Bee movement across heterogeneous tropical forests: multi-paternal analyses reveal the importance of neighborhood composition for pollen dispersal

Megan C. O'Connell[1] (iD), Antonio R. Castilla[1,2], Leticia X. Lee[1,3], and Shalene Jha[1]

[1] Department of Integrative Biology, University of Texas at Austin, 1 University Station, Austin, TX 78712, USA

[2] Centro de Ecologia Aplicada Prof. Baeta Neves/InBIO, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisbon, Portugal

[3] Department of Earth and Environment, Boston University, 685 Commonwealth Avenue, Boston, MA 02215, USA

ABSTRACT

Animal pollination is critical for maintaining the reproduction and genetic diversity of many plant species, especially those in tropical ecosystems. Despite the threat to pollination posed by tropical deforestation, it remains an understudied process. In particular, little is known about these dynamics in multi-paternal, successional plant species whose fruits can contain substantial genetic diversity. Given the importance of successional plants in reforestation, quantifying the factors that impact their reproduction is essential for understand- ing plant gene flow in the context of global change. In this study, we investigated pollen-mediated gene flow at the multi-paternal fruit level to quantify how tropical pollinators navigate and mediate gene flow in altered forests. Utilizing microsatellite genotyping and pater- nity analyses, we revealed that distinct plant neighborhood and individual factors drive pollen dispersal at the intra-fruit scale. Variance in pollen dispersal distances was greater for neighborhoods with higher conspecific density, indicating that density dependent reproduc- tive patterns play a role at this scale. Additionally, both the diversity and evenness of sires mediated by a single pollinator were affected by the size of the mother tree, that is, larger mothers received pollen from a less diverse, less even pool of sires per fruit. Pollinator body size was not found to be a significant driver of pollen dispersal, indicating that both small- and large-bodied pollinators were equally important pollen dispersers at this scale. By exploring patterns of variation at the intra-fruit level, we show that conspecific den- sity and tree size significantly impact multi-paternal pollen-mediated gene flow, reinforcing the importance of investigating intraspecific, intra-individual variance in plant reproduction.

Abstract in Spanish is available with online material.

THE TROPICS ARE HOME TO UNPARALLELED LEVELS OF BIODIVERSITY and complex networks of plant−animal interactions, much of which may be threatened by increasing levels of deforestation and land alteration. Given that in most studied tropical forest com- munities, more than 90 percent of tropical plant species are ani- mal-pollinated (Ollerton *et al.* 2011), the disruption of pollen dispersal services represents one of the greatest threats to tropical biodiversity posed by anthropogenic land conversion (Aizen & Feinsinger 1994, Hadley & Betts 2012, Hansen *et al.* 2013) . Many animal-pollinated plants are self-incompatible and obligately dependent on their pollen dispersers (Aguilar *et al.* 2006); there- fore, pollen-mediated gene flow and the subsequent diversity of sired offspring are critically impacted by a pollinator's ability to effectively disperse pollen between individual plants. As deforesta- tion alters the spatial aggregation and size of plants, these changes to the arrangement of individuals within plant neighbor- hoods can influence mutualistic interactions (Kunin 1997, Jones & Comita 2008); thus, it is possible that neighborhood and

individual traits could influence not only seed production but also pollen-mediated gene flow. At the neighborhood level, positive density dependence is a ubiquitous phenomenon in plant repro- duction and is thought to be a strong driver of pollen dispersal and siring patterns (Ghazoul 2005, Dick *et al.* 2008). At the indi- vidual level, traits, such as plant and pollinator size, may also act to influence seed set and pollen dispersal (Lowe *et al.* 2015, Cas- tilla *et al.* 2017). Despite the potential importance of such neigh- borhood- and individual-level traits in mediating plant reproduction across human-altered regions, much remains unknown about the drivers of pollen-mediated gene flow.

Understanding pollen dispersal and siring patterns is particu- larly critical for tropical pioneer plants, as these species are often dominant in secondary forests, which are becoming increasingly common across the tropics. Such species often have very differ- ent life strategies compared to primary forest species (Snow 1965), the latter of which have been the primary focus of many past plant gene flow studies (reviewed in Dick *et al.* 2008). Specif- ically, many pioneer species have multi-seeded fruits that could potentially be fertilized by several sires; thus, the post-fertilization dispersal unit could transfer very different genetic information

than that of a single-seeded fruit. Therefore, pioneer species may display unique, but overlooked patterns of pollen movement and siring across altered forests (Rhodes *et al.* 2017). The role of pollinators may also be different for multi-seeded pioneer plant species, as the spatial scales at which pollinator foraging affects pollen-mediated gene flow include not only the population and individual tree levels (Vranckx *et al.* 2011), but also the individual fruit, potentially yielding different gene flow patterns depending on the ecological scale at which they are measured (Breed *et al.* 2015). Despite the high occurrence of multi-paternity in pioneer plant species, and the growing importance of understanding the regeneration of tropical forests in global conservation efforts, few studies have examined the variation in siring patterns or pollen dispersal distances within multi-paternal fruits in these forests (Davies *et al.* 2015, Ramos *et al.* 2016) and fewer have explored its relationship to attributes of (1) the pollinator, (2) the plant, and (3) the plant neighborhood (Breed *et al.* 2015, Castilla *et al.* 2017, Rhodes *et al.* 2017).

To begin, while we know that pollinator traits can influence seed production in multi-paternal plants (Castilla *et al.* 2015, 2017, Cranmer *et al.* 2012), our understanding of how pollinator attributes influence pollen-mediated gene flow in multi-paternal species is limited (Breed *et al.* 2015, Hasegawa *et al.* 2015). Past work has suggested that pollinator traits such as body size and foraging behavior can be important drivers of plant reproductive output (Sahli & Conner 2007, Mitchell *et al.* 2013). Assertions about optimal foraging and pollinator efficacy based on body size have led to the general assumption that larger-bodied pollinators should perform better at various efficacy metrics such as dispers- ing pollen further distances and depositing more pollen (Lindauer 1957, Stout 2000, Greenleaf *et al.* 2007). While some studies support this theory (Hasegawa *et al.* 2015), many have historically compared pollinators that are functionally and morphologically very different (Breed *et al.* 2015, Rhodes *et al.* 2017); or when comparing more functionally similar pollinators, studies have found a more complicated relationship between size, behavior, and effectiveness (Stout 2000). In tropical forests, Castilla *et al.* (2017) found that although larger-bodied pollinators set more seeds per visit, smaller-bodied pollinators visited plants more fre- quently and dispersed pollen similar distances when compared to large-bodied pollinators. Across studies, what remains unknown is how morphologically and functionally similar tropical pollina- tors contribute to siring and pollen dispersal within multi-paternal fruits, particularly in ecosystems where individual plant traits and neighborhood composition may be variable.

At the scale of the individual plant, floral display size could be a critical trait in determining pollen dispersal distance and sir- ing diversity. For example, past work shows that pollinators may visit larger trees for longer foraging durations within a tree, possi- bly increasing the transfer of self-pollen or pollen from nearby, closely related individuals, and likely promoting greater levels of geitonogamy and inbreeding (Karron & Mitchell 2012, Mitchell *et al.* 2013). Alternately, trees with larger floral displays could receive more occasional floral visitors than smaller trees and thus could support long-distance dispersal events and act to increase

genetic admixture (Makino *et al.* 2007). For tree species that have developed complete self-incompatibility, these influences on within-tree foraging may have significant impacts on the genetic variability of new cohorts. In tropical forests, past research has indicated that larger trees may produce more fruits and seeds per tree but can have a lower proportion of viable seeds per fruit compared to smaller trees (Castilla *et al.* 2015). These patterns suggest that individual plant traits may contribute to variance in pollinator foraging behavior, differentially impacting seed compo- sition at the fruit, individual, or regional level.

In addition to individual plant traits, plant neighborhood fea- tures may also influence pollen dispersal and siring in plants. Plant conspecific density has been found to play a critical role in the probability of successful outcrossed mating events, and the survival of future, genetically diverse plant cohorts (Ghazoul 2005, Ismail *et al.* 2012, Comita *et al.* 2014). Spatially isolated trees may engage in fewer mating opportunities and are often assumed to be more genetically distinct; thus, the ability of pollen dispersers to facilitate long-distance dispersal events from less dense patches may be particularly important in the movement of novel genetic diversity to and from spatially isolated plant neigh- borhoods (Nei 1972, Hutchison and Templeton 1999, Vekemans & Hardy 2004). Mechanistically, spatial isolation of plants may reduce pollinator efficacy and plant fitness through increased transference of self-pollen and biparental inbreeding, which can lead to increases in seed abortion rates or seed cohorts with reduced fitness (Hufford & Hamrick 2003, Breed *et al.* 2012, 2014, Rhodes *et al.* 2017). Interestingly, this may lead to neigh- borhoods of high local kinship, a critical trait where neighboring individuals exhibit high levels of relatedness (Loiselle *et al.* 1995). In combination with conspecific density, two tropical studies have found that high local kinship in dense neighborhoods can coun- teract the benefits of proximity to reproductive output, some- times increasing the transfer of pollen that is too genetically similar, consequently increasing the number of aborted seeds and/or fruits in those neighborhoods (Jones & Comita 2008, Castilla *et al.* 2015). Overall, the combination of these spatial and genetic patterns may be particularly relevant in fragmented tropi- cal forests where conspecific density and kinship can be highly variable (Jha & Dick 2010) and natural population densities tend to be low (Duminil *et al.* 2016).

Finally, while a number of fields now emphasize the impor- tance of analyzing ecological variance and measures of intra-indi- vidual and intraspecific variation in ecological systems (Violle *et al.* 2012), many studies still focus on maximal, mean, or single- seed measures and sire diversity indices to describe pollen disper- sal processes (Lowe 2005, Lowe *et al.* 2015) . Such analyses may not be able to capture the true breadth of genetic information transferred to each new generation via pollen dispersal, particu- larly in the case of plants with reproductive strategies that involve multi-seeded, multi-paternal fruits (Ghazoul 2005, Lowe *et al.* 2015). Regarding pollinators, mean and maximal foraging mea- sures may not provide an accurate depiction of a pollinator's typi- cal foraging behavior (Roubik 1989, van Nieuwstadt & Iraheta 1996), nor the variation that exists in an individual pollinator's

response to landscape alteration (Wenner et al. 1991). Given these factors, it is likely that changes in pollen dispersal patterns due to land-use and global change described at the plant species or regional scale may exhibit a very different distribution from those described at the individual or intra-individual scale. Thus, to more completely capture patterns of genetic diversity and the reproduc- tive strategies of functionally different plant species, it will be nec- essary to explicitly analyze variance, diversity, and evenness, especially at finer ecological scales such as the intra-individual, and even intra-reproductive unit level (Bolnick et al. 2011, Breed et al. 2012, Breed et al. 2014, Breed et al. 2015).

In this study, we investigate the impacts of pollinator, plant, and plant neighborhood traits on pollen-mediated gene flow for the tropical pioneer tree, Miconia affinis. Specifically, we ask whether pollen dispersal distances and the transfer of genetic diversity per pollinator visit to a multi-seeded, multi-paternal fruit differ across different pollinator body sizes, plant sizes, conspeci- fic tree densities, and local kinship levels. To take advantage of the multi- paternal fruit structure of this species, we utilize a unique approach and measure several statistical moments and multiple diversity indices per pollen dispersal event. In particular, we examine three facets of pollen dispersal and three facets of sire diversity per pollinator visit: (a) the mean pollen dispersal dis- tance, maximum pollen dispersal distance, and standard deviation of the pollen dispersal distance and (b) the raw sire counts, the Chao estimated sire diversity, and sire evenness. We hypothesize that (1) variation in pollen dispersal distances exhibited in a single visit is enhanced by plant conspecific density, local kinship, mother size, and pollinator body size. Particularly, we predict that variation in pollen dispersal distance will be greatest in more iso- lated trees that are visited by larger-bodied pollinators. We also hypothesize that (2) sire diversity exhibited in a single visit is dri- ven by plant local kinship and pollinator body size, but not by plant conspecific density or mother size. Specifically, we predict that sire diversity will be greatest in trees within lower kinship neighborhoods and for visits mediated by larger-bodied pollinators.

METHODS

Study system and sample collection.—For much of the past two centuries, the moist lowland forests of the Panama Canal watershed have been heavily impacted by anthropogenic develop- ment; in 2001, it was estimated that only 54 percent of the origi- nal forest remained while more than 43 percent had been converted to pasture or shrubland (Condit et al. 2001). Our study system includes three study regions that exist across a ~3370-ha area and include 1157 individuals of the tropical pioneer tree M. affinis (Melastomataceae; 3–6 m in height). This tree species has an extensive distribution ranging from Mexico to Brazil and inhabits a wide range of habitats, from primary and unaltered secondary-growth forest to highly fragmented forest and grass- land systems (further system description can be found in Table S1). M. affinis is an ideal study species due to its well-stu- died ecology (Luck & Daily 2003, Jha & Dick 2010, Castilla et al.

2015), known colonization history in the area (Castilla et al. 2016), and availability of genetic tools to examine pollen dispersal (Jha & Dick 2009). The species is dependent on buzz pollination conducted by a suite of native bee species, is hermaphroditic, and is self-incompatible (Jha & Dick 2010) (Fig. 1A). Mature trees of M. affinis display 1–3 flowering events during the Panamanian dry season (January to June), blooming for ~2 d per floral event. The globose fruits of M. affinis develop to maturity between May and September and are mainly dispersed by native small-bodied fru- givorous birds (Luck & Daily 2003, Jha & Dick 2008, 2010) (Fig. 1A).

Single-visit pollination experiments were conducted in 2013 (as described in Castilla et al. 2017): Five focal inflorescences on randomly selected mother trees (N = 75 mother trees; N = 375 inflorescences) were bagged until the day of flowering and the bags were removed to allow a single pollinator to visit each inflo- rescence. Each pollinator was allowed to visit several flowers within one focal inflorescence; when the pollinator departed, it was collected and stored on 70 percent ethanol for subsequent identification and to measure its intertegular distance as a proxy for body size (ITD; sensu Cane 1987). After a single pollinator visit, each inflorescence was re-bagged until fertilized fruits matured, at which point all fertilized fruits were collected. Dissec- tions on the collected fruits and seed viability counts were per- formed to determine the seed set (the proportion of viable seeds out of the total number of seeds) produced by each observed single pollinator visit. Leaf tissue was collected from all adult M. affinis trees (N = 1157) within a 2 km radius of each study region's geographic centroid for use in paternity analyses, as this distance has been shown to capture a large portion of the pollen dispersal kernel for the species (Jha & Dick 2010).

Two individual traits and two plant neighborhood traits were measured per pollination event. Individual traits investigated included the diameter at breast height of the mother trees (dbh_{mother}) as a proxy of floral display size (correlation validated for this species in Castilla et al. 2017, sensu Kettle et al. 2011) and the size of each pollinator as measured by their intertegular dis- tance ($ITD_{pollinator}$), which is often correlated with some indices of dispersal ability (Greenleaf et al. 2007). The plant neighbor- hood traits included nearest neighbor distance (NND_{mother}; the average spatial distance to the ten nearest conspecific trees to the measured mother) as an index of conspecific density, and local kinship of the mother tree, defined as the mean Loiselle kinship coefficient (F_{ij}; the pairwise comparisons between the mother tree and all its neighbors within a 400 m radius sensu Castilla et al. 2017, Hardy et al. 2006). In previous analyses in our study system (Castilla et al. 2016), a 400 m radius was determined to be an important threshold within which mother trees displayed positive fine-scale spatial genetic structure, indicating higher kinship values within this area than expected (Fig. S3). Past work in this system (Castilla et al. 2015, 2016) and others (Jones & Comita 2008) indicates that these specific individual and neighborhood traits have the potential to influence pollen movement. While forest cover was evaluated in our study regions (Table S1), it correlated highly with NND; therefore, we opted to retain NND instead as

FIGURE 1. (A) A *Melipona panamica* bee visiting an *M. affinis* inflorescence (*Photograph:* Antonio R. Castilla), (B) an *M. affinis* infructescence, fertilized fruits turn dark purple when mature, (C) mature *M. affinis* fruits can contain ~30–50 seeds, the number of viable seeds per fruit is highly variable among and within mothers, (D) a map detailing the sires where pollen was collected (black) and the mother where the pollen was deposited (pink) during a single pollination event by a *Melipona panamica* individual in Gamboa, Panama.

we know from past work in the region that NND can influence seed viability and pollen dispersal, even when forest cover is relatively homogeneous (Castilla *et al.* 2016).

GENETIC ANALYSES.—In order to quantify variation in sire diver- sity and pollen dispersal distances at the fruit level, we subsam- pled fruits from the single-visit experiments, only including visits that resulted in >10 viable seeds per fruit, from which ten seeds were randomly selected for the genetic analysis. We also randomly subsampled the resulting fruits to only include one fruit per tree, to minimize resampling of individual mother trees. For our paternity analyses, we only utilized fruits for which we were able to successfully genotype ten viable seeds (N = 34 single fruits; N = 340 seeds). We set the threshold at ten viable seeds, as previous work in this system has indicated that *M. affinis* exhibits high sire diversities at the inflorescence level; therefore, we wanted to ensure that we had an even and sufficient sample size to estimate sire diversity at the fruit level (Castilla *et al.* 2015). Further, given that more than 60 percent

of all fruits visited by a single pollinator had greater than ten viable seeds, setting this minimum threshold did not preclude us from describing a large portion of fruits in our study sys- tem. These visits were conducted primarily by social bee species within the Meliponini tribe; however, the 14 species varied greatly in size, exhibiting ITD measures that ranged from 0.91– 7.72 mm (x = 2.19 mm, SD = 1.09 mm). We did not explore pollinator species-level effects given our small per-species sam- ple size and the inability to detect species-level effects in larger data sets from the study region (See Fig. S1, Castilla *et al.* 2017). DNA was extracted from individual seeds using the DNAzol Genomic DNA Isolation Reagent extraction protocol, and from leaves sampled from all 1157 adult trees using the CTAB protocol, and then PCR processed using Qiagen Multi- plex PCR Master Mix (Castilla *et al.* 2015). The seeds and leaves were genotyped at 12 highly polymorphic microsatellite loci (Le Roux & Wieczorek 2008, Jha & Dick 2009) and later narrowed to eight polymorphic markers (Micaff-5, Micaff-7, Micaff-8, Micaff-14, Micaff-16, Micaff-19, B102, B109) to

remove markers with inconsistent PCR recovery or possible presence of null alleles.

Using the genotypes from all ten seeds per visit, we used HP-RARE (Kalinowski 2005) to measure the allelic richness and private allelic richness of each fruit (Table S2). We then conducted likelihood-based paternity assignments using CERVUS, where sire assignments were only made to seeds with a confidence criterion of >0.80 (Marshall *et al.* 1998). Paternities were also assigned at 90 percent confidence, but because this reduced our sample size to less half of the original dataset, we focused our analyses on data from the >80 percent confidence assignments (282 seeds), as per other past plant dispersal studies (*e.g.,* Hardesty *et al.* 2006, Bittencourt & Sebbenn 2007, Lander *et al.* 2010). Given that sampling efforts are finite and limited to a 2 km radius in this study, unassigned parentage is likely the result of dispersal events outside of the sampled plant neighborhoods, as assumed in past studies (Bittencourt & Sebbenn 2007, Bacles & Ennos 2008, Dick *et al.* 2008). From these assignments, two groups of measures were generated: (a) three dispersal distance moments and (b) three sire diversity measures. For the dispersal distance moments, we calculated the linear distances between mother and the assigned pollen donor from the CERVUS analysis (Fig. 1D), and these were measured at three statistical moments: the mean, the standard deviation, and the maximum pollen dispersal distances per fruit. For the sire diversity measures, we measured three values: the raw sire counts per fruit from the CERVUS output (*sensu* Pelabon *et al.* 2015), the Chao diversity estimator (Chao 1984) for the CERVUS output (to account for small sample sizes), and sire evenness, defined as the proportional representation of individual sires within a seed set, to account for the likely uneven representation of sires in our progeny arrays (Mitchell *et al.* 2013). As a second index of sire counts and diversity, we also calculated the number of full versus half sibships per fruit using COLONY (Jones and Wang 2010) and similarly calculated half sibship Chao diversity; we found the two quantifications of sire diversity to be very similar and therefore present the COLONY results in the Supplemental Information (Fig. S1) and focused on the CERVUS-based paternity analysis when discussing our results. Finally, for descriptive purposes, we also used GenAlEx (Peakall & Smouse 2006) to generate a study region-level genetic summary including: N (the total number of seeds that were successfully assigned fathers per study region), N_a (the average number of alleles per locus), H_e (the expected heterozygosity), and H_o (the observed heterozygosity) (Table S2).

STATISTICAL ANALYSES.—We used linear mixed-effects models to analyze the influence of individual explanatory variables (dbh and ITD) and plant neighborhood explanatory variables (NND and local Kinship) on the two groups of response variables: (a) dispersal distance moments (mean, maximum, standard deviation) and (b) sire diversity measures (raw count, Chao diversity, evenness). We also included study region and mother tree as random factors, with mother tree nested within study region. All our response variables and NNDs were log-transformed. We found no interactions between variables in our models (Table S3).

RESULTS

Our CERVUS analysis successfully assigned fathers to 83 percent of the 340 sampled seeds with a confidence criterion of >0.80 (282 seeds). The subsequent dispersal distances measured from these assignments ranged from 2.8 m to 3600 m ($\bar{1}$ = 905 m, SD = 953 m) with 66 percent of the pollen dispersal events coming from fathers that were 1 km or closer to the mother trees (Fig. S2). Pollinator body size did not affect pollen dispersal distances in our study system (Fig. 2, Table 1). We found a negative relationship between NND and the standard deviation in pollen dispersal distances per visit/fruit (Table 1, Fig. 3A), but no significant relationships between any of the explanatory variables and either the mean or maximum pollen dispersal distance (Table 1). In other words, the multiple pollen dispersal distances from a single visit were more similar to one another (lower in standard deviation) in spatially isolated trees than in spatially aggregated trees. The raw sire counts from the CERVUS paternity analysis yielded anywhere from 3 to 9 fathers per visit/fruit ($\bar{1}$ = 6.4, SD = 1.74), the Chao sire diversities estimated from the same output ranged from 4 to 10 fathers per visit/fruit ($\bar{1}$ = 7.3, SD = 1.77), and sire evenness ranged from 0.55 to 1.0 ($\bar{1}$ = 0.92, SD = 0.1). Both the raw sire counts and Chao sire diversities were significantly affected by the size of the mother tree (dbh), with raw sire counts and sire diversity significantly decreasing as the mother dbh increased (Table 1, Fig. 3B). Sire evenness per dispersal event also showed a negative relationship with dbh (Table 1, Fig. 3C).

DISCUSSION

In this study, by investigating multiple measures of pollen dispersal and siring in a multi-paternal species, we determined key drivers of pollen-mediated gene flow for a ubiquitous successional plant species across heterogeneous study regions. Specifically, we found that variation in pollen dispersal distances per visit was significantly greater for trees that were more spatially aggregated. We also show that measures of sire diversity and evenness are most influenced by the size of the mother tree, with sire diversity and evenness significantly decreasing as mother tree size increases. None of our measures of multi-paternal reproductive dynamics were significantly affected by the body size of the pollinator, indicating that in the pollinator community we measured, both large-bodied and small-bodied pollinators are contributing similarly to pollen-mediated gene flow across fragmented tropical landscapes.

First, we found that for our study species, a tropical pioneer tree, there was tremendous variation in pollen dispersal and siring mediated by a single pollinator visit, irrespective of pollinator size. In fact, many of the smaller-bodied pollinators travelled just as far, if not further, than some of the larger-bodied pollinators, depositing pollen from a wide variety of distances and from multiple fathers in a single floral visit. For example, we found a single small-bodied *Trigona muzoensis* (ITD = 1.72 mm) deposited pollen collected from an estimated nine sires that were anywhere from 10 m to 2700 m away from the mother. A much larger
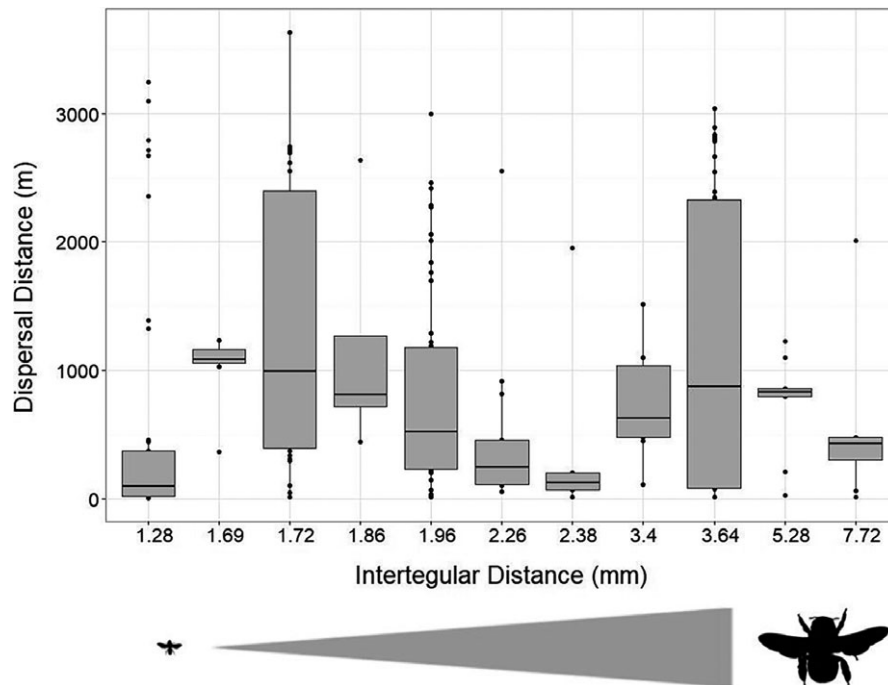
FIGURE 2. Dispersal distances for all observed species of pollinators listed in increasing body size (ITD) order. From smallest to largest species (mm): *Tetrago- nisca angustula* (1.28), *Halictidae sp.* (1.69), *Trigona muzoensis* (1.72), *Paratetrapedia lineata* (1.86), *Trigona fuscipennis* (1.96), *Trigona fulviventris* (2.26), *Pseudochloropsis schrottky* (2.38), *Trigona buyssoni* (3.4), *Melipona panamica* (3.64), *Centris dichootricha* (5.28), *Xylocopa* fimbriata (7.72).

bee, *Xylocopa* fimbriata (ITD = 7.72 mm), deposited pollen from an estimated seven sires that were similarly about 12 to 2000 m away from the visited mother. In terms of traditional perspectives regarding pollinator body size and maximal foraging behaviors, these results contradict the assumption that large-bodied pollinators will always travel further distances than small-bodied pollinators and that, despite forest features, larger-bodied pollinators will consistently travel the longest distances (Greenleaf *et al.* 2007). These results support our previous findings that pollinator body size is not the primary driver of pollen dispersal distance in our study system (Castilla *et al.* 2017). Other studies have also begun to present mixed findings with regard to pollinator body size, indicating that it may be too simplistic of a portrayal of a pollinator species' biology to accurately describe their foraging response to landscape change. Stout (2000), for example, found that although larger bees visited more flowers, smaller bees were more effective at triggering floral mechanisms that release pollen in Scotch broom (*Cystisus scoparius*). Our results resonate with a number of studies that indicate that individual functional traits, such as pollinator body size (ITD), are not the best predictors of pollinator behavior and pollen movement in heterogeneous land- scapes (Roubik 1989, Stout 2000, Makino *et al.* 2007), and that future studies may benefit from including more detailed data on pollinator foraging behaviors.

Instead, we found that the variance in distances from which pollen was dispersed per visit was significantly predicted by the degree of spatial isolation of the mother tree; specifically, our data reveal that variation in the sire distances per pollinator visit

increased in neighborhoods with higher conspecific density (lower NND). In other words, more spatially aggregated mother trees received pollen from sires that were located at more variable distances from the mother tree. This finding is in contrast to Dumi- nil *et al.* (2016), which found that pollen dispersal distances consistently increased with decreasing conspecific density, but corroborates patterns found by Ismail *et al.* (2012) which indicate that while plants in more dense patches tend to receive pollen from donors within their own patch, they receive this pollen from a larger number of donors from a variety of distances within the patch. From the plant's perspective, spatially aggregated mothers may benefit from this variance in pollen dispersal distance, as they are likely receiving pollen from a higher variety of conspeci- fic neighborhoods. Past work has indicated that both the kinship and the density of different conspecific neighborhoods can impact fruit production (Jones & Comita 2008); specifically, they found that high conspecific density can interact negatively with high local relatedness by counteracting positive density dependent forces in fruit set with higher biparental inbreeding and thus higher fruit abortion rates. Previous research in our study system has also found a negative interaction between local kinship and conspecific density, which also acted to reduce reproductive out- put and increase variance in mean seed viability with increasing conspecific density and local kinship (Castilla *et al.* 2015). While we did not find a similar interaction between density and kinship in our analysis of multiple seeds per fruit, we did document high variance in pollen dispersal within a fruit, and we reveal that den- sity dependent patterns also exist for variance in pollen dispersal,

TABLE 1. *Full outputs of the linear mixed-effects models run for the two response variable groups: (1) dispersal distance moments (standard deviation, mean, maximum) and (2) diversity metrics (the raw counts and Chao extrapolated sire diversity measures, sire evenness).*

	Individual factors		Neighborhood factors	
	dbh	ITD	NND	Mother Kinship
Dispersal distance moments				
SD				
Est.	0.195	0.149	0.325	0.025
t	1.529	1.167	2.573	0.200
P	0.136	0.251	0.015*	0.842
Mean				
Est.	0.070	0.070	0.192	0.009
t	0.520	0.517	1.433	0.072
P	0.606	0.608	0.161	0.943
Max				
Est.	0.007	0.072	0.041	0.026 *t*
	0.057	0.632	0.329	0.232 *P*
	0.955	0.533	0.746	0.818
Diversity metrics				
Raw				
Est.	0.131	0.019	0.041	0.052 *t*
	2.613	0.380	0.822	1.098 *P*
	0.013*	0.706	0.417	0.280
Chao				
Est.	0.095	0.023	0.030	0.047 *t*
	2.192	0.524	0.704	1.139 *P*
	0.035*	0.592	0.486	0.263
Evenness				
Est.	0.059	0.007	0.009	0.031 *t*
	2.951	0.354	0.446	1.633 *P*
	0.006*	0.726	0.659	0.112

Independent variables tested include nearest neighbor distance (m) (NND), the local kinship of the mother tree, the size of the mother tree (cm) (dbh) and the intertegular distance of the pollinator (mm)(ITD). Results are pre- sented as the estimated strength and direction of the relationship, and the *t*- and *P*-values. df = 34.

*Significant relationships in are bold.

with potential impacts on post-fertilization gene flow in other multi-paternal systems.

This density dependent relationship with pollen dispersal variation may also be due to the way pollinators forage in resource-dense versus resource-poor patches in the short 2-d, mass-flowering period of our study species (Delmas *et al.* 2015). From the pollinator's perspective, spatially aggregated patches may act as epicenters of foraging activity, attracting pollinators, from both near and far, specifically those who have noted them as reward centers in their spatial memory (as seen in flight cages, Burns & Thomson 2005). Also, breakdowns in predicted nearest neighbor foraging patterns have been observed to depend not only on conspecific densities in remnant patches, but the spatial

aggregation of pollen sources within these patches (Dick *et al.* 2008). Interestingly, our pollen dispersal data, which documented a few long-distance and several short-range foraging events for almost all of the observed pollinators, provide rare individual- level tracking and support for previous hypotheses that the majority (>75%) of bee foraging activity occurs within the lower 40 percent of a species' maximum foraging range (Roubik 1989). Several of these previous theories had either been based on con- trolled, experimental conditions (van Nieuwstadt & Iraheta 1996, Zurbuchen *et al.* 2010), or field-based observations that were designed to measure only maximum foraging distances (Roubik & Aluja 1983, Knight *et al.* 2005), but no studies were aimed at capturing the breadth of distances pollinators travel during nor- mal foraging bouts for multiple species, particularly in heteroge- neous forests. Although we were unable to separate the potential contribution of secondary pollen transfer to the long-distance dis- persal events we measured, its impacts may be limited due to the short time window (~2-d flowering events) pollinators have to forage at *M. affinis*. Overall, our measures of variance in dispersal distances bolster previous findings of density dependence, detect patterns that were not apparent via mean and maximum mea- sures, and help provide a more complete depiction of how polli- nators are navigating these heterogeneous landscapes.

Third, we found that the diversity and evenness of sires per pollinator visit were significantly influenced by the size of the mother tree (dbh): Both sire diversity and sire evenness decreased as the size of the mother tree increased. Given that the number of inflorescences per tree strongly correlates with the size of the tree in this system (Castilla *et al.* 2017), dbh of *M. affinis* mothers serves as a proxy for floral display size. Taken together, these results indicate that larger *M. affinis* individuals receive less diverse pollen from a less even group of sires compared to smaller trees. Pollinators have been found to respond to tree floral display size independent of local conspecific density (Makino *et al.* 2006); therefore, potential explanations for this pattern could be that flo- ral display size independently alters pollinator foraging patterns at the individual tree and fruit level. Specifically, larger floral displays may promote longer foraging bouts at a single tree, increasing the transfer of self or non-legitimate pollen, thereby decreasing female reproductive success (Ghazoul 2005, Jones & Comita 2008, Brys & Jacquemyn 2010). Previous findings in other sys- tems have also indicated that increased floral display sizes may lead to increases in near-neighbor matings and reductions in the number of sires due to changes in pollinator foraging patterns, regardless of pollinator species or size (Stout 2000). More specifi- cally, Mitchell *et al.* (2004) and Karron *et al.* (2003) found that several species of bees that pollinate *Mimulus ringens* strongly responded to increased floral display size: Trees with larger floral display sizes attracted more floral visitors, but these visitors stayed at individual trees for longer and visited more flowers per plant as floral display size increased, effectively reducing siring success and increasing self-fertilization. Our results could simi- larly indicate that *M. affinis* pollinators are spending more time foraging at trees with larger floral displays, visiting fewer potential pollen donors, resulting in reduced sire diversity and evenness
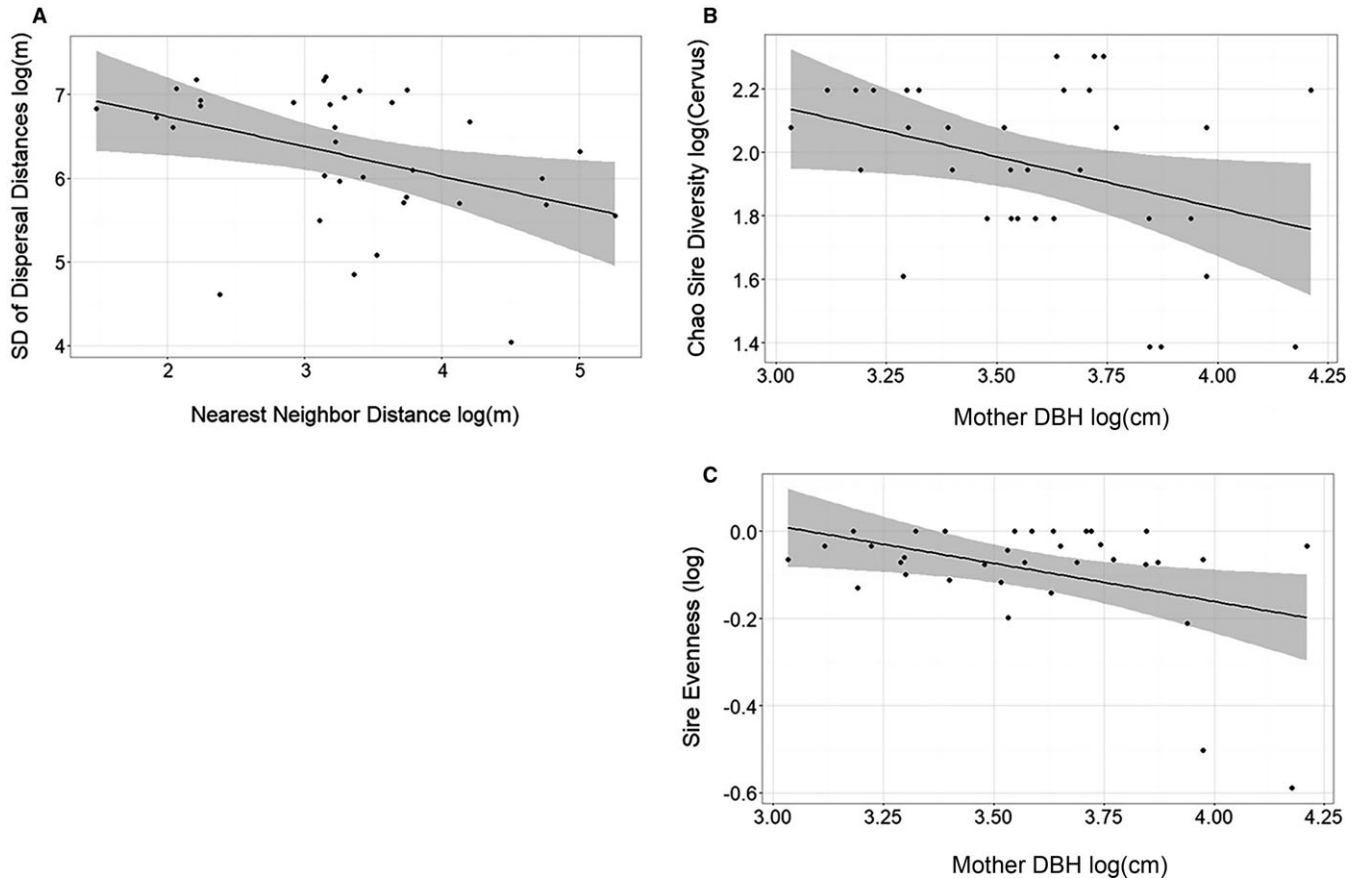
FIGURE 3. (A) The negative relationship between the standard deviation in dispersal distances per pollination event and nearest neighbor distance (m), (B) the negative relationship between Chao sire diversity and mother tree diameter at breast height (DBH) (cm), (C) the negative relationship between sire evenness and the size of the mother tree diameter at breast height (cm)

within fruits at such trees. Therefore, similar to our conspecific density and dispersal distance findings, if larger trees act as resource epicenters for pollinator foraging, we may be detecting a trade-off between attracting sufficient pollinators to an area to promote gene flow and receiving sufficiently diverse pollen per pollinator visit (Mitchell *et al.* 2013). Additionally, our findings support previous suggestions by Stout (2000) that while floral display size may alter pollinator visitation and pollen dispersal rates, it does so independently of pollinator body size, indicating that bees of all sizes respond similarly to floral display size. Over- all, these results highlight the importance of diverse tree sizes to promote variation in pollen dispersal and reproductive processes and to prevent erosion of genetic diversity in fragmented tropical plant communities (Ellstrand 2014).

As secondary and fragmented forest structures are becoming increasingly common across tropical regions, it is critical that we improve our understanding of how forest structure impacts species interactions and the reproductive dynamics of successional plant communities (Girão *et al.* 2007, Magrach *et al.* 2014, Lowe *et al.* 2015). In this study, we found that two key factors, NND and mother tree size, significantly influenced pollen dispersal and

sire diversity at the within-fruit level and thus should be taken into consideration when assessing pollinator-mediated gene flow. This finding is novel as it highlights the relevance of both plant neighborhood (conspecific density) and individual traits (tree size) as drivers of within-individual variance in pollen dispersal and sire diversity across heterogeneous forests. We specifically note that maintaining an array of conspecific tree sizes, not just large trees, in a forest can support high levels of sire genetic diversity and evenness. We also found that pollinators of all body sizes promoted high multi-paternity levels and long-distance gene flow, indicating that pollinator conservation decisions should not necessarily rank species prioritization based on size alone. Importantly, given that we found different drivers of pollen distance and sire diversity when examining multiple seeds in multi-paternal fruit, it is important that we begin to explore a broader array of plant reproductive systems and life history strategies in our investigation of landscape genetics processes for conservation. (Kremen 2005). Finally, our results also illuminate the need to evaluate dispersal and genetic indices beyond mean values and highlight the importance of future work to develop additional methods that capture variation in ecological function.

ACKNOWLEDGMENTS

DATA AVAILABILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.j1344c7 (O'Connell *et al.* 2018).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

TABLE S1. *Environmental metrics of study regions, their Miconia affinis neighborhoods, and their pollinator communities across the Panama Canal watershed.*

TABLE S2. *Genetic summary of M. affinis seeds in the three study regions.*

TABLE S3. *Correlation tests between response variables.*

FIGURE S1. Raw sire count, and Chao extrapolated sire diversity.

FIGURE S2. Density distribution of all pollen dispersal events.

FIGURE S3. Correlograms from spatial autocorrelation analysis using the Ritland kinship coefficient and even 100 m distance classes in the three *M. affinis* focal study regions.

LITERATURE CITED

AGUILAR, R., L. ASHWORTH, L. GALETTO, AND M. AIZEN. 2006. Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis. Ecol. Lett. 9: 968–980.

AIZEN, M. A., AND P. FEINSINGER. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75: 330–351.

BACLES, C. F. E., AND R. A. ENNOS. 2008. Paternity analysis of pollen-mediated gene flow for fraxinus excelsior L. in a chronically fragmented landscape. Heredity 101: 368–380.

BITTENCOURT, J., AND A. SEBBENN. 2007. Patterns of pollen and seed dispersal in a small, fragmented population of the wind-pollinated tree *Araucaria angustifolia* in southern Brazil. Heredity 99: 580–591.

BOLNICK, D. I., P. AMARASEKARE, M. S. ARAUJO, R. BÜRGER, J. M. LEVINE, M. NOVAK, AND D. A. VASSEUR. 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol. Evol. 26: 183–192.

BREED, M. F., M. J. CHRISTMAS, AND A. J. LOWE. 2014. Higher levels of multiple paternities increase seedling survival in the long-lived tree *Eucalyptus gracilis*. PLoS One 9: e90478.

BREED, M. F., M. H. K. MARKLUND, K. M. OTTEWELL, M. G. GARDNER, J. B. C. HARRIS, AND A. J. LOWE. 2012. Pollen diversity matters: Revealing the neglected effect of pollen diversity on fitness in fragmented land-scapes. Mol. Ecol. 21: 5955–5968.

BREED, M., K. OTTEWELL, M. GARDNER, M. MARKLUND, E. DORMONTT, AND A. LOWE. 2015. Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. Heredity 115: 108–114.

BRYS, R., AND H. JACQUEMYN. 2010. Floral display size and spatial distribution of potential mates affect pollen deposition and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). Plant Biol. 12: 597–603.

BURNS, J. G., AND J. D. THOMSON. 2005. A test of spatial memory and movement patterns of bumblebees at multiple spatial and temporal scales. Behav. Ecol. 17: 48–55.

CANE, J. H. 1987. Estimation of bee size using intertegular span (Apoidea). J. Kans. Entomol. Soc. 60: 145–147.

CASTILLA, A. R., N. POPE, R. JAFFE, AND S. JHA. 2016. Elevation, not deforestation, promotes genetic differentiation in a pioneer tropical tree. PLoS One 11: e0156694.

CASTILLA, A., N. POPE, AND S. JHA. 2015. Positive density-dependent reproduction regulated by local kinship and size in an understorey tropical tree. Ann. Bot. 117: 319–329.

CASTILLA, A. R., N. S. POPE, M. O'CONNELL, M. F. RODRIGUEZ, L. TREVIÑO, A. SANTOS, AND S. JHA. 2017. Adding landscape genetics and individual traits to the ecosystem function paradigm reveals the importance of species functional breadth. Proc. Natl Acad. Sci. USA 114: 12761–12766.

CHAO, A. 1984. Nonparametric estimation of the number of classes in a population. Scand. Stat. Theory Appl. 11: 265–270.

COMITA, L. S., S. A. QUEENBOROUGH, S. J. MURPHY, J. L. ECK, K. XU, M. KRISHNADAS, N. BECKMAN, Y. ZHU, AND L. GOMEZ-APARICIO. 2014. Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. J. Eco. 102: 845–856.

CONDIT, R., W. D. ROBINSON, R. IBAÑEZ, S. AGUILAR, A. SANJUR, R. MARTINEZ, AND S. J. WRIGHT. 2001. The Status of the Panama Canal Watershed and Its Biodiversity at the Beginning of the 21st Century: Long-term ecological studies reveal a diverse flora and fauna near the Panama Canal, harbored within a corridor of forest stretching from the Caribbean to the Pacific, but deforestation, land degradation, erosion, and overhunting remain threats. AIBS Bull. 51: 389–398.

CRANMER, L., D. MCCOLLIN, AND J. OLLERTON. 2012. Landscape structure influences pollinator movements and directly affects plant reproductive success. Oikos 121: 562–568.

DAVIES, S. J., S. CAVERS, B. FINEGAN, A. WHITE, M. F. BREED, AND A. J. LOWE. 2015. Pollen flow in fragmented landscapes maintains genetic diversity following stand-replacing disturbance in a Neotropical pioneer tree, *Vochysia ferruginea* Mart. Heredity 115: 125129.

DELMAS, C. E. L., N. ESCARAVAGE, P. O. CHEPTOU, O. CHARRIER, S. RUZAFA, P. WINTERTON, AND A. PORNON. 2015. Relative impact of mate versus pollinator availability on pollen limitation and outcrossing rates in a mass-flowering species. Plant Biol. 17: 209–218.

DICK, C. W., O. J. HARDY, F. A. JONES, AND R. J. PETIT. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. Trop. Plant Biol. 1: 20–33.

DUMINIL, J., K. DAÏNOU, D. K. KAVIRIRI, P. GILLET, J. LOO, J. L. DOUCET, AND O. J. HARDY. 2016. Relationships between population density, fine-scale genetic structure, mating system and pollen dispersal in a timber tree from African rainforests. Heredity 116: 295.

ELLSTRAND, N. C. 2014. Is gene flow the most important evolutionary force in plants? Am. J. Bot. 101: 737–753.

GHAZOUL, J. 2005. Pollen and seed dispersal among dispersed plants. Biol. Rev. 80: 413–443.

GIRÃO, L. C., A. V. LOPES, M. TABARELLI, AND E. M. BRUNA. 2007. Changes in tree reproductive traits reduce functional diversity in a fragmented Atlantic forest landscape. PLoS One 2: e908.

GREENLEAF, S. M., N. WILLIAMS, R. WINFREE, AND C. KREMEN. 2007. Bee foraging ranges and their relationship to body size. Oecologia 153: 589–596.

HADLEY, A. S., AND M. G. BETTS. 2012. The effects of landscape fragmenta- tion on pollination dynamics: Absence of evidence not evidence of absence. Biol. Rev. 87: 526–544.

HANSEN, M. C., P. POTAPOV, R. MOORE, M. HANCHER, S. TURUBANOVA, A. TYUKAVINA, D. THAU, S. STEHMAN, S. GOETZ, T. LOVELAND, A. KOMMAREDDY, A. EGOROV, L. CHINI, C. O. JUSTICE, AND J. R. TOWNSHEND. 2013. High-resolution global maps of 21st-century forest cover change. Science 342: 850–853.

HARDESTY, B. D., S. P. HUBBELL, AND E. BERMINGHAM. 2006. Genetic evidence of frequent long-distance recruitment in a vertebrate-dispersed tree. Ecol. Lett. 9: 516–525.

HARDY, O. J., L. MAGGIA, E. BANDOU, P. BREYNE, H. CARON, M. CHEVALLIER, A. DOLIGEZ, C. DUTECH, A. KREMER, C. LATOUCHE-HALLE, V. TROISPOUX, V. VERON, AND B. DEGEN. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 neotropical tree species. Mol. Ecol. 15: 559–571.

HASEGAWA, Y., Y. SUYAMA, AND K. SEIWA. 2015. Variation in pollen-donor composition among pollinators in an entomophilous tree species, *Castanea crenata*, revealed by single-pollen genotyping. PLoS One 10: e0120393.

HUFFORD, K., AND J. L. HAMRICK. 2003. Viability selection at three early life stages of the tropical tree, Platypodium elegans (Fabaceae, Papil- ionoideae). Evolution 57: 518–26.

HUTCHISON, D., AND A. TEMPLETON. 1999. Correlation of Pairwise Genetic and Geographic Distance Measures: Inferring the Relative Influences of Gene Flow and Drift on the Distribution of Genetic Variability. Evolution 53: 1898–1914.

ISMAIL, S. A., J. GHAZOUL, G. RAVIKANTH, R. UMA SHAANKER, C. G. KUSHALAPPA, AND C. J. KETTLE. 2012. Does long-distance pollen dispersal preclude inbreeding in tropical trees? Fragmentation genetics of *Dysoxylum malabaricum* in an agro-forest landscape. Mol. Ecol. 21: 5484–5496.

JHA, S., AND C. W. DICK. 2008. Shade coffee farms promote genetic diversity of native trees. Curr. Biol. 18: 1126–1128.

JHA, S., AND C. W. DICK. 2009. Isolation and characterization of nine microsatellite loci for the tropical understory tree *Miconia affinis* Wurdack (Melastomataceae). Mol. Ecol. Resour. 9: 344–345.

JHA, S., AND C. W. DICK. 2010. Native bees mediate long-distance pollen dispersal in a shade coffee landscape mosaic. Proc. Natl Acad. Sci. USA 107: 13760–13764.

JONES, O. R., AND J. WANG. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol. Ecol. Resour. 10: 551–555.

JONES, F. A., AND L. S. COMITA. 2008. Neighbourhood density and genetic relatedness interact to determine fruit set and abortion rates in a continuous tropical tree population. Proc. R. Soc. B 275: 2759– 2767.

KALINOWSKI, S. T. 2005. HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. Mol. Ecol. Resour. 5: 187–189.

KARRON, J. D., AND R. J. MITCHELL. 2012. Effects of floral display size on male and female reproductive success in *Mimulus ringens*. Ann. Bot. 109: 563–570.

KARRON, J. D., R. J. MITCHELL, K. G. HOLMQUIST, J. M. BELL, AND B. FUNK. 2003. The influence of floral display size on selfing rates in *Mimulus ringens*. Heredity 92: 242–248.

KETTLE, C. J., C. R. MAYCOCK, J. GHAZOUL, P. M. HOLLINGSWORTH, E. KHOO, R. S. SUKRI, AND D. F. BURSLEM. 2011. Ecological implications of a

flower size/number trade-off in tropical forest trees. PLoS One 6: e16111.

KNIGHT, M. E., A. P. MARTIN, S. BISHOP, J. L. OSBORNE, R. J. HALE, A. SANDERSON, AND D. GOULSON. 2005. An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Mol. Ecol. 14: 1811–1820.

KREMEN, C. 2005. Managing ecosystem services: What do we need to know about their ecology? Ecol. Lett. 8: 468–479.

KUNIN, W. 1997. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of Brassica kaber. J. Ecol. 85: 225–234.

LANDER, T. A., D. H. BOSHIER, AND S. A. HARRIS. 2010. Fragmented but not isolated: Contribution of single trees, small patches and long-distance pollen flow to genetic connectivity for Gomortega keule, an endangered Chilean tree. Biol. Cons. 143: 2583–2590.

LE ROUX, J. J. AND A. M. WIECZOREK. 2008. Isolation and characterization of polymorphic microsatellite markers from the velvet tree, *Miconia calvescens* DC. (Melastomataceae). Mol. Ecol. Resour. 8:961–964.

LINDAUER, M. 1957. Communication among the honeybees and stingless bees of India. Bee World 38: 3–14.

LOWE, A. 2005. Population genetics of neotropical trees focus issue. Heredity 95: 243–245.

LOISELLE, B., V. SORK, J. NASON, AND C. GRAHAM. 1995. Spatial Genetic Structure of a Tropical Understory Shrub, Psychotria officinalis (Rubiaceae). Am. J. Bot. 82: 1420–1425.

LOWE, A. J., S. CAVERS, D. BOSHIER, M. F. BREED, AND P. M. HOLLINGSWORTH. 2015. The resilience of forest fragmentation genetics – No longer a paradox – We were just looking in the wrong place. Heredity 115: 97–99.

LUCK, G. W., AND G. C. DAILY. 2003. Tropical countryside bird assemblages: Richness, composition, and foraging differ by landscape context. Ecol. Appl. 13: 235–247.

MAGRACH, A., W. F. LAURANCE, A. R. LARRINAGA, AND L. SANTAMARIA. 2014. Meta-analysis of the effects of forest fragmentation on interspecific interactions. Conserv. Biol. 28: 1342–1348.

MAKINO, T. T., K. OHASHI, AND S. SAKAI. 2007. How do floral display size and the density of surrounding flowers influence the likelihood of bumble bee revisitation to a plant? Funct. Ecol. 21: 87–95.

MARSHALL, T. C., J. B. K. E. SLATE, L. E. B. KRUUK, AND J. M. PEMBERTON. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. 7: 639–655.

MITCHELL, R. J., J. D. KARRON, K. G. HOLMQUIST, AND J. M. BELL. 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. Funct. Ecol. 18: 116–124.

MITCHELL, R. J., W. G. WILSON, K. G. HOLMQUIST, AND J. D. KARRON. 2013. Influence of pollen transport dynamics on sire profiles and multiple paternity in flowering plants. PLoS One 8: e76312.

NEI, M. 1972. Genetic Distance between Populations. Amer. Nat. 106: 283–292.

van NIEUWSTADT, M., AND C. R. IRAHETA. 1996. Relation between size and foraging range in stingless bees (Apidae, Meliponinae). Apidologie 27: 219–228.

O'CONNELL, M. C., A. R. CASTILLA, L. X. LEE, AND S. JHA. 2018. Data from: Bee movement across heterogeneous tropical forests: Multi-paternal analyses reveal the importance of neighborhood composition for pollen dispersal. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.j1344c7.

OLLERTON, J., R. WINFREE, AND S. TARRANT. 2011. How many flowering plants are pollinated by animals? Oikos 120: 321–326.

PEAKALL, R. AND P. E. SMOUSE 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6: 288–295.

PELABON, C., E. ALBERTSEN, M. FALAHATI-ANBARAN, J. WRIGHT, AND W. S. ARMBRUSTER. 2015. Does multiple paternity affect seed mass in

angiosperms? An experimental test in *Dalechampia scandens*. J. Evol. Biol. 28: 1719–1733.

RAMOS, S. L. F., G. DEQUIGIOVANNI, A. M. SEBBENN, M. T. G. LOPES, P. Y. KAGEYAMA, J. L. V. de MACÊDO, M. KIRST, AND E. A. VEASEY. 2016. Spatial genetic structure, genetic diversity and pollen dispersal in a harvested population of *Astrocaryum aculeatum* in the Brazilian Amazon. BMC Genet. 17: 63.

RHODES, M. K., J. B. FANT, AND K. A. SKOGEN. 2017. Pollinator identity and spatial isolation influence multiple paternity in an annual plant. Mol. Ecol. 26: 4296–4308.

ROUBIK, D. W. 1989. Ecology and natural history of tropical bees. Cambridge University Press, Cambridge, UK.

ROUBIK, D., AND M. ALUJA. 1983. Flight ranges of Melipona and Trigona in tropical forest. J. Kans. Entomol. Soc. 56: 217–222.

SAHLI, H., AND J. CONNER. 2007. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). Am. J. Bot. 94: 203–209.

SNOW, D. 1965. A possible selective factor in the evolution of fruiting seasons in tropical forest. Oikos 15: 274–281.

STOUT, J. C. 2000. Does size matter? Bumblebee behavior and the pollination of *Cystisus scoparius* L. (Fabaceae). Apidologie 31: 129–139.

VIOLLE, C., B. ENQUIST, B. MCGILL, L. JIANG, C. ALBERT, C. HULSHOF, V. JUNG, AND J. MESSIER. 2012. The return of the variance: Intraspeci- fic variability in community ecology. Trends Ecol. Evol. 27: 244– 52.

VEKEMANS, X. AND O.J. HARDY. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Molec. Ecol. 13: 921–935.

VRANCKX, G., H. JACQUEMYN, B. MUYS, AND O. HONNAY. 2011. Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. Conserv. Biol. 26: 228–237.

WENNER, A. M., D. E. MEADE, AND L. J. FRIESEN. 1991. Recruitment, search behavior, and flight ranges of honey bees. Am. Zool. 31: 768–782.

ZURBUCHEN, A., L. LANDERT, J. KLAIBER, A. MÜLLER, S. HEIN, AND S. DORN. 2010. Maximum foraging ranges in solitary bees: Only few individuals have the capability to cover long foraging distances. Biol. Cons. 143: 669–676.