & Betts, M.G. (2009). Tropical deforestation alters hummingbird movement patterns. *Biology Letters*, 5, 207–210.

Hadley, A.S. & Betts, M.G. (2012). The effects of landscape fragmentation on pollination dynamics: Absence of evidence not evidence of absence. *Biological Reviews*, 87, 526–544.

Janzen, D.H. (1971). Euglossine Bees as Long-Distance Pollinators of Tropical Plants. *Science*, 171, 203–205.

Jin, Y. & Qian, H. (2019). V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography*, 42, 1353–1359.

Kells, A.R. & Goulson, D. (2003). Preferred nesting sites of bumblebee queens (Hymenoptera: Apidae) in agroecosystems in the UK. *Biological Conservation*, 109, 165–174.

Kormann, U., Scherber, C., Tscharntke, T., Klein, N., Larbig, M., Valente, J.J., *et al.* (2016). Corridors restore animal-mediated pollination in fragmented tropical forest landscapes. *Proceedings of the Royal Society B: Biological Sciences*, 283.

Krauss, S.L., Phillips, R.D., Karron, J.D., Johnson, S.D., Roberts, D.G. & Hopper, S.D. (2017). Novel Consequences of Bird Pollination for Plant Mating. *Trends in Plant Science*, 22, 395–410.

Mitchell, R.J., Wilson, W.G., Holmquist, K.G. & Karron, J.D. (2013). Influence of Pollen Transport Dynamics on Sire Profiles and Multiple Paternity in Flowering Plants. *PLoS ONE*, 8.

Mola, J.M., Hemberger, J., Kochanski, J., Richardson, L.L. & Pearse, I.S. (2021). The importance of forests in bumble bee biology and conservation. *BioScience*, 71, 1234–1248.

Quesada, M., Fuchs, E.J. & Lobo, J.A. (2001). Pollen load size, reproductive success, and progeny kinship of naturally pollinated flowers of the tropical dry forest tree Pachira quinata (Bombacaceae). *American Journal of Botany*, 88, 2113–2118.

Quesada, M., Stoner, K.E., Lobo, J.A., Herrerías-Diego, Y., Palacios- Guevara, C., Munguía-Rosas, M.A., *et al.* (2004). Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated Bombacaceous trees. *Biotropica*, 36, 131–138.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., *et al.* (2000). Global biodiversity scenarios for the year 2100. *Science*, 287, 1770–1774.

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

Smouse, P.E., Dyer, R.J., Westfall, R.D. & Sork, V.L. (2001). Two-generation analysis of pollen flow across a landscape. I. male gamete heterogeneity among females. *Evolution*, 55, 260–271.

Templeton, A., Shaw, K., Routman, E. & Davis, S. (1990). The Genetic Consequences of Habitat Fragmentation Author (s): Alan R . Source : Annals of the Missouri Botanical Garden , Vol . 77 , No . 1 , (1990), pp . 13-27 Published by : Missouri Botanical Garden Press Stable URL : http://www.jstor.org/stable/23. *Annals of the Missouri Botanical Garden*, 77, 13–27.

Tonhasca, A., Blackmer, J.L. & Albuquerque, G.S. (2002). Abundance and diversity of euglossine bees in the fragmented landscape of the Brazilian Atlantic Forest. *Biotropica*, 34, 416–422.

Volpe, N.L., Robinson, W.D., Frey, S.J.K., Hadley, A.S. & Betts, M.G. (2016). Tropical forest fragmentation limits movements, but not occurrence of a generalist pollinator species. *PLoS ONE*, 11, 1–13.

Winfree, R., Aguilar, R., Vásquez, D.P., LeBuhn, G. & Aizen, M.A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, 90, 2068–2076.

Young, A., Boyle, T. & Brown, T. (1996). The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, 11, 413–418.

Zurbuchen, A., Cheesman, S., Klaiber, J., Müller, A., Hein, S. & Dorn, S. (2010). Long foraging distances impose high costs on offspring production in solitary bees. *Journal of Animal Ecology*, 79, 674–681.

Figures

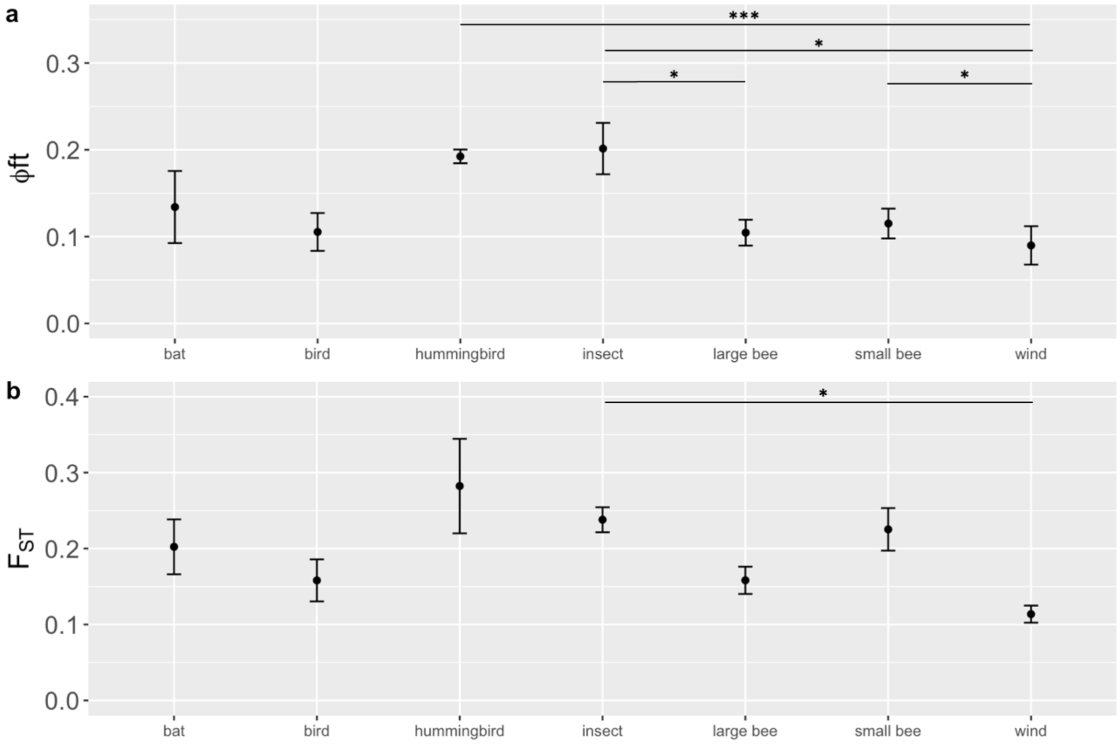

Figure 1. Mean values and standard errors of **a** Φ_{FT} and **b** F_{ST} for species with different pollen vectors. Mean values and standard errors were calculated from raw Φ_{FT} and F_{ST} values. Horizontal lines (*FDR $p < 0.05$, ***FDR $p < 0.001$) indicate significant differences between groups.

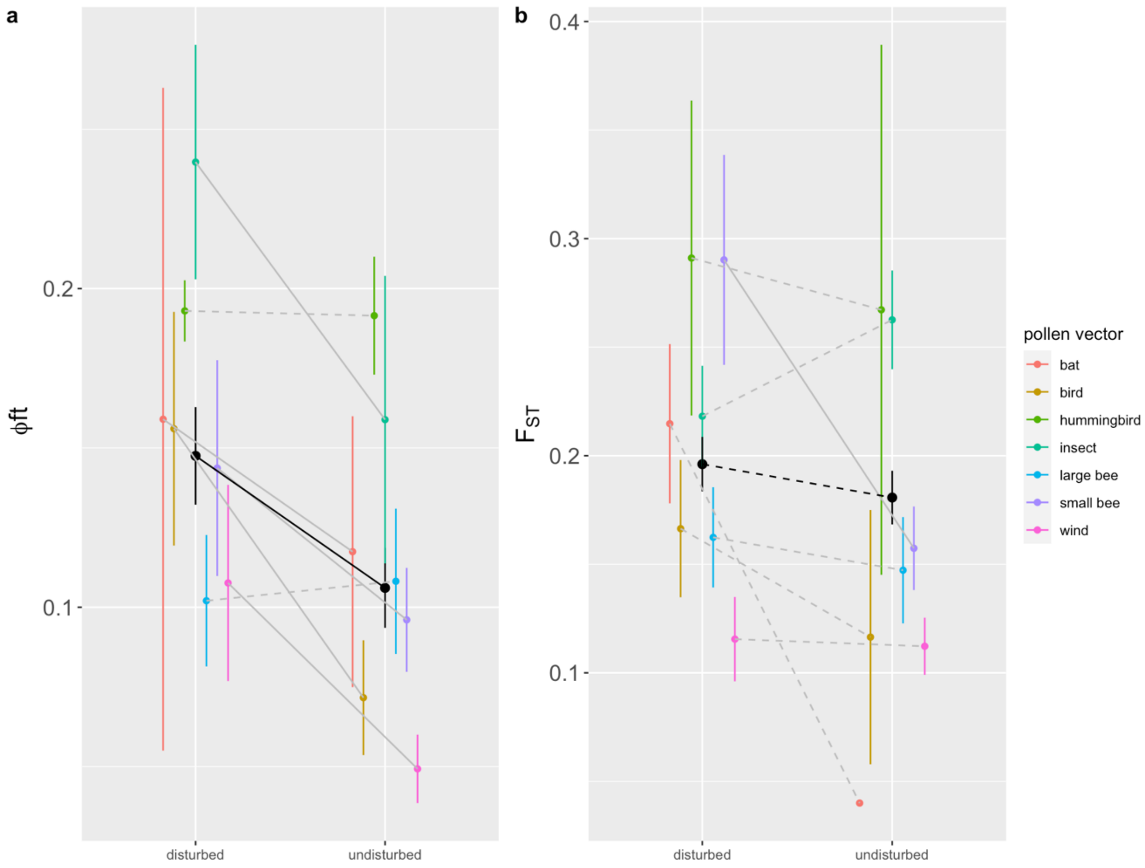

Figure 2. Mean values and standard errors of **a** Φ_{FT} and **b** F_{ST} for disturbed and continuous populations. Mean values and standard errors were calculated from raw Φ_{FT} and F_{ST} values. Overall mean value for disturbed and continuous populations are shown in black. Mean values for species with different pollen vectors are shown in different colors. Significant differences between continuous and disturbed values are connected with solid lines, non-significant differences are connected with dashed lines.

Tables

Table 1. Results of phylogenetic mixed-effect regressions testing the effect of habitat disturbance on Φ_{FT} and F_{ST} for plant species with different pollen vectors. Values reported below are based on Φ_{FT} and F_{ST} transformed by Tukey's ladder of powers, with transformed values indicated for each group. Significant p-values are in bold.

	Φ_{FT}			
	Estimate	SE	Z-score	P-value
(a) Bat $\Phi_{FT}^{0.725}$				
Intercept	0.418	0.081	5.182	**<0.001**
Disturbed	0.089	0.001	96.683	**<0.001**
(b) Bird $\Phi_{FT}^{0.500}$				
Intercept	0.380	0.050	7.621	**<0.001**
Disturbed	0.146	0.050	2.948	**0.003**
(c) Hummingbird $\Phi_{FT}^{9.975}$				
Intercept	0.5633	0.0112	50.4191	**<0.001**
Disturbed	-0.0084	0.0062	-1.3645	0.172
(d) Insect $\Phi_{FT}^{0.500}$				
Intercept	0.4989	0.037	13.478	**<0.001**
Disturbed	0.0612	0.0022	27.426	**<0.001**
(e) Large bee $\Phi_{FT}^{0.750}$				
Intercept	0.4271	0.0442	9.665	**<0.001**
Disturbed	-0.0029	0.0421	-0.0686	0.945
(f) Small bee $\Phi_{FT}^{0.275}$				
Intercept	0.4203	0.4557	9.2206	**<0.001**
Disturbed	0.0611	0.0196	3.1183	**0.002**
(g) Wind $\Phi_{FT}^{0.200}$				
Intercept	0.3313	0.1022	4.3157	**<0.001**
Disturbed	0.0132	0.0007	18.5103	**<0.001**

Table 1 continued

	F_{ST}			
	Estimate	SE	Z-score	P-value
(a) Bat $F_{ST}^{0.125}$				
Intercept	0.433	0.115	3.766	<**0.001**
Disturbed	0.183	0.106	1.722	0.085
(b) Bird $F_{ST}^{-0.225}$				
Intercept	0.498	0.093	5.348	<**0.001**
Disturbed	0.096	0.079	1.225	0.221
(c) Hummingbird $F_{ST}^{0.250}$				
Intercept	0.593	0.100	5.972	<**0.001**
Disturbed	0.056	0.097	0.574	0.566
(d) Insect $F_{ST}^{0.450}$				
Intercept	0.603	0.043	14.049	<**0.001**
Disturbed	-0.009	0.036	-0.245	0.810
(e) Large bee $F_{ST}^{0.250}$				
Intercept	0.502	0.045	11.107	<**0.001**
Disturbed	0.015	0.050	0.304	0.761
(f) Small bee $F_{ST}^{0.200}$				
Intercept	0.553	0.046	11.958	<**0.001**
Disturbed	0.097	0.040	2.418	**0.016**
(g) Wind $F_{ST}^{0.225}$				
Intercept	0.549	0.094	5.823	<**0.001**
Disturbed	0.016	0.028	0.557	0.578

Appendix

Appendix S1. List of publications used to collect Φ_{FT} data

Araújo, M.R.G., Melo Júnior, A.F.D., Menezes, E.V., Brandão, M.M., Cota, L.G., Oliveira, D.A.D., *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.

Austerlitz, F., Dutech, C., Smouse, P.E., Davis, F. & Sork, V.L. (2007). Estimating anisotropic pollen dispersal: a case study in Quercus lobata. *Heredity*, 99, 193–204.

Azevedo, V.C.R., Kanashiro, M., Ciampi, A.Y. & Grattapaglia, D. (2007). Genetic structure and mating system of Manilkara huberi (Ducke) A. Chev., a heavily logged Amazonian timber species. *Journal of Heredity*, 98, 646–654.

Barriball, K., Goodell, K. & Rocha, O.J. (2014). Mating patterns and pollinator communities of the invasive shrub Lonicera maackii: A comparison between interior plants and edge plants. *International Journal of Plant Sciences*, 175, 946–954.

Bessega, C., Pometti, C., Campos, C., Saidman, B.O. & Vilardi, J.C. (2017). Implications of mating system and pollen dispersal indices for management and conservation of the semi-arid species Prosopis flexuosa (Leguminosae). *Forest Ecology and Management*, 400, 218–227.

Bessega, C., Pometti, C.L., Ewens, M., Saidman, B.O. & Vilardi, J.C. (2012). Strategies for conservation for disturbed Prosopis alba (Leguminosae, Mimosoidae) forests based on mating system and pollen dispersal parameters. *Tree Genetics & Genomes*, 8, 277–288.

Braga, A.C. & Collevatti, R.G. (2011). Temporal variation in pollen dispersal and breeding structure in a bee-pollinated Neotropical tree. *Heredity*, 106, 911–919.

Breed, M.F., Ottewell, K.M., Gardner, M.G., Marklund, M.H.K., Stead, M.G., Harris, J.B.C., *et al.* (2015). Mating system and early viability resistance to habitat fragmentation in a bird-pollinated eucalypt. *Heredity*, 115, 100–107.

Byrne, M., Elliott, C.P., Yates, C. & Coates, D.J. (2007). Extensive pollen dispersal in a bird-pollinated shrub, Calothamnus quadrifidus, in a fragmented landscape. *Molecular Ecology*, 16, 1303–1314.

Cloutier, D., Hardy, O.J., Caron, H., Ciampi, A.Y., Degen, B., Kanashiro, M., *et al.* (2007a). Low inbreeding and high pollen dispersal distances in populations of two Amazonian forest tree species. *Biotropica*, 39, 406–415.

Cloutier, D., Kanashiro, M., Ciampi, A.Y. & Schoen, D.J. (2007b). Impact of selective logging on inbreeding and gene dispersal in an Amazonian tree population of Carapa guianensis Aubl. *Molecular Ecology*, 16, 797–809.

Colabella, F., Gallo, L.A., Moreno, A.C. & Marchelli, P. (2014). Extensive pollen flow in a natural fragmented population of Patagonian cypress Austrocedrus chilensis. *Tree Genetics & Genomes*, 10, 1519–1529.

Collevatti, R.G., Olivatti, A.M., Telles, M.P.C. & Chaves, L.J. (2016). Gene flow among Hancornia speciosa (Apocynaceae) varieties and hybrid fitness. *Tree Genetics & Genomes,* 12, 74.

Cuartas-Hernández, S., Núñez-Farfán, J. & Smouse, P.E. (2010). Restricted pollen flow of Dieffenbachia seguine populations in fragmented and continuous tropical forest. *Heredity,* 105, 197–204.

Cuartas-Hernández, S.E. & Moreno-Betancur, D.J. (2020). Contrasting population genetic structure of two sympatric species of Anthurium (Araceae) along elevation in an Andean mountain forest. *Biotropica*, 52, 636–650.

Da Silva Carneiro, F., Magno Sebbenn, A., Kanashiro, M. & Degen, B. (2007). Low interannual variation of mating system and gene flow of Symphonia globulifera in the Brazilian Amazon. *Biotropica*, 39, 628–636.

Deacon, N.J. & Cavender-Bares, J. (2015). Limited pollen dispersal contributes to population genetic structure but not local adaptation in Quercus oleoides forests of Costa Rica. *PLoS ONE*, 10, e0138783.

Degen, B., Bandou, E. & Caron, H. (2004). Limited pollen dispersal and biparental inbreeding in Symphonia globulifera in French Guiana. *Heredity,* 93, 585–591.

Dick, C.W., Etchelecu, G. & Austerlitz, F. (2003). Pollen dispersal of tropical trees (Dinizia excelsa: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Mol Ecol*, 12, 753–764.

Dunphy, B.K. & Hamrick, J.L. (2007). Estimation of gene flow into fragmented populations of Bursera simaruba (Burseraceae) in the dry-forest life zone of Puerto Rico. *American Journal of Botany,* 94, 1786–1794.

Dyer, R.J., Chan, D.M., Gardiakos, V.A. & Meadows, C.A. (2012). Pollination graphs: quantifying pollen pool covariance networks and the influence of intervening landscape on genetic connectivity in the North American understory tree, Cornus florida L. *Landscape Ecol*, 27, 239–251.

Eduardo, A., De Lacerda, B., Kanashiro, M. & Sebbenn, A.M. (2008). Long-pollen movement and deviation of random mating in a low-density continuous population of a tropical tree Hymenaea courbaril in the Brazilian Amazon. *Biotropica,* 40, 462–470.

Fernández-M., J.F. & Sork, V.L. (2005). Mating patterns of a subdivided population of the Andean oak (Quercus humboldtii Bonpl., Fagaceae). *Journal of Heredity*, 96, 635–643.

Fernández-Manjarrés, J.F., Idol, J. & Sork, V.L. (2006). Mating patterns of black oak Quercus velutina (Fagaceae) in a Missouri oak-hickory forest. *Journal of Heredity*, 97, 451–455.

Gonzales, E., Hamrick, J.L., Smouse, P.E. & Dyer, R.J. (2006). Pollen-mediated gene dispersal within continuous and fragmented populations of a forest understorey species, Trillium cuneatum. *Molecular Ecology*, 15, 2047–2058.

Guidugli, M.C., Nazareno, A.G., Feres, J.M., Contel, E.P.B., Mestriner, M.A. & Alzate-Marin, A.L. (2016). Small but not isolated: a population genetic survey of the tropical tree Cariniana estrellensis (Lecythidaceae) in a highly fragmented habitat. *Heredity*, 116, 339–347.

Hardy, O.J., González-Martínez, S.C., Colas, B., Fréville, H., Mignot, A. & Olivieri, I. (2004). Fine-scale genetic structure and gene dispersal in Centaurea corymbosa (Asteraceae). II. Correlated paternity within and among sibships. *Genetics*, 168, 1601–1614.

Hirao, A.S., Kameyama, Y., Ohara, M., Isagi, Y. & Kudo, G. (2006). Seasonal changes in pollinator activity influence pollen dispersal and seed production of the alpine shrub Rhododendron aureum (Ericaceae). *Molecular Ecology*, 15, 1165–1173.

Hmeljevski, K.V., Wolowski, M., Forzza, R.C. & Freitas, L. (2017). High outcrossing rates and short-distance pollination in a species restricted to granitic inselbergs. *Aust. J. Bot.*, 65, 315.

Hufford, M.B., Gepts, P. & Ross-Ibarra, J. (2011). Influence of cryptic population structure on observed mating patterns in the wild progenitor of maize (Zea mays ssp. parviglumis). *Molecular Ecology*, 20, 46–55.

Irwin, A.J., Hamrick, J.L., Godt, M.J.W. & Smouse, P.E. (2003). A multiyear estimate of the effective pollen donor pool for Albizia julibrissin. *Heredity*, 90, 187–194.

Kassa, A., Konrad, H. & Geburek, T. (2018). Mating pattern and pollen dispersal in the wild olive tree (Olea europaea subsp. cuspidata). *Tree Genetics & Genomes*, 14, 3.

Kettle, C.J., Maycock, C.R., Ghazoul, J., Hollingsworth, P.M., Khoo, E., Sukri, R.S.H., *et al.* (2011). Ecological implications of a flower size/number trade-off in tropical forest trees. *PLoS ONE*, 6, e16111.

Kikuchi, S., Shibata, M. & Tanaka, H. (2015). Effects of forest fragmentation on the mating system of a cool-temperate heterodichogamous tree Acer mono. *Global Ecology and Conservation*, 3, 789–801.

Lepais, O. & Gerber, S. (2011). Reproductive patterns shape introgression dynamics and species succession within the European white oak species complex. *Evolution*, 65, 156–170.

Llorens, T.M., Byrne, M., Yates, C.J., Nistelberger, H.M. & Coates, D.J. (2012). Evaluating the influence of different aspects of habitat fragmentation on mating patterns and pollen dispersal in the bird-pollinated Banksia sphaerocarpa var. caesia. *Molecular Ecology*, 21, 314–328.

Lompo, D., Vinceti, B., Konrad, H., Duminil, J. & Geburek, T. (2020). Fine-scale spatial genetic structure, mating, and gene dispersal patterns in Parkia biglobosa populations with different levels of habitat fragmentation. *Am J Bot*, 107, 1041–1053.

Marchelli, P., Smouse, P.E. & Gallo, L.A. (2012). Short-distance pollen dispersal for an outcrossed, wind-pollinated southern beech (Nothofagus nervosa (Phil.) Dim. et Mil.). *Tree Genetics & Genomes*, 8, 1123–1134.

Matter, P. (2013). Estimating contemporary pollen-flow and associated outbreeding effects on herbaceous plant populations along altitudinal gradients. ETH Zurich.

Melo, A.T.D.O. & Franceschinelli, E.V. (2016). High genetic diversity and strong spatial genetic structure in Cabralea canjerana (Vell.) Mart. (Meliaceae): implications to Brazilian Atlantic Forest tree conservation. *Natureza & Conservação*, 32, 135–145.

Mimura, M., Barbour, R.C., Potts, B.M., Vaillancourt, R.E. & Watanabe, K.N. (2009). Comparison of contemporary mating patterns in continuous and fragmented Eucalyptus globulus native forests. *Molecular Ecology*, 18, 4180–4192.

Moraes, M.A., Gaino, A.P.S., Moraes, M.L.T., Freitas, M.L.M. & Sebbenn, A.M. (2012). Estimating coancestry within open-pollinated progenies of a dioecious species: the case study of Myracrodruon urundeuva. *Silvae Genetica*, 61, 256–264.

Nakanishi, A., Takeuchi, T., Ueno, S., Nishimura, N. & Tomaru, N. (2020). Spatial variation in bird pollination and its mitigating effects on the genetic diversity of pollen pools accepted by Camellia japonica trees within a population at a landscape level. *Heredity*, 124, 170–181.

Nakanishi, A., Tomaru, N., Yoshimaru, H., Manabe, T. & Yamamoto, S. (2005). Interannual genetic heterogeneity of pollen pools accepted by Quercus salicina individuals. *Molecular Ecology*, 14, 4469–4478.

Nakanishi, A., Yoshimaru, H., Tomaru, N., Miura, M., Manabe, T. & Yamamoto, S.I. (2012). Patterns of pollen flow in a dense population of the insect-pollinated canopy tree species Castanopsis sieboldii. *Journal of Heredity*, 103, 547–556.

Ngeve, M.N., Koedam, N. & Triest, L. (2020). Runaway fathers? Limited pollen dispersal and mating system in Rhizophora racemosa populations of a disturbed mangrove estuary. *Aquatic Botany*, 165, 103241.

O'Connell, L.M., Mosseler, A. & Rajora, O.P. (2006). Impacts of forest fragmentation on the mating system and genetic diversity of white spruce (Picea glauca) at the landscape level. *Heredity*, 97, 418–426.

Ojeda-Camacho, M., Kjær, E.D. & 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.

Ortego, J., Bonal, R., Muñoz, A. & Aparicio, J.M. (2014). Extensive pollen immigration and no evidence of disrupted mating patterns or reproduction in a highly fragmented holm oak stand. *Journal of Plant Ecology*, 7, 384–395.

Ottewell, K., Grey, E., Castillo, F. & Karubian, J. (2012). The pollen dispersal kernel and mating system of an insect-pollinated tropical palm, Oenocarpus bataua. *Heredity*, 109, 332–339.

Oyama, K., Herrera-Arroyo, M.L., Rocha-Ramírez, V., Benítez-Malvido, J., Ruiz-Sánchez, E. & González-Rodríguez, A. (2017). Gene flow interruption in a recently human-modified landscape: The value of isolated trees for the maintenance of genetic diversity in a Mexican endemic red oak. *Forest Ecology and Management*, 390, 27–35.

Pakkad, G., Ueno, S. & Yoshimaru, H. (2008). Gene flow pattern and mating system in a small population of Quercus semiserrata Roxb. (Fagaceae). *Forest Ecology and Management*, 255, 3819–3826.

Pluess, A.R., Sork, V.L., Dolan, B., Davis, F.W., Grivet, D., Merg, K., *et al.* (2009). Short distance pollen movement in a wind-pollinated tree, Quercus lobata (Fagaceae). *Forest Ecology and Management*, 258, 735–744.

Quesada, M., Herrerías-Diego, Y., Lobo, J.A., Sánchez-Montoya, G., Rosas, F. & Aguilar, R. (2013). Long-term effects of habitat fragmentation on mating patterns and gene flow of a tropical dry forest tree, Ceiba aesculifolia (Malvaceae: Bombacoideae). *American Journal of Botany*, 100, 1095–1101.

Riba-Hernández, P., Segura, J.L. & Muñoz-Valverde, J. (2016). Female fruit production depends on female flower production and crown size rather than male density in a continuous population of a tropical dioecious tree (Virola surinamensis). *American Journal of Botany*, 103, 1990–1999.

Ritchie, A.L., Nevill, P.G., Sinclair, E.A. & Krauss, S.L. (2017). Does restored plant diversity play a role in the reproductive functionality of Banksia populations? *Restor Ecol*, 25, 414–423.

Robledo-Arnuncio, J.J., Smouse, P.E., Gil, L. & Alía, R. (2004). Pollen movement under alternative silvicultural practices in native populations of Scots pine (Pinus sylvestris L.) in central Spain. *Forest Ecology and Management*, 197, 245–255.

Rong, J., Janson, S., Umehara, M., Ono, M. & Vrieling, K. (2010). Historical and contemporary gene dispersal in wild carrot (Daucus carota ssp. carota) populations. *Annals of Botany*, 106, 285–296.

Rosas, F., Quesada, M., Lobo, J.A. & Sork, V.L. (2011). Effects of habitat fragmentation on pollen flow and genetic diversity of the endangered tropical tree Swietenia humilis (Meliaceae). *Biological Conservation*, 144, 3082–3088.

Saro, I., Robledo-Arnuncio, J.J., González-Pérez, M.A. & Sosa, P.A. (2014). Patterns of pollen dispersal in a small population of the Canarian endemic palm (Phoenix canariensis). *Heredity*, 113, 215–223.

Silva, M.B., Kanashiro, M., Ciampi, A.Y., Thompson, I. & Sebbenn, A.M. (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.

Slavov, G.T., Leonardi, S., Burczyk, J., Adams, W.T., Strauss, S.H. & Difazio, S.P. (2009). Extensive pollen flow in two ecologically contrasting populations of Populus trichocarpa. *Molecular Ecology*, 18, 357–373.

Suarez-Gonzalez, A. & Good, S.V. (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.

Suarez-Gonzalez, A.R. (2011). Reproductive and genetic consequences of fragmentation in chokecherry (Prunus virginiana, L.). University of Winnipeg.

Suárez-Montes, P., Chávez-Pesqueira, M. & Núñez-Farfán, J. (2016). Life history and past demography maintain genetic structure, outcrossing rate, contemporary pollen gene flow of an understory herb in a highly fragmented rainforest. *PeerJ*, 4, e2764.

Tarazi, R., Sebbenn, A.M., Kageyama, P.Y. & Vencovsky, R. (2013). Edge effects enhance selfing and seed harvesting efforts in the insect-pollinated Neotropical tree Copaifera langsdorffii (Fabaceae). *Heredity*, 110, 578–585.

Thomasset, M., Hodkinson, T.R., Restoux, G., Frascaria-Lacoste, N., Douglas, G.C. & Fernández-Manjarrés, J.F. (2014). Thank you for not flowering: conservation genetics and gene flow analysis of native and non-native populations of Fraxinus (Oleaceae) in Ireland. *Heredity*, 112, 596–606.

Turchetto, C., Lima, J.S., Rodrigues, D.M., Bonatto, S.L. & Freitas, L.B. (2015). Pollen dispersal and breeding structure in a hawkmoth-pollinated Pampa grasslands species Petunia axillaris (Solanaceae). *Ann Bot*, 115, 939–948.

Uchiyama, K., Goto, S. & Ide, Y. (2009). Effects of population density on male and female reproductive success in the wind-pollinated, wind-dispersed tree species Betula maximowicziana. *Conserv Genet*, 10, 1265–1275.

Veron, V., Caron, H. & Degen, B. (2005). Gene flow and mating system of the tropical tree Sextonia rubra. *Silvae Genetica*, 54, 275–280.

Wang, J., Kang, M., Gao, P. & Huang, H. (2010). Contemporary pollen flow and mating patterns of a subtropical canopy tree Eurycorymbus cavaleriei in a fragmented agricultural landscape. *Forest Ecology and Management*, 260, 2180–2188.

Wendt, S.N., de Sousa, V.A., Sebbenn, A.M., Sturion, J.A., Luís, F. & Quoirin, M. (2007). Polinização efetiva em pomar de Ilex paraguariensis St. Hil. In: *Fenologia: ferramenta para conservação, melhoramento e manejo de recursos vegetais arbóreos* (eds. Rego, G.M., Negrelle, R.R.B. & Morellato, L.P.C.). Embrapa Florestas, Colombo, Brazil, pp. 345–362.

Widiyatno, Indrioko, S., Na'iem, M., Purnomo, S., Hosaka, T., Uchiyama, K., *et al.* (2017). Effects of logging rotation in a lowland dipterocarp forest on mating system and gene flow in Shorea parvifolia. *Tree Genetics & Genomes*, 13, 85.

Appendix S2. List of publications used to collect F_{ST} data

de Abreu Moreira, P., Brandão, M.M., de Araujo, N.H., de Oliveira, D.A. & Fernandes, G.W. (2015). Genetic diversity and structure of the tree Enterolobium contortisiliquum (Fabaceae) associated with remnants of a seasonally dry tropical forest. *Flora*, 210, 40–46.

Addisalem, A.B., Bongers, F., Kassahun, T. & Smulders, M.J.M. (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, J.D. (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, M.N., *et al.* (2008). Genetic structure of Tunisian natural carob tree (Ceratonia siliqua L.) populations inferred from RAPD markers. *Annals of Forest Science*, 65.

Alvarez-Buylla, E.R. & Garay, A.A. (1994). Population genetic structure of Cecropia obtusifolia a tropical pioneer tree species. *Evolution*, 48, 437–453.

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

Amat, M.E., 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, G.C., Vidal-Russell, R., Aizen, M.A. & 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, S.E. (2007). Regional population dynamics define the local genetic structure in Sorbus torminalis. *Molecular Ecology*, 16, 1291–1301.

Barbará, T., Martinelli, G., Fay, M.F., Mayo, S.J. & 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, M.F., 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, R.S., Estill, J.C. & Cruzan, M.B. (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.

Beland, J.D., Krakowski, J., Ritland, C.E., Ritland, K. & El-Kassaby, Y.A. (2005). Genetic structure and mating system of northern Arbutus menziesii (Ericaceae) populations. *Canadian Journal of Botany*, 83, 1581–1589.

Bessega, C., Pometti, C.L., Ewens, M., Saidman, B.O. & Vilardi, J.C. (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., Ravikanth, G., Ismail, S.A., Patel, M.K., Spanu, I., Vasudeva, R., *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.

Boisselier-Dubayle, M.C., Leblois, R., Samadi, S., Lambourdie're, 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, J.M. (2005). Genetic diversity and population structure of an insular tree, Santalum austrocaledonicum in New Caledonian archipelago. *Molecular Ecology*, 14, 1979–1989.

Brandão, M.M., Vieira, F. de A., Nazareno, A.G. & 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, 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, L.M. (2011). Genetic diversity and population genetic structure in fragmented Allocasuarina verticillata (Allocasuarinaceae) implications for restoration. *Australian Journal of Botany*, 59, 770–780.

Broadhurst, L.M. & Coates, D.J. (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, L.M., Young, A.G. & Murray, B.G. (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.

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, L.E. (2016). Population genetic structure of a widespread bat-pollinated columnar cactus. *PLoS ONE*, 11, e0152329.

Butcher, P.A., McDonald, M.W. & Bell, J.C. (2009). Congruence between environmental parameters, morphology and genetic structure in Australia’s most widely distributed eucalypt, Eucalyptus camaldulensis. *Tree Genetics and Genomes*, 5, 189–210.

Caetano, S., Prado, D., Pennington, R.T., Beck, S., Oliveira-Filho, A., Spichiger, R., *et al.* (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, M.S. & Premoli, A.C. (2005). Isozyme diversity in large and isolated populations of Luma apiculata (Myrtaceae) in north-western Patagonia, Argentina. *Australian Journal of Botany*, 53, 781–787.

Campbell, D.R. & Dooley, J.L. (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, M.M., Forzza, R.C., Veasey, E.A., Zucchi, M.I. & Oliveira, G.C.X. (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.O. & Jones, F.A. (2012). Comparative genetic structure of two mangrove species in Caribbean and Pacific estuaries of Panama. *BMC Evolutionary Biology*, 12, 205.

Chase, M.R., Boshier, D.H. & Bawa, K.S. (1995). Population genetics of Cordia alliodora (Boraginaceae), a neotropical tree. 1. Genetic variation in natural populations. *American Journal of Botany*, 82, 468–475.

Cheng, J., Lyu, L.S., Shen, Y.B., Li, K.X., Liu, Z.H., Wang, W.X., *et al.* (2016). Population structure and genetic diversity of Lithocarpus litseifolius (Fagaceae) assessed using microsatellite markers. *Nordic Journal of Botany*, 34, 752–760.

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

Chung, M.G., Chung, J.M., Chung, M.Y. & Eppersonà, B.K. (2000). Spatial distribution of allozyme polymorphisms following clonal and sexual reproduction in populations of Rhus javanica (Anacardiaceae). *Heredity*, 84, 178–185.

Chung, M.Y., Kim, K.J., Pak, J.H., Park, C.W., Sun, B.Y., Myers, E.R., *et al.* (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, M.Y., Nason, J., Chung, M.G., Kim, K.J., Park, C.W., Sun, B.Y., *et al.* (2002). Landscape-level spatial genetic structure in Quercus acutissima (Fagaceae). *American Journal of Botany*, 89, 1229–1236.

Cloutier, D., Hardy, O.J., Caron, H., Ciampi, A.Y., Degen, B., Kanashiro, M., *et al.* (2007). Low inbreeding and high pollen dispersal distances in populations of two Amazonian forest tree species. *Biotropica*, 39, 406–415.

Coart, E., Vekemans, X., Smulders, M.J.M., Wagner, I., van Huylenbroeck, J., van Bockstaele, E., *et al.* (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, C.T. & Biesboer, D.D. (1992). Monomorphism, reduced gene flow, and cleistogamy in rare and common species of Lespedeza (Fabaceae). *American Journal of Botany*, 79, 567–575.

Collevatti, R.G., Grattapaglia, D. & Hay, J.D. (2001). Population genetic structure of the endangered tropical tree species Caryocar brasiliense, based on variability at microsatellite loci. *Molecular Ecology*, 10, 349–356.

Collevatti, R.G., Telles, M.P.C., Lima, J.S., Gouveia, F.O. & Soares, T.N. (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, A.R.O., Ruas, E.A., Vieira, B.G., Rodrigues, L.A., Costa, B.F., Bianchini, E., *et al.* (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, R.E., Steane, D.A., Jones, R.C. & Marques, C. (2017). Microsatellite analysis of population structure in Eucalyptus globulus. *Genome*, 60, 770–777.

Cota, L.G., Moreira, P.A., Brandão, M.M., Royo, V.A., Melo Junior, A.F., Menezes, E.V., *et al.* (2017). Structure and genetic diversity of anacardium humile (Anacardiaceae): A tropical shrub. *Genetics and Molecular Research*, 16, gmr16039778.

Culley, T.M. & Wolfe, A.D. (2001). Population genetic structure of the cleistogamous plant species Viola pubescens Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity*, 86.

Daïnou, K., Mahy, G., Duminil, J., Dick, C.W., Doucet, J.L., Donkpégan, A.S.L., *et al.* (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, J.O., Ruas, E.A., Rodrigues, L.A., Ruas, C.F., Bianchini, E., Pimenta, J.A., *et al.* (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, O.G. & Gudu, S. (2000). Allozyme variation in 16 natural populations of Fuidherbiu albida (Del.) A. Chev. *Hereditas*, 133, 133–145.

De, F. & Vieira, A. (2009). Genetic differentiation and temporal aspects of the fine-scale genetic structure in fragments-vegetation corridors: inferences from a dioecious-dominant neotropical tree. Universidade Federal de Lavras.

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

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

Degen, B., Bandou, E. & Caron, H. (2004). Limited pollen dispersal and biparental inbreeding in Symphonia globulifera in French Guiana. *Heredity*, 93, 585–591.

Demenou, B.B., Doucet, J.L. & Hardy, O.J. (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.

Doligez, A. & Joly, H.I. (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.J. & Burdon, J.J. (1986). Genetic structure of natural populations of wild sunflowers (Helianthus annuus L.) in Australia. *Australian Journal of Biological Sciences*, 39, 255–270.

Dunphy, B.K., Hamrick, J.L. & Schwagerl, J. (2004). A comparison of direct and indirect measures of gene flow in the bat-pollinated tree Hymenaea courbaril in the dry forest life zone of southwestern Puerto Rico. *International Journal of Plant Sciences*, 165, 427–436.

Dutech, C., Joly, H.I. & 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, R.J. (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, P.R., Usher, A.V., Whelan, R.J. & Ayre, D.J. (2002). Microsatellite diversity and genetic structure of fragmented populations of the rare, fire-dependent shrub Grevillea macleayana. *Molecular Ecology*, 11, 967–977.

Fant, J.B., Havens, K., Keller, J.M., Radosavljevic, A. & Yates, E.D. (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, C.B., Vekemans, X. & Hardy, O.J. (2003). Quantifying gene flow from spatial genetic structure data in a metapopulation of Chamaecrista fasciculata (Leguminosae). *Evolution*, 57, 995–1007.

Fontaine, C., Lovett, P.N., 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, P.F. & Sork, V.L. (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. & Kesselià, R. (1999). Population structure and gene flow of the Brazilian shrub Helicteres brevispira. *Heredity*, 82, 355–363.

Frascaria, N., Santi, F. & Gouyon, P.H. (1992). Genetic differentiation within and among populations of chestnut (Castanea sativa Mill.) and wild cherry (Prunus avium L.). *Heredity*, 70, 634–641.

Fu, Y. & Dane, F. (2003). Allozyme variation in endangered Castanea pumila var. pumila. *Annals of Botany*, 92, 223–230.

Fuchs, E.J. & Hamrick, J.L. (2010). Genetic diversity in the endangered tropical tree, guaiacum sanctum (zygophyllaceae). *Journal of Heredity*, 101, 284–291.

Ganzhorn, S.M., Thomas, W.W., Gaiotto, F.A. & Lewis, J.D. (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, J.P., 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, M.J.W. & Hamrick, J.L. (1993). Genetic diversity and population structure in Tradescantia hirsuticaulis (Commelinaceae). *American Journal of Botany*, 80, 959–966.

Godt, M.J.W. & Hamrick, J.L. (1999). Population genetic analysis of Elliottia racemosa (Ericaceae), a rare Georgia shrub. *Molecular Ecology*, 8, 75–82.

Goetze, M., Büttow, M.V., Zanella, C.M., Paggi, G.M., Bruxel, M., Pinheiro, F.G., *et al.* (2015). Genetic variation in Aechmea winkleri, a bromeliad from an inland Atlantic rainforest fragment in Southern Brazil. *Biochemical Systematics and Ecology*, 58, 204–210.

González-Astorga, J., Cruz-Angón, A., Flores-Palacios, A. & Vovides, A.P. (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, S.C., Dubreuil, M., Riba, M., Vendramin, G.G., 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, M.A., Caujapé-Castells, J. & Sosa, P.A. (2004). Allozyme variation and structure of the Canarian endemic palm tree Phoenix canariensis (Arecaceae): Implications for conservation. *Heredity*, 93, 307–315.

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

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

Hahn, C.Z., Michalski, S.G., Fischer, M. & Durka, W. (2017). Genetic diversity and differentiation follow secondary succession in a multi-species study on woody plants from subtropical China. *Journal of Plant Ecology*, 10, 213–221.

Hahn, T., Kettle, C.J., Ghazoul, J., Frei, E.R., Matter, P. & Pluess, A.R. (2012). Patterns of genetic variation across altitude in three plant species of semi-dry grasslands. *PLoS ONE*, 7, e41608.

Hall, P., Chase, M.R. & Bawa, K.S. (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, O.K., Changtragoon, S., Ponoy, B., Kjær, E.D., Minn, Y., Finkeldey, R., *et al.* (2015). Genetic resources of teak (Tectona grandis Linn. f.)—strong genetic structure among natural populations. *Tree Genetics and Genomes*, 11, 802.

Hardesty, B.D., Dick, C.W., Hamrick, J.L., Degen, B., Hubbell, S.P. & Bermingham, E. (2010). Geographic influence on genetic structure in the widespread Neotropical tree Simarouba amara (Simaroubaceae). *Tropical Plant Biology*, 3, 28–39.

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

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, E.K.V., Albrecht, L., García-Villacorta, R., Staeps, F.C., Herre, E.A., *et al.* (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.

Heuertz, M., Hausman, J.F., Hardy, O.J., Vendramin, G.G., 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, B.F., Selbach-Schnadelbach, A., Galetto, L. & Kevan, P.G. (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, K.V., Nazareno, A.G., Leandro Bueno, M., dos Reis, M.S. & Forzza, R.C. (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.

Hoey, M.T. & Parks, C.R. (1994). Genetic Divergence in Liquidambar styraciflua, L. formosana, and L. acalycina (Hamamelidaceae). *Systematic Botany*, 19, 308–316.

Hughes, M. (2002). Population structure and speciation in Begonia L. Ph.D. University of Glasgow.

Hughes, M., MacMaster, G., Möller, M., Bellstedt, D.U. & Edwards, T.J. (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., Möller, M., Edwards, T.J., Bellstedt, D.U. & De Villiers, M. (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, M.K. (1999). Genetic diversity and population structure of Korean alder (Alnus japonica; Betulaceae). *Canadian Journal of Forest Research*, 29, 1311–1316.

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

Izquierdo, L.Y. & Piñero, D. (2000). High genetic diversity in the only known population of aechmea tuitensis (Bromeliaceae). *Australian Journal of Botany*, 48, 645–650.

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. *Molecular Ecology*, 14, 4479–4488.

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

Jeong, J., Park, Y.J. & Kim, Z.S. (2007). Genetic diversity and spatial structure of Symplocarpus renifolius on Mt. Cheonma, Korea. *Korean Journal of Plant Research*, 20, 530–539.

Jia, H. min, Jiao, Y., Wang, G. yun, Li, Y. hui, Jia, H. juan, Wu, H. xia, *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., Agúndez, D., Alía, R. & Gil, L. (1999). Genetic variation in central and marginal populations of Quercus suber L. *Silvae Genetica*, 48, 278–284.

Juárez, L., Montaña, C. & Ferrer, M.M. (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., 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, S.H., Jang, Y.S., Han, J.G., Chung, H.G., Lee, S.W. & Cho, K.J. (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., *et al.* (2005). Spatial genetic structure among and within populations of Primula sieboldii growing beside separate streams. *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, S.E. & Waller, D.M. (1987). Genetic consequences of outcrossing in the cleistogamous annual, Impatiens capensis. I. Population-genetic structure. *Evolution*, 41, 969–978.

Kramer, A.T., Fant, J.B. & Ashley, M.V. (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, D.F. (1997). Microgeographic genetic structure and gene flow in Hibiscus moscheutos (Malvaceae) populations. *American Journal of Botany*, 84, 1285–1293.

Kuss, P., Pluess, A.R., Aegisdottir, H.H. & 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, A.E., Hardy, O.J., Kaka'Ï, R.G., Sinsin, B., Van Damme, P., *et al.* (2009). Spatial genetic structuring of baobab (Adansonia digitata, Malvaceae) in the traditional agroforestry systems of west africa. *American Journal of Botany*, 96, 950–957.

Lamont, R.W., Conroy, G.C., Reddell, P. & Ogbourne, S.M. (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, J.W. & 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, C.P.Y., Saunders, R.M.K. & 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-LaPegue, 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, F.T. (2000). Founder Effects and the Genetic Structure of Coulter Pine. *The Journal of Heredity*, 91, 307–315.

Lee, C.T., Lee, S.L., Ng, K.K.S., Salwana, H.S., Norwati, M. & Saw, L.G. (2007). Allozyme diversity of Koompassia malaccensis (Leguminosae) in Peninsular Malaysia. *Journal of Tropical Forest Science*, 19, 73–78.

Lee, S.L. (2000). Mating system parameters of Dryobalanops aromatica Gaertn. f. (Dipterocarpaceae) in three different forest types and a seed orchard. *Heredity*, 85, 338–345.

Lee, S.L., Ng, K.K.S., Saw, L.G., Norwati, A., Salwana, M.H.S., Lee, C.T., *et al.* (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, S.L., Wickneswari, R., Mahani, M.C. & Zakri, A.H. (2000). Genetic Diversity of a Tropical Tree Species, Shorea leprosura Miq. (Dipterocarpaceae), in Malaysia: Implications for Conservation of Genetic Resources and Tree Improvement'. *Biotropica*, 32, 213–224.

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.

Lemos, R.P.M., D'Oliveira, C.B. & Stefenon, V.M. (2015). Genetic structure and internal gene flow in populations of Schinus molle (Anacardiaceae) in the Brazilian Pampa. *Tree Genetics and Genomes*, 11, 75.

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

Ley, A.C. & Hardy, O.J. (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, E.J., 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 micvotlzeca F. Muell. populations. *Hereditas*, 132, 15–16.

Liengsiri, C., Yeh, F.C. & Boyle, T.J.B. (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, S.G., 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, 20150290.

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, 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.

Loveless, M.D., Hamrick, J.L. & Foster, R.B. (1998). Population structure and mating system in Tachigali versicolor, a monocarpic neotropical tree. *Heredity*, 81, 134–143.

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

de Lucas, A.I., GonzÁlez-MartÍnez, S.C., Vendramin, G.G., Hidalgo, E. & Heuertz, M. (2009). Spatial genetic structure in continuous and fragmented populations of Pinus pinaster Aiton. *Molecular Ecology*, 18, 4564–4576.

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

Maguire, T.L., 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.

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

Mariette, S., Cottrell, J., Csaikl, U.M., Goikoechea, P., König, A., Lowe, A.J., *et al.* (2002). Comparison of levels of genetic diversity detected with AFLP and microsatellite markers within and among mixed Q. petrae (Matt.) Liebl. and Q. robur L. stands. *Silvae Genetica*, 51, 72–79.

Mariot, A., Stasi, L.C.D. & Dos Reis, M.S. (2002). Genetic diversity in natural populations of Piper cernuum. *The Journal of Heredity*, 93, 365–369.

Marquardt, P.E. & Epperson, B.K. (2004). Spatial and population genetic structure of microsatellites in white pine. *Molecular Ecology*, 13, 3305–3315.

Marsico, T.D., Hellmann, J.J. & 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, M.A., Mattioni, C., Molina, J.R., Alvarez, J.B., Cherubini, M., Herrera, M.A., *et al.* (2012). Landscape genetic structure of chestnut (Castanea sativa Mill.) in Spain. *Tree Genetics and Genomes*, 8, 127–136.

Matolweni, L.O., 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, C.J., Ghazoul, J. & Pluess, A.R. (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, J.A., González-Marítnez, S.C., 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, M.W., Rawlings, M., Butcher, P.A. & Bell, J.C. (2003). Regional divergence and inbreeding in Eucalyptus cladocalyx (Myrtaceae). *Australian Journal of Botany*, 51, 393–403.

Mcgranahanl, M., Bell, J.C., Moranz, G.F. & Slee, M. (1997). High genetic divergence between geographic regions in the highly outcrossing species Acacia aulacocarpa (Cunn. ex Benth.). *Forest Genetics*, 4, 1–13.

Meirmans, P.G., Goudet, J. & Gaggiotti, O.E. (2011). Ecology and life history affect different aspects of the population structure of 27 high-alpine plants. *Molecular Ecology*, 20, 3144–3155.

Michaud, H., Lumaret, R. & Romane, F. (1992). Variation in the genetic structure and reproductive biology of holm oak populations. *Vegetatio*, 99–100, 107–113.

Montalvo, A.M., Conard, S.G., Conkle, M.T. & Hodgkiss, P.D. (1997). Population structure, genetic diversity, and clone formation in Quercus chrysoilepis (Fagaceae). *American Journal of Botany*, 84, 1553–1564.

Moran, G.F., Bell, J.C. & Turnbull, J.W. (1989a). A cline in genetic diversity in river she-oak Casuarina cunninghamiana. *Australian Journal of Botany*, 37, 169–180.

Moran, G.F., Muona, O. & Bell, J.C. (1989b). Acacia mangium: A tropical forest tree of the coastal lowlands with low genetic diversity. *Evolution*, 43, 231–235.

Moreira, P.A., Fernandes, G.W. & Collevatti, R.G. (2009). Fragmentation and spatial genetic structure in Tabebuia ochracea (Bignoniaceae) a seasonally dry Neotropical tree. *Forest Ecology and Management*, 258, 2690–2695.

Moreira, R.G., McCauley, R.A., Cortés-Palomec, A.C., Fernandes, G.W. & 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., Petit, R.J., White, L. & Abernethys, K. (2000). Chloroplast DNA variation in a rainforest tree (Aucoumea klaineana , Burseraceae) in Gabon. *Molecular Ecology*, 9, 359–363.

Murawski, D.A. & Bawa, K.S. (1994). Genetic structure and mating system of Stemonoporus oblongifolius (Dipterocarpaceae) in Sri Lanka. *American Journal of Botany*, 81, 155–160.

Murawski, D.A. & Hamrick, J.L. (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, R.S. & 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, T.P.T., Tran, T.H., Nguyen, M.D., 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., *et al.* (2012). Spatial genetic structure of Campanula sabatia, a threatened narrow endemic species of the Mediterranean Basin. *Folia Geobotanica*, 47, 249–262.

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, E.D. & 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, N.H., von Wettberg, E.J.B. & Negrón-Ortiz, V. (2014). Population genetics of the Federally Threatened Miccosukee gooseberry (Ribes echinellum), an endemic North American species. *Conservation Genetics*, 15, 749–755.

de Oliveira Melo, A.T. & Franceschinelli, E.V. (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.

de Oliveira Wadt, L.H. & Kageyama, P.Y. (2004). Estrutura genética e sistema de acasalamento de Piper hispidinervum. *Pesquisa Agropecuária Brasileira*, 39, 151–157.

Omondi, S.F., Kireger, E., Dangasuk, O.G., Chikamai, B., Odee, D.W., Cavers, S., *et al.* (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 Forest*, 27, 189–200.

Palma-Silva, C., Lexer, C., Paggi, G.M., Barbará, T., Bered, F. & Bodanese-Zanettini, M.H. (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, O.P. (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*, 5, 159–175.

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

Parks, C.R., Wendel, J.F., Sewell, M.M. & 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, K.G., Dvorak, W.S., Janse, B.J.H. & Myburg, A.A. (2008). Microsatellite diversity and genetic structure of the commercially important tropical tree species Eucalyptus urophylla, endemic to seven islands in eastern Indonesia. *Tree Genetics and Genomes*, 4, 519–530.

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

Peakall, R. & Beattie, A.J. (1996). Ecological and genetic consequences of pollination by sexual deception in the orchid Caladenia tentactulata. *Evolution*, 50, 2207–2220.

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

Playford, J., Bell, J.C. & Moran, G.F. (1993). A major disjunction in genetic diversity over the geographic range of Acacia melanoxylon R. Br. *Australian Journal of Botany*, 41, 355–68.

Pluess, A.R. (2011). Pursuing glacier retreat: Genetic structure of a rapidly expanding Larix decidua 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, A.C. (1997). Genetic variation in a geographically restricted and two widespread species of South American Nothofagus. *Journal of Biogeography*, 24, 883–892.

Prober, S.M. & Brown, A.H.D. (1994). Conservation of the grassy white box woodlands: Population genetics and fragmentation of Eucalyptus albens. *Conservation Ecology*, 8, 1003–1013.

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

Quevedo, A.A., 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., Raspé, R. & Jacquemart, A.-L. (1998). Allozyme diversity and genetic structure of European populations of Sorbus aucuparia L. (Rosaceae: Maloideae). *Heredity*, 81, 537–545.

Reif, B.P., Mathiasen, R.L., Kenaley, S.C. & Allan, G.J. (2015). Genetic structure and morphological differentiation of three western North American dwarf mistletoes (Arceuthobium: Viscaceae). *Systematic Botany*, 40, 191–207.

Ribeiro, F.E., Baudouin, L., Lebrun, P., Chaves, L.J., Brondani, C., Zucchi, M.I., *et al.* (2010). Population structures of Brazilian tall coconut (Cocos nucifera L.) by microsatellite markers. *Genetics and Molecular Biology*, 33, 696–702.

Ritchie, A.L., Nevill, P.G., Sinclair, E.A. & Krauss, S.L. (2017). Does restored plant diversity play a role in the reproductive functionality of Banksia populations? *Restoration Ecology*, 25, 414–423.

Robertson, A., Newton, A.C. & Ennos, R.A. (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, O.J. & Lobo, J.A. (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, K.E. (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, J.L. & Wood, B.W. (1999). Genetic diversity within provenance and cultivar germplasm collections versus natural populations of pecan (Carya illinoinensis). *The Journal of Heredity*, 90, 521–528.

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

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, J.L. (1990a). Comparative analysis of population genetic structure in Quercus macrocarpa and Q. gambelii (Fagaceae). *Systematic Botany*, 15, 240–251.

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

Searle, S.D., Bell, J.C. & Moran, G.F. (2000). Genetic diversity in natural populations of Acacia mearnsii. *Australian Journal of Botany*, 48, 279–286.

Sgorbati, S., Labra, M., Grugni, E., Barcaccia, G., Galasso, G., Boni, U., *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, 663–686.

Shapcott A. (1998). The patterns of genetic diversity in Carpentaria acuminata (Arecacea), 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, D.L. & Meagher, T.R. (1996). Genetic diversity in micronesian island populations of the tropical tree campnosperma brevipetiolata (anacardiaceae). *American Journal of Botany*, 83, 1571–1579.

Sherman-Broyles, S.L., Broyles, S.B. & Hamrick, J.L. (1992). Geographic distribution of allozyme aariation in Ulmus crassifolia. *Systematic Botany*, 17, 33–41.

Slavov, G.T., Leonardi, S., Adams, W.T., Strauss, S.H. & DiFazio, S.P. (2010). Population substructure in continuous and fragmented stands of Populus trichocarpa. *Heredity*, 105, 348–357.

Sochor, M., Vašut, R.J., 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 and Genomes*, 9, 1193–1205.

Soltis, D.E., Gilmartin, A.J., 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., *et al.* (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.L., Huang, S. & Wiener, E. (1993). Macrogeographic and fine-scale genetic structure in a North American oak species, Quercus rubra L. *Annals of Forestry Science*, 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, T.N., Gadek, K.E., Yoder, J.B., Flatz, R. & Smith, C.I. (2013). Asymmetric hybridization and gene flow between Joshua trees (Agavaceae: Yucca) reflect differences in pollinator host specificity. *Molecular Ecology*, 22, 437–449.

Stefenon, V.M., 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 understorey herb of the Brazilian Atlantic rain forest. *Journal of Tropical Ecology*, 30, 199–208.

Suarez-Gonzalez, A. & Good, S.V. (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, P.S., Martins, K., Wadt, L.H. de O., Azevedo, V.C.R. & Solferini, V.N. (2015). Genetic structure of Bertholletia excelsa populations from the Amazon at different spatial scales. *Conservation Genetics*, 16, 955–964.

Suma, T.B. & Balasundaran, M. (2003). Isozyme variation in five provenances of Santalum album in India. *Australian Journal of Botany*, 51, 243–249.

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, K.M. (2013). Restricted gene flow within and between rapidly diverging Neotropical plant species. *Molecular Ecology*, 22, 4931–4942.

Swift, J.F., Smith, S.A., Menges, E.S., Bassüner, B. & Edwards, C.E. (2016). Analysis of mating system and genetic structure in the endangered, amphicarpic plant, Lewton’s polygala (Polygala lewtonii). *Conservation Genetics*, 17, 1269–1284.

Tambarussi, E.V., Sebbenn, A.M., Alves-Pereira, A., Vencovsky, R., Cambuim, J., da Silva, A.M., *et al.* (2017). Dipteryx alata Vogel (Fabaceae), a neotropical tree with high levels of selfing: Implications for conservation and breeding programs. *Annals of Forest Research*, 60, 243–261.

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, 1189–1199.

Tinio, C.E., Finkeldey, R., Prinz, K. & Fernando, E.S. (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. *Molecular Ecology*, 14, 3929–3941.

Turchetto, C., Lima, J.S., Rodrigues, D.M., Bonatto, S.L. & Freitas, L.B. (2015). Pollen dispersal and breeding structure in a hawkmoth-pollinated Pampa grasslands species Petunia axillaris (Solanaceae). *Annals of Botany*, 115, 939–948.

Twyford, A.D., Kidner, C.A. & Ennos, R.A. (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. (2005). 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, S., Sousa, D. & 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, M.A., Soltis, D.E. & Soltis, P.S. (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, E.R., Glaubitz, J.C., Rhodes, O.E. & Woeste, K.E. (2006). Genetic homogeneity in Juglans nigra (Juglandaceae) at nuclear microsatellites. *American Journal of Botany*, 93, 118–126.

Walisch, T.J., 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, H., Pei, D., Gu, R.-S. & Wang, B.-Q. (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.

Wang, R., Compton, S.G., 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.

Wickneswari, R. & Norwati, M. (1993). Genetic diversity of natural-populations of Acacia auriculiformis. *Australian Journal of Botany*, 41, 65–77.

Williams, C.F. & Guries, R.P. (1994). Genetic consequences of seed dispersal in three sympatric forest herbs. I. Hierarchical population-genetic structure. *Evolution*, 48, 791–805.

Williams, C.F. & Waser, N.M. (1999). Spatial genetic structure of Delphinium nuttallianum populations: inferences about gene flow. *Heredity*, 83, 541–550.

Williams, J.H. & Arnold, M.L. (2001). Sources of genetic structure in the woody perennial Betula occidentalis. *International Journal of Plant Sciences*, 162, 1097–1109.

Xie, C.-Y., El-Kassaby, Y.A. & Ying, C.C. (2002). Genetics of red alder (Alnus Rubra Bong.) populations in British Columbia and its implications 1 for gene resources management 1, 2 1. *New Forests*, 24, 97–112.

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, C.W., 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.

Zucchi, M.I., Baldin Pinheiro, J.B., Chaves, L.J., Coelho, A.S.G., Couto, M.A., de Morais, L.K., *et al.* (2005). Genetic structure and gene flow of Eugenia dysenterica natural populations. *Pesquisa Agropecuária Brasileira*, 40, 975–980.

Supplementary materials

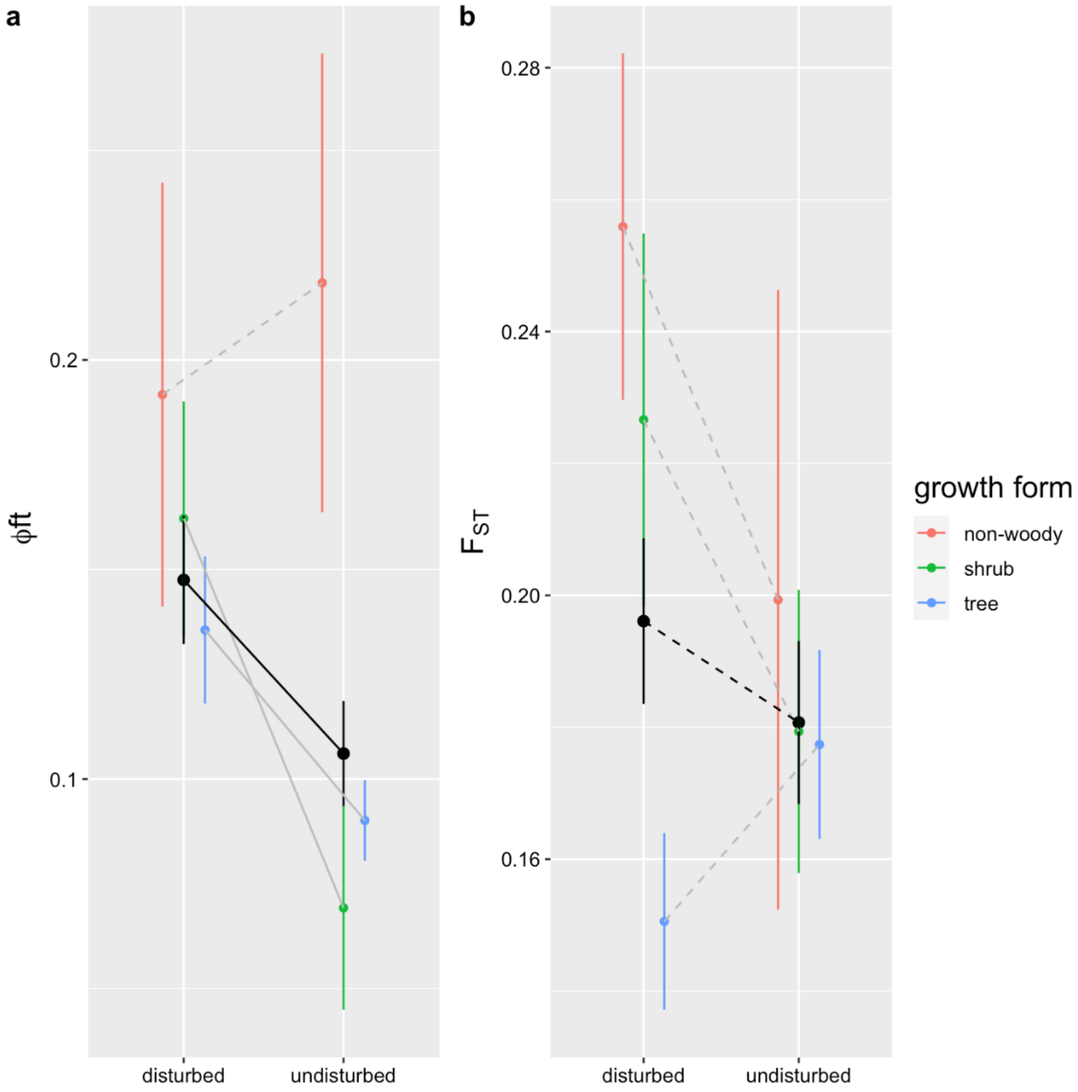

Figure S1. Mean values and standard errors of **a** Φ_{FT} and **b** F_{ST} for disturbed and continuous populations separated by growth form. Mean values and standard errors were calculated from raw Φ_{FT} and F_{ST} values. Overall mean value for disturbed and continuous populations are shown in black. Mean values for species with different growth forms are shown in different colors. Significant differences between continuous and disturbed values are connected with solid lines, non-significant differences are connected with dashed lines.

Table S1. Results of phylogenetic mixed-effect regressions testing the effect of pollination mode on Φ_{FT}. Each regression (a-g) has a different pollination mode category set as the reference variable. Reported values are based on Φ_{FT} transformed by Tukey's ladder of powers which resulted in values of $\Phi_{FT}^{0.350}$. False Discovery Rate (FDR) P-values are P-values corrected for false discovery rates due to multiple comparisons.

	Estimate	S.E.	Z-score	P-value	FDR P-value
(a) Intercept (bat)	0.543	0.108	5.04	4.65E-07	--
bird	-0.025	0.070	-0.366	0.714	0.714
hummingbird	0.067	0.079	0.852	0.394	0.493
insect	0.083	0.063	1.309	0.191	0.287
large bee	-0.032	0.065	-0.490	0.625	0.662
small bee	0.015	0.067	0.225	0.822	0.740
wind	-0.099	0.063	-1.579	0.114	0.248
(b) Intercept (bird)	0.518	0.101	5.113	3.18E-07	--
bat	0.026	0.070	0.366	0.714	0.714
hummingbird	0.096	0.067	1.375	0.169	0.277
insect	0.108	0.050	2.162	0.031	0.111
large bee	0.006	0.048	-0.129	0.898	0.770
small bee	0.041	0.053	0.775	0.438	0.493
wind	-0.074	0.048	-1.522	0.128	0.248
(c) Intercept (hummingbird)	0.61	0.107	5.695	1.23E-08	--
bat	-0.067	0.079	-0.852	0.394	0.493
bird	-0.093	0.067	-1.375	0.169	0.277
insect	0.046	0.057	0.280	0.780	0.739
large bee	-0.099	0.062	-1.601	0.109	0.248
small bee	-0.058	0.064	-0.806	0.420	0.493
wind	-0.166	0.059	-2.808	0.005	**0.042**
(d) Intercept (insect)	0.626	0.096	6.509	7.57E-11	--
bat	-0.083	0.063	-1.309	0.191	0.287
bird	-0.108	0.050	-2.162	0.031	0.111
hummingbird	-0.016	0.057	-0.280	0.780	0.739
large bee	-0.115	0.042	-2.711	0.007	**0.042**
small bee	-0.068	0.046	-1.484	0.138	0.248
wind	-0.182	0.038	-4.958	7.12E-07	**0.000**

Table S1 continued

(e) Intercept (large bee)	0.511	0.098	5.24	1.60E-07	--
bat	0.032	0.065	0.490	0.625	0.662
bird	0.006	0.048	0.129	0.898	0.770
hummingbird	0.099	0.062	1.601	0.109	0.248
insect	0.115	0.042	2.711	0.007	**0.042**
small bee	0.047	0.045	1.051	0.293	0.406
wind	-0.067	0.040	-1.690	0.091	0.248
(f) Intercept (small bee)	0.558	0.099	5.619	1.92E-08	--
bat	-0.015	0.067	-0.225	0.822	0.74
bird	-0.041	0.053	-0.775	0.438	0.493
hummingbird	0.052	0.064	0.806	0.420	0.493
insect	0.068	0.046	1.484	0.138	0.248
large bee	-0.047	0.045	-1.051	0.293	0.406
wind	-0.114	0.044	-2.578	0.010	**0.045**
(g) Intercept (wind)	0.444	0.095	4.693	2.70E-06	--
bat	0.099	0.063	1.579	0.114	0.248
bird	0.074	0.048	1.522	0.128	0.248
hummingbird	0.167	0.059	2.808	0.005	**0.042**
insect	0.182	0.037	4.958	7.12E-07	**0.000**
large bee	0.067	0.040	1.690	0.091	0.248
small bee	0.114	0.044	2.578	0.010	**0.045**

Table S2. Results of phylogenetic mixed-effect regressions testing the effect of pollination mode on F_{ST}. Each regression (a-g) has a different pollination mode category set as the reference variable. Reported values are based on F_{ST} transformed by Tukey's ladder of powers which resulted in values of $F_{ST}^{0.300}$. False Discovery Rate (FDR) P-values are P-values corrected for false discovery rates due to multiple comparisons.

	Estimate	S.E.	Z-score	P-value	FDR P-value
(a) Intercept (bat)	0.605	0.055	10.929	<2E-16	--
bird	-0.070	0.060	-1.157	0.248	0.473
hummingbird	0.018	0.049	0.369	0.712	0.921
insect	0.010	0.041	0.251	0.802	0.921
large bee	-0.046	0.043	-1.077	0.281	0.492
small bee	0.014	0.044	0.323	0.747	0.921
wind	-0.059	0.044	-1.317	0.188	0.395
(b) Intercept (bird)	0.535	0.059	9.136	<2E-16	--
bat	0.070	0.060	1.157	0.248	0.473
hummingbird	0.088	0.058	1.535	0.125	0.292
insect	0.080	0.046	1.748	0.081	0.239
large bee	0.054	0.048	0.503	0.615	0.921
small bee	0.084	0.050	1.690	0.091	0.239
wind	0.011	0.047	0.241	0.809	0.921
(c) Intercept (hummingbird)	0.623	0.052	11.989	<2E-16	--
bat	-0.018	0.049	-0.369	0.712	0.921
bird	-0.088	0.057	-1.535	0.125	0.292
insect	-0.008	0.037	-0.208	0.835	0.921
large bee	-0.064	0.038	-1.696	0.090	0.239
small bee	-0.004	0.040	-0.099	0.921	0.921
wind	-0.077	0.041	-1.888	0.059	0.239
(d) Intercept (insect)	0.615	0.042	14.601	<2.2E-16	--
bat	-0.010	0.041	-0.251	0.802	0.921
bird	-0.080	0.460	-1.748	0.081	0.239
hummingbird	0.008	0.037	0.208	0.835	0.921
large bee	-0.056	0.022	-2.568	0.010	0.098
small bee	0.004	0.026	0.145	0.884	0.921
wind	-0.069	0.021	-3.276	0.001	**0.021**

Table S2 continued

(e) Intercept (large bee)	0.559	0.043	13.002	<2E-16	--
bat	0.046	0.043	1.077	0.281	0.492
bird	-0.024	0.048	-0.503	0.615	0.921
hummingbird	0.064	0.038	1.696	0.090	0.239
insect	0.056	0.022	2.568	0.010	0.098
small bee	0.060	0.028	2.176	0.030	0.158
wind	-0.013	0.025	-0.515	0.607	0.921
(f) Intercept (small bee)	0.619	0.045	13.795	<2E-16	--
bat	-0.014	0.044	-0.323	0.747	0.921
bird	-0.084	0.050	-1.689	0.091	0.239
hummingbird	0.004	0.040	0.099	0.921	0.921
insect	-0.004	0.026	-0.145	0.884	0.921
large bee	-0.060	0.028	-2.176	0.030	0.158
wind	-0.073	0.030	-2.460	0.014	0.098
(g) Intercept (wind)	0.546	0.044	12.394	<2.2E-16	--
bat	0.059	0.044	1.317	0.188	0.395
bird	-0.011	0.047	-0.241	0.809	0.921
hummingbird	0.077	0.041	1.888	0.059	0.239
insect	0.069	0.021	3.276	0.001	**0.021**
large bee	0.013	0.025	0.515	0.607	0.921
small bee	0.073	0.030	2.460	0.014	0.098

Table S3. Results of phylogenetic mixed-effect regressions testing the effect of habitat disturbance on Φ_{FT} and F_{ST} for plant species with different growth forms. Values reported below are based on Φ_{FT} and F_{ST} transformed by Tukey's ladder of powers, with the transformed values indicated for each group. Significant p-values are in bold.

	Φ_{FT}			
	Estimate	SE	Z-score	P-value
(a) non-woody $\Phi_{FT}^{0.475}$				
Intercept (undisturbed)	0.363	0.071	5.138	**<0.001**
disturbed	0.026	0.033	0.779	0.436
(b) shrub $\Phi_{FT}^{0.675}$				
Intercept (undisturbed)	0.159	0.05	3.202	**0.001**
disturbed	0.128	0.049	2.63	**0.009**
(c) tree $\Phi_{FT}^{0.375}$				
Intercept (undisturbed)	0.477	0.09	5.271	**<0.001**
disturbed	0.055	0.021	2.622	**0.009**
	F_{ST}			
	Estimate	SE	Z-score	P-value
(a) non-woody $F_{ST}^{0.350}$				
Intercept (undisturbed)	0.524	0.077	6.781	**<0.001**
disturbed	0.084	0.046	1.816	0.070
(b) shrub $F_{ST}^{0.575}$				
Intercept (undisturbed)	0.400	0.057	6.985	**<0.001**
disturbed	0.027	0.041	0.668	0.504
(c) tree $F_{ST}^{0.200}$				
Intercept (undisturbed)	0.638	0.045	14.271	**<0.001**
disturbed	0.021	0.018	1.179	0.283

Chapter 2: Pollinators' tolerance to habitat disturbance and differences in their pollination services shape plant mating dynamics

Rieka Yu[1] and Nathan Muchhala[1]

[1]University of Missouri – St. Louis
One University Blvd. 223R Research Hall, Biology Department, St. Louis, MO 63121

Abstract

In the face of land use change, plant populations undergo changes in population genetic patterns and suffer decreased genetic diversity due to reduced population sizes and mate availability. However, growing evidence suggests that pollinators contribute to the degree to which habitat loss negatively impacts plant mating patterns. Here, we used *Campanula americana*, which is pollinated by bumblebees, the bellflower resin bee *Megachile campanulae*, and small bees, and investigated variations in pollen deposition, seed set, haplotype diversity, and inbreeding in order to explore the drivers of plant population genetic change. We used piecewise structural equation modeling (SEM) to determine whether habitat loss or differences in pollinator visits by different types of bees is the main driver of plant mating patterns. We found that not all pollinators are susceptible to habitat loss and that pollinator identity is the main driver of plant mating patterns. We specifically found that *M. campanulae* and small bees visit flowers equally regardless of the amount of habitat loss. *M. campanulae* increased seed set and haplotype diversity while visits by small bees decreased haplotype diversity. In this study we showed that the

pollinator community is critical to consider in any study focusing on habitat loss. Understanding the role of pollinators in both responding to habitat disturbance and influencing plant population dynamic has implications for future conservation and restoration practices.

Introduction

The majority of flowering plants depend on animal vectors for successful mating (Ollerton et al., 2011), making the maintenance of plant-pollinator interactions critical for the persistence of plant populations. Unfortunately, land use change threatens both plant and pollinator populations, paving the way for changes in plant-pollinator interactions (Sala et al., 2000; Fischer and Lindenmayer, 2007; Hadley and Betts, 2012; Aguilar et al., 2019). With land use change, plant populations suffer increased patch isolation and a reduction in population size, leading to limited mate availability and reductions in plant mating quality (Ellstrand and Elam, 1993; Young et al., 1996; Fahrig, 2003). Limited mate availability can lead to fewer pollen grains deposited on stigmas and a decrease in the diversity of the available pollen pool, leading to lower seed sets and reduced genetic diversity of offspring (Eckert et al., 2010; Vanbergen et al., 2014). With smaller and more isolated populations, the frequency of mating with related individuals will increase, leading to an increase in inbreeding which may also affect seed set (Aguilar et al., 2008; Eckert et al. 2010; Vanbergen et al. 2014; Aguilar et al., 2019). These changes in plant mating patterns would then lead to the loss of genetic diversity which has severe implications for population persistence.

Many studies have found the expected decrease in genetic diversity in disturbed populations, but some show differences in responses linked to pollinator identity. Emerging evidence suggests pollinators differ in their response to disturbance due to variation in behaviors, habitat preferences, and mobility, which will then indirectly influence plant mating patterns. For example, studies have shown bird pollinators deposit more diverse pollen loads and maintain gene flow in disturbed plant populations compared to insects (Breed et al., 2015; Krauss et al., 2017). Among insects, larger-bodied pollinators have been found to deposit larger pollen loads and facilitate greater genetic connectivity between plant populations (Gamba and Muchhala, 2020; Földesi et al., 2021; Wessinger, 2021). Sociality also plays an important aspect as social species tend to have larger foraging ranges and are more ecologically flexible in different habitats (Wenzel et al., 2020; Grüter and Hayes, 2022).

While habitat loss can reduce the populations of pollinators, the overall composition of land use types at the local and landscape scale are also important factors of pollinator community composition. In addition to floral resources, the presence of nesting habitats is important for maintaining pollinator populations. For example, in urban areas soil nesting bees become less abundant due to the amount of impervious surface while above-ground nesters increase in abundance due to the presence of cavities in buildings (Wilson and Jamieson, 2019; Wenzel et al., 2020). For bumblebees, the availability of nesting and overwintering habitat are important environmental factors influencing where queens establish their nests (Mola et al., 2021).

Many studies have documented the effects of habitat loss on plant population genetics, but the true drivers of these effects are still unclear. We are aware of only one study to date which has explored the direct and indirect drivers of population genetic change in a tropical system (Torres-Vanegas et al., 2021). This study showed that forest patch size had no direct effect on genetic diversity but did lead to changes in hummingbird community composition which was associated with plant mating quality (Torres-Vanegas et al., 2021).

Here, we examine the role of direct and indirect effects of habitat loss on plant mating patterns in a temperate system, using path analyses to assess how both floral and nesting resources affect pollinator communities, plant mating success (pollen deposition and seed set) and quality (haplotype diversity and inbreeding). We hypothesize that changes in plant population genetics in disturbed habitats is driven by changes in the pollinator community, and thus predict direct relationships between habitat loss and pollinators as well as between pollinators and plant mating success and quality. Alternatively, habitat loss can lead to changes in plant population genetics due to changes in floral availability. In this instance we would expect to see a direct relationship between disturbance and plant mating quality.

Methods

Study system

Campanula americana is an annual or biennial herb native to eastern North America and is found in open forests. It is self-compatible and protandrous, with anthers dehiscing before flowers open and presenting pollen on pollen-collecting hairs along the style (Koski et al. 2018). Pollinators remove pollen within the first day, after which the stigmatic lobes open to receive pollen (Koski et al., 2018). The main visitors of *C. americana* are bumblebees, the bellflower resin bee *Megachile campanulae*, and small solitary bees in Halictidae and Apidae (Koski et al., 2018). Of the three groups of pollinators, bumblebees are the most efficient pollinators, depositing the greatest number of pollen grains per visit and leading to the greatest seed set (Koski et al., 2018). *M. campanulae* and small bees deposit similar levels of pollen but *M. campanulae* facilitates greater seed sets than small bees (Koski et al., 2018). Because small bees remove pollen, they increase pollen limitation with each visit.

Study design

We studied *C. americana* populations in St. Louis, St. Charles, Jefferson, and Franklin County in Missouri. We filmed one flower per 63 focal *C. americana* individuals across nine study sites (Figure 1). Flowers were filmed during its female phase for one day during peak pollinator activity (11:00 – 14:45). We bagged flowers before and after filming to ensure no visits outside this period. From videos we recorded the number of visits/flower by bumblebees, *M. campanulae*, and small bees. The resulting fruits from focal flowers were collected after four weeks for genotyping. We additionally collected maternal leaves for genetic analyses. From fruits we counted seed set then planted up to 10 seeds per maternal plant in a growth chamber with a 12 hours cycle of 21^{o}C and 14^{o}C. This resulted in 1-7 seedlings from 24 maternal families for DNA extraction. We also collected stigmas from the filmed flowers to count the number of deposited *C. americana* pollen grains. Stigmas were collected 24 hours after filming ended and were placed in Farmer’s solution for 24 hours to fix stigmas and then transferred to 70% ethanol until slide preparation. Stigmas were affixed to a slide with clear tape and stained with fuchsin gel after which we counted pollen grains under a microscope. Some stigmas were damaged during processing and therefore we have pollen counts for 59 out of the 63 focal flowers we sampled.

Because the local floral community can influence pollen loads, we counted the number of conspecific and heterospecific flowers in a 10-m radius around focal individuals. Linear mixed effects models with site as a random factor showed no effect of the number of conspecific flowers and that the number of heterospecific flowers only influenced the number of bumblebee visits (Table S1). We therefore included the number of heterospecific flowers as a predictor variable of bumblebee visits for analyses.

As a measure of habitat loss, we calculated the amount of *C. americana* habitat and proportion of forest in a 150-m and 1-km radius around each focal individual. Forest cover was calculated from the National Landcover Database (NLCD) in QGIS and included deciduous forest, evergreen forest, and mixed forest (Dewitz and U.S. Geological Survey, 2021) (Figure S1; Table S2). We used personal and GBIF observations of *C. americana* and Landsat-8 bands 1-7 to model the habitat distribution for *C. americana* using Maxent (Phillips and Dudík, 2008). The output of Maxent assigns cells a value of habitat suitability ranging from 0-1, with 1 being the most suitable (Figure S2). As a measure of the amount of *C. americana* habitat we took the sum of these cells in a 150-m and 1-km radius around each focal individual. The availability of four different types of nesting areas (cultivated, developed, barren, and grassland) was also calculated from the NLCD in QGIS. We separately measured the proportion of cultivated, developed, barren, and grassland areas in a 150-m and 1-km radius. Because bees have a diversity of nesting behaviors (e.g. ground-nesting or nesting in hollow stems), each of these four land types were considered as a different type of nesting habitat.

Genetic analyses

For maternal plants and offspring, we extracted DNA following the CTAB method (Doyle and Doyle, 1987). We genotyped individuals at eight microsatellite loci using polymerase chain reaction (PCR) in two multiplex PCR assays according to Koski et al. 2018. PCRs were run using 25uL Bioline MyTaq PCR mix as well as dH_2O, fluorescently tagged forward primers, and reverse primers, with a final concentration of 0.4uM of each forward and reverse primer. The amplification procedure followed Koski et al. (2018). Fragment analyses were conducted by the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign. Samples were run on an Applied Biosystems 3730xl DNA Analyzer with a Liz500 size standard. We identified alleles through GeneMapper, and binned alleles with Tandem2. Tandem2 bins alleles based on the length of microsatellite repeat motifs. We inferred tetraploid genotypes with an R script calculating the relative area of allele peaks (Esselink et al., 2004; Koski et al., 2019).

As measures of plant mating quality, we estimated the haplotype diversity of paternal donors of the seedlings that we germinated in the growth chamber and counted the number of seeds per fruit. From maternal and offspring genotypes, we calculated pollen haplotypes by subtracting the maternal genotype from the corresponding offspring genotype using the R package GSTUDIO (Dyer, 2014). With the available R code from Torres-Vanegas et al. (2021) we calculated the haplotype diversity as the probability that the paternal alleles of two randomly chosen offspring are different (Nei, 1987) and averaged this value across all loci. Individuals that did not set seed were given a haplotype diversity value of zero because the pollen donors effectively made no contribution to the next generation’s population. However, we also repeat these analyses for only those individuals that set seed. We used SPAGeDi to calculate individual inbreeding coefficients per loci, calculated as the kinship coefficient between gene copies between individuals (Hardy and Vekemans, 2003). We then averaged the inbreeding coefficients across loci for each individual. Because only 24 out of 63 individuals set seed we do not have inbreeding coefficients for the whole data set.

Statistical analyses

To determine the effects of floral and nesting resources as well as pollinator visits on haplotype diversity and seed set we performed piecewise structural equation modeling (SEM). Piecewise SEMs consist of multiple linear equations, each representing a hypothesized causal relationship (Shipley, 2000; Lefcheck, 2015).

Prior to performing piecewiseSEMS we estimated variance inflation factors (VIF) to evaluate correlations among predictor variables. Using a value of VIF ≥ 5 to detect collinearity, we found all predictor variables were below 5 and could be included together in our models (Table S3). We also ran linear mixed effects models to determine which land use categories at which scales were significant predictors of pollinator visits. Significant variables were then included in the hypothesized relationships of the piecewise SEM. These mixed effect models showed that for bumblebees, but not other the other pollinators, the proportion of cultivated land in a 150-m radius positively influenced the number of visits (Table S4-S9). We therefore included the proportion of cultivated land in a 150-m as a predictor variable.

Using the R package piecewiseSEM, we evaluated the direct and indirect effects of floral and nesting resources on the number of visits by each type of bee, number of deposited pollen grains, seed set, and haplotype diversity separately for three separate piecewiseSEMs (Lefcheck, 2015). We tested path models both with and without direct effects of habitat loss on plant mating quality at the 150-m and 1-km scale separately

(Table S9). For each path model, we used linear mixed-effects models with site included as a random variable.

We tested if the hypothesized relationships in our piecewiseSEMs was missing any additional relationships using Shipley's test of directed separation, also known as the d-separation test (Shipley, 2000, 2013). The d-separation test showed a missing positive relationship between the number of *M. campanulae* and small bee visits for all models. However, because we do not have previous knowledge to hypothesize the causal relationship between these variables, we did not include this relationship in our path models. We selected final path models that had the greatest number of significant explanatory variables with the lowest AIC score. We used Fisher's C statistic to evaluate the goodness-of-fit for each path model for which a non-significant P value indicates the hypothesized relationships match the data (Lefcheck, 2015). For each model we also estimated the standardized regression coefficient and the conditional R^2 from the piecewiseSEM package. Because we were missing inbreeding coefficients for a large part of the dataset, we ran separate linear mixed-effects models testing the effect of habitat loss, land use type, and pollinator on inbreeding coefficients. Site was set as a random effect in these models.

Results

Based on videos of focal *C. americana* flowers bumblebees had visitation rates of 0.17 visits/hour (SE = 0.06), *M. campanulae* had visitation rates of 3.36 visits/hour (SE = 0.88), and small bees had visitation rates of 5.86 visits/hour (SE = 1.40). The average number of heterospecific flowers in a 10-m radius around focal *C. americana* flowers was 163.5flowers (SE = 42.1). The average number of deposited pollen grains was

160.42 grains (SE 18.11), and average seed set was 12.75 seeds per flower (SE = 2.13) (Figure S3 and S4). Average haplotype diversity was 0.12 (SE = 0.02) and average individual inbreeding coefficients was 0.05 (SE = 0.02) (Figure S5 and S6).

Our best fit piecewise SEM showed no direct effect of the number of heterospecific flowers, the amount of *C. americana* habitat, or the amount of forest area on haplotype diversity, seed set, or the number of deposited pollen grains by. There was evidence of indirect effects of the number of heterospecific flowers and the amount of forested area and cultivated area on plant mating patterns through changes in pollinator visits (Figure 2; Table S9). When repeating analyses using haplotype diversity values for only the individuals that set seed, we found that the number of pollinator visits by any type of bee did not affect haplotype diversity (Figure S7). We found no effects of the amount of forested area and land use type or number of pollinator visits on inbreeding coefficients (Table 1).

Discussion

Our results support the hypothesis that changes in mating patterns in disturbed plant populations are caused by changes in the pollinator community rather than the availability of potential mates. We find no direct effects of the amount of forest or suitable habitat on the number of deposited pollen grains, seed set, or haplotype diversity in *C. americana*. Instead, we find that the mating dynamics of *C. americana* is affected by the identity of pollinators that visit it, which in turn is affected by land use type.

We show that floral resources are important predictors of bumblebee visits to *C. americana*. Specifically, increased number of heterospecific flowers in a 10-m radius around focal *C. americana* individuals led to increases in the number of bumblebee visits.

Evidence suggests that both floral abundance and diversity are significant attractants of bumblebees (Hegland and Boeke, 2006; Hülsmann et al., 2015; Martínez-Bauer et al., 2021). In fact, bumblebees show low floral constancy in the field and actively forage on different plant species (Martínez-Bauer et al., 2021). Although our data on the number of heterospecific flowers does not have information on species richness, the fact that the number of conspecific flowers did not affect bumblebee visits to focal *C. americana* individuals indicates bumblebees may have been responding to floral diversity in our system.

Unexpectedly, our results show floral resources around focal flowers had no effect on the number of visits by small bees or *M. campanulae*. For small bees like *Lasioglossum* and *Halictus*, research suggests the stability of floral abundance in previous months is an important predictor of visits (Guezen and Forrest, 2021). However, in our study measures of conspecific and heterospecific flowers represented current floral abundance at the time of visitation while the species distribution model and the proportion of forest were measures of habitat suitability for *C. americana* only. This lack of temporal information may explain why we found no relationship between floral resources and small bees. *Megachile* bees on the other hand respond to landscape level floral resources but are dispersed across the landscape (Guezen and Forrest, 2021). It is possible we did not find landscape level effects because there was not enough variation in the amount of suitable *C. americana* habitat in our study.

In addition to responding to local floral diversity, bumblebees also respond to land use types at a local scale, with visits to *C. americana* increasing with the proportion of cultivated and forested area in a 150-m radius. The exact nesting preferences of bumblebees are unknown, but their nests are frequently found in grass-dominated field boundaries of agriculture and forest (Svensson et al., 2000). This potentially explains why bumblebee visitation increases with both cultivated and forested areas. Although bumblebees will not nest in areas where active tilling occurs, they will nest in cultivated areas that have grasses for livestock grazing in addition to forests (Svensson et al., 2000; Liczner and Colla, 2019). Because there was a positive relationship between the number of bumblebee visits and cultivated areas, the much of the cultivated area in our study area are likely no-tilling areas. We did not expect land use type to be significant only at a local scale for bumblebees because they are able to forage several kilometers from their nest (Osborne et al., 2008; Redhead et al., 2016). Despite the ability to fly long distances, average bumblebee foraging ranges vary from ~200-600m (Darvill et al., 2004; Wolf and Moritz, 2008; Redhead et al., 2016), which is likely why we only see effects at the 150-m scale.

We did not find correlations between any land use type and *M. campanulae* visits. *M. campanulae* appears to be flexible in its nest use, and they have even been observed using plastic bags as nesting material (MacIvor and Moore, 2013). This ability to use novel nest materials may indicate that *M. campanulae* are able to nest in multiple environments and are not associated with any particular land use type. The lack of correlation between land use type and *M. campanulae* visits highlights the fact that not all pollinators are susceptible to habitat disturbance and that *M. campanulae* can be important for maintaining pollination services to *C. americana* when disturbance decreases visits by other bees. We also found that small bee visits were not correlated with land use types, however, this may be because the grouping of small bees in this study include multiple groups that differ in nesting behavior. By grouping all small bees into a single group, we likely lost fine-grain effects of land use on particular species.

We found pollinators directly affected plant mating patterns, with the number *M. campanulae* visits positively correlated with seed set and haplotype diversity. This is likely because *M. campanulae* is oligolectic, preferentially visiting flowers from *Campanula* (Michener, 2000), and is less likely to carry and deposit heterospecific pollen grains. This deposition of a greater number of pollen grains is then translated to greater seed set and haplotype diversity. We expected to find similar patterns for bumblebees but instead found no relationship between the number of bumblebee visits and plant mating quality. This may be because bumblebees visit multiple species of flowers and are not depositing a large enough number of pollen grains to make statistical inferences.

For small bees, we found that their visits were negatively correlated with haplotype diversity. Small bees visited multiple flowers within the same individual (personal observation), which may have increased the amount of self-pollen on their bodies. Small bees also have relatively small bodies, which has been shown to be correlated with depositing smaller pollen loads (Földesi et al., 2021). With smaller pollen loads, visits to within-individual flowers likely displace greater proportions of outcross pollen, leading to decreased haplotype diversity with greater visits by small bees. Small bees also mostly collect pollen rather than depositing pollen when visiting *C. americana* (Koski et al., 2018), and by removing pollen grains on stigmas, they can reduce the genetic diversity of the deposited pollen. This is supported by previous work in *C. americana* that shows greater visits by small bees leads to greater pollen limitation (Koski et al., 2018).

To our knowledge, this is the first study to investigate the role of pollinators in mediating the effects of habitat disturbance on plant mating patterns in a temperate system. In this study we disentangled the mechanisms of plant population genetic change in disturbed habitats. We found that pollinators, not habitat loss, have direct effects on plant mating quality. We specifically showed that different types of pollinators both differentially respond to habitat characteristics and differentially affect plant mating patterns. We also identified foraging resources and nesting habitats as important predictors of pollinator availability, which is not always included in investigations of habitat loss and plant population genetics.

Our results contribute to growing evidence that pollinators are key components of how plant populations respond to changes in the environment (Hadley and Betts, 2012; Breed et al., 2015; Aguilar et al., 2019). The relationship between the habitat, pollinators, and plant mating patterns ultimately has implications for plant population persistence, the maintenance of genetic diversity, and understanding the selective pressures on plant populations. The pollination system of *C. americana* is unique in that we can compare three different functional groups of pollinators and by studying other generalist pollination systems with multiple functional groups researchers can make additional comparisons of pollinator groups. Repeating a study like this one in multiple systems also allows for better generalizations of pollinator performance, and therefore a better ability to understand mechanisms of genetic change. We therefore encourage future research to better understand how pollinator communities respond to their environments and how it impacts plant populations in different systems throughout the globe.

Author contribution

All authors contributed to study design. R.Y. collected data, performed analyses, and wrote the manuscript. All authors contributed substantially to revisions.

Acknowledgements

This work was funded by the Whitney R. Harris World Ecology Center, the Botanical Society of America, and the Missouri Botanical Garden. We would like to thank the Litzsinger Road Ecology Center and Shaw Nature Reserve of the Missouri Botanical Garden, the Missouri state park system, and the Missouri Department of Conservation for allowing us to conduct field work on their property. We thank Timothy Zhang, Kelly Toohey, Raj Prasai, Vivian Congleton, and Bree Carlton for help with field work. We also thank Nicole Koleas and Pearl Abecia for help with both field and lab work.

Literature Cited

Aguilar, R., E. J. Cristóbal-Pérez, F. J. Balvino-Olvera, M. Aguilar-Aguilar, N. Aguirre-Acosta, L. Ashworth, J. A. Lobo, et al. 2019. Habitat fragmentation reduces plant progeny quality: a global synthesis J. M. Gomez [ed.],. *Ecology Letters* 22: 1163–1173.

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

Breed, M. F., K. M. Ottewell, M. G. Gardner, M. H. K. Marklund, E. E. Dormontt, and A. J. Lowe. 2015. Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. *Heredity* 115: 108–114.

Darvill, B., M. E. Knight, and D. Goulson. 2004. Use of genetic markers to quantify bumblebee foraging range and nest density. *Oikos* 107: 471–478.

Dewitz, J. and U.S. Geological Survey. 2021. National Land Cover Database (NLCD) 2019 Products (ver. 2.0, June 2021): U.S. Geological Survey data release.

Doyle, J., and J. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*.

Dyer, R. J. 2014. An introduction to the gstudio Package: R package version 1.2. Website https://dyerlab.github.io/gstudio/.

Eckert, C.G., Kalisz, S., Geber, M.A., Sargent, R., Elle, E., Cheptou, P.-O., *et al.* (2010). Plant mating systems in a changing world. *Trends in Ecology & Evolution*, 25, 35–43.

Ellstrand, N. C., and D. R. Elam. 1993. Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. 24.

Esselink, G. D., H. Nybom, and B. Vosman. 2004. Assignment of allelic configuration in polyploids using the MAC-PR (microsatellite DNA allele counting—peak ratios) method. *Theoretical and Applied Genetics* 109: 402–408.

Fahrig, L. 2003. Effects of Habitat Fragmentation on Biodiversity. *Annual Review of Ecology, Evolution, and Systematics* 34: 487–515.

Fischer, J., and D. B. Lindenmayer. 2007. Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography* 16: 265–280.

Földesi, R., B. G. Howlett, I. Grass, and P. Batáry. 2021. Larger pollinators deposit more pollen on stigmas across multiple plant species—A meta-analysis A. Magrach [ed.],. *Journal of Applied Ecology* 58: 699–707.

Gamba, D., and N. Muchhala. 2020. Global patterns of population genetic differentiation in seed plants. *Molecular Ecology* 29: 3413–3428.

Grüter, C., and L. Hayes. 2022. Sociality is a key driver of foraging ranges in bees. *Current Biology* 32: 5390-5397.e3.

Guezen, J. M., and J. R. K. Forrest. 2021. Seasonality of floral resources in relation to bee activity in agroecosystems. *Ecology and Evolution* 11: 3130–3147.

Hadley, A. S., and M. G. Betts. 2012. The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. *Biological Reviews* 87: 526–544.

Hardy, O.J. & 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.

Hegland, S. J., and L. Boeke. 2006. Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology* 31: 532–538.

Hülsmann, M., H. Von Wehrden, A.-M. Klein, and S. D. Leonhardt. 2015. Plant diversity and composition compensate for negative effects of urbanization on foraging bumble bees. *Apidologie* 46: 760–770.

Koski, M. H., L. F. Galloway, and J. W. Busch. 2019. Pollen limitation and autonomous selfing ability interact to shape variation in outcrossing rate across a species range. *American Journal of Botany* 106: 1240–1247.

Koski, M. H., J. L. Ison, A. Padilla, A. Q. Pham, and L. F. Galloway. 2018. Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism. *Proceedings of the Royal Society B: Biological Sciences* 285: 20180635.

Krauss, S. L., R. D. Phillips, J. D. Karron, S. D. Johnson, D. G. Roberts, and S. D. Hopper. 2017. Novel Consequences of Bird Pollination for Plant Mating. *Trends in Plant Science* 22: 395–410.

Lefcheck, J. S. 2015. PIECEWISESEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*.

Liczner, A. R., and S. R. Colla. 2019. A systematic review of the nesting and overwintering habitat of bumble bees globally. *Journal of Insect Conservation* 23: 787–801.

MacIvor, J. S., and A. E. Moore. 2013. Bees collect polyurethane and polyethylene plastics as novel nest materials. *Ecosphere* 4: art155.

Martínez-Bauer, A. E., F. J. Chadwick, A. J. Westmoreland, and T. A. Lander. 2021. Novel pollen analogue technique shows bumblebees display low floral constancy and prefer sites with high floral diversity. *Landscape Ecology* 36: 3231–3247.

Michener, C. D. 2000. The bees of the world. Johns Hopkins University Press, Baltimore, Md.

Mola, J. M., J. Hemberger, J. Kochanski, L. L. Richardson, and I. S. Pearse. 2021. The Importance of Forests in Bumble Bee Biology and Conservation. *BioScience* 71: 1234–1248.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? *Oikos* 120: 321–326.

Osborne, J. L., A. P. Martin, N. L. Carreck, J. L. Swain, M. E. Knight, D. Goulson, R. J. Hale, and R. A. Sanderson. 2008. Bumblebee flight distances in relation to the forage landscape. *Journal of Animal Ecology* 77: 406–415.

Phillips, S. J., and M. Dudík. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* 31: 161–175.

Redhead, J. W., S. Dreier, A. F. G. Bourke, M. S. Heard, W. C. Jordan, S. Sumner, J. Wang, and C. Carvell. 2016. Effects of habitat composition and landscape structure on worker foraging distances of five bumble bee species. *Ecological Applications* 26: 726–739.

Sala, O. E., F. Stuart Chapin, Iii, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, et al. 2000. Global Biodiversity Scenarios for the Year 2100. *Science* 287: 1770–1774.

Shipley, B. 2000. A New Inferential Test for Path Models Based on Directed Acyclic Graphs. *Structural Equation Modeling: A Multidisciplinary Journal* 7: 206–218.

Shipley, B. 2013. The AIC model selection method applied to path analytic models compared using a d-separation test. *Ecology* 94: 560–564.

Svensson, B., J. Lagerlöf, and B. G. Svensson. 2000. Habitat preferences of nest-seeking bumble bees (Hymenoptera: Apidae) in an agricultural landscape. *Agriculture, Ecosystems & Environment* 77: 247–255.

Torres-Vanegas, F., A. S. Hadley, U. G. Kormann, F. A. Jones, M. G. Betts, and H. H. Wagner. 2021. Tropical deforestation reduces plant mating quality by shifting the functional composition of pollinator communities R. Phillips [ed.],. *Journal of Ecology* 109: 1730–1746.

Vanbergen, A.J. (2014). Landscape alteration and habitat modification: impacts on plant–pollinator systems. *Current Opinion in Insect Science*, 5, 44–49.

Wenzel, A., I. Grass, V. V. Belavadi, and T. Tscharntke. 2020. How urbanization is driving pollinator diversity and pollination – A systematic review. *Biological Conservation* 241: 108321.

Wessinger, C. A. 2021. From pollen dispersal to plant diversification: genetic consequences of pollination mode. *New Phytologist* 229: 3125–3132.

Wilson, C. J., and M. A. Jamieson. 2019. The effects of urbanization on bee communities depends on floral resource availability and bee functional traits M. (Gee) G. Chapman [ed.],. *PLOS ONE* 14: e0225852.

Wolf, S., and R. F. A. Moritz. 2008. Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). *Apidologie* 39: 419–427.

Young, A., T. Boyle, and T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution* 11: 413–418.

Figures

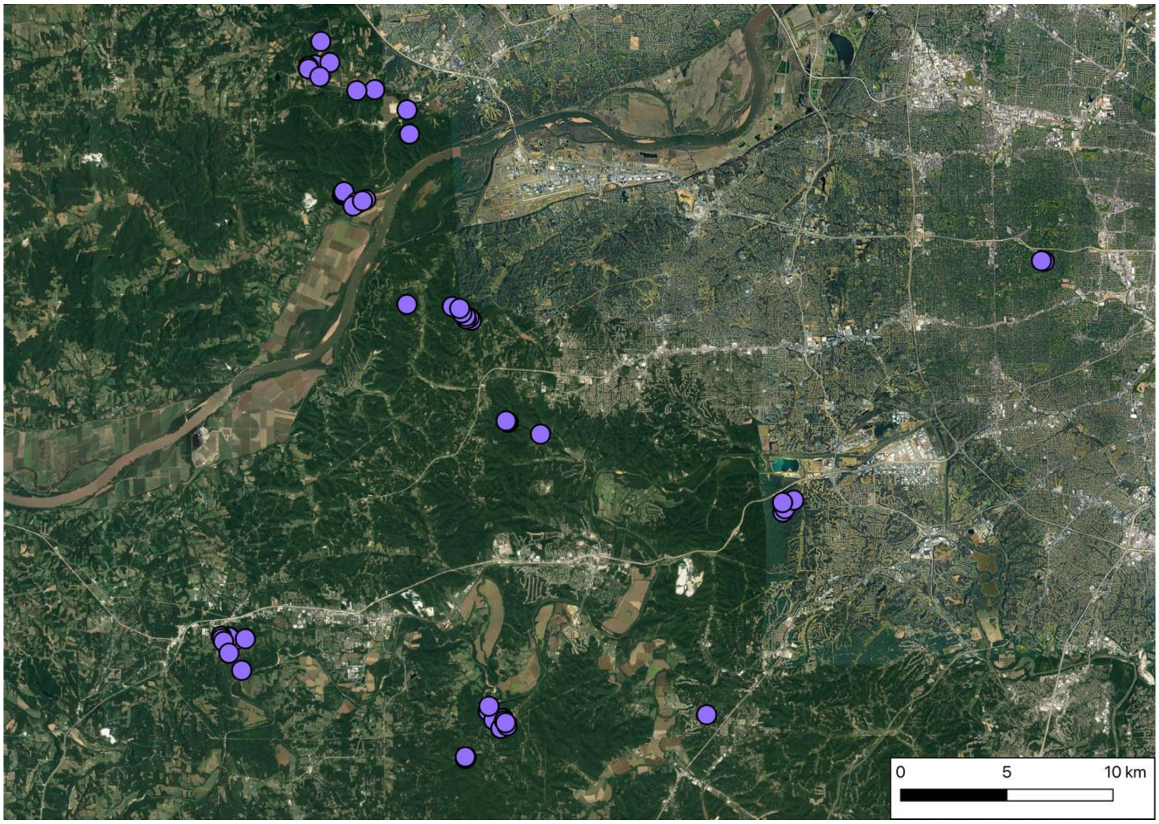

Figure 1. Locations of focal *Campanula americana* individuals across study sites. This study's focal individuals are shown as purple points. Points are shown on a map of Google Terrain.

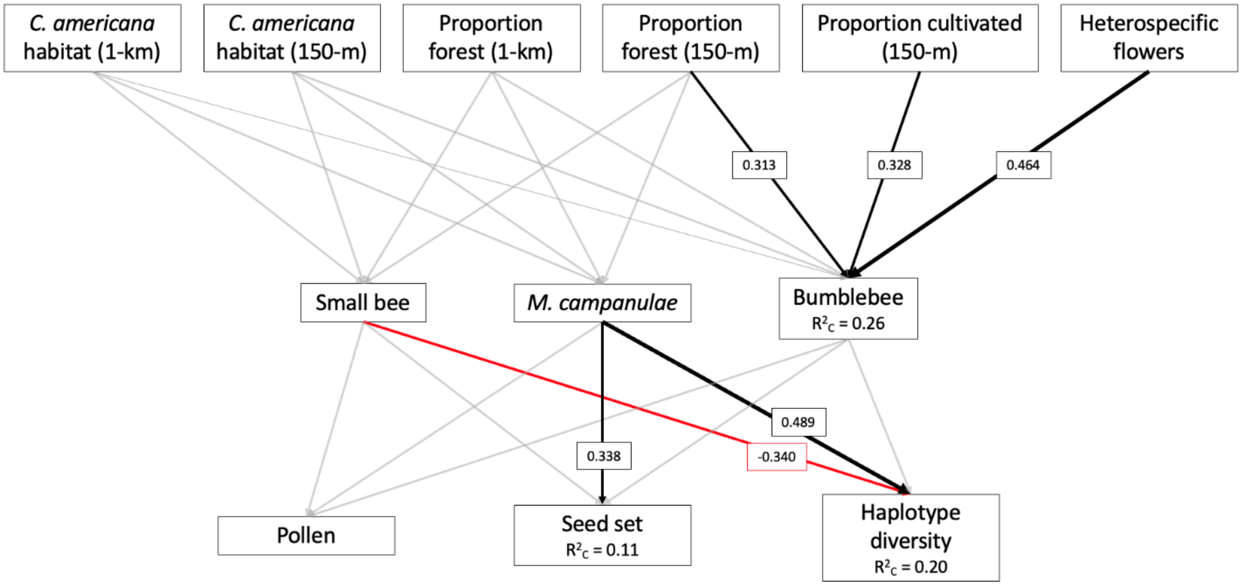

Figure 2. Relationships between floral resource, land use type, pollinator, and plant mating quality. All arrows show hypothesized effects tested with piecewise SEM. Significant effects are bold and non-significant effects are gray. Negative effects are shown in red and positive effects are shown in black. For significant effects standardized path coefficients are shown in boxes with arrows and the conditional R^2_C are shown with response variables. Arrows are scaled to the magnitude of the standardized path coefficient.

Tables

Table 1. Results of the linear mixed effects models analyzing the effect of habitat loss and pollinator visits on individual inbreeding coefficients. We ran three separate models testing the effect of habitat loss at the 1-km scale and the 150-m scale and the effect of pollinator visits. Site was included as a random variable.

1-km scale

	β	SE	df	t-value	p-value
Intercept	-3.282	2.392	6	-1.372	0.219
Forest	-0.016	0.388	5	-0.041	0.969
C. americana habitat	0.444	0.331	5	1.342	0.237

150-m scale

	β	SE	df	t-value	p-value
Intercept	-0.946	0.870	6	-1.087	0.319
Forest	0.048	0.157	5	0.305	0.773
C. americana habitat	0.246	0.231	5	1.062	0.337

Pollinator

	β	SE	df	t-value	p-value
Intercept	0.108	0.098	6	1.101	0.313
Bumblebee	-0.048	0.082	4	-0.587	0.589
M. campanulae	-2.00E-04	0.040	4	-0.005	0.996
Small bee	-0.130	0.032	4	-0.418	0.697

Supplementary materials

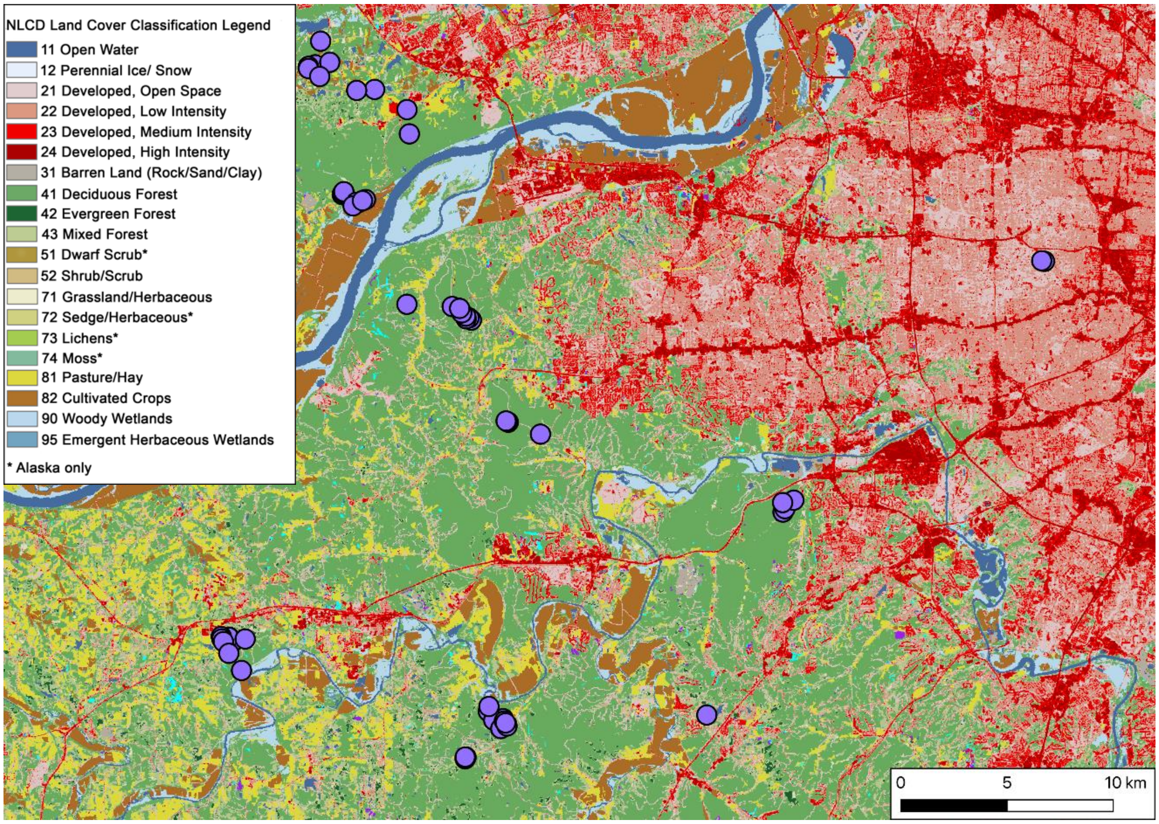

Figure S1. Focal individuals of *C. americana* mapped on the National Land Cover Database. Individuals are shown as purple points. The legend shows the color classifications of land type, which was used to calculate the amount of forest and other land use types.

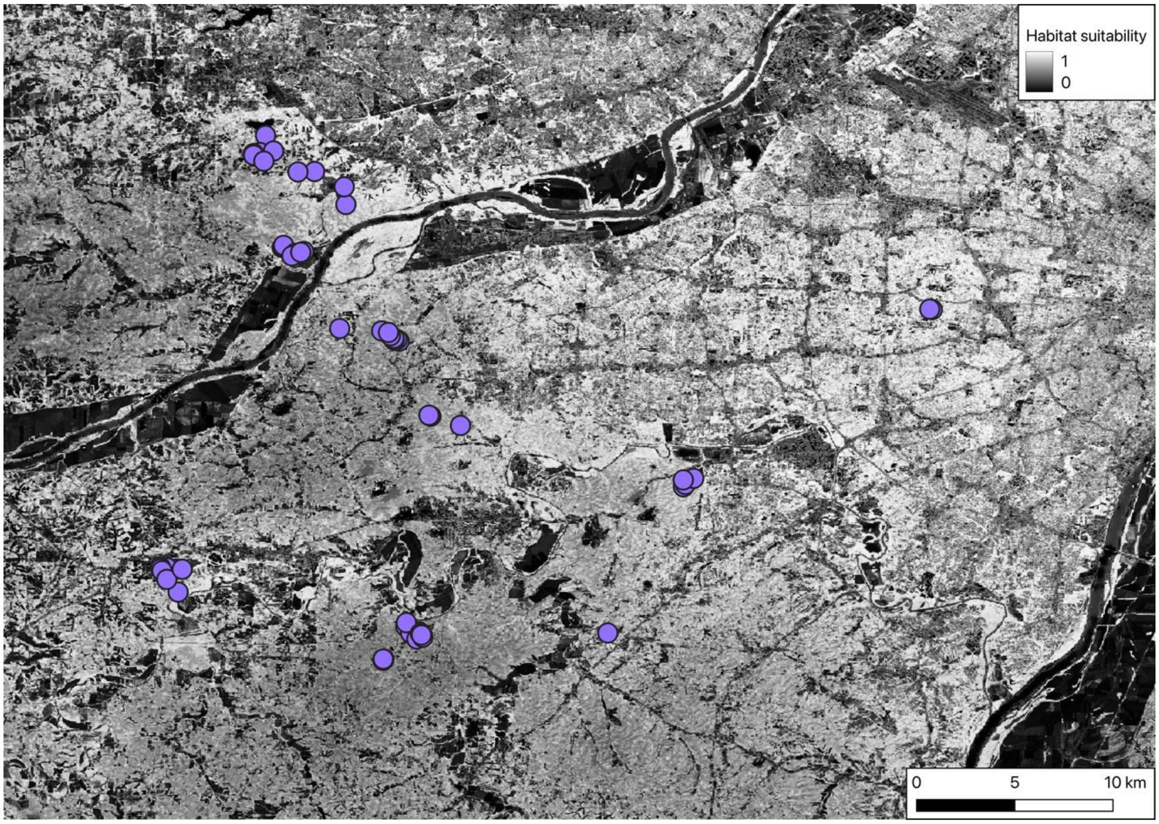

Figure S2. The results of the species distribution model for *C. americana*. The species distribution model was created in Maxent, using Landsat-8 bands 1-7 as well as *C. americana* observations from GBIF and this study. To model the species distribution, we performed multiple runs with our data using the bootstrapping resampling method with 1000 replicates. The mean AUC, a measure of the accuracy of prediction, was 0.815.

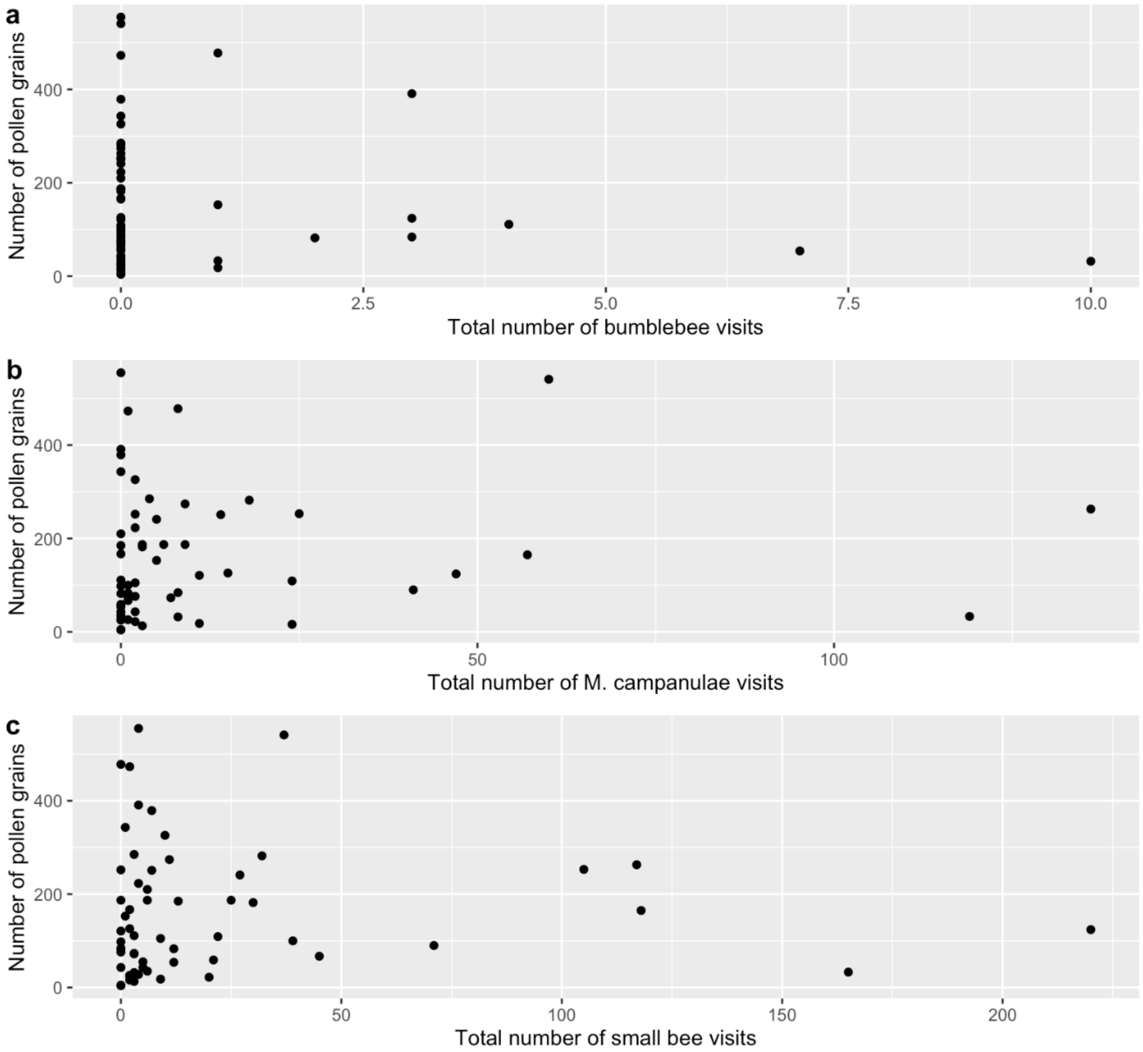

Figure S3. The number of deposited pollen grains as a function of pollinator visits. Points show the number of pollen grains counted on stigmas (n = 59) that were collected from focal *C. americana* flowers. Values are shown as a function of the total number of visits to focal flowers by **a** bumblebees, **b** *M. campanulae*, and **c** small bees.

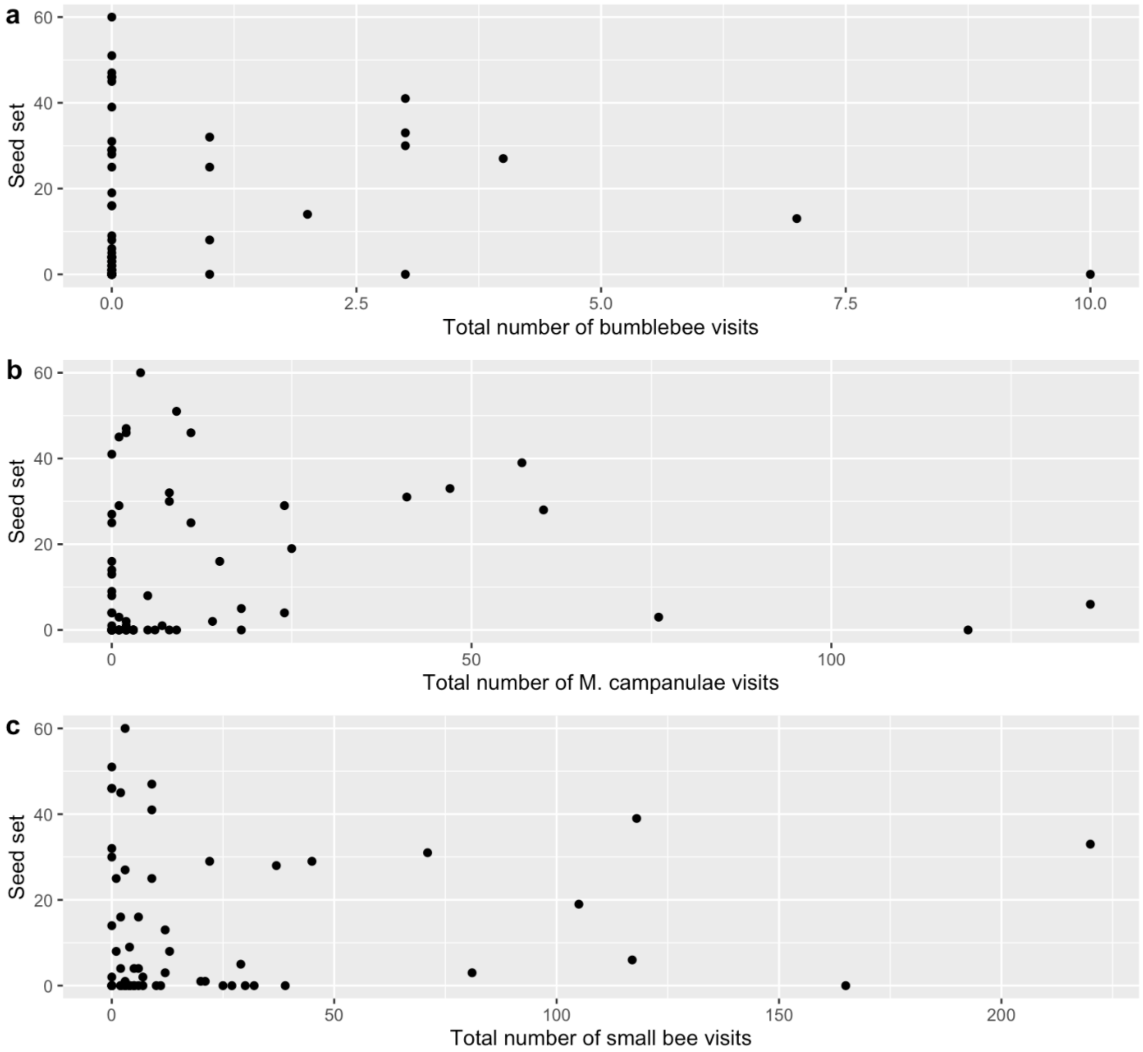

Figure S4. Seed set as a function of pollinator visits. Points show the number of seeds from fruits (n = 63) that were collected from focal *C. americana* flowers. Values are shown as a function of the total number of visits to focal flowers by **a** bumblebees, **b** *M. campanulae*, and **c** small bees.

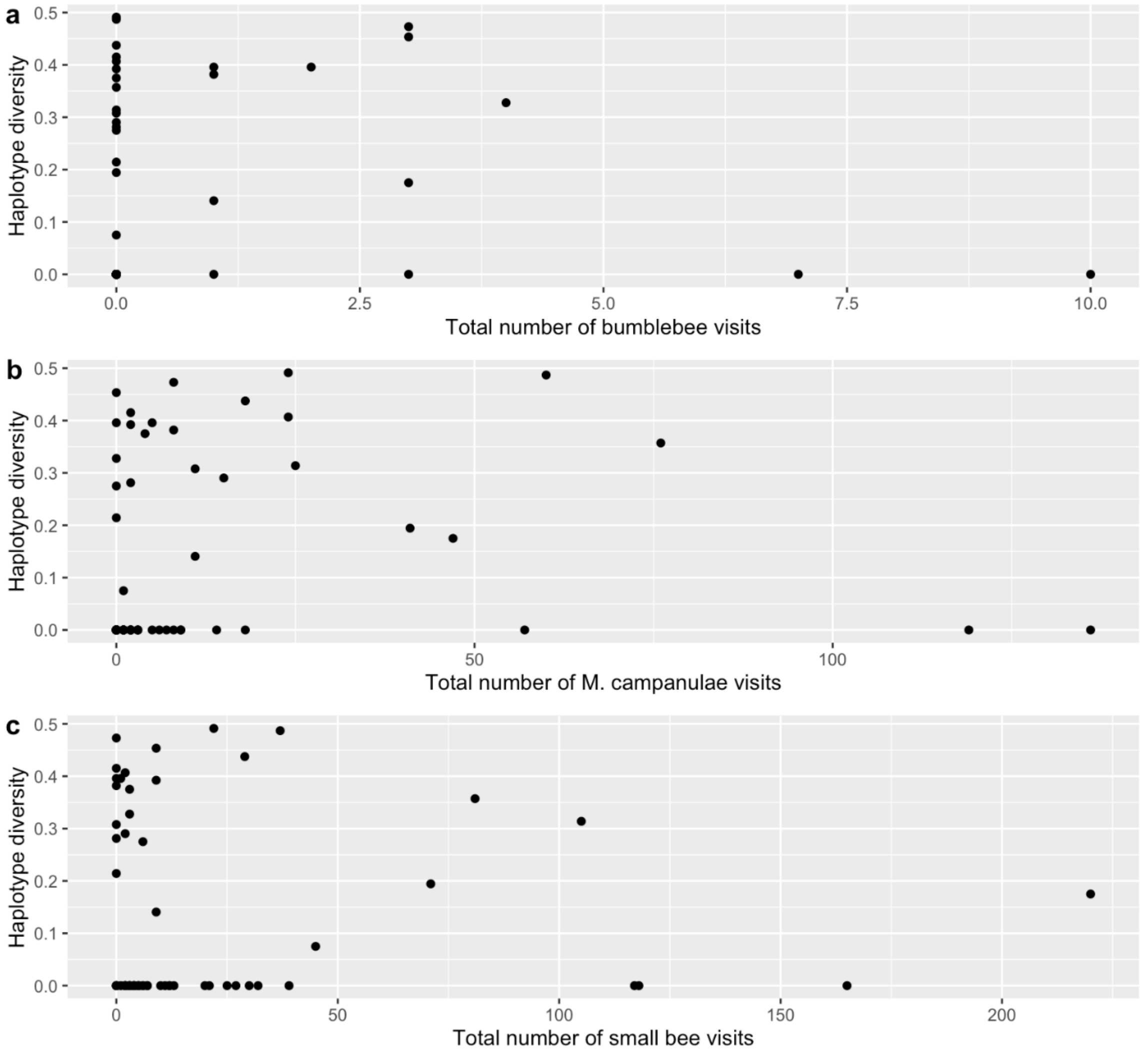

Figure S5. Haplotype diversity of *C. americana* offspring as a function of pollinator visits. Points show the haplotype diversity of seedlings (n = 63) grown from the fruits collected from focal *C. americana* flowers. Values are shown as a function of the total number of visits to focal flowers by **a** bumblebees, **b** *M. campanulae,* and **c** small bees.

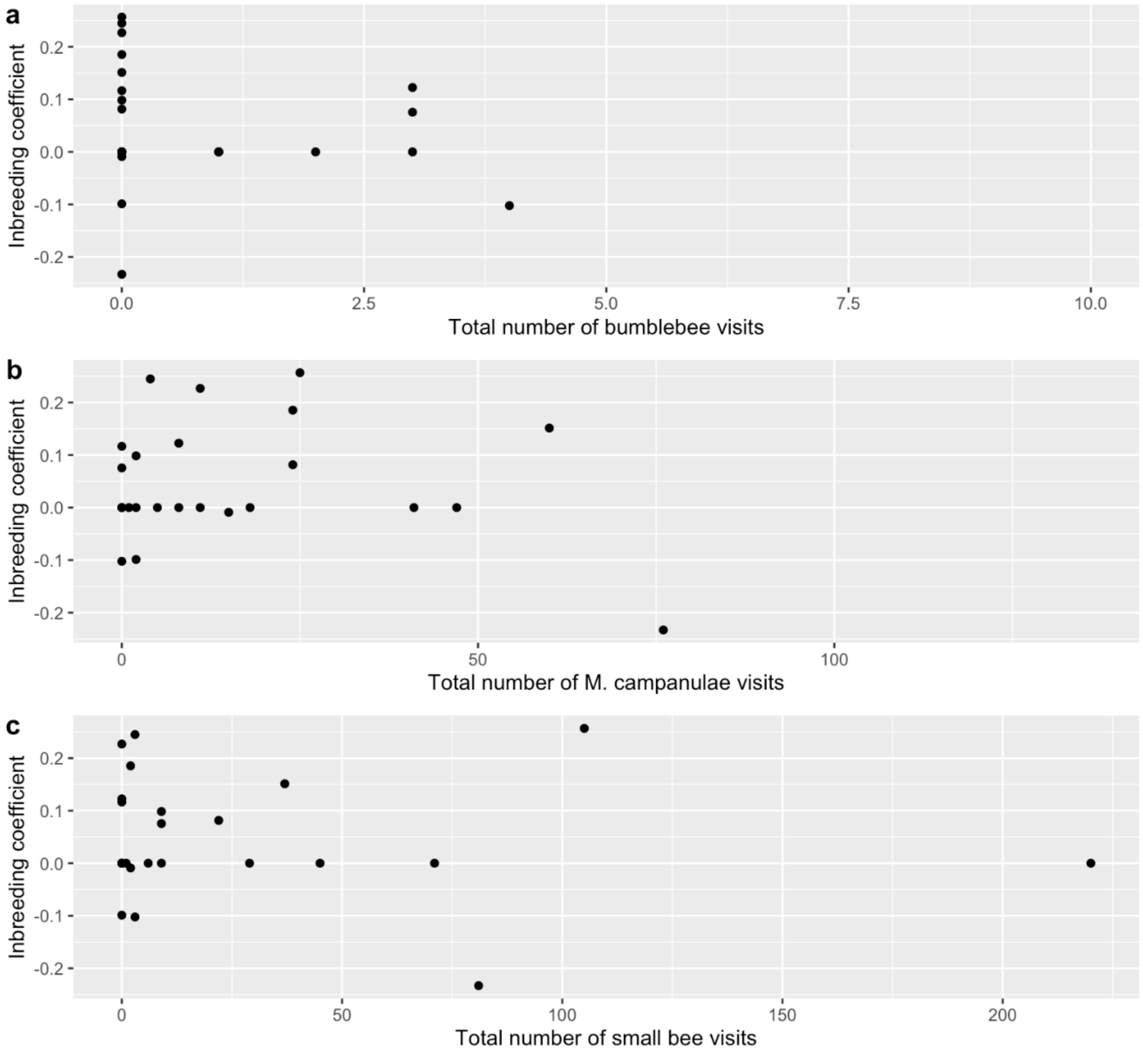

Figure S6. Inbreeding coefficients as a function of pollinator visits. Points show the inbreeding coefficients of seedlings (n = 24) grown from the fruits that were collected from focal *C. americana* flowers. Values are shown as a function of the total number of visits to focal flowers by **a** bumblebees, **b** *M. campanulae*, and **c** small bees.

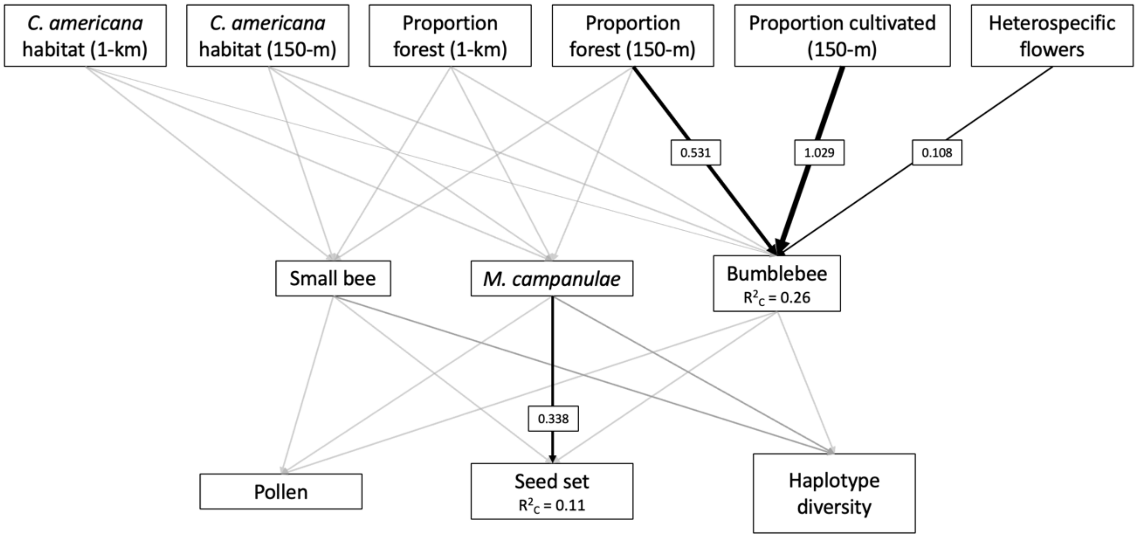

Figure S7. Relationships between floral resource, land use type, pollinator, and plant mating quality. The dataset used in this SEM includes haplotype diversity values (n = 24) for only flowers that set seed. All arrows show hypothesized effects tested with piecewise SEM. Significant effects are bold and non-significant effects are gray. Positive effects are shown in black. For significant effects standardized path coefficients are shown in boxes with arrows and the conditional R^2_C are shown with response variables. Arrows are scaled to the magnitude of the standardized path coefficient.

Table S1. Results of linear mixed-effects models analyzing the effect of the number of conspecific and heterospecific flowers in a 10-m radius around focal *C. americana* flowers. Site was included as a random factor. We modeled effects on (a) haplotype diversity, (b) seed set, (c) number of bumblebee visits, (d) number of *M. campanulae* visits, (e) number of small bee visits, and (f) number of pollen grains

(a) haplotype diversity

	β	SE	df	t-value	p-value
Intercept	0.228	0.109	51	2.084	0.04
conspecific	-0.013	0.038	51	-0.35	0.728
heterospecific	0.018	0.018	51	1.044	0.302

(b) seed set

	β	SE	df	t-value	p-value
Intercept	1.843	0.528	51	3.489	0.001
conspecific	-0.198	0.187	51	-1.059	0.295
heterospecific	0.134	0.085	51	1.576	0.121

(c) bumblebee

	β	SE	df	t-value	p-value
Intercept	-0.078	0.189	51	-0.414	0.68
conspecific	0.04	0.065	51	0.62	0.538
heterospecific	0.081	0.03	51	2.708	0.009

(d) *M. campanulae*

	β	SE	df	t-value	p-value
Intercept	1.028	0.569	51	1.806	0.077
conspecific	0.314	0.147	51	2.131	0.038
heterospecific	-0.049	0.07	51	-0.71	0.481

(e) small bee

	β	SE	df	t-value	p-value
Intercept	1.028	0.569	51	1.806	0.077
conspecific	0.314	0.147	51	2.131	0.397
heterospecific	-0.049	0.07	51	-0.71	0.481

Table S1 continued.

(f) number of pollen grains

	β	SE	df	t-value	p-value
Intercept	5.129	0.389	47	13.346	0
conspecific	-0.199	0.136	47	-1.463	0.15
heterospecific	-0.005	0.061	47	-0.076	0.94

Table S2. A correlation matrix of land type area at a 150-m and 1-km scale. Each correlation matrix shows Spearman's rank coefficient. Coefficients with a p-value less than 0.05 are bolded.

150-m

	forest	developed	cultivated	barren	grassland
forest	1	**-0.61**	**-0.54**	-0.2	0
developed	**-0.61**	1	**0.26**	0.15	-0.07
cultivated	**-0.54**	**0.26**	1	0.22	-0.13
barren	-0.2	0.15	0.22	1	-0.07
grassland	0	-0.97	-0.13	-0.07	1

1-km

	forest	developed	cultivated	barren	grassland
forest	1	0.07	**-0.67**	**-0.43**	**-0.41**
developed	0.07	1	**-0.49**	**-0.39**	-0.05
cultivated	-0.67	-0.49	1	0.56	0.54
barren	**-0.43**	**-0.39**	**0.56**	1	**0.36**
grassland	**-0.41**	-0.05	**0.54**	**0.36**	1

Table S3. Variance inflation factors (VIF) for models testing effects on plant mating quality. VIF was calculated separately for variables at the (a) 1-km scale and (b) 150-m scale.

(a)	
Variable	**VIF**
C. americana habitat (1-km)	1.657
Forest (1-km)	3.196
Developed (1-km)	3.915
Barren (1-km)	1.482
Grassland (1-km)	1.297
Bumblebee	1.098
Megachile	1.631
Small bee	1.693
(b)	
Variable	**VIF**
C. americana habitat (150-m)	1.209
Forest (150-m)	2.957
Developed (150-m)	2.606
Barren (150-m)	1.103
Grassland (150-m)	1.128
Bumblebee	1.019
Megachile	1.462
Small bee	1.701

Table S4. Results of the linear mixed-effects model on the effect of land use type at a 1-km scale on the number of bumblebee visits. Site was included as a random effect.

	β	SE	df	t-value	p-value
Intercept	0.074	0.306	50	0.241	0.810
cultivated (1-km)	-0.212	0.648	50	-0.327	0.745
developed (1-km)	0.124	0.369	50	0.336	0.738
barren (1-km)	-1.195	1.772	50	-0.674	0.503
grassland (1-km)	2.617	2.083	50	1.256	0.215

Table S5. Results of the linear mixed-effects model on the effect of land use type at a 150-m scale on the number of bumblebee visits. Site was included as a random effect.

	β	SE	df	t-value	p-value
Intercept	0.111	0.13	50	0.855	0.397
cultivated (150-m)	0.960	0.42	50	2.284	0.027
developed (150-m)	0.049	0.233	50	0.210	0.835
barren (150-m)	-3.106	3.53	50	-0.880	0.383
grassland (150-m)	-0.221	0.61	50	-0.362	0.719

Tables S6. Results of the linear mixed-effects model on the effect of land use type at a 1-km scale on the number of *Megachile campanulae* visits. Site was included as a random effect.

	β	SE	df	t-value	p-value
Intercept	3.130	1.016	50	3.082	0.003
cultivated (1-km)	-2.001	1.923	50	-1.041	0.303
developed (1-km)	-1.207	1.168	50	-1.034	0.306
barren (1-km)	-3.471	5.542	50	-0.626	0.534
grassland (1-km)	-1.976	7.063	50	-0.280	0.781

Table S7. Results of the linear mixed-effects model on the effect of land use type at a 150-m scale on the number of *Megachile campanulae* visits. Site was included as a random effect.

	β	SE	df	t-value	p-value
Intercept	1.767	0.515	50	3.431	0.001
cultivated (150-m)	0.012	0.972	50	0.013	0.990
developed (150-m)	-0.085	0.705	50	-0.121	0.904
barren (150-m)	3.476	7.643	50	0.455	0.651
grassland (150-m)	1.068	1.343	50	0.795	0.430

Table S8. Results of the linear mixed-effects model on the effect of land use type at a 1-km scale on the number of small bee visits. Site was included as a random effect.

	β	SE	df	t-value	p-value
Intercept	3.331	0.980	50	3.399	0.001
cultivated (1-km)	-1.579	1.877	50	-0.841	0.404
developed (1-km)	-1.513	1.123	50	-1.347	0.184
barren (1-km)	1.254	5.382	50	0.233	0.817
grassland (1-km)	-0.671	6.849	50	-0.098	0.922

Table S9. Results of the linear mixed-effects model on the effect of land use type at a 150-m scale on the number of small bee visits. Site was included as a random effect.

	β	SE	df	t-value	p-value
Intercept	2.488	0.508	50	4.900	0.000
cultivated (150-m)	-0.729	0.925	50	-0.788	0.435
developed (150-m)	-0.042	0.682	50	-0.920	0.362
barren (150-m)	-6.696	7.266	50	0.920	0.362
grassland (150-m)	0.725	1.277	50	0.568	0.573

Table S10. Statistics of the tested piecewise SEM models. For each model we provide the tested relationships with the SEM's Fisher's C statistic, corresponding p-value, and AIC score. The best model for each mating quality variable is bolded.

Path models	Fisher's C	p-value	AIC
haplotype ~ bumblebee + megachile + small bee **bumblebee ~ *C. americana* habitat (150-m) + forest (150-m) + cultivated (150-m) + heterospecific flowers** **megachile ~ C. americana habitat (150-m) + forest (150-m)** **small bee ~ C. americana habitat (150-m) + forest (150-m)**	**34.838**	**0.616**	**580.658**
haplotype ~ bumblebee + megachile + small bee bumblebee ~ *C. americana* habitat (1-km) + forest (1-km) + heterospecific flowers megachile ~ C. americana habitat (1-km) + forest (1-km) small bee ~ C. americana habitat (1-km) + forest (1-km)	19.933	0.233	579.485
haplotype ~ bumblebee + megachile + small bee + *C. americana* habitat (150-m) + forest (150-m) bumblebee ~ *C. americana* habitat (150-m) + forest (150-m) + heterospecific flowers megachile ~ C. americana habitat (150-m) + forest (150-m) small bee ~ C. americana habitat (150-m) + forest (150-m)	31.764	0.578	586.508
haplotype ~ bumblebee + megachile + small bee + C. americana habitat (1-km) + forest (1-km) bumblebee ~ C. americana habitat (1-km) + forest (1-km) + heterospecific flowers megachile ~ C. americana habitat (1-km) + forest (1-km) small bee ~ C. americana habitat (1-km) + forest (1-km)	17.933	0.118	584.795

Table S10 continued

seed set ~ bumblebee + megachile + small bee **bumblebee ~ *C. americana* habitat (150-m) + forest (150-m) + cultivated (150-m) + heterospecific flowers** **megachile ~ C. americana habitat (150-m) + forest (150-m)** **small bee ~ C. americana habitat (150-m) + forest (150-m)**	**32.445**	**0.724**	**777.512**
seed set ~ bumblebee + megachile + small bee bumblebee ~ *C. americana* habitat (1-km) + forest (1-km) + heterospecific flowers megachile ~ C. americana habitat (1-km) + forest (1-km) small bee ~ C. americana habitat (1-km) + forest (1-km)	20.982	0.179	776.339
seed set ~ bumblebee + megachile + small bee + *C. americana* habitat (150-m) + forest (150-m) bumblebee ~ *C. americana* habitat (150-m) + forest (150-m) + heterospecific flowers megachile ~ C. americana habitat (150-m) + forest (150-m) small bee ~ C. americana habitat (150-m) + forest (150-m)	32.978	0.518	778.153
seed set ~ bumblebee + megachile + small bee + C. americana habitat (1-km) + forest (1-km) bumblebee ~ C. americana habitat (1-km) + forest (1-km) + heterospecific flowers megachile ~ C. americana habitat (1-km) + forest (1-km) small bee ~ C. americana habitat (1-km) + forest (1-km)	18.042	0.114	774.853

Table S10 continued

pollen ~ bumblebee + megachile + small bee **bumblebee ~ *C. americana* habitat (150-m) + forest (150-m) + cultivated (150-m) + heterospecific flowers** **megachile ~ C. americana habitat (150-m) + forest (150-m)** **small bee ~ C. americana habitat (150-m) + forest (150-m)**	**38.585**	**0.443**	**727.979**
pollen ~ bumblebee + megachile + small bee bumblebee ~ *C. americana* habitat (1-km) + forest (1-km) + heterospecific flowers megachile ~ C. americana habitat (1-km) + forest (1-km) small bee ~ C. americana habitat (1-km) + forest (1-km)	18.947	0.271	726.806
pollen ~ bumblebee + megachile + small bee + *C. americana* habitat (150-m) + forest (150-m) bumblebee ~ *C. americana* habitat (150-m) + forest (150-m) + heterospecific flowers megachile ~ C. americana habitat (150-m) + forest (150-m) small bee ~ C. americana habitat (150-m) + forest (150-m)	38.338	0.279	728.285
pollen ~ bumblebee + megachile + small bee + C. americana habitat (1-km) + forest (1-km) bumblebee ~ C. americana habitat (1-km) + forest (1-km) + heterospecific flowers megachile ~ C. americana habitat (1-km) + forest (1-km) small bee ~ C. americana habitat (1-km) + forest (1-km)	17.126	0.145	726.93

Chapter 3: Greater number of pollen donors improves female reproductive success but not progeny vigor in *Allium stellatum*

Rieka Yu[1] and Nathan Muchhala[1]

[1]University of Missouri – St. Louis
One University Blvd. 223R Research Hall, Biology Department, St. Louis, MO 63121

Abstract

Plant-pollinator interactions have important ecological implications as pollination dynamics influence the identity and amount of pollen arriving to stigmas. The composition of deposited pollen loads ultimately affects plant population genetics and fitness. Here, we test the pollen competition hypothesis, which states that pollen competition will select for higher quality offspring, and evaluate how greater pollen competition from donor diversity influences offspring fitness. We performed hand-pollinations on *Allium stellatum*, depositing pollen mixtures from either one, two, or three donors while controlling for pollen load size. We collected and germinated seeds from hand-pollinations and evaluated how donor diversity influenced seed and seedling characteristics. Using generalized linear mixed-effects models, we found a marginally significant positive effect of the number of donors on seed set. This positive relationship was associated with an overall trade-off between seed number and seed size, such that these seeds were smaller and consequently grew slower as seedlings. The flowers that received pollen from a greater number of donors also had a greater number of seeds that

germinated. We found support for the pollen competition hypothesis, with pollen competition between different pollen donors benefitting female reproductive success, but with a possible trade-off in progeny vigor. The degree to which pollen competition benefits progeny can potentially be influenced by life history strategies that determine resource allocation to offspring. Our results clearly show donor diversity of pollen loads is an important component of reproductive and offspring fitness. Therefore, it is important to study how pollinators differentially deposit pollen loads to understand drivers of plant population dynamics.

Introduction

Animal pollinators are necessary for successful reproduction in 80% of angiosperm species, making plant-pollinator interactions an important ecological interaction (Ollerton *et al.* 2011). Variation in visit frequency, pollinator community, pollinator functional group, and pollinator behavior can influence the amount and composition of deposited pollen (Bernasconi 2003; Pannell & Labouche 2013; Krauss *et al.* 2017; Minnaar *et al.* 2019; Torres-Vanegas *et al.* 2021). How the amount and composition of deposited pollen influences plant population dynamics is therefore a crucial research topic. For example, a survey of 482 plant species showed 63% suffer from pollen limitation (Knight *et al.* 2005), which reduces a plant's reproductive capacity, potentially influencing population fitness and persistence (Ashman *et al.* 2004; Aizen & Harder 2007). In terms of deposited pollen composition, quality is an important factor as the degree of self or closely related pollen influences seed set (Knight *et al.* 2005; Aizen & Harder 2007). Additionally, the genetic diversity of deposited pollen is also an important component of plant population genetics because it influences the number of fertilized ovules and the number of sires within a single fruit (Ellstrand 1984; Montalvo 1992; Rhodes *et al.* 2017).

Variation in the amount and composition of pollen received by a stigma also creates different selection pressures through pollen competition. Pollen competition occurs when the number of grains deposited on the stigma is greater than the number of ovules. The pollen competition hypothesis states that pollen competition leads to selection of faster growing pollen tubes and results in greater progeny vigor (Mulcahy 1979). Under the pollen competition hypothesis variation in pollen tube growth rate is assumed to be the driver of progeny vigor, with a genetic basis causing both faster growing pollen tubes and seedlings (Janzen 1977; Mulcahy 1979; Stephenson 1981; Stephenson & Bertin 1983; Lee 1984). Studies have shown a large overlap in gene expression of both microgametophytes and sporophytes (Pedersen *et al.* 1987; Honys & Twell 2003; Beaudry *et al.* 2020), with evidence of a relationship between genes expressed in pollen and seedling vigor (Warman *et al.* 2020). Pollen pools with a greater number of donors are also more likely to contain more high quality microgametophytes, leading to a larger number of seed with higher quality.

There is some support for the relationship between pollen competition and offspring quality with studies testing effects of pollen load, pollen size, and location of pollen placement on the stigma (Baskin and Baskin 2015; Baskin and Baskin 2019). In multiple species, larger pollen loads have been shown to decrease germination time and increase seed set, fruit set, seed mass, germination success, and seedling growth rate (Winsor *et al.* 1987; Bertin 1990; Palmer & Zimmerman 1994; Niesenbaum 1999; Holland *et al.* 2009). Pollen competition between large and small pollen grains in *Ipomoea purpurea* leads to larger pollen grains having higher siring success, although this study did not investigate offspring fitness (McCallum & Chang 2016). In *Dianthus chinensis*, which has an elongated stigmatic surface, pollen that was deposited farthest from the ovary led to seeds with greater mass and earlier germination times (Mckenna 1983; Mulcahy & Mulcahy 1987).

In addition to pollen load, size, and placement, the donor diversity of deposited pollen, in terms of the number of potential fathers represented in the pollen load, can also mediate pollen competition. In the few studies on the role of donor diversity in pollen competition, there have been mixed results, with evidence for both positive and negative effects of increased diversity on reproductive success and offspring fitness (Snow 1990; Niesenbaum 1999; Paschke *et al.* 2002). Snow (1990) found no effects of donor diversity on reproductive output of *Rapahnus raphanistrum*, however, flowers in the study came from an "intense" competition line which was expected to already have high fitness, and treatments were repeated on the same maternal plant, which would not have controlled for effects related to that specific maternal individual. Niesenbaum (1999) showed an increase in donor number increased fruit set for *Mirabilis jalapa*, however treatments were also repeated on the same maternal individual. Paschke *et al.* (2002) found that a higher number of donor numbers in *Cochlearia bavarica* improved reproductive success, but also led to lower offspring fitness. Pollen donors however, were all selected 0.5 m from the recipient plant which may have led to confounding effects of relatedness.

Evidence suggests different pollinator types deposit pollen with a varying degree of quality. A comparison of bird- and insect-pollinated plants shows fruits from bird-pollinated plants are sired by almost twice the number of fathers as fruits from insect-pollinated plants (Krauss *et al.* 2017). Additionally, a meta-analysis on plants with pollinators with differing mobilities show more mobile pollinators facilitate higher proportions of half-sibs, meaning they are depositing pollen from a greater number of donors (Breed *et al.* 2015). These differences in pollinators are an important yet overlooked selective factor on the flowers they visit and require experimental evidence from multiple systems to test if pollen competition between multiple donors improves fitness (Krauss *et al.* 2017).

In this study we used hand-pollinations to test the pollen competition hypothesis in *Allium stellatum* and to determine the implications of multiple mating on offspring fitness. We specifically tested if the number of donors deposited on a recipient flower influences seed set and progeny vigor. We expect greater donor diversity from a greater number of donors will increase the chance of fertilization by higher quality pollen resulting in higher offspring fitness.

Methods

Study system

Allium stellatum (prairie onion) is a perennial herb found on limestone glades in central Canada from Ontario to Saskatchewan and in central United States from Illinois to Texas. Flowers are protandrous and obligate outcrossers, and are arranged in umbels of 25-30 flowers, with about six ovules per flower (Molano-Flores *et al.* 1999; Weiherer *et al.* 2020). Our study took place at the Missouri Botanical Garden's Shaw Nature Reserve (SNR) in Gray Summit, Missouri (38°27'56.9"N 90°49'23.7"W) where the population has approximately 2,000 individuals (Weiherer *et al.* 2020). Flowers at SNR are primarily visited by beetles and bees in the families Apidae, Halictidae, and Megachilidae (Weiherer *et al.* 2020). In this population, 46% of *A. stellatum* flowers set fruit and stigmas receive on average 2.8 pollen grains and with each flower producing on average 3.0 seeds from open pollination (Weiherer *et al.* 2020).

Pollination treatments

To determine effects of donor diversity on progeny fitness, we implemented treatments of open-pollination, geitonogamous selfing, and pollination by one, two, and three donors. Geitonogamous selfing and one-, two-, and three-donor treatments were performed with hand-pollinations. For n = 150 individuals, four individual flowers received the open pollination and one-, two-, and three-donor treatments, with the four treatments nested within an individual. For an additional n = 150 individuals, one individual flower received the geitonogamous selfing treatment. One hundred nineteen individuals were chosen as donors from a different location at SNR about 200-300 meters away, separated by walkways and a patch of wooded area. We rotated through these donors ensuring no individual was used as a donor for separate treatments within the same individual.

To prepare flowers for hand-pollinations, we cut off excess flowers from umbels and emasculated flowers receiving treatment pollen before stigma receptivity. For individuals receiving the open pollination and donor treatments, all but four flowers were cut off, one for the open pollination treatment and three for the one-, two-, and three-donor treatments. For the open pollination treatment, we chose flowers that were past stigma receptivity as indicated by wilted stigmas, and therefore could have received both self and outcross pollen. For the geitonogamous selfing treatment on separate individuals, we left one emasculated flower as well as one unmanipulated flower that was the source of self-pollen. After emasculating flowers, we covered umbels with organza bags for two days until stigma receptivity. Once stigmas were receptive on flowers for each treatment, we deposited self-pollen for geitonogamous treatments and deposited a pollen mixture from one, two, or three donors for the remaining hand-pollination treatments. Hand-pollinations were performed by touching the plastic ball tip of a sewing pin to a dehisced anther of a donor individual. To make donor mixtures, we tapped the plastic tip of the sewing pin to different anthers on different areas of the plastic tip. We then used the metal tip of another sewing pin to mix pollen together on the surface of the plastic tip. Mixtures were then deposited by tapping the plastic tip to stigmas, maintaining similar amounts of pollen transfer across all treatments. We checked for any differences in pollen load size in our one-, two-, and three-donor treatments by collecting stigmas from every fifth individual and counting fuchsin-stained pollen grains with a microscope. On average, 26.41 grains (SE = 8.04) were deposited in the one-donor treatment, 29.23 grains (SE = 6.56) in the two- donor treatment, and 20.96 grains (SE = 4.11) in the three-donor treatment. An ANOVA showed no difference in pollen number between the one-, two-, and three-donor treatments ($F_{2,85} = 0.043$, $P = 0.958$).

Resulting seeds were collected four weeks after pollinations. Seeds were cold stratified in damp sand at 4^{o}C for 60 days, and then planted in Black Gold® seedling mix (a mixture of peat moss and perlite) and watered once a week. Planted seeds were grown in an incubator at 18^{o}C and monitored weekly for six months.

Fitness metrics

As measures of offspring fitness, we measured seed and seedling characteristics. For seeds, we measured seed set (the number of seeds per flowers) and seed mass. For seedlings, we recorded number of weeks to germination, as well as seedling height and number of leaves each week. We also calculated seedling growth rates as change in height (cm) per week. After 24 weeks, we dried all seedlings in an oven for 48 hours and measured their biomass. We also calculated the germination rate of seeds as well as the number of germinated seeds per mother.

Statistical analysis

We used generalized linear mixed-effects models (GLMMs) to test the effect of pollination treatment on seed set, seed mass, number of weeks to germination, seedling height 12 and 24 weeks after planting, number of leaves 12 and 24 weeks after planting, seedling biomass, number of germinated seeds per flower, and the proportion of germinated seeds per flower. In the GLMMs we used a quasipoisson distribution to analyze seed set and used a Gamma distribution to analyze seed mass, number of weeks to germination, seedling height 12 and 24 weeks after planting, number of leaves 12 and 24 weeks after planting, seedling biomass, and the number of seeds germinated per flower. We used a binomial distribution to analyze the proportion of germinated seeds per flower.

To compare seedling growth rate between treatments with different donor diversities, we performed a linear mixed effect regression analyzing the effect of pollination treatment and week after planting on seedling height. Pollination treatment and week after planting were included as interaction terms and maternal individual was included as a random factor. Because seed mass can influence seedling growth rate, we also repeated the same analysis with seed mass added as another random factor.

We tested for correlations in different fitness metrics of offspring that resulted from pollen loads with different number of donors. We performed a Pearson's correlation test to determine correlations between seed set and seed mass along with correlations between seed mass and seedling height at 12 weeks, height at 24 weeks, number of leaves at 12 weeks, and number of leaves at 24 weeks.

Results

Open-pollinated flowers had an average of 3.44 seeds (SE = 0.14) and self-pollinated flowers had an average of 2.31 seeds (SE = 0.31). Seeds from open-pollinated flowers weighed an average of 2.97 mg (SE = 0.07) and seeds from self-pollinated flowers weighed an average of 2.86 mg (SE = 0.29). Open-pollinated flowers had on average 0.78 seeds that germinated (SE = 0.10) and self-pollinated flowers had on average 0.92 seeds that germinated (SE = 0.26).

Donor number had a marginally significant effect on seed set with 3-donor treatments having larger seed sets than 1-donor treatments (Table 1; Fig. 1A). There was no effect of donor number on seed mass (Fig. 1B), number of weeks to germination, seedling height at 12 and 24 weeks, number of leaves at 12 and 24 weeks, or number of germinated seeds per flower (Table S1). Treatment had a significant effect on seedling biomass with seedlings from self-pollination treatments weighing less that those from 1-donor treatments (Table S1). There was a significant effect of donor number on the proportion of germinated seeds per flower, with flowers from 3-donor treatments being more likely to have germinated seeds than those from 1-donor treatments (Table 2; Fig. 2).

Our linear mixed effect regressions showed significant interactions between number of donors and seedling growth rate (cm/week) (F4,1987 = 4.5127, P = 0.001; Table 3; Fig. 3); one-donor treatments grew significantly faster than three-donor treatments (P = 0.051) but not two-donor treatments (P = 0.151). Two-donor treatments did not grow faster than three-donor treatments (P = 0.997). When seed mass was added as a random effect, there was still a significant interaction between number of donors and seedling growth rate (cm/week) (F4,1965 = 4.7522, P < 0.001). One-donor treatments grew marginally faster than three-donor treatments (P = 0.082) but not two-donor treatments (P = 0.140). Two-donor treatments also did not grow faster than three-donor treatments (P = 0.999).

There was a significant negative correlation between seed set and seed mass (r = -0.270, df = 81, P = 0.014). Twelve weeks after planting, there was a significant positive correlation between seed mass and height (r = 0.355, df = 48, P = 0.012) and between seed mass and number of leaves (r = 0.325, df = 48, P = 0.021). Twenty-four weeks after planning there was no correlation between seed mass and seedling height (r = 0.097, df = 50, P = 0.500) nor number of leaves (r = 0.600, df = 50, P = 0.819). There was no correlation between seedling height 12 weeks after planting and seedling biomass (r = 0.115, df = 45, p = 0.442), but there was a positive correlation between seedling height at 24 weeks and biomass (r = 0.515, df = 50, p = 0.028). There was no correlation between biomass and number of leaves 12 weeks after planting (r = 0.148, df = 45, p = 9.289) and number of leaves 24 weeks after planting (r = -0.071, df = 50, p = 0.618). Seedling height 12 and 24 weeks after planting were positively correlated (r = 0.515, df = 45, p < 0.001). Number of leaves 12 and 24 weeks after planting were not correlated (r = 0.223, p = 0.132). Seedling height and number of leaves were positively correlated 12 weeks after planting (r = 0.517, df = 48, p < 0.001) and 24 weeks after planting (r = 0.600, df = 50, p < 0.001).

Discussion

Our study shows that the donor diversity of deposited pollen, in terms of the number of pollen donors, differentially affected separate metrics of fitness in *A. stellatum*. Pollination by a greater number of donors led to increased reproductive success through a higher likelihood of having germinating offspring, with some evidence that the number of pollen donors also increases seed set. There was an apparent decrease in seedling growth rate with an increase in number of donors, however, this is likely due to the overall pattern across all treatments of greater seed set per fruit leading to smaller and therefore lower quality seeds.

We expected increased pollen competition to improve progeny vigor because evidence suggests pollen tube growth rate is positively correlated with offspring quality, with faster-growing pollen tubes fertilizing ovules first and producing faster-growing seedlings (Mulcahy & Mulcahy 1975; Winsor *et al.* 1987; Bertin 1990; Richardson & Stephenson 1992). However, these studies do not provide direct evidence that pollen tube growth rate is connected genetically to offspring quality (Walsh & Charlesworth 1992). Studies looking at the effect of pollen diversity on pollen competition have found mixed results with no consistent patterns. In *Cochlearia bavarica*, (Paschke *et al.* 2002) found an increase in donor diversity from three to nine donors increased reproductive success but found that offspring vigor was highest at intermediate levels of donor diversity. Donor diversity increased fruit set in *Mirabilis jalapa* but had no effect on seedling growth (Niesenbaum 1999). Our study shows that increased donor diversity (more fathers) actually leads to decreased seedling growth rate, thus similarly failing to support the idea that greater pollen competition will lead to increased offspring quality.

However, growth rate of seedlings is not solely determined by the quality of the pollen fathering them – it is also determined by maternal investment. In fact, we suspect that the negative correlation we found between number of fathers and growth rate of the seedlings is actually due to a trade-off between number of seeds and seed quality.

Specifically, the greater number of fathers led to increased seed set, which led to the mother investing less resources per seed. A trade-off between seed set and seed size can occur if plants have limited resources to invest in seeds (Smith & Fretwell 1974; Shipley & Dion 1992; Leishman *et al.* 2000), and seed size is considered an indicator of nutrient availability and therefore seedling vigor (Westoby *et al.* 1992; Milberg & Lamont 1997; Leishman *et al.* 2000). We found that seeds from larger seed sets in our study were significantly smaller. In turn, these smaller seeds produced slower growing seedlings. We note that these differences disappeared 24 weeks after planting, which is expected as the relationship between seedling growth and seed size disappears with time as seedling growth becomes less dependent on embryo size (Westoby *et al.* 1992).

In perennial species such as *A. stellatum*, a trade-off between seed number and quality may be important for survival of the mother plant and future reproduction. Evidence suggests that high investment in reproduction has a cost to reproduction in future years (Ehrlén 1992; Obeso 2002). In fact, pollen competition studies show larger pollen loads on stigmas leads to decreased seed germination and growth rates for perennial species, but increased germination and growth rates for annual species (Winsor *et al.* 1987; Richardson & Stephenson 1992; Quesada *et al.* 1996; Niesenbaum 1999; Paschke *et al.* 2002), but further research is necessary to test this pattern. Because perennials must invest in resources for future reproduction and annuals do not, effects of pollen competition may differ based on maternal investment. The need for perennials to invest in future reproduction therefore would explain a potential trade-off between seed set and seed quality in *A. stellatum*.

Although an increased number of pollen donors leads to decreased seedling vigor in *A. stellatum*, it still benefits female reproductive success in terms of increased seed set and an increased number of germinating offspring. The fact that there was no difference in the number of deposited pollen grains across our treatments suggests that competition between the multiple fathers leads to fertilization by pollen grains that are more likely to lead to seed germination, in support of the pollen competition hypothesis. These results also indicate that multiple mating is beneficial for females in terms of producing a greater number of progeny. In plants, the benefits of multiple mating are often discussed in the context of genetic benefits; here, we show reproductive success is also an important consideration.

Here we test the pollen competition hypothesis, however, female choice may be an important factor. With female choice, females can recognize good quality pollen at the stigma (Valdivia *et al.* 2009; Chae & Lord 2011; Rejón *et al.* 2016; Goring 2018), style (Lind *et al.* 1996; Wu *et al.* 2000; Chae & Lord 2011), or after ovule fertilization and selectively abort seeds and fruit or stop pollen tube growth from low quality donors (Stephenson & Bertin 1983). It is also possible that females preferentially invest resources in multiply sired ovules. Female selection would then also lead to a greater number of higher quality seeds. While we are unable to test which mechanism is at play here, it is likely that a combination of both male-male competition and female choice influence the fertilization success and quality of offspring.

The results of this study suggest that multiple mating is beneficial for female reproductive success but not for offspring growth rates in *A. stellatum*. These results are consistent with the pollen competition hypothesis but leave us with a complex picture of the role of pollen competition in population dynamics. The pollen competition hypothesis often discusses the benefits of competition on offspring fitness but not maternal fitness, while our results show that maternal fitness is also an important component. We unexpectedly found that progeny vigor declined with the number of pollen donors, however this may be explained by a trade-off between seed number and quality, an idea which warrants further investigation. Whether or not negative effects on offspring vigor are mediated by a trade-off, the donor diversity of deposited pollen plays a significant role in offspring fitness. Hand pollination experiments from this study should be repeated on other species to investigate how life history trade-offs interact with the effects of pollen competition for a more robust test of the pollen competition hypothesis. Future work should also add a genetic component to separate effects of pollen competition versus female choice. Ultimately, the result that increased donor diversity while holding pollen load size constant leads to an increase the number of germinating seedlings stresses the importance of understanding how pollinators differ in the number of donors represented in the pollen loads they carry.

Acknowledgements

The authors thank the Missouri Botanical Garden for allowing us to conduct our field research at Shaw Nature Reserve. We would also like to thank Kelly Toohey for her help with field work. Funding for this project was provided by the Whitney R. Harris World Ecology Center.

Author contributions

R.Y.: Conceptualization, methodology, data collection, data analysis, investigation, project administration, writing. N.M.: Conceptualization, methodology, supervision, validation, review and editing.

Literature cited

Aizen, M.A. & Harder, L.D. (2007). Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality, 88, 271–281.

Ashman, T.L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., *et al.* (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, 85, 2408–2421.

Baskin, J.M., & Baskin, C.C. (2015). Pollen (microgametophyte) competition: an assessment of its significance in the evolution of flowering plant diversity, with particular reference to seed germination. *Seed Science Research*, 25, 1-11.

Baskin, J.M., & Baskin, C.C. (2019). How much influence does the paternal parent have on seed germination? *Seed Science Research*, 29, 1-11.

Beaudry, F.E.G., Rifkin, J.L., Barrett, S.C.H. & Wright, S.I. (2020). Evolutionary Genomics of Plant Gametophytic Selection. *Plant Communications*, 1, 100115.

Bernasconi, G. (2003). Seed paternity in flowering plants: An evolutionary perspective. *Perspectives in Plant Ecology, Evolution and Systematics*, 6, 149–158.

Bertin, R.I. (1990). Effects of Pollination Intensity in Campsis radicans. *American Journal of Botany*, 77, 178–187.

Breed, M.F., Ottewell, K.M., Gardner, M.G., Marklund, M.H.K., Dormontt, E.E. & Lowe, A.J. (2015). Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. *Heredity*, 115, 108–114.

Chae, K. & Lord, E.M. (2011). Pollen tube growth and guidance: roles of small, secreted proteins. *Annals of Botany*, 108, 627–636.

Ehrlén, J. (1992). Proximate limits to seed production in a herbaceous perennial legume, Lathyrus vernus. *Ecology*, 73, 1820–1831.

Ellstrand, N.C. (1984). Multiple Paternity within the Fruits of the Wild Radish, Raphanus sativus. *The American Naturalist*, 123, 819–828.

Goring, D.R. (2018). Exocyst, exosomes, and autophagy in the regulation of Brassicaceae pollen-stigma interactions. *Journal of Experimental Botany*, 69, 69–78.

Holland, J.N., Chamberlain, S.A., Waguespack, A.M. & Kinyo, A.S. (2009). Effects of pollen load and donor diversity on seed and fruit mass in the columnar cactus, pachycereus schottii (Cactaceae). *International Journal of Plant Sciences*, 170, 467–475.

Honys, D. & Twell, D. (2003). Comparative Analysis of the Arabidopsis Pollen Transcriptome. *Plant Physiology,* 132, 640–652.

Janzen, D.H. (1977). A Note on Optimal Mate Selection by Plants. *The American Naturalist*, 111, 365–371.

Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., *et al.* (2005). Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, 36, 467–497.

Krauss, S.L., Phillips, R.D., Karron, J.D., Johnson, S.D., Roberts, D.G. & Hopper, S.D. (2017). Novel Consequences of Bird Pollination for Plant Mating. *Trends in Plant Science,* 22, 395–410.

Lee, T.D. (1984). Patterns of Fruit Maturation: A Gametophyte Competition Hypothesis. *The American Naturalist*, 123, 427–432.

Leishman, M.R., Wright, I.J., Moles, A.T. & Westoby, M. (2000). The evolutionary ecology of seed size. In: *Seeds: the ecology of regeneration in plant communities* (ed. Fenner, M.). pp. 31–57.

Lind, J.L., Bönig, I., Clarke, A.E. & Anderson, M.A. (1996). A style-specific 120-kDa glycoprotein enters pollen tubes ofNicotiana alata in vivo. *Sexual Plant Reprod,* 9, 75–86.

McCallum, B. & Chang, S.M. (2016). Pollen competition in style: Effects of pollen size on siring success in the hermaphroditic common morning glory, Ipomoea purpurea. *American Journal of Botany*, 103, 460–470.

Mckenna, M. (1983). Ecological aspects of gametophytic competition in Dianthus chinensis. In: *Pollen: Biology and Implications in Plant Breeding* (eds. Mulcahy, D. & Ottaviano, E.). Elsevier, New York, pp. 419–424.

Milberg, P. & Lamont, B.B. (1997). Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. *New Phytologist*, 137, 665–672.

Minnaar, C., Anderson, B., De Jager, M.L. & Karron, J.D. (2019). Plant-pollinator interactions along the pathway to paternity. *Annals of Botany*, 123, 225–245.

Molano-Flores, B., Hendrix, S.D. & Heard, S.B. (1999). The Effect of Population Size on Stigma Pollen Load , Fruit Set , and Seed Set in Allium. *International Journal of Plant Sciences*, 160, 753–757.

Montalvo, A.M. (1992). Relative Success of Self and Outcross Pollen Comparing Mixed- and Single-Donor Pollinations in Aquilegia Caerulea . *Evolution*, 46, 1181–1198.

Mulcahy, D.L. (1979). The Rise of the Angiosperms: A Genecological Factor. *Science,* 206, 20–23.

Mulcahy, D.L. & Mulcahy, G.B. (1975). The influence of gametophytic competition on sporophytic quality in Dianthus chinensis. *Theoretical and Applied Genetics*, 46, 277–280.

Mulcahy, D.L. & Mulcahy, G.B. (1987). The effects of pollen competition. *American Scientist*, 75, 44–50.

Niesenbaum, R.A. (1999). The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in Mirabilis jalapa (Nyctaginaceae). *American Journal of Botany*, 86, 261–268.

Obeso, J.R. (2002). The costs of reproduction in plants. *New Phytologist*, 155, 321–348.

Ollerton, J., Winfree, R. & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120, 321–326.

Palmer, T.M. & Zimmerman, M. (1994). Pollen Competition and Sporophyte Fitness in Brassica campestris: Does Intense Pollen Competition Result in Individuals with Better Pollen? *Oikos*, 69, 80.

Pannell, J.R. & Labouche, A.M. (2013). The incidence and selection of multiple mating in plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368.

Paschke, M., Abs, C. & Schmid, B. (2002). Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic Cochlearia bavarica (Brassicaceae). *American Journal of Botany*, 89, 1250–1259.

Pedersen, S., Simonsen, V. & Loeschcke, V. (1987). Overlap of gametophytic and sporophytic gene expression in barley. *Theoret. Appl. Genetics*, 75, 200–206.

Quesada, M., Stephenson, A.G. & Winsor, J.A. (1996). Effects of pollen competition on the reproductive performance in cucurbit hybrids (Cucurbitaceae): F_1 and backcross generations. *Can. J. Bot.*, 74, 1113–1118.

Rejón, J., Delalande, F., Schaeffer-Reiss, C., Alché, J., Rodríguez-García, M., Van Dorsselaer, A., *et al.* (2016). The Pollen Coat Proteome: At the Cutting Edge of Plant Reproduction. *Proteomes*, 4, 5.

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, 4296–4308.

Richardson, T.E. & Stephenson, A.G. (1992). Effects of parentage and size of the pollen load on progeny performance in Campanula americana. *Evolution*, 46, 1731–1739.

Shipley, B. & Dion, J. (1992). The allometry of seed production in herbaceous angiosperms. *The American Naturalist*, 139, 467–483.

Smith, C.C. & Fretwell, S.D. (1974). The Optimal Balance between Size and Number of Offspring. *The American Naturalist*, 108, 499–506.

Snow, A.A. (1990). Effects of Pollen-Load Size and Number of Donors on Sporophyte Fitness in Wild Radish (Raphanus raphanistrum), 136, 742–758.

Stephenson, A.G. (1981). Flower and Fruit Abortion: Proximate Causes and Ultimate Functions. *Annual Review of Ecology and Systematics*, 1, 253–279.

Stephenson, A.G. & Bertin, R.I. (1983). Male competition, female choice, and sexual selection in plants. In: *Pollination Biology* (ed. Real, L.). Academic Press Inc.

Torres-Vanegas, F., Hadley, A.S., Kormann, U.G., Jones, F.A., Betts, M.G. & Wagner, H.H. (2021). Tropical deforestation reduces plant mating quality by shifting the functional composition of pollinator communities. *Journal of Ecology*, 109, 1730–1746.

Valdivia, E.R., Stephenson, A.G., Durachko, D.M. & Cosgrove, D. (2009). Class B β-expansins are needed for pollen separation and stigma penetration. *Sex Plant Reprod*, 22, 141–152.

Walsh, N.E. & Charlesworth, D. (1992). Evolutionary Interpretations of Differences in Pollen Tube Growth Rates, 67, 19–37.

Warman, C., Panda, K., Vejlupkova, Z., Hokin, S., Unger-Wallace, E., Cole, R.A., *et al.* (2020). High expression in maize pollen correlates with genetic contributions to pollen fitness as well as with coordinated transcription from neighboring transposable elements. *PLoS Genet*, 16, e1008462.

Weiherer, D., Eckardt, K. & Bernhardt, P. (2020). Comparative floral ecology and breeding systems between sympatric populations of Nothoscordum bivalve and Allium stellatum (Amaryllidaceae). *Journal of Pollination Ecology*, 26, 16–31.

Westoby, M., Jurado, E. & Leishman, M. (1992). Comparative evolutionary ecology of seed size. *Trends in Ecology and Evolution*, 7, 368–372.

Winsor, J.A., Davis, L.E. & Stephenson, A.G. (1987). The Relationship between Pollen Load and Fruit Maturation and the Effect of Pollen Load on Offspring Vigor in Cucurbita pepo. *The American Naturalist*, 129, 643–656.

Wu, H., Wong, E., Ogdahl, J. & Cheung, A.Y. (2000). A pollen tube growth-promoting arabinogalactan protein from Nicotiana alata is similar to the tobacco TTS protein. *Plant J*, 22, 165–176.

Figures

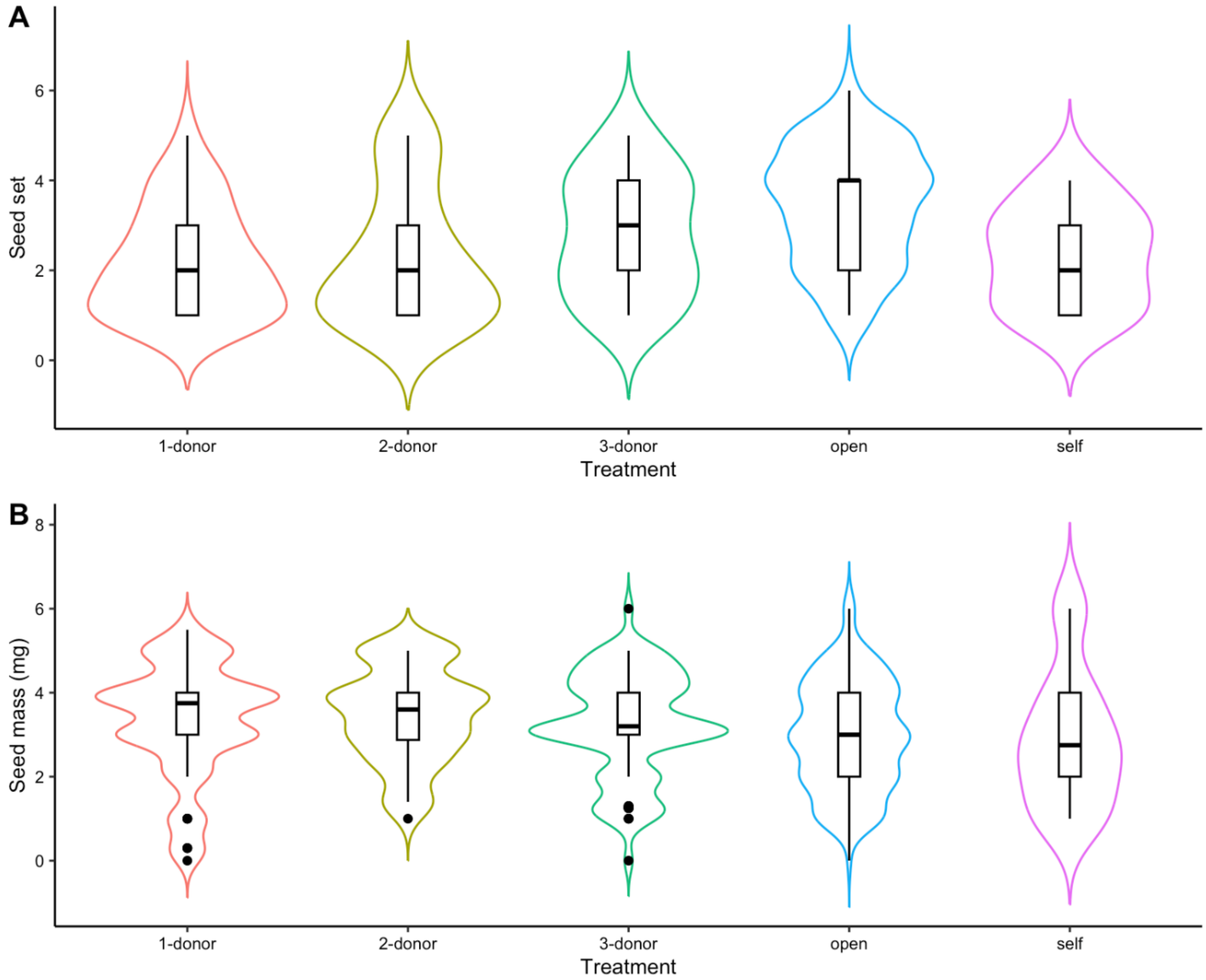

Figure 1. Violin and box plots of (A) seed set per flower and (B) seed mass of fruits from each pollination treatment in *A. stellatum*. 1-, 2-, and 3-donor treatments consisted of hand pollinations with the number of deposited donors manipulated. Open treatments were unmanipulated flowers and self treatments were geitonogamous hand pollinations.

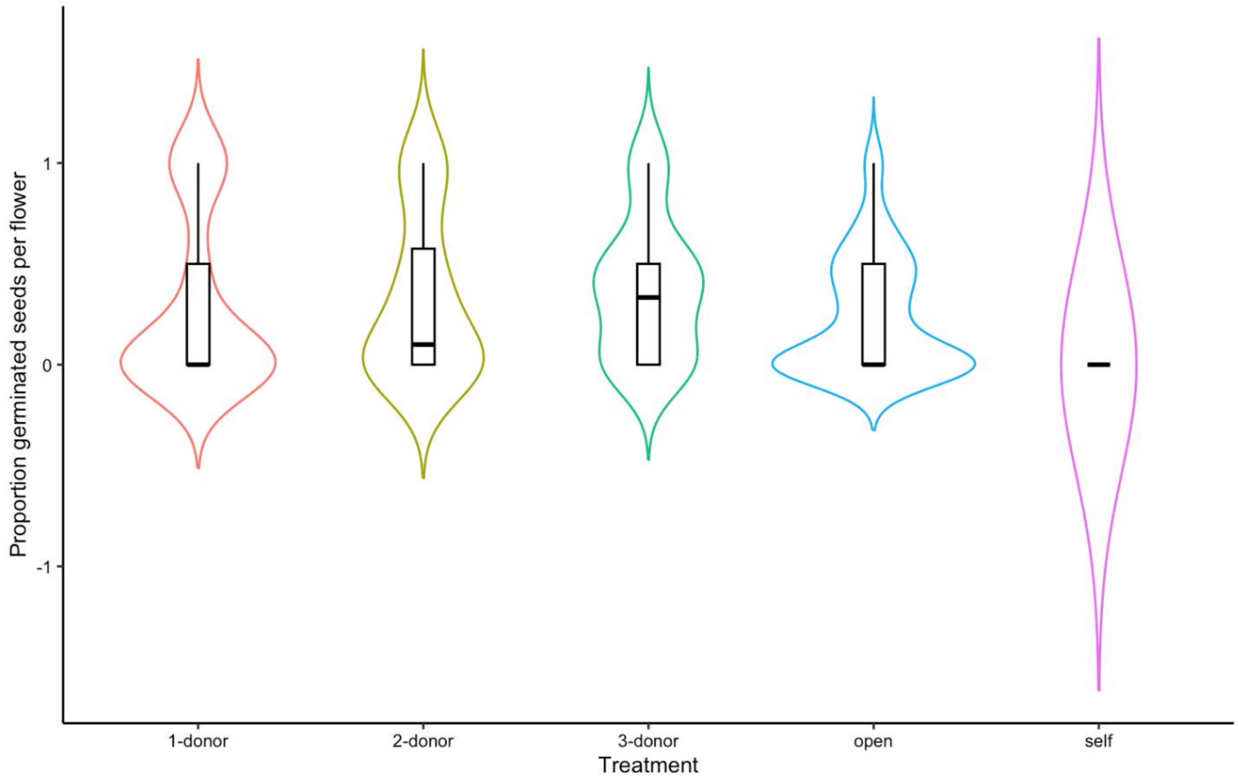

Figure 2. Violin and box plot of the proportion of germinated seeds per flower from each pollination treatment in *A. stellatum*. 1-, 2-, and 3-donor treatments consisted of hand pollinations with the number of deposited donors manipulated. Open treatments were unmanipulated flowers and self treatments were geitonogamous hand pollinations.

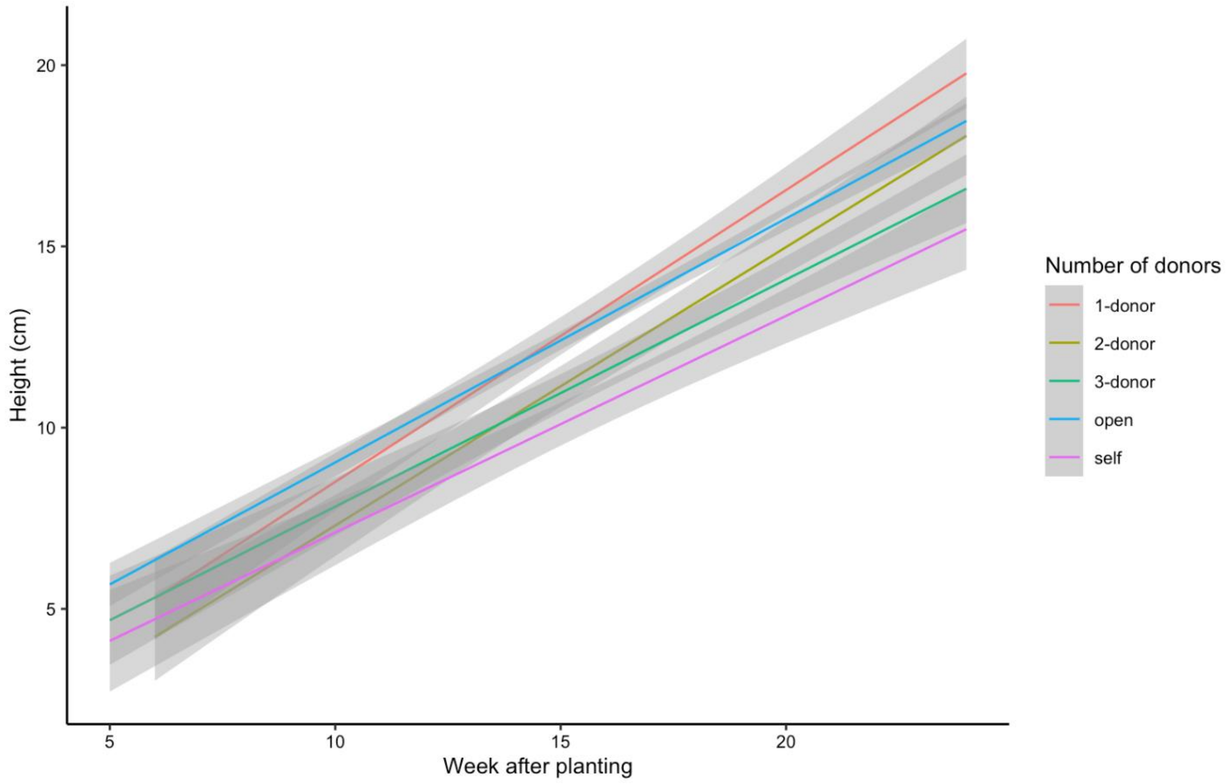

Figure 3. Growth rate, measured as the heigh (cm) of seedlings each week, of *A. stellatum* seedlings from pollination treatments. Slopes were calculated with a linear regression. Shaded areas show the 95% confidence interval. 1-, 2-, and 3-donor treatments consisted of hand pollinations with the number of deposited donors manipulated. Open treatments were unmanipulated flowers and self treatments were geitonogamous hand pollinations.

Tables

Table 1. Results of the generalized mixed-effect model testing the effect of pollination treatment on seed set. We ran the model using a quasipoisson distribution with maternal individual set as a random factor. •p < 0.1, *p < 0.05, ** p <0.01, *** p < 0.001.

	β	SE	t-value	p-value
Intercept (1-donor)	0.776	0.099	7.853	<1E-12***
2-donor	0.060	0.141	0.427	0.670
3-donor	0.249	0.133	1.873	0.063•
open	0.459	0.108	4.222	<1E-12***
self	0.060	0.174	0.347	0.728

Table 2. Results of the generalized mixed-effects model testing the effect of pollination treatment on the proportion of germinated seeds per flower. We ran the model using a binomial distribution with maternal individual set as a random factor. •p < 0.1, *p < 0.05, ** p <0.01, *** p < 0.001.

	β	SE	t-value	p-value
Intercept (1-donor)	-0.505	0.404	-1.250	0.212
2-donor	0.481	0.572	0.841	0.401
3-donor	1.293	0.586	2.204	0.028**
open	0.402	0.460	0.872	0.383
self	0.669	0.716	0.935	0.350

Table 3. Results of the linear mixed-effects model testing the interacting effect of pollination treatment and number of weeks after planting on seedling height. Pollination treatment and the number of weeks after planting were coded as interaction terms with maternal individual set as a random factor. $^{\bullet}$p < 0.1, *p < 0.05, ** p <0.01, *** p < 0.001.

	β	SE	df	t-value	p-value
Intercept (1-donor)	-1.346	0.925	1987	-1.455	0.146
Week after planting	0.835	0.046	1987	17.962	0.000^{***}
2-donor	2.064	1.103	1987	1.871	$0.062^{\bullet}$
3-donor	1.898	1.089	1987	1.742	$0.082^{\bullet}$
open	1.795	0.918	1987	1.956	$0.051^{\bullet}$
self	3.401	1.226	1987	2.775	0.006^{**}
Week after planting x 2-donor	-0.145	0.063	1987	-2.283	0.023^{*}
Week after planting x 3-donor	-0.167	0.061	1987	-2.721	0.007^{**}
Week after planting x open	-0.157	0.052	1987	-3.039	0.002^{**}
Week after planting x self	-0.299	0.072	1987	-4.153	0.000^{***}

Supplementary materials

Table S1. Results of the generalized linear mixed-effects models for the effect of pollination treatments on different offspring fitness metrics. Models were run using a Gamma distribution with maternal individual set as a random factor. $^{\bullet}$p < 0.1, *p < 0.05, ** p <0.01, *** p < 0.001

	β	SE	t-value	p-value
Seed mass (mg)				
Intercept (1-donor)	0.38	0.028	13.538	<1E-15***
2-donor	-0.03	-0.025	-1.167	0.243
3-donor	-0.004	0.025	-0.169	0.866
open	0.031	0.023	1.358	0.174
self	0.023	0.060	0.392	0.695
Weeks to germination				
Intercept (1-donor)	0.007	0.005	1.321	0.187
2-donor	9.61E-07	0.003	0.000	1.000
3-donor	0.004	0.005	0.668	0.504
open	0.002	0.005	0.431	0.666
self	0.002	0.007	0.287	0.774
Height at 12 weeks				
Intercept (1-donor)	0.099	0.013	7.59	<1E-13**
2-donor	0.013	0.016	0.866	0.386
3-donor	0.001	0.015	0.067	0.947
open	0.001	0.012	0.120	0.905
self	0.011	0.018	0.657	0.511
Height at 24 weeks				
Intercept (1-donor)	0.063	0.006	10.031	<1E-15***
2-donor	0.007	0.008	0.901	0.368
3-donor	0.006	0.007	0.811	0.417
open	0.004	0.006	0.702	0.483
self	0.014	0.009	1.606	0.108
Number of leaves at 12 weeks				
Intercept (1-donor)	0.569	0.06	9.436	<1E-15***
2-donor	0.041	0.075	0.554	0.579
3-donor	-0.006	0.073	-0.086	0.931
open	-0.014	0.062	-0.229	0.819
self	-0.016	0.074	-0.213	0.832

Table S1 continued

Number of leaves at 24 weeks				
Intercept (1-donor)	0.332	0.028	11.922	<1E-15***
2-donor	-0.005	0.030	-0.171	0.864
3-donor	0.020	0.031	0.657	0.511
open	0.005	0.025	0.200	0.841
self	0.014	0.035	0.398	0.690
Seedling biomass (mg)				
Intercept (1-donor)	-2.77	0.193	-14.328	<1E-15***
2-donor	-0.246	0.240	-1.027	0.305
3-donor	-0.094	0.230	-0.407	0.684
open	-0.220	0.200	-1.120	0.263
self	-0.613	0.258	-2.375	0.018*
Number of germinated seeds per flower				
Intercept (1-donor)	-0.645	0.264	-2.444	0.015*
2-donor	0.248	0.348	0.712	0.476
3-donor	0.454	0.330	1.379	0.168
open	0.314	0.288	1.090	0.276
self	0.488	0.406	1.204	0.229