

Tropical deforestation reduces plant mating quality by shifting the functional composition of pollinator communities

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Abstract

1. Deforestation can impact the quality of pollen received by target plants (i.e. delivery of incompatible pollen, self-pollen or pollen from closely related individuals). Such reductions in plant mating quality may be direct, when deforestation reduces plant population size and the availability of pollen donors, or indirect, when decreased mating quality results, for example, from shifts in the composition of the pollinator community. As most flowering plants depend on animal pollinators for reproduction, there is a need to understand the direct and indirect links between deforestation, pollinator community composition and plant mating quality.
2. We quantified the direct, pollen donor-mediated and indirect, pollinator-mediated effects of deforestation on mating quality in *Heliconia tortuosa*, a tropical herb pollinated by low- and high-mobility hummingbirds. We used a confirmatory path analysis to test the hypothesis that deforestation (amount of forest cover and forest patch size) influenced mating quality (haplotype diversity of pollen pools, outcrossing and biparental inbreeding) directly and indirectly through functional shifts in the composition of pollinator communities (proportion of high-mobility hummingbirds).
3. We found that deforestation triggered functional shifts in the composition of pollinator communities, as the proportion of high-mobility hummingbirds increased significantly with the amount of forest cover and forest patch size. The composition of the pollinator community affected mating quality, as the haplotype diversity of pollen pools increased significantly with the proportion of high-mobility hummingbirds, while biparental inbreeding decreased significantly. Although we did not detect any significant direct, pollen donor-mediated effects of deforestation on mating quality, reductions in the amount of forest cover and forest patch size resulted in functional shifts that filtered out high-mobility hummingbirds from the pollinator community, thereby reducing mating quality indirectly.
4. *Synthesis*. Deforestation primarily influenced plant mating quality through a cascading effect mediated by functional shifts in the composition of the pollinator community. Our results indicate that plant mating quality strongly depends on the composition of local pollinator communities. Functional shifts that filter out highly

mobile and effective pollinators may reduce the transfer of genetically diverse pollen loads from unrelated plants. Such shifts may have pronounced effects on plant population dynamics and disrupt genetic connectivity.

KEYWORDS

biparental inbreeding, confirmatory path analysis, genetic diversity, *Heliconia tortuosa*, hummingbirds, outcrossing, pollinator functional groups, tropical forest

1 | INTRODUCTION

Most species of flowering plants rely on animal pollinators for effective mating (Ollerton et al., 2011), often interacting with diverse communities of floral visitors (Ollerton et al., 2007; Waser et al., 1996). However, many species commonly receive incompatible pollen, self-pollen or pollen from closely related individuals, thus influencing the quality of pollen that reaches target plants (Aizen & Harder, 2007; Ashman et al., 2004). Such reductions in plant mating quality (i.e. quality of pollen received by a plant) can decrease the likelihood of beneficial genotype combinations among the progeny (Karron et al., 2012; Paschke et al., 2002) and limit female choice for more genetically diverse and compatible pollen (Breed et al., 2012, 2015; Skogsmyr & Lankinen, 2002). This may result in less vigorous offspring with lower germination rates (Aguilar et al., 2019; Marshall & Ellstrand, 1986), competitive abilities (Gómez, 2000; Herrera, 2000) and survival probabilities (Breed et al., 2014; Karron & Marshall, 1990). As such, characterizing the mechanisms that govern variation in plant mating quality is essential to understand the ecological and evolutionary dynamics of plant reproduction.

Changes in the amount and spatial configuration of habitat (hereafter deforestation) can negatively affect mating quality and result in reduced plant reproductive success (Aguilar et al., 2006; Eckert et al., 2010; Leimu et al., 2010). Deforestation can substantially impact the genetic diversity of pollen loads received by plants (Barrett & Harder, 2017; Pannell & Labouche, 2013), the rate of self-fertilization (Brunet & Holmquist, 2009; Brunet & Sweet, 2006) and the frequency of mating among related individuals (Griffin & Eckert, 2003; Herlihy & Eckert, 2004) by altering three main components of plant–pollinator interactions (Hadley & Betts, 2012; Xiao et al., 2016): plant demography (e.g. population size, flowering phenology), pollinator availability and pollinator behaviour.

Accumulating evidence suggests that deforestation directly influences plant mating quality by reducing plant abundance and population size (Aguilar & Galetto, 2004; Eckert et al., 2010; Harder & Aizen, 2010; Wagenius et al., 2007). At local and landscape scales, these reductions result in a lower number of conspecific pollen donors, limiting both pollen availability (i.e. reduced pollen quantity) and potential options for outcrossing (Aizen & Harder, 2007; Knight, 2003; Ward & Johnson, 2005). Furthermore, deforestation can also affect plant mating quality directly by altering important microenvironmental conditions that regulate the phenological patterns of plants (Xiao et al., 2016). Asynchronous flower production and shortened flowering periods can also limit pollen donor availability

and reduce options for cross-pollination (Barrett & Harder, 2017; Nason & Hamrick, 1997).

In addition to these direct, pollen donor-mediated effects, deforestation can also influence plant mating quality indirectly, through cascading, pollinator-mediated effects driven by shifts in the composition of the pollinator community (Kremen et al., 2007). Owing to morphological and foraging behavioural differences, distinct functional groups of floral visitors can have important and divergent effects on mating quality for a particular plant species (Castilla et al., 2017; Rhodes et al., 2017; Rodríguez-Rodríguez et al., 2013; Valverde et al., 2019). For example, pollinators that forage across long distances to acquire resources (hereafter highly mobile pollinators) may be more efficient at transferring genetically diverse pollen from multiple sources (Krauss et al., 2017; Pannell & Labouche, 2013), thus facilitating outcrossing (Bezemer et al., 2019; Byrne et al., 2007; Llorens et al., 2012; Ohashi & Thomson, 2009) and mating among unrelated individuals (Barrett & Harder, 2017). Deforestation has been shown to reduce the availability of highly mobile pollinators (Hadley & Betts, 2012; Hadley et al., 2017) by restricting their daily movement patterns (Kormann et al., 2016; Kremen et al., 2007; Volpe et al., 2014, 2016) and limiting their access to floral resources (Brosi & Briggs, 2013; Fenster et al., 2004; Kremen et al., 2002), thus shifting the functional composition of the pollinator community with which plants interact (Aizen & Feinsinger, 2003; Ashworth et al., 2004; Brosi et al., 2008). Accumulating evidence suggests that even small changes in the functional composition of the pollinator community can substantially impact plant mating quality (Brosi & Briggs, 2013; Fründ et al., 2013).

Studies assessing the effects of deforestation on the functional composition of pollinator communities and how these shifts influence plant mating quality are generally lacking, especially in tropical plants. In this paper, we use a path analysis approach to quantify the direct (i.e. pollen donor-mediated) and indirect (i.e. pollinator-mediated) effects of tropical deforestation on mating quality in *Heliconia tortuosa*. This tropical forest herb is pollinated by two functional groups (sensu Fenster et al., 2004) of hummingbirds that differ in their movement patterns (Betts et al., 2015). Territorial hummingbirds aggressively defend small areas (<100 m in diameter) that contain a high density of floral resources (Betts et al., 2015; Dobkin, 1984). This 'low-mobility' foraging strategy results in restricted movement patterns that facilitate self-fertilization and mating among nearby individuals (Linhart, 1973; Stiles, 1975). In contrast, traplining hummingbirds typically forage across long-distance routes (up to 1 km per day) to acquire nectar resources (Gill, 1988; Volpe et al., 2014), potentially enhancing the transfer of pollen across the

landscape. This 'high-mobility' foraging strategy can potentially increase outcrossing and mate diversity by facilitating the receipt of non-self and unrelated pollen (Ohashi & Thomson, 2009). Our recent work showed that hierarchical patterns of pollen pool differentiation (quantified among forest patches and among neighbouring *H. tortuosa* individuals within forest patches) were consistent with the foraging strategy of high-mobility traplining hummingbirds, as neighbouring *H. tortuosa* individuals were found to sample genetically distinct pollen pools (Torres-Vanegas et al., 2019). This suggests that these species are largely responsible for effective pollen transfer in *H. tortuosa* (Torres-Vanegas et al., 2019). As such, the next important step is to understand how deforestation and reduced abundance of high-mobility traplining hummingbirds impact plant mating quality.

As surrogates for mating quality, we estimated the genetic diversity of pollen grains that resulted in successful pollination events in individual plants that were sampled across a human-modified landscape, as well as their corresponding outcrossing rates and degree of biparental inbreeding (i.e. extent of mating among related individuals). Combined with data on the local species composition of pollinator communities, the path analysis approach used here allowed us to evaluate the hypothesis that deforestation, quantified by the amount of forest cover and forest patch size, affects plant mating quality directly through reduced availability of conspecific pollen donors and indirectly through functional shifts in the composition of the pollinator community.

Specifically, we predict that deforestation will directly reduce the genetic (i.e. haplotype) diversity of pollen loads received by individual plants, lower outcrossing rates and increase biparental inbreeding, as reductions in the amount of forest cover and forest patch size will limit

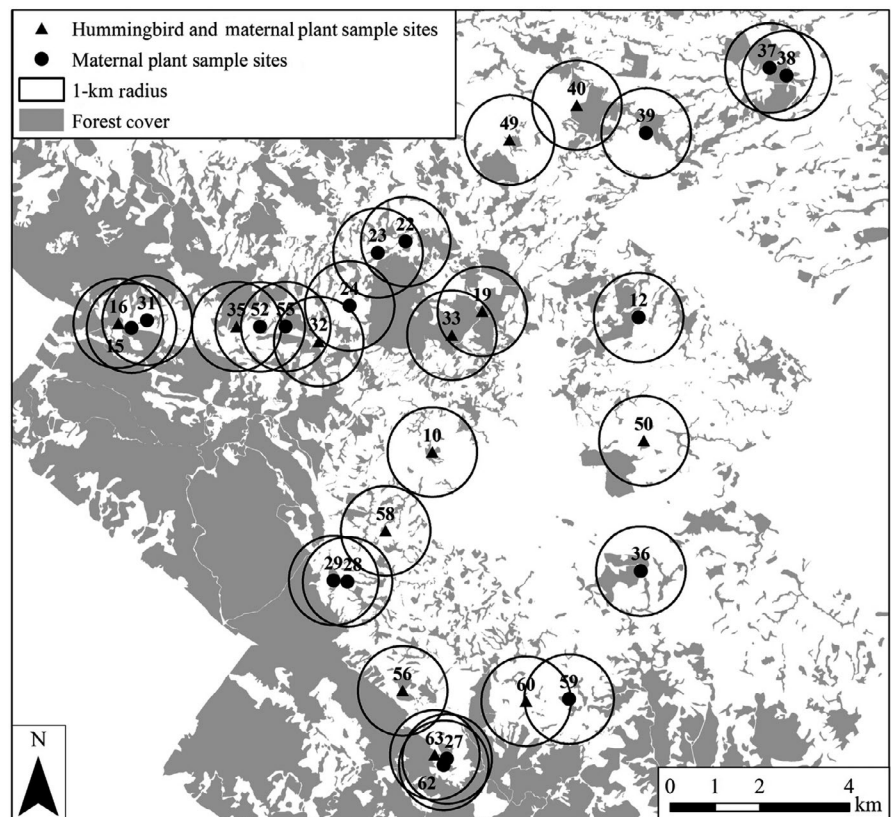
the number of conspecific pollen donors (i.e. plant abundance, pollen availability) and decrease options for cross-pollination. Beyond these direct effects, we predict that deforestation will also have indirect, cascading effects. Specifically, we expect that reductions in the amount of forest cover and forest patch size will induce shifts in the functional composition of the pollinator community by filtering out high-mobility hummingbird species with the capacity to transfer pollen from multiple sources across long distances. In turn, we predict that these functional shifts will negatively affect mating quality by reducing the genetic diversity of the pollen loads received by individual plants, lowering outcrossing rates and increasing biparental inbreeding.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in an (approx. 310 km²) area surrounding the Organization for Tropical Studies Las Cruces Biological Station in southern Costa Rica (8°47'7"N, 82°57'32"W). The study area included an elevation gradient from 850 to 1,500 m a.s.l. and was originally covered by Pacific premontane tropical forest. Today, only about 30% of the forest cover remains (Zahawi et al., 2015). The existing forest patches (approx. 2,200) range from <1 to >1,300 ha in size and span a gradient from 1% to 99% in the amount of forest cover within a 1-km radius (Figure 1). The non-forested matrix is dominated by pasture and agriculture, with some recent regrowth of secondary forest (Hadley et al., 2014; Zahawi et al., 2015).

FIGURE 1 Map of the study area in southern Costa Rica. Sampling locations within focal forest patches are shown ($n = 30$). Patches including sampling of maternal plants only are represented by a solid circle ($n = 17$); patches including hummingbird captures and sampling of maternal plants are shown by a solid triangle ($n = 13$). Numbers represent the ID of each forest patch. Open circles correspond to a 1-km radius within which the proportion of forested area was calculated. The minimum distance between all sampling locations was approx. 160 m; the minimum distance between sampling locations with hummingbird captures was approx. 1,600 m



2.2 | Study system

The focal species of this study, *H. tortuosa* Griggs (Heliconiaceae), is a perennial, hermaphroditic herb that is exclusively found in the understorey of premontane tropical forests, where it occurs individually or in small clonal clumps (Stiles, 1975). This species is one of the most common hummingbird-pollinated plants in the study area (Hadley et al., 2014). Previous research suggests that *H. tortuosa* is a hub species in the pollination network, as it interacts with multiple species of hummingbirds that exhibit important variation in bill length and curvature (Betts et al., 2015; Borgella et al., 2006). Although this herb also interacts with a common species of butterfly, *Anartia fatima*, pollination experiments have shown that this floral visitor fails to deliver any kind of pollination service (Betts et al., 2015). During the peak flowering season (February–May), *H. tortuosa* individuals typically produce one or two inflorescences, although clonal plants with three or more inflorescences have been observed. Each inflorescence holds up to 12 bracts, each subtending up to 15 curved and tubular flowers that open sequentially and are fertile for a single day (Stiles, 1975). Across the study area, *H. tortuosa* is patchily distributed within forest patches, with a mean density of 127 inflorescences per ha. (Hadley et al., 2014). *Heliconia tortuosa* can reproduce clonally by rhizomatous growth and is partially self-compatible (Kress, 1983). However, pollinator exclusion experiments have shown an absence of self-fertilization within the same flower (autogamy), which suggests that hummingbirds are required for successful pollination (Betts et al., 2015). Self-fertilization is possible between different flowers of the same individual (geitonogamy). Upon successful pollination, *H. tortuosa* produces fleshy fruits with up to three seeds. Seed dispersal is mediated by several species of generalist frugivore birds, in particular the clay-coloured thrush, *Turdus grayi* (L.A. Arias-Medellin et al., unpublished data).

2.3 | Study design

Our sampling design was based on previous work in the study area. Hadley et al. (2014) used a stratified-random sampling design to select 40 focal forest patches that represented two independent gradients in the amount of forest cover and forest patch size. We used a subset of 30 focal forest patches that were selected for long-term research in the study system. Within this subset, focal forest patches ranged from 0.6 ha to more than 1,300 ha in size and from 7.8% to 74.4% in amount of forest cover, measured as the proportion of forested area within 1-km radius (Figure 1; Table S1). This distance corresponds to the maximum daily movement range of traplining hummingbirds (Hadley & Betts, 2009; Volpe et al., 2016). Due to the stratified-random sampling design, forest patches with close to 100% in amount of forest cover were not selected, as the proportion of forested area within 1 km and forest patch size are highly confounded, and because deforestation effects are expected to occur most strongly at lower amounts of forest cover (Andr n, 1994; Betts et al., 2007).

During the 2013 flowering season, we selected 25 focal patches for sampling. In 2016, understorey disturbance and restricted access prevented us from sampling 12 focal forest patches sampled in 2013. Thus, during the 2016 flowering season, we resampled 13 focal patches and included five additional forest patches to compensate. Therefore, our dataset comprised 30 focal forest patches (2013: $n = 25$; 2016: $n = 18$).

In each forest patch, we identified a road access point from which we randomly selected a location (hereafter sampling site) anywhere within a distance of up to 500 m (Hadley et al., 2014). This distance was selected to limit confounding between forest patch size and potential edge effects, as only very large forest patches have distances >500 m from their edge (Hadley et al., 2014). From each random sampling site, we selected and marked the nearest five flowering *H. tortuosa* individuals (hereafter maternal plants). To avoid marking clonal individuals, we required a minimum distance of 1 m among the selected maternal plants (distances ranged from 1 to 162 m; mean = 28.40 m; median = 20.39 m). At the end of each flowering season, we sampled leaf tissue from each maternal plant and covered a single inflorescence to avoid fruit removal (Hadley et al., 2014). Once fruits were mature, we randomly selected two bracts per inflorescence and collected the seeds from all fruits. However, fruit abortion prevented us from collecting a sufficient number of seeds (≥ 5) from all sampled maternal plants. In 2013, we sampled 87 maternal plants, while 71 new maternal plants were sampled in 2016. Combined, the final sampled materials comprised seeds from 158 maternal plants sampled across 30 focal forest patches (Table S1). We haphazardly selected an average of 10 seeds per maternal plant (range 5–21) for DNA extraction and genotyping, resulting in a total of 1,584 seeds (2013: 770 seeds from 87 maternal plants; 2016: 814 seeds from 71 maternal plants; Table S1). These selected samples represented, on average, 5.6 fruits per maternal plant, 5.1 seeds per bract and 1.8 seeds per fruit.

Since local flower density has been shown to be one of the most important confounds influencing plant mating quality (Knight et al., 2005; Kremen et al., 2007), we counted all *H. tortuosa* inflorescences within 20 m of each maternal plant and calculated the average as an estimate of the mean conspecific flower density per sampling site. Measures of conspecific flower density were log-transformed to normalize the distribution.

2.4 | Genotyping

We extracted genomic DNA from all sampled maternal plants (leaf tissue from 158 individuals) and selected seeds (1,584 embryos were carefully dissected from the selected seeds). All DNA extractions were completed using the QIAGEN DNeasy Plant Mini Kit following the manufacturer's protocol (QIAGEN). All samples were amplified and genotyped at 11 microsatellite loci (Hac_C7, Hb_C115, Hac_D1, Hb_B9, Hac_B4, Hac_B6, Hac_C114, Hac_A103, Hc_C7, Hac_A116, Hc_C126) and markers were tested for departures from Hardy–Weinberg equilibrium, linkage disequilibrium and null alleles

(for details see Torres-Vanegas et al., 2019). Genotyping errors were scored as the per locus rate of genotype mismatches observed in the maternal plant-offspring arrays. To account for the possibility of sampling the same maternal plant across flowering seasons, we calculated the probability of observing identical multilocus genotypes between the sampled maternal plants (P_{ID} ; Waits et al., 2001) using CERVUS 3.0.7. (Kalinowski et al., 2007).

2.5 | Plant mating quality

As surrogates for plant mating quality, we estimated the genetic diversity of pollen haplotypes sampled by each maternal plant, the corresponding outcrossing rate and the degree of biparental inbreeding. Pollen haplotypes correspond to the set of alleles present in pollen grains that resulted in successful pollination (i.e. paternal multilocus genotype of each seed). As such, they do not represent the complete array of pollen grains received by each maternal plant.

We obtained pollen haplotypes by subtracting the genetic contribution of each maternal plant from the multilocus genotype of each corresponding seed using the *minus.mom* function in the R package *GSTUDIO* (Dyer, 2014). This function resolves ambiguous cases (where a mother-offspring pair has the same heterozygous genotype) by estimating the posterior paternal-maternal gametic likelihood for each allele, given the allele frequencies from all genotyped seeds (Smouse et al., 2001). The allele frequencies of multilocus pollen haplotypes were estimated for each maternal plant using the *allele.frequencies* function in the R package *GSTUDIO* (Dyer, 2014). These were used to estimate the haplotype diversity (h) of pollen pools sampled by each maternal plant. This measure corresponds to the probability (averaged across all loci) that the paternal alleles of two randomly chosen seeds from the same maternal plant are different (Nei, 1987) and is expected to be positively correlated with the effective number of pollen donors (N_{EP} ; Smouse et al., 2001). We developed an R function that allowed for the estimation of this measure (see associated digital repository). Corresponding standard errors were calculated by performing 1,000 bootstrap replicates. All analyses were performed in R 3.5.0 (R Development Core Team, 2017).

We used MLTR 3.4 (Ritland, 2002) to estimate the multilocus outcrossing rate (t_m) and biparental inbreeding ($t_m - t_s$) for the seeds sampled from each maternal plant. The multilocus outcrossing rate (t_m) is an estimate of the proportion of outcrossed seeds produced by a single maternal plant, including mating among related and unrelated individuals. The single-locus outcrossing rate (t_s) differs in that only mating among unrelated individuals is considered as an outcrossing event (Ritland, 2002). Thus, the difference between these two estimates ($t_m - t_s$) corresponds to a measure of the frequency of mating among related individuals (biparental inbreeding). The above parameters were calculated using the Newton-Raphson method and by allowing pollen pool allele frequencies to equal those of the ovule. Standard errors were calculated based on 1,000 bootstraps with resampling conducted at the family level.

2.6 | Pollinator community composition

Hummingbird capture data from previous work in the study area (Hadley et al., 2014, 2017) were used to describe the functional composition of the pollinator community within a subset of 13 focal forest patches (Figure 1; Table S2). Within each of these patches, 10 mist nets were separately placed in front of understory, hummingbird-pollinated flowers (Hadley et al., 2017). Note that these do not necessarily correspond to flowers from our sampled maternal plants. Therefore, mist net captures were intended to provide data at the forest-patch level, not at the maternal plant level. Hummingbird captures were conducted between 05:30 and 12:30 hr during the peak flowering season of *H. tortuosa* in 2010 and 2011. The sampling protocol had a consistent effort across all forest patches: total net hours were approx. 1,906 with an average of approx. 136 hr per forest patch (Hadley et al., 2017). Captured hummingbirds were identified to the species level and marked to avoid counting recaptures as new individuals. We selected mist nets over direct observation methods (e.g. point counts or walkabout surveys), as rapid hummingbird movements and low lighting conditions in the forest understory make the identification of hummingbird species challenging.

Using a variety of methods (e.g. radio-transmitter tracking, radio-frequency identification, field observations), previous work in the study area calculated the median daily foraging distance of each hummingbird species and categorized them as either low- or high-mobility pollinators (Betts et al., 2015). Specifically, hummingbird species with median daily foraging distances of more than 0.5 km were classified as high-mobility pollinators (*Campylopterus hemileucurus*, *Phaethornis guy* and *Phaethornis longirostris*). All other species (*Amazilia decora*, *Amazilia edward*, *Amazilia tzacatl*, *Heliodoxa jacula*, *Phaeochroa cuvierii* and *Phaethornis striigularis*) were classified as low-mobility pollinators, as their median daily foraging distances are less than 0.5 km (Betts et al., 2015). Note that the latter group contains one short-distance traplining hummingbird (*P. striigularis*) and five territorial hummingbird species, whereas the first group contains long-distance traplining hummingbirds exclusively (Betts et al., 2015; Stiles & Freeman, 1993). Aviary experiments have shown that high-mobility traplining hummingbirds are more effective as pollinators compared to territorial species (Betts et al., 2015).

We used the raw number of hummingbird captures to estimate the proportion of high-mobility hummingbirds (P_{hmh}) at each focal forest patch. We applied a small-sample correction that has been shown to produce unbiased proportion estimates and improve the performance of standard errors (Agresti & Caffo, 2000; Agresti & Coull, 1998). This correction involves adding four pseudo-observations, two successes (high-mobility hummingbirds) and two failures (low-mobility hummingbirds) respectively:

$$P_{hmh} = \frac{\text{High mobility hummingbird captures} + 2}{\text{Hummingbird captures} + 4}$$

Our main interest here was to assess the relative availability of hummingbird functional groups that were present at capture sites and

therefore available to pollinate nearby flowers (Hadley et al., 2017). Thus, the 'proportion of high-mobility hummingbirds' is more biologically meaningful than absolute hummingbird abundance when describing the local functional composition of pollinator communities. Also, this metric accounts for the possibility of interspecific agonistic interactions between low- and high-mobility hummingbirds (Linhart, 1973; Stiles, 1975). Note that the path analysis approach used here was also performed using the proportion of high-mobility hummingbirds without the small-sample correction. The interpretation did not differ between the small-sample corrected and uncorrected estimates; thus, we only present the former.

2.7 | Total effect of deforestation on plant mating quality

We examined the total effect of deforestation (proportion of forested area within 1 km and forest patch size) on plant mating quality by performing separate analyses of data from the complete set of sampled forest patches ($n = 30$) and from the subset of patches with hummingbird captures ($n = 13$). This allowed us to determine how representative data from this subset are for the complete set of sampled forest patches. Note that the subsequent linear and linear mixed-effects models fitted for data from the subset of forest patches with hummingbird captures were later incorporated into our confirmatory path analysis.

Across all 30 focal forest patches, the proportion of forested area within 1 km and forest patch size (log-transformed) were not significantly correlated (Pearson's $r = 0.304$, $p = 0.102$). However, these variables were highly correlated (Pearson's $r = 0.879$, $p < 0.05$) for the subset of patches with hummingbird capture data. Further, we found that local conspecific flower density (estimated for each sampling site as the mean number of *H. tortuosa* inflorescences within 20 m of each maternal plant) was not significantly correlated with the proportion of forested area within 1 km (Pearson's $r = 0.086$, $p = 0.649$), forest patch size (Pearson's $r = 0.253$, $p = 0.177$), or with the proportion of high-mobility hummingbirds (Pearson's $r = 0.313$, $p = 0.297$). Also, the total number of *H. tortuosa* inflorescences within 20 m of each maternal plant was not significantly correlated with the haplotype diversity (h) of pollen pools (Pearson's $r = 0.034$, $p = 0.663$), the multilocus outcrossing rate (t_m) (Pearson's $r = 0.072$, $p = 0.367$) or biparental inbreeding ($t_m - t_s$) (Pearson's $r = 0.051$, $p = 0.519$). In this study, local conspecific flower density was not confounded with plant mating quality surrogates and was thus excluded from further analysis.

We evaluated the total effect of the proportion of forested area within a 1-km radius and forest patch size (forest-patch level predictor variables) on the haplotype diversity (h) of pollen pools, multilocus outcrossing rates (t_m) or biparental inbreeding ($t_m - t_s$) (maternal plant-level response variables) with separate linear mixed-effects models (LMM), each with one deforestation variable (predictor) and one plant mating quality surrogate (response). To account for multiple, non-independent sampling of maternal plants within each forest patch, we used forest patch ID as a random effect. This

random effect also removed potential non-independence caused by repeated sampling of forest patches across both years. All other variables were treated as fixed effects. We statistically controlled for potential differences among sampling years by including this variable as a fixed effect (binary factor: 2013 = 0, 2016 = 1) in each linear mixed-effects model. All variables were scaled ($mean = 0$, $SD = 1$) to allow comparison of regression coefficients. We also estimated the variance explained by fixed and random effects (R_M^2 and R_C^2) using the *r.squaredGLMM* function of the *MuMIn* package (Barton, 2020) in R 3.5.0.

To explicitly account for spatial autocorrelation, each LMM was re-fitted with a spatial correlation structure. We used five different spatial correlation structures (*corExp*, *corGaus*, *corSpher*, *corRatio*, *corLin*) by specifying the *correlation* argument in the *lme* function of the R package *NLME* (Pinheiro et al., 2021). We fitted all LMMs with restricted maximum likelihood (REML) and compared the associated AICc score of each LMM that did not account for spatial autocorrelation with the five corresponding LMMs that included a spatial correlation structure. We then selected the LMM with the lowest AICc score as the best-supported model. We only detected spatial autocorrelation for LMMs that included the multilocus outcrossing rate (t_m) as a response variable. Within these, the *corExp* spatial correlation structure was best-supported for the complete set of 30 focal forest patches, while the *corGaus* was best-supported for the subset of 13 forest patches (Table S3). All other best-supported LMMs did not include a spatial correlation structure, suggesting that accounting for spatial autocorrelation was not needed (Table S3).

2.8 | Direct versus indirect effects of deforestation on plant mating quality

To evaluate the direct, pollen donor-mediated and indirect, pollinator-mediated effects of tropical deforestation on the haplotype diversity (h) of pollen pools, multilocus outcrossing rates (t_m) and biparental inbreeding ($t_m - t_s$), we performed a confirmatory path analysis based on a piecewise structural equation modelling (SEM) approach (Lefcheck, 2016). This analysis was based on samples from the 13 forest patches that included hummingbird capture data, resulting in 761 seeds sampled from 73 maternal plants (2013: 338 seeds from 37 maternal plants; 2016: 423 seeds from 36 maternal plants). Note that the linear mixed-effects models described in the previous section (based on 13 forest patches) were incorporated into the confirmatory path analysis.

In confirmatory path analysis, previous knowledge of the ecological system is used to build a path model that represents the hypothetical causal links between multiple variables (Li, 1975; Shipley, 1997). Thus, variables can act as both predictors and responses, and multiple hypotheses can be tested simultaneously within a single causal path model, allowing for the quantification of direct and indirect effects (Shipley, 2009). Unlike traditional SEM, piecewise SEM relies on individual tests for each hypothesized causal link within the path model (Grace et al., 2012;

Shiple, 2000). This local estimation has the advantage that different model types can be separately defined for each causal link within the path model, allowing to appropriately account for non-independence among samples by using linear mixed-effects models (Lefcheck, 2016; Shipley, 2009). This piecewise SEM approach decomposes the total effect of any one variable on another into the corresponding direct effect and indirect effect (Lefcheck, 2016). The indirect effect is mediated by a third variable that is also included in the path model (Shipley, 2009). Note that while confirmatory path analysis is referred to as 'causal modeling', the causality is merely hypothesized in an a-priori path model and cannot be established from observational data.

Prior to defining a path model, all predictor variables (proportion of forested area within 1 km, forest patch size (log-transformed) and proportion of high-mobility hummingbirds) were examined for collinearity by estimating variance inflation factors (VIF) using the *vif* function in the R package *CAR* (Fox & Weisberg, 2019). A conservative value of $VIF > 2.5$ (Myers, 1990) was chosen to detect collinearity among predictors. Since the proportion of forested area within 1 km and forest patch size were found to be highly correlated ($VIF = 4.55$, *Pearson's* $r = 0.879$, $p < 0.05$) for the subset of patches with hummingbird capture data, these predictors were not included in the same path model. Thus, we investigated the direct and indirect effects of tropical deforestation in two separate piecewise SEMs: one corresponding to the amount of forest cover (proportion of forested area within 1 km) and another representing forest patch size.

The initial path models ($n = 6$: each combination of two alternative deforestation variables and three alternative plant mating quality surrogates) included all hypothesized effects (Figure S1). Each model postulated that the haplotype diversity (h) of pollen pools, multilocus outcrossing rates (t_m) or biparental inbreeding ($t_m - t_s$) are directly and indirectly influenced by deforestation. Specifically, we expected that the haplotype diversity (h) of pollen pools, multilocus outcrossing rates (t_m) and the proportion of high-mobility hummingbirds would increase, and biparental inbreeding ($t_m - t_s$) decrease, with the proportion of forested area within 1 km and with forest patch size. In addition, we expected to find indirect effects of deforestation on plant mating quality mediated through functional shifts in the composition of the pollinator community. As such, we expected that the haplotype diversity (h) of pollen pools and multilocus outcrossing rates (t_m) would increase, and biparental inbreeding ($t_m - t_s$) decrease, with the proportion of high-mobility hummingbirds, as the long-distance foraging routes of these pollinators potentially increase mate diversity and enhance the delivery of genetically diverse pollen loads (Betts et al., 2015).

The piecewise SEMs tested each pathway within each initial path model (Figure S1) by combining linear models (LM) and linear mixed-effects models (LMM). Linear models tested the effect of deforestation on the proportion of high-mobility hummingbirds (forest-patch level response variable). Linear mixed-effects models tested the effects of deforestation, the proportion of high-mobility hummingbirds and sampling year on the haplotype diversity (h) of pollen pools,

multilocus outcrossing rates (t_m) or biparental inbreeding ($t_m - t_s$) (maternal plant-level response variables). Forest patch ID was included as a random effect to account for multiple, non-independent maternal plants sampled within each forest patch. Residuals from all models were checked to verify assumptions of normality, constant variance and the absence of influential points. To meet model assumptions, all response variables that were bounded between 0 and 1 (proportion of high-mobility hummingbirds, haplotype diversity (h) of pollen pools, multilocus outcrossing rates (t_m)) were arcsine square-root transformed. This transformation was chosen over the binomial link function because our initial path models included the proportion of high-mobility hummingbirds both as a predictor and as a response variable. Patch size was log-transformed in order to normalize the distribution and linearize relationships. All models were fit in R 3.5.0 (R Development Core Team, 2017) with either the *lm* or *lme* (NLME package, Pinheiro et al., 2021) functions and combined into a SEM using the *PIECEWISESEM* package (Lefcheck, 2016).

For each initial path model (Figure S1), we used a *d*-separation test to evaluate the significance of the direct pathway between the corresponding deforestation variable and each surrogate of plant mating quality, and to assess whether including this direct effect improved model performance as indicated by AICc (Shipley, 2009, 2013). If the direct pathway was not statistically significant and its inclusion did not result in a lower AICc score, it was eliminated from the piecewise SEM and the initial path model was pruned accordingly (Shipley, 2009). Note that both conditions had to be met in order to eliminate the direct pathway. Fisher's C statistic was used to evaluate the goodness-of-fit of each piecewise SEM, where non-significant values indicate good model fit (Lefcheck, 2016; Shipley, 2009).

The magnitude of the direct effects was quantified by the standardized path coefficient between any two variables, whereas indirect effects were quantified by multiplying the standardized path coefficients of the two pathways linking deforestation variables and plant mating quality surrogates through the proportion of high-mobility hummingbirds. Finally, we estimated R^2_{adj} for linear models, and R^2_C and R^2_M for linear mixed-effects models.

2.9 | Fine-scale spatial genetic structure among maternal plants

The extent to which mating among nearby individuals facilitates biparental inbreeding largely depends on their relatedness (i.e. fine-scale spatial genetic structure). To evaluate the degree of relatedness among maternal plants, we estimated the pairwise kinship coefficient (F_{ij} ; Loiselle et al., 1995) among all pairs of maternal plants separated by up to 1,000 m (pooled across all forest patches) using SPAGeDi 1.5 (Hardy & Vekemans, 2002). This coefficient measures the extent of genetic similarity among individuals, where positive F_{ij} values indicate that pairs of individuals are more closely related than expected by chance. We used eight distance classes that maximized the number of pairwise comparisons (breaks: 10, 25, 50, 100, 250, 500, 750, 1,000 m). Note that the

last distance class corresponds to the maximum daily movement range of high-mobility hummingbirds. Each distance class included more than 50 pairwise comparisons. Mean F_{ij} values were obtained for each distance class by averaging all pairs of maternal plants within each interval and plotted against each distance class (log-transformed to linearize relationship; Vekemans & Hardy, 2004) to visualize spatial autocorrelation. Based on 10,000 permutations of individual geographic locations, statistical significance and standard errors were calculated for mean F_{ij} values across each distance class. In order to account for multiple testing, we applied a progressive Bonferroni correction (Hewitt et al., 1997) where the first distance class was tested against the $\alpha = 0.05$ significance level, the second distance class was tested against $\alpha' = \alpha/2$ and so forth until the k -th distance class which was tested against $\alpha' = \alpha/k$. Testing stopped after the first non-significant distance class. We also estimated the Sp statistic (Vekemans & Hardy, 2004) as $Sp = -b/(1 - F_1)$, where b is the slope of the regression of F_{ij} across all distance classes and F_1 is the mean F_{ij} value at the first distance class. Given that we sampled a limited number of maternal plants within each forest patch (≤ 5), our primary focus was to evaluate overall fine-scale spatial genetic structure, which is not intended to represent site-specific patterns.

3 | RESULTS

3.1 | Genetic markers and plant mating quality

The genotyping of 158 maternal plants and 1,584 seeds across 11 microsatellite loci yielded a total of 105 alleles (Table S4). Within the subset of patches with hummingbird capture data ($n = 13$), maternal plants ($n = 73$) and seeds ($n = 761$) yielded a total of 102 alleles. Most loci showed high levels of polymorphism, as the number of alleles per locus ranged from 4 to 17 (4 to 15 within the subset of patches with hummingbird capture data) and observed heterozygosity varied between 0.11 and 0.79 (0.15 and 0.85 within the subset of patches with hummingbird capture data). All markers showed some departures from Hardy-Weinberg equilibrium, but there were no consistent departures across loci or forest patches (Table S5). Also, loci did not show any significant linkage (Table S6) and the rate of genotype mismatches in our maternal plant-offspring arrays varied between 0.00 and 0.06 (Table S4). Therefore, all markers were retained. Loci provided a multilocus probability of identity $P(I_D)$ of < 0.00001 , suggesting that the probability of sampling the same maternal plant across flowering seasons was low.

Across maternal plants from all 30 focal forest patches, the haplotype diversity (h) of pollen pools ranged from 0.247 to 0.620 (0.266 to 0.620 for maternal plants from patches with hummingbird capture data), while multilocus outcrossing rates (t_m) and biparental inbreeding ($t_m - t_s$) varied from 0.493 to 1.0 and from -0.082 to 0.648 respectively (0.571 to 1.0 and -0.082 to 0.588 for maternal plants from patches with hummingbird capture data) (Table S7).

3.2 | Pollinator community composition

Across the 13 focal forest patches with mist net data, a total of 305 unique individual hummingbirds were captured, representing nine species of legitimate floral visitors of *H. tortuosa*. Within each forest patch, the total number of unique captures ranged between 12 and 40 (5–25 high-mobility hummingbirds) (Table S2). After applying the small-sample correction, the estimated proportion of high-mobility hummingbirds per forest patch ranged from 0.285 to 0.710 (Table S2). These proportions were significantly correlated with the number of high-mobility hummingbird captures per forest patch (Pearson's $r = 0.604$, $p = 0.022$).

3.3 | Total effect of deforestation on plant mating quality

For all 30 focal forest patches, both the haplotype diversity (h) of pollen pools and the multilocus outcrossing rate (t_m) showed a strong, significant positive relationship with forest patch size (LMM: haplotype diversity (h): $\hat{\beta} = 0.502$, $SE = 0.150$, $p = 0.002$, $R_M^2 = 0.233$, $R_C^2 = 0.715$, Figure S2a; LMM: outcrossing (t_m): $corExp\hat{\beta} = 0.224$, $SE = 0.102$, $p = 0.037$, $R_M^2 = 0.048$, $R_C^2 = 0.048$, Figure S2b), even after accounting for potential differences among sampling years. However, these variables were not significantly associated with the proportion of forested area within 1 km (LMM: haplotype diversity (h): $\hat{\beta} = 0.196$, $SE = 0.166$, $p = 0.248$, $R_M^2 = 0.042$, $R_C^2 = 0.718$, Figure S2d; LMM: outcrossing (t_m): $corExp\hat{\beta} = 0.034$, $SE = 0.102$, $p = 0.740$, $R_M^2 = 0.001$, $R_C^2 = 0.001$; Figure S2e). Biparental inbreeding ($t_m - t_s$) showed a strong, significant negative relationship with forest patch size (LMM: $\hat{\beta} = -0.309$, $SE = 0.084$, $p = 0.001$, $R_M^2 = 0.249$, $R_C^2 = 0.320$, Figure S2c), even after accounting for sampling year, but was not significantly associated with the proportion of forested area within 1 km (LMM: $\hat{\beta} = -0.197$, $SE = 0.097$, $p = 0.052$, $R_M^2 = 0.179$, $R_C^2 = 0.327$, Figure S2f).

For the subset of 13 focal forest patches with hummingbird capture data, the haplotype diversity (h) of pollen pools also showed a strong, significant positive relationship with forest patch size (LMM: $\hat{\beta} = 0.641$, $SE = 0.187$, $p = 0.005$, $R_M^2 = 0.370$, $R_C^2 = 0.671$, Figure S3a), even after controlling for potential differences among sampling years, but was not significantly associated with the proportion of forested area within 1 km (LMM: $\hat{\beta} = 0.392$, $SE = 0.232$, $p = 0.118$, $R_M^2 = 0.135$, $R_C^2 = 0.671$, Figure S3d). The multilocus outcrossing rate (t_m) was not significantly associated with the proportion of forested area within 1 km (LMM: $corGaus\hat{\beta} = 0.074$, $SE = 0.132$, $p = 0.585$, $R_M^2 = 0.006$, $R_C^2 = 0.006$, Figure S3e) or with forest patch size (LMM: $corGaus\hat{\beta} = 0.134$, $SE = 0.130$, $p = 0.321$, $R_M^2 = 0.019$, $R_C^2 = 0.019$, Figure S3b). Biparental inbreeding ($t_m - t_s$) showed a strong, significant negative relationship with both the proportion of forested area within 1 km (LMM: $\hat{\beta} = -0.363$, $SE = 0.135$, $p = 0.021$, $R_M^2 = 0.237$, $R_C^2 = 0.335$, Figure S3f) and forest patch size (LMM: $\hat{\beta} = -0.423$, $SE = 0.125$, $p = 0.006$, $R_M^2 = 0.289$, $R_C^2 = 0.357$, Figure S3c), even after accounting for sampling year.

3.4 | Effect of deforestation on pollinator community composition

The proportion of high-mobility hummingbirds showed a strong, significant positive relationship with the proportion of forested area within 1 km (LM: $\hat{\beta} = 0.638$, $SE = 0.232$, $p = 0.018$, $R_{adj}^2 = 0.354$, Figure 2a) and forest patch size (LM: $\hat{\beta} = 0.744$, $SE = 0.201$, $p = 0.003$, $R_{adj}^2 = 0.513$, Figure 2b).

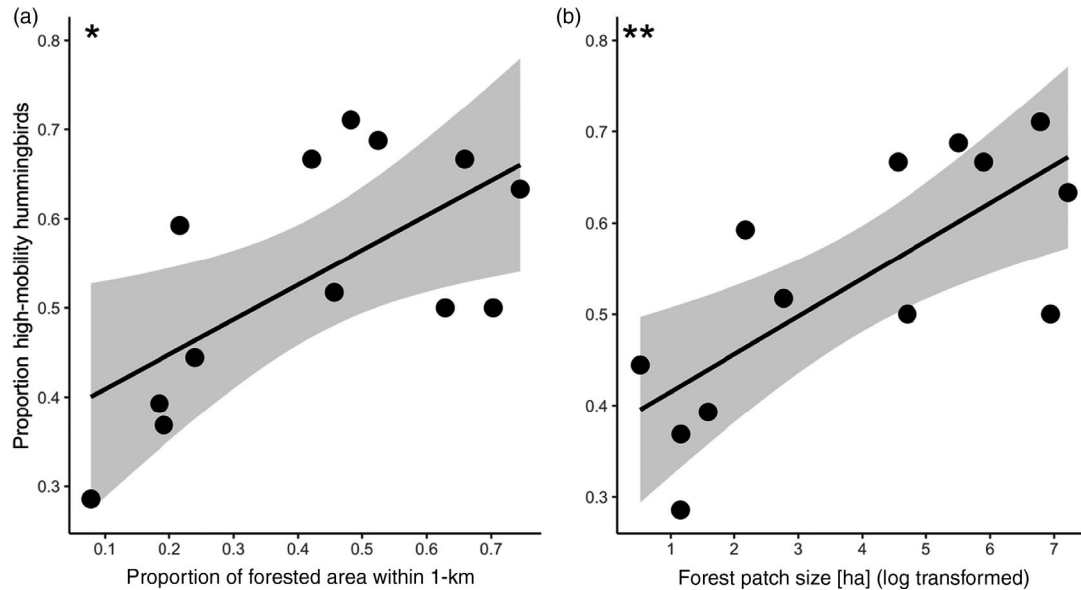


FIGURE 2 Effect of deforestation variables on the functional composition of pollinator communities. The proportion of high-mobility hummingbirds as a function of (a) the proportion of forested area within a 1-km radius of sampling site and (b) forest patch size (log-transformed). Each solid circle represents one forest patch. The solid lines indicate the modelled relationship between the variables, while the shaded area corresponds to the estimated 95% confidence interval. Both the proportion of forested area within 1 km and forest patch size strongly influenced the proportion of high-mobility hummingbirds. Significance levels: * $p < 0.05$, ** $p < 0.01$

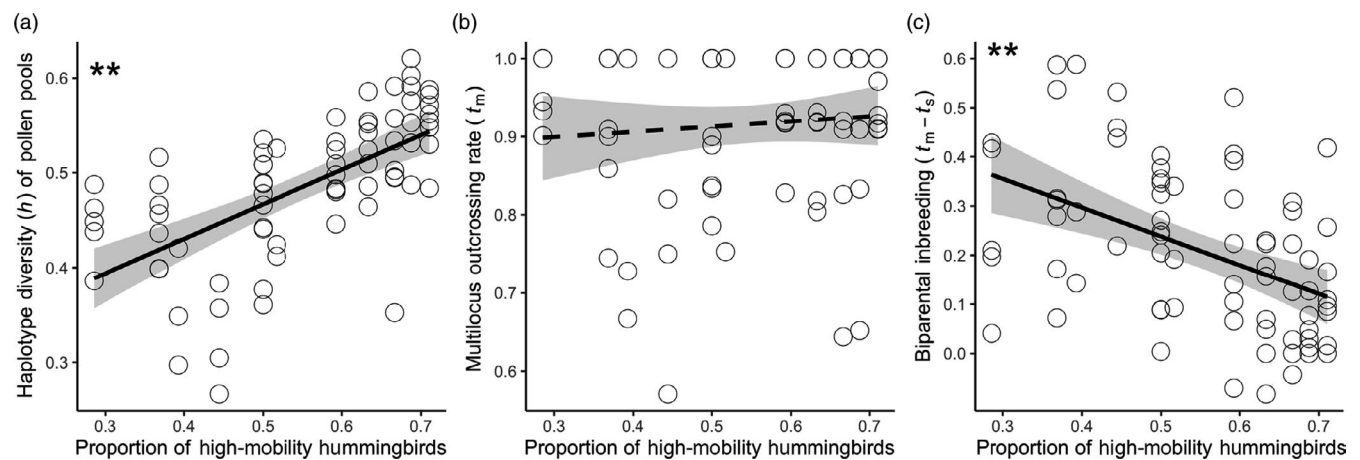


FIGURE 3 Effect of the functional composition of the pollinator community on plant mating quality. (a) The haplotype diversity (h) of pollen pools, (b) multilocus outcrossing rates (t_m) and (c) biparental inbreeding ($t_m - t_s$) as a function of the proportion of high-mobility hummingbirds. Each open circle denotes one maternal plant. The solid ($p < 0.05$) and dashed ($p > 0.05$) lines indicate the modelled relationship between the variables, while the shaded areas represent the estimated 95% confidence intervals. The proportion of high-mobility hummingbirds had a significant positive effect on the haplotype diversity (h) of pollen pools and a significant negative effect on biparental inbreeding ($t_m - t_s$). Multilocus outcrossing rate (t_m) was not significantly associated with the proportion of high-mobility hummingbirds. Significance level: ** $p < 0.01$

3.5 | Effect of pollinator community composition on plant mating quality

The haplotype diversity (h) of pollen pools showed a strong, significant positive relationship with the proportion of high-mobility hummingbirds (LMM: $\hat{\beta} = 0.639$, $SE = 0.168$, $p = 0.003$, $R_M^2 = 0.385$, $R_C^2 = 0.652$, Figure 3a). The multilocus outcrossing rate (t_m) was not significantly associated with the proportion of high-mobility pollinators (LMM:

$corGaus\hat{\beta} = 0.065$, $SE = 0.124$, $p = 0.526$, $R_M^2 = 0.005$, $R_C^2 = 0.005$, Figure 3b). Biparental inbreeding ($t_m - t_s$) showed a strong, significant negative relationship with the proportion of high-mobility pollinators (LMM: $\hat{\beta} = -0.472$, $SE = 0.096$, $p < 0.001$, $R_M^2 = 0.337$, $R_C^2 = 0.337$, Figure 3c). These results remained significant after accounting for potential differences among sampling years.

3.6 | Direct versus indirect effects of deforestation on plant mating quality

All our initial path models (Figure S1) testing the direct, pollen donor-mediated and indirect, pollinator-mediated effects of tropical deforestation on plant mating quality were well supported by the data, as Fisher's C statistic provided non-significant p -values (Table S8). However, the best-fit path models (Figure 4; Figure S4), as indicated by $\Delta AICc$ (Table S8), did not include direct, pollen donor-mediated effects of deforestation on plant mating quality. This was supported

by the d -separation tests showing that these direct pathways were not statistically significant (Table 1). Rather, our best-fit path models (Figure 4; Figure S4) supported a strong indirect relationship between deforestation variables and plant mating quality surrogates, mediated through functional shifts in the composition of the pollinator community (Table 2). Since our deforestation variables were highly correlated and their direct and indirect effects on plant mating quality were fundamentally similar (Table 2), we present the best-fit path model for one alternative deforestation variable (forest patch size) in Figure 4. The direct and indirect effects of the proportion of forested area within 1 km are shown in Figure S4.

3.7 | Fine-scale spatial genetic structure among maternal plants

Mean pairwise kinship coefficients (F_{ij}) among maternal plants declined with increasing distance (Figure 5), varying from 0.052 to -0.005

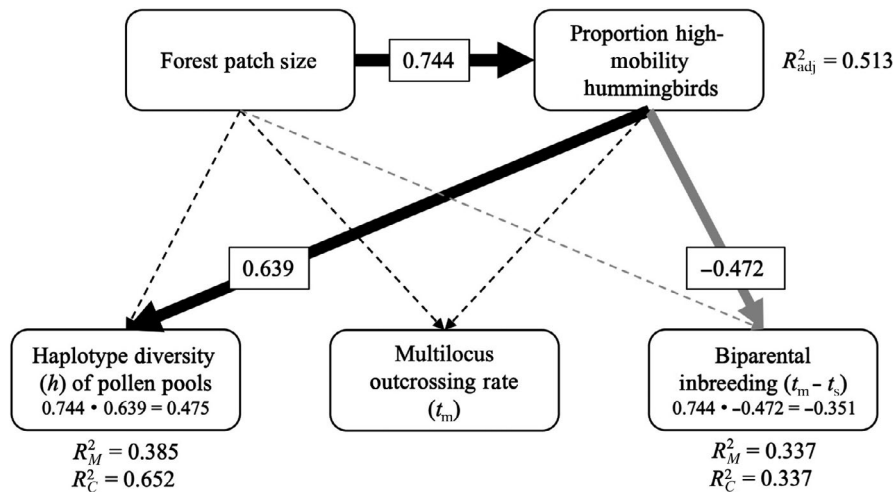


FIGURE 4 Effects of a deforestation variable (log forest patch size) on the functional composition of the pollinator community (proportion of high-mobility hummingbirds) and plant mating quality (haplotype diversity (h) of pollen pools, multilocus outcrossing rate (t_m) and biparental inbreeding ($t_m - t_s$)). Black arrows show positive and grey arrows show negative effects derived from piecewise SEM analysis. Standardized path coefficients and variance explained (R_{adj}^2 , R_M^2 and R_C^2) are only given for statistically significant paths (solid arrows; $p < 0.05$). Dashed arrows show non-significant paths. The widths of the arrows have been scaled to indicate the magnitude of the standardized path coefficients. Arrows between the alternative deforestation variable and plant mating quality surrogates indicate direct effects, while indirect effects are shown as the product of the standardized path coefficients linking deforestation and mating quality through the proportion of high-mobility hummingbirds

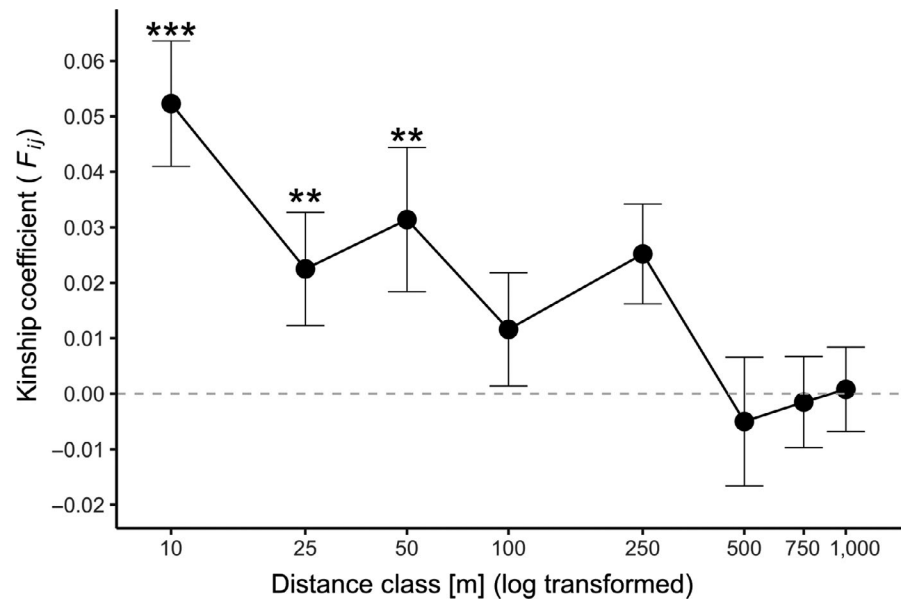
TABLE 1 Results from d -separation tests evaluating the statistical significance of the direct pathways between deforestation variables (proportion of forested area within 1 km and forest patch size) and plant mating quality surrogates (haplotype diversity (h) of pollen pools, multilocus outcrossing rate (t_m) and biparental inbreeding ($t_m - t_s$))

Independence claim	Critical value	p -value
Haplotype diversity (h) of pollen pools ~ proportion of forested area within 1 km	-0.058	0.954
Haplotype diversity (h) of pollen pools ~ forest patch size	1.307	0.220
Multilocus outcrossing rate (t_m) ~ proportion of forested area within 1 km	0.481	0.640
Multilocus outcrossing rate (t_m) ~ forest patch size	1.188	0.262
Biparental inbreeding ($t_m - t_s$) ~ proportion of forested area within 1 km	-1.167	0.270
Biparental inbreeding ($t_m - t_s$) ~ forest patch size	-1.236	0.244

TABLE 2 Direct and indirect effects of deforestation variables (proportion of forested area within 1 km and log forest patch size) on plant mating quality surrogates (haplotype diversity (h) of pollen pools, multilocus outcrossing rate (t_m) and biparental inbreeding ($t_m - t_s$)). The magnitude of the effects was derived from the best-fit path models. Direct effects were quantified by the standardized path coefficient between deforestation variables and mating quality surrogates, whereas indirect effects were quantified by multiplying the standardized path coefficient linking deforestation variables and the proportion of high-mobility hummingbirds (first term in parenthesis) by the standardized path coefficient linking the proportion of high-mobility hummingbirds and mating quality surrogates (second term in parenthesis)

Deforestation variable	Mating quality variable	Direct	Indirect	Total
Proportion of forested area within 1 km	Haplotype diversity (h) of pollen pools	-0.015	0.407 (0.638 • 0.639)	0.392
	Multilocus outcrossing rate (t_m)	0.033	0.041 (0.638 • 0.065)	0.074
	Biparental inbreeding ($t_m - t_s$)	-0.062	-0.301 (0.638 • -0.472)	-0.363
Forest patch size	Haplotype diversity (h) of pollen pools	0.166	0.475 (0.744 • 0.639)	0.641
	Multilocus outcrossing rate (t_m)	0.086	0.048 (0.744 • 0.065)	0.134
	Biparental inbreeding ($t_m - t_s$)	-0.072	-0.351 (0.744 • -0.472)	-0.423

FIGURE 5 Relationship between mean pairwise kinship coefficients (F_{ij}) of maternal plants and distance class (log-transformed). Solid circles represent the mean F_{ij} values for each distance class. Error bars represent the 95% confidence intervals for mean F_{ij} values. Maternal plants separated by up to 10, 25 and 50 m presented greater kinship than expected by chance, revealing positive fine-scale spatial genetic structure. For each distance class, mean F_{ij} values that differ significantly from zero are indicated with asterisks (** $p < 0.01$, *** $p < 0.001$). A progressive Bonferroni correction was applied to account for multiple testing



(Table S9). Mean F_{ij} values were highest at the first distance class (0–10 m), while negative values occurred at distances greater than 500 m. After applying a progressive Bonferroni correction, permutation tests indicated that mean positive F_{ij} values were significantly different from zero at the first (0–10 m), second (10–25 m) and third (25–50 m) distance class (Figure 5). The Sp statistic had a value of 0.011.

4 | DISCUSSION

In this study, we used a confirmatory path analysis to quantify the direct, pollen donor-mediated and indirect, pollinator-mediated effects of tropical deforestation on mating quality in *H. tortuosa*. Contrary to our initial predictions, we found that the direct effects of deforestation on mating quality were weak and not statistically significant, indicating that the number of conspecific pollen donors in the neighbouring landscape is not the main driver of variation in plant mating quality. Instead, we found that deforestation had a strong indirect effect on plant mating quality through a cascading effect mediated by functional shifts in the composition of the

pollinator community. Thus, our results indicate that plant mating quality strongly depends on the functional composition of the local pollinator community, which in turn is sensitive to deforestation.

4.1 | Effect of deforestation on pollinator community composition

Our results show that deforestation can have pronounced effects on the functional composition of pollinator communities. We found that the proportion of high-mobility hummingbirds increased significantly with the proportion of forested area within 1 km (Figure 2a) and log forest patch size (Figure 2b), making these deforestation variables important determinants of the functional composition of pollinator communities in our study system. Thus, our results support the hypothesis that deforestation results in functional shifts in the composition of pollinator communities by filtering out high-mobility hummingbird species with high energetic requirements and long-distance foraging strategies. However, we could not assess the relative importance of habitat loss versus fragmentation due to the

confounding of the proportion of forested area within 1 km and forest patch size for the subset of forest patches with hummingbird capture data.

Deforestation can negatively affect high-mobility pollinators in three major ways. First, deforestation has been shown to constrain the daily movement patterns of high-mobility pollinators and reduce their ability to move across the landscape (Hadley & Betts, 2009; Kormann et al., 2016; Volpe et al., 2016). These effects on the movement behaviour of pollinators may lead to the decline of species that forage across long distances (Hadley et al., 2017) and thus trigger functional shifts in the composition of the pollinator community. Second, deforestation can alter the spatial and temporal distribution of floral resources, altering the relative diversity of foraging niches available for different functional groups of pollinators (Fenster et al., 2004). The resulting changes in overall resource diversity may induce functional shifts in the composition of the pollinator community by filtering out species with high energetic demands or requiring large foraging areas (Kremen et al., 2002; Williams et al., 2010). Third, deforestation can increase direct interspecific competition for floral resources among territorial and high-mobility hummingbirds (Gill, 1988). Increased abundance of territorial hummingbirds in highly disturbed landscapes may heighten the frequency of aggressive interactions (Linhart, 1973; Stiles, 1975), altering the behaviour of high-mobility hummingbirds and limiting their access to floral resources (Brosi & Briggs, 2013; Fründ et al., 2013).

Deforestation-mediated shifts in the functional composition of pollinator communities may have detrimental effects for plant reproductive success even if total visitation rates are unaffected (Bruckman & Campbell, 2014). As such, the loss of particular species from the pollinator community may not necessarily lower overall visitation rates or limit the amount of pollen received by flowers (i.e. pollen-quantity limitation) if plants attract multiple functional groups of pollinators. However, functional shifts towards a less effective pollinator community do have the potential to impact plant mating quality (Karron et al., 2012). Thus, plant reproductive success may be limited by the quality of pollen received, and not necessarily by the quantity of pollen arriving at flowers (Aizen & Harder, 2007; Moore & Pannell, 2011).

4.2 | Effect of pollinator community composition on plant mating quality

We found strong effects of the functional composition of the pollinator community on mating quality in *H. tortuosa*: (a) the haplotype diversity (h) of pollen pools increased significantly with the proportion of high-mobility hummingbirds (Figure 3a), while (b) biparental inbreeding ($t_m - t_s$) decreased significantly (Figure 3c). Primarily, we attribute these results to the foraging strategy and morphological specialization of these pollinators.

Previous research in *H. tortuosa* has hypothesized that floral visits by high-mobility hummingbirds may facilitate the delivery of genetically diverse pollen loads (Betts et al., 2015; Hadley et al., 2014),

as these highly vagile species forage across long-distance routes (up to 1 km per day; Volpe et al., 2014). Our results support this hypothesis, as we found that pollinator communities with a greater proportion of high-mobility hummingbirds enhanced the delivery of genetically diverse pollen loads and reduced the frequency of mating among related individuals (i.e. biparental inbreeding). Also, our results revealed positive fine-scale spatial genetic structure among maternal plants, as individuals separated by up to 50 m exhibited significantly greater kinship than expected by chance (Figure 5). Thus, functional shifts in the composition of the pollinator community that filter out high-mobility hummingbirds may increase local pollination by territorial hummingbirds and heighten the frequency of mating among related individuals.

Experimental evidence in *H. tortuosa* has shown that the specialized bills (i.e. long and curved) of high-mobility hummingbirds, which closely match the morphology of flowers, increase their nectar extraction capacity and effectiveness as pollinators, as pollen tubes occur almost exclusively when flowers are fully depleted of nectar (Betts et al., 2015). In combination, the foraging strategy of high-mobility hummingbirds and their highly specialized bills maximize mating with genetically diverse pollen loads from unrelated individuals. We suggest that high-mobility hummingbird species act as genetic linkers between patchily distributed habitats (Lundberg & Moberg, 2003; Sekercioglu, 2006), as they can visit multiple forest patches during a single foraging bout (Volpe et al., 2014) and enhance the transfer of pollen well beyond the range where maternal plants exhibit positive fine-scale spatial genetic structure. Thus, high-mobility hummingbirds promote plant mating quality and likely play a substantial role in the maintenance of genetic connectivity.

Contrary to our initial predictions, the multilocus outcrossing rate (t_m) did not increase with the proportion of high-mobility hummingbirds (Figure 3b). This suggests that even pollinator communities with a low proportion of high-mobility hummingbirds are able to provide outcrossed pollen. Although pollinator communities with increased abundance of territorial hummingbirds may enhance the transfer of self-pollen (Hadley et al., 2017; Linhart, 1973; Stiles, 1975), pollination experiments have shown that these floral visitors are not effective pollinators of *H. tortuosa*, as their bill morphology (i.e. short and straight) does not mirror flower shape, thus resulting in a low nectar extraction capacity (Betts et al., 2015). Therefore, territorial hummingbirds are not expected to contribute significantly to pollination success, even in the absence of high-mobility hummingbirds. Importantly, previous research has shown that seed set in *H. tortuosa* declines with reduced availability of high-mobility hummingbirds (Hadley et al., 2014), likely a consequence of the transfer of self-pollen and pollen from closely related individuals by territorial hummingbirds. Also, the maintenance of high multilocus outcrossing rates (t_m), independent of the functional composition of pollinator communities, may indicate the presence of a self-incompatibility mechanism in *H. tortuosa* (Kress, 1983) that significantly reduces the success of self-fertilization.

We argue that mating quality in plant species that attract multiple functionally distinct groups of pollinators may indeed be tightly

linked to the functional composition of the pollinator community and therefore sensitive to the loss of highly mobile and effective floral visitors. Coupled with the negative fitness consequences associated with mating with low-quality pollen (Aguilar et al., 2019; Karron et al., 2012; Paschke et al., 2002), shifts in the composition of the pollinator community may have pronounced effects on the population dynamics of outcrossing plants, potentially reducing population growth, jeopardizing long-term viability and ultimately leading to local extinction (Aguirre & Dirzo, 2008; Knight et al., 2005).

4.3 | Direct versus indirect effects of deforestation on plant mating quality

Our confirmatory path analysis decomposed the overall (total) effects of two alternative deforestation variables on three alternative plant mating quality surrogates to quantify their corresponding direct and indirect effects, the latter of which were mediated by shifts in the functional composition of pollinator communities. We did not detect any significant direct, pollen donor-mediated effects of deforestation on the haplotype diversity (h) of pollen pools, multilocus outcrossing rate (t_m) or biparental inbreeding ($t_m - t_s$) (Figure 4; Figure S4; Table 2). Although our results suggest that deforestation (in particular reductions in forest patch size) had a significant total effect on the haplotype diversity (h) of pollen pools and biparental inbreeding ($t_m - t_s$), our best-fit path models (Figure 4; Figure S4) showed that this effect is not due to a direct influence of deforestation on plant mating quality, but rather to an indirect, pollinator-mediated effect triggered by functional shifts in the composition of pollinator communities (Table 2). This finding is consistent with another study (Cusser et al., 2016) demonstrating that the direct influence of natural land cover loss on mating quality in a bee-pollinated cotton agroecosystem is weak, only affecting the pollination service indirectly through shifts in the composition of the pollinator community. Note that in our study, the subset of 13 forest patches with hummingbird capture data showed increased total effects of deforestation on plant mating quality compared to data from all 30 forest patches. This makes it unlikely that the lack of direct effects found here is an artefact due to this specific subset of forest patches, as the chances of finding a significant direct effect were higher for this subset.

One important caveat of this study is that our results are exclusively based on successful pollination events and do not consider deforestation effects on earlier stages of the mating process. Previous work in *H. tortuosa* has shown that reductions in forest patch size lowered seed set by 40% (Hadley et al., 2014). This suggests that significant direct deforestation effects on pollination success may occur before viable seeds are produced (*i.e.* progamic process, seed development; Barrett & Harder, 2017). Moreover, our results are consistent with several reviews (Bacles & Jump, 2011; Hamrick, 2004; Kramer et al., 2008; Lowe et al., 2005) that have documented a lack of significant direct deforestation effects on the genetic diversity of pollen loads, outcrossing and biparental inbreeding. However, it is incorrect to assume that the absence of these direct effects

translates into resilience of plant mating quality to deforestation, as our results show that the negative consequences of deforestation can occur indirectly and may only be detectable when considering effects driven by the pollinator community. Therefore, we suggest that future studies evaluating the effects of deforestation on plant mating quality should explicitly test how changes in the amount and spatial configuration of habitat influence the functional composition of the pollinator community.

5 | CONCLUSIONS

To our knowledge, this study is the first to decompose the direct, pollen donor-mediated and indirect, pollinator-mediated effects of deforestation on plant mating quality within a tropical landscape. We have shown that deforestation can influence the functional composition of pollinator communities and indirectly affect plant mating quality by filtering out highly mobile and effective pollinators. Our study contributes to a growing body of work arguing that maintaining or increasing landscape connectivity is important for plant reproductive success (Diekötter et al., 2007; Valdés & García, 2011), as this will protect the long-distance foraging routes of genetic linker species and facilitate the transfer of genetically diverse pollen loads from unrelated individuals. To further understand the ecological and evolutionary forces acting on plant populations, we emphasize the need for additional research on how plant mating outcomes are affected by particular functional groups of pollinators and by shifts in the composition of the pollinator community.

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AUTHORS' CONTRIBUTIONS

This study contributes to a larger research project initiated by A.S.H., M.G.B. and F.A.J., and was conceptualized by F.T.-V. with guidance from H.H.W., A.S.H. and U.G.K. F.T.-V. conducted the laboratory work, analysed the data and led the writing of the manuscript. All authors contributed substantially to the critical revision and editing of this manuscript.

DATA AVAILABILITY STATEMENT

Datasets generated for this study and R code used in the analyses are available in the Dryad Digital Repository <https://doi.org/10.5061/dryad.rfj6q578w> (Torres-Vanegas et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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