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A missing link: pollinators as drivers of plant population structure

Grace Burgin & Robin Hopkins

A tremendous amount of empirical evidence emphasizes the significance of plant-pollinator interactions in flowering plant evolution. For example, there are numerous cases of associations between floral traits and diversification rate, and parallel evolution of floral character combinations in unrelated groups that use similar pollinator types (Kay and Sargent, 2009). These macroevolutionary patterns highlight pollinators as selective agents driving floral trait variation, reproductive isolation, and diversification. However, the role of pollinators is even more fundamental to the plant life cycle than this adaptative framework might suggest – pollinators are key dispersal agents.

Many plants rely on external vectors for pollen and seed dispersal. Data suggest that dispersal via seed is often limited, particularly among plants without adaptations for long-distance seed dispersal (Ennos, 1994). Therefore, the movement of pollen via pollinators represents a significant mechanism through which alleles move across the landscape (Sork and Smouse, 2006; Browne and Karubian, 2018). One theoretical model emerging from the plant speciation literature explicitly reframes the role of pollinators in plant evolution to include non-adaptive demographic processes like dispersal and gene flow (Loveless and Hamrick, 1984; Wessinger, 2021). This so-called 'dispersal dependent speciatio that plants have evolved suites of traits that enhance reproduction using a specific pollinator type. As plants adapt to optimize reproduction using particular pollinators (e.g. bees versus hummingbirds), they also commit to the patterns of pollen-mediated gene flow driven by their pollinator's movement behaviors. For example, highl long distances while less mobile pollinators move pollen locally. These differences are assumed to scale up to affect population genetic structure which in turn affects the likelihood of speciation and extinction (Harvey et al., 2019; Wessinger et al., 2019).

Much of the existing research testing the pollen dispersal dependent speciation hypothesis compares diversification rates across lineages or average dispersal distance between species (Schmidt-Lebuhn et al., 2019; Wessinger, 2021). If we continue to scale down biological levels, do these patterns of pollinator mediated dispersal hold? Does the distribution of genetic variation within a flowering plant species reflect the movement behavior of its pollinator(s)? Here we explore the within species predictions of the pollen dispersal dependent speciation hypothesis and suggest how population genetic tools can be leveraged to investigate the non-adaptive demographic relationships between plants and their pollinators.

Within species predictions of the pollen dispersal dependent speciation hypothesis

If pollinators are an important factor controlling gene dispersal in plants, plant population genetic structure will reflect variation in pollinator movement (Figure 1). Specifically, variation in pollen dispersal across space might result from differences in the frequency of pollinator visitation to each population, the pattern of pollinator movement among populations, and pollinator responses to the environment.

Heterogeneity in the absolute amount of pollinator visitation across populations will affect how genetic variation is distributed both within and between populations. Enhanced attraction of pollinators will increase the likelihood of pollen movement between a wider number of individuals within a population (Harder and Barrett, 1996). For individuals in populations that receive relatively few visits, the opportunity for diverse mating events is limited and inbreeding is more likely, reducing effective population size and promoting population differentiation (Charlesworth, 2003). Simultaneously, populations that receive relatively few pollinator visits will have infrequent opportunities for pollen import and/or export between populations and tend to be more genetically isolated.

The frequency of pollinator trips among populations will affect plant genetic structure. Because plants are sessile, we might assume that genetic structure directly reflects geographic location and therefore follows a pattern of isolation by distance (Loveless and Hamrick, 1984; Cruzan and Hendrickson, 2020). However, when pollinators repeatedly move between two spatially isolated populations, these populations will be more genetically similar than predicted by geographic distance alone (Sork and Smouse, 2006). For example, intensifying human land-use in the Southeastern United States has resulted in spatially isolated populations of the prairie endemic, *Oenothera harringtonii*, and yet, measures of genetic distance suggest population connectivity facilitated by long-distance movement of hawkmoth pollinators (Skogen et al., 2019). This example demonstrates how the movement patterns of pollinators define pollen dispersal and contribute to plant genetic structure beyond isolation by distance.

While much of our current understanding of pollinator-mediated dispersal focuses on average movement distance, patterns of movement across the environment are similarly important. Pollinators are distributed heterogeneously across the landscape due to nesting site preferences, adaptation to environmental gradients, territoriality behaviors, or physiological/morphological limits on movement distance. When individuals search for plants to forage on, they move in response to environmental signals including physical obstacles, habitat fragmentation, and human development (Stephens et al., 2008). Collectively, these factors will affect not only pollinator foraging behaviors but also population sizes, migration patterns, and local adaptation of pollinators. These microevolutionary forces will determine the distribution of genetic variation within pollinator species and could also have profound impacts on how pollinators move pollen across the environment. Whether the impact of pollinator microevolutionary processes on pollen flow in turn affects the genetic structure of flowering plant species remains unexplored.

There are many reasons why intraspecific variation in pollinator movement behaviors may not explain variation in plant genetic structure. Selection during the post-pollination and establishment phase may have a dominant role in determining which alleles and allelic combinations persist in a population and thus could obscure patterns of gene flow via pollen. In some plant species, seed/propagule dispersal may contribute significantly more to population connectivity than pollen dispersal (Nazareno et al., 2021). For plant species using multiple pollinator types, the individual impact of each pollinator may be swamped by a collective effect not attributable to any given pollinator. Because of these possibilities, exploring the role of intraspecific pollinator movement behavior in driving plant genetic structure remains an exciting future direction for the study of plant-pollinator interactions.

Applying a population genetic perspective to the study of plant-pollinator interactions

To determine if and how plant population connectivity responds to pollinator movement in any specific biological system, two patterns must be described: 1) patterns of pollen movement by pollinators and 2) the distribution of genetic variation within the plant species.

A variety of experimental approaches can be used to quantify pollinator movement. Historically, pollinator dispersal kernels were estimated using direct observations of individual pollinators (e.g. Levin and Kerster, 1974). Pollinator mark-recapture or tagging studies allow researchers to estimate movement over larger geographic distances (Kays et al., 2015). The use of pollen labels or pollen grain analogues has the additional advantage of incorporating variation in effectiveness of pollen pickup and deposition (e.g. Schmidt-Lebuhn et al., 2019).

At the broadest scale, pollinator genetic structure reveals how pollinators are distributed across the environment. While habitat and/or mating preferences likely also contribute to patterns of genetic structure, genetic measures of pollinator population connectivity infer how frequently individuals move between populations and across geography (Lowe and Allendorf, 2010). When these movements correlate with foraging behaviors, the genetic structure of a pollinator will reveal potential patterns of pollen flow. Given the increased accessibility of genomic sequencing and advancements in analyzing sequence data, population genetic tools are increasingly tractable for exploring how pollinators move across the environment. These approaches infer the connectivity of pollinator populations and form hypotheses about pollinator movement patterns. Researchers can then use these data to generate species specific predictions relating pollen dispersal to plant population connectivity.

There are several experimental approaches researchers can use to test hypotheses about plant population connectivity. Parentage analyses and assignment tests can reveal pollen movement within a single reproductive season (Sork and Smouse, 2006; Bode et al., 2018). Estimates of genetic distance between populations describes the cumulative impact of pollen dispersal and subsequent gene flow across generations (Cruzan and Hendrickson, 2020). Using pollinator movement patterns as an explanatory variable for plant genetic structure, researchers can then quantitatively test the prediction that pollinators affect the distribution of genetic variation within a plant species beyond isolation by distance (as is done with other environmental variables, e.g. Bradburd et al., 2013).

Significance and conclusions

Quantifying the impact of pollinator movement on plant genetic structure will expand our understanding of the ecological and evolutionary consequences of plant-pollinator interactions and generate predictions that can inform species management. If pollinators provide key dispersal services, the predicted impacts of pollinator loss will affect not only plant reproductive output but also gene flow between plant populations. Environmental disturbances that affect pollinators will also affect the genetic structure of plants. Alternatively, if habitat disturbance fragments plant populations, highly mobile pollinators may be able to maintain gene flow despite geographic distance.

Emphasizing the role of pollinators as dispersal vectors links patterns of pollinator driven diversification at the macroevolutionary scale to within species demographic processes (Harvey et al., 2019). When plant genetic structure reflects pollinator movement behaviors, pollinator mediated gene flow may contribute to the likelihood of population isolation and species extinction, persistence, or divergence. Although the relationship between dispersal dynamics, metapopulation structure, and diversification is complex, growing empirical evidence for existing theoretical models supports an association across diverse taxonomic groups (Harvey et al., 2019; Wessinger et al., 2019; Wessinger, 2021). While pollinator mediated selection on adaptive floral traits is certainly important to flowering plant evolution, understanding pollinators as dispersal agents can offer an additional path towards reconciling macroevolutionary patterns of floral diversity with microevolutionary processes.

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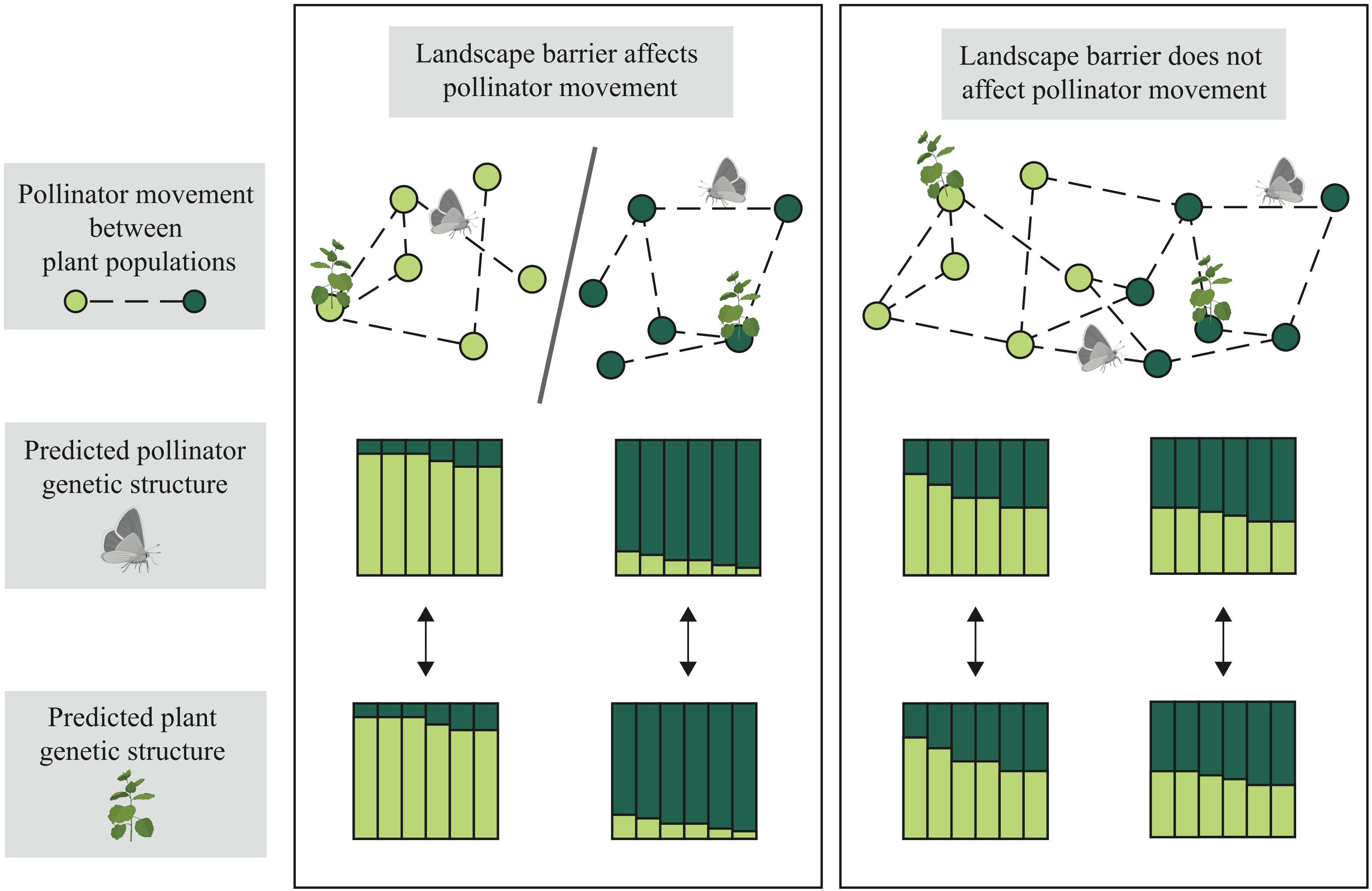

Figure 1 Pollinator movement pattern affects both pollinator and plant genetic structure. Green dots represent plant populations and pollinator movement is indicated with dashed black lines. In the example on the left, pollinators are unable to cross a hypothetical landscape barrier resulting in structured genetic variation in the polllinator species. Because gene flow via pollen dispersal is also obstructed, the plant genetic structure closely reflects pollinator genetic structure. On the right, no barrier to movement predicts a pattern of isolation by distance in both plant and pollinator species. Elements of the figure were drawn using Biorender.com