ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Habitat loss and fragmentation reduce effective gene flow by disrupting seed dispersal in a neotropical palm

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Abstract

Habitat loss and fragmentation often reduce gene flow and genetic diversity in plants by disrupting the movement of pollen and seed. However, direct comparisons of the contributions of pollen vs. seed dispersal to genetic variation in fragmented landscapes are lacking. To address this knowledge gap, we partitioned the genetic diversity contributed by male gametes from pollen sources and female gametes from seed sources within established seedlings of the palm *Oenocarpus bataua* in forest fragments and continuous forest in northwest Ecuador. This approach allowed us to quantify the separate contributions of each of these two dispersal processes to genetic variation. Compared to continuous forest, fragments had stronger spatial genetic structure, especially among female gametes, and reduced effective population sizes. We found that within and among fragments, allelic diversity was lower and genetic structure higher for female gametes than for male gametes. Moreover, female gametic allelic diversity in fragments decreased with decreasing surrounding forest cover, while male gametic allelic diversity did not. These results indicate that limited seed dispersal within and among fragments restricts genetic diversity and strengthens genetic structure in this system. Although pollen movement may also be impacted by habitat loss and fragmentation, it nonetheless serves to promote gene flow and diversity within and among fragments. Pollen and seed dispersal play distinctive roles in determining patterns of genetic variation in fragmented landscapes, and maintaining the integrity of both dispersal processes will be critical to managing and conserving genetic variation in the face of continuing habitat loss and fragmentation in tropical landscapes.

KEYWORDS
allelic alpha, beta and gamma diversity, Chocó rainforest, habitat loss and fragmentation, *Oenocarpus bataua*, population genetics

1 | INTRODUCTION

Habitat loss and fragmentation, in which formerly continuous landscapes are reduced to isolated patches (Fahrig, 2003), can disrupt or modify the movement of pollen and seeds, with potentially negative genetic consequences for plants (Aguilar, Quesada, Ashworth, Herrerias-Diego, & Lobo, 2008; Haddad et al., 2015; Hamrick, 2004; Lowe, Boshier, Ward, Bacles, & Navarro, 2005; Sork & Smouse,

2006; Vranckx, Jacquemyn, Muys, & Honnay, 2012; Young, Boyle, & Brown, 1996). Limited movement of pollen or seeds, or both, restricts gene flow within and among forest patches, potentially leading to decreased genetic diversity, increased inbreeding and decreased population viability in fragmented landscapes (Young et al., 1996). Pollen and seed dispersal may respond differently to habitat loss and fragmentation, as they often occur via different modes (e.g., wind-dispersed vs. animal-dispersed) or dispersal vectors (e.g.,

pollen dispersed by insects vs. seeds dispersed by vertebrates). In addition, fundamental differences in these dispersal processes—seed dispersal carries twice as much genetic information and directly impacts demographic processes such as colonization, while pollen dispersal carries only male haploid genotypes (Crawford, 1984; García & Grivet, 2011; Hamilton, 1999; Nathan & Muller-Landau, 2000; Wang & Smith, 2002)—may also lead to differential outcomes. As such, changes to either or both dispersal processes could serve to restrict or promote levels of gene flow in fragmented populations. For these reasons, our understanding of the ecology, evolution and conservation of plant populations in fragmented landscapes would be improved by explicitly accounting for the separate impacts of these distinct dispersal processes. However, progress towards this goal has been impeded because of challenges associated with isolating and comparing the specific contributions of pollen and seed movement to patterns of genetic variation in fragmented landscapes (Sork & Smouse, 2006). This in turn hinders management of plant populations in landscapes impacted by habitat loss and fragmentation (Hamrick, 2004, 2010; Sork, Nason, Campbell, & Fernandez, 1999; Sork & Smouse, 2006; Young et al., 1996).

Habitat loss and fragmentation are particularly acute in the tropics, where it has been identified as a primary threat to biodiversity loss (Haddad et al., 2015; Hansen et al., 2013). In the tropics, most tree species depend on animals for both pollen and seed dispersal (Herrera, 2002; Ollerton, Winfree, & Tarrant, 2011) and declines in these dispersal agents are expected to directly impact the genetic diversity and evolutionary potential of plant populations (Dirzo et al., 2014). Consistent with this idea, pollen dispersal and pollination services by insects are often negatively impacted by habitat loss and fragmentation (Finger et al., 2011, 2012; Opedal et al., 2017; Rathcke & Jules, 1993; Rosas, Quesada, Lobo, & Sork, 2011; Sebbenn et al., 2011). However, in other cases, insect pollinators have been shown to be resistant to habitat loss and fragmentation and able to continue to provide pollen dispersal services across large expanses of nonforest habitat (Dick, 2001, 2003; Hamrick, 2010; Hanson, Brunsfeld, Finegan, & Waits, 2008; Ismail et al., 2012; White, Boshier, & Powell, 2002). Similarly, some vertebrate frugivores are negatively impacted by habitat loss and fragmentation, leading to compromised seed dispersal services (Fontúrbel et al., 2015; Galetti & Dirzo, 2013; Galetti, Guevara, & Côrtes, 2013; Giombini, Bravo, Sica, & Tosto, 2017; McConkey et al., 2012; Sodhi, Liow, & Bazzaz, 2004), while others are more resilient and capable of long-distance movement connecting isolated patches of forest (Abedi-Lartey, Dechmann, Wikelski, Scharf, & Fahr, 2016; Lenz et al., 2011; Mueller, Lenz, Caprano, Fiedler, & Böhning-Gaese, 2014). This variation in responses of pollen and seed disperser populations to habitat loss and fragmentation has led to uncertainty about the relative roles of pollen and seed movement for gene flow of plants in fragmented landscapes and the resulting impacts on patterns of genetic diversity.

Nevertheless, several studies have hypothesized that extensive pollen dispersal is responsible for providing high levels of genetic diversity and weakening genetic structure in fragmented landscapes, while restricted seed dispersal constrains genetic diversity and creates strong genetic structure (Bittencourt & Sebbenn, 2007; Hamilton, 1999; Hamrick, 2004, 2010; Hanson et al., 2008; Sork & Smouse, 2006), similar to what has been found in continuous forest (Ennos, 1994; Grivet, Robledo-Arnuncio, Smouse, & Sork, 2009; Nakanishi, Tomaru, Yoshimaru, Manabe, & Yamamoto, 2009; Sork, Smouse, Grivet, & Scofield, 2015). The alternative hypothesis that extensive seed dispersal provides as much or more genetic connectivity than pollen dispersal has also been shown to be relevant in some scenarios (Abedi-Lartey et al., 2016; Bacles, Lowe, & Ennos, 2006; Mueller et al., 2014). It is important to note that genetic structure either within or among forest fragments will arise as consequence of limited seed dispersal, even if pollen dispersal is extensive (Wang, Compton, & Chen, 2011), but limited pollen dispersal does not lead to increased genetic structure if seed dispersal remains extensive. Our ability to quantify the contribution of pollen vs. seed movement to genetic variation has been limited in part by the fact that direct measures of pollen and seed flow for animal-dispersed plant species in fragmented landscapes are lacking. Instead, many studies have compared pollen and seed flow rates across species (Hamrick, 2010), which may limit our ability to predict how any single species will be impacted by habitat loss and fragmentation due to the wide variation in disperser population responses across species (see above). Other studies have used indirect approaches that make untested assumptions about parentage (Bacles et al., 2006; Guidugli et al., 2016; Ismail et al., 2017; Moraes & Sebbenn, 2011), or compared different sets of molecular markers (Ennos, 1994; Hamilton & Miller, 2002; Petit et al., 2005). More broadly, even when the movement of pollen or seed is estimated via direct or indirect methods (Bittencourt & Sebbenn, 2007; Guidugli et al., 2016; Ismail et al., 2017; Sebbenn et al., 2011), the ways in which this movement influences standing patterns of genetic variation (e.g., allelic diversity and genetic structure) in established seedlings remain poorly resolved. Therefore, directly comparing the relative contributions of male gametes from pollen sources and female gametes from seed sources to resulting patterns of genetic diversity and structure in the same individual seedlings (e.g., Ozawa, Watanabe, Uchiyama, Saito, & Ide, 2013; Sork et al., 2015) would provide a useful perspective on these long-standing hypotheses concerning impacts of habitat loss and fragmentation on plant populations.

A major challenge in isolating the relative contribution of male and female gametes to patterns of genetic variation is that, without further information, it is currently impossible to disentangle the amount of genetic diversity contributed by male and female parents if only biparentally inherited genotypes are available. However, it is possible to overcome this limitation for species where the seed remains attached to recruited seedlings, because the seed pericarp tissue contains the maternal genotype (Godoy & Jordano, 2001). This information, when combined with biparentally inherited leaf tissue, permits gametic assays that decompose the relative contributions of male gametes from pollen sources and female gametes from seed sources to patterns of allelic diversity and spatial genetic

structure (Smouse, Dyer, Westfall, & Sork, 2001; Sork et al., 2015). The seedling/seed coat assay also enables the estimation of parental correlations (i.e., the probability of two seedlings within or among patches sharing the same mother or father) and effective parental numbers (Grivet et al., 2009; Robledo-Arnuncio, Grivet, Smouse, & Sork, 2012). This approach was first applied to established *Quercus lobata* seedlings in continuous habitat in California (Grivet et al., 2009), but has not been applied in fragmented landscapes, outside of a recent study on the shrub *Pistacia lentiscus* in forest fragments in Spain (Parejo-Farnés, Robledo-Arnuncio, Albaladejo, Rubio-Pérez, & Aparicio, 2017). Both studies focused on wind-pollinated plants and found evidence that limited seed movement relative to pollen movement constrains genetic diversity and effective population sizes. However, it remains unclear whether animal-dispersed species that may experience long-distance seed dispersal relatively frequently (Holbrook & Loiselle, 2007; Karubian, Durães, Storey, & Smith, 2012) will show similar patterns.

In this study, we directly compared the relative contribution of male gametes from pollen sources and female gametes from seed sources to patterns of genetic diversity and structure of established seedlings of the canopy palm *Oenocarpus bataua* across a fragmented landscape in northwest Ecuador experiencing ongoing habitat loss and fragmentation. We compared seedlings in fragments to seedlings in nearby continuous forest reserve (Browne, Ottewell, Sork, & Karubian, in press). Seeds of *O. bataua* are dispersed by large-bodied vertebrates capable of long-distance seed dispersal, whereas pollen is dispersed by beetles and other insects (Karubian et al., 2012; Núñez-Avellaneda & Rojas-Robles, 2008). A previous study of *O. bataua* seedlings found increased levels of fine-scale spatial genetic structure in forest fragments compared to a nearby continuous forest reserve (Browne, Ottewell, & Karubian, 2015). However, because this earlier study only sampled biparentally inherited leaf tissue, the relative contribution of male vs. female gametes to the observed increase in spatial genetic structure remains unclear, as such an increase could be caused by a restriction to seed movement or both pollen and seed movement. In this study, we used a seedling/seed coat gametic assay to partition the relative contributions of male and female gametes to within and across fragment allelic diversity, the probability of seedlings sharing the same parents, effective parental sizes and spatial genetic structure and how the degree of habitat loss and fragmentation surrounding each fragmented is related to patterns of genetic variation. We used this approach to evaluate the hypotheses that in fragmented landscapes: (a) Male gametes from pollen sources contribute higher genetic diversity than do female gametes from seed sources due to extensive pollen movement relative to seed movement; (b) female gametes contribute higher genetic diversity than male gametes due to extensive seed dispersal relative to pollen dispersal, or (c) male and female gametes contribute equally to genetic diversity. We expected to observe significant spatial genetic structure if both seed and pollen dispersal are limited, or if seed dispersal is limited, but not if seed dispersal is extensive and pollen dispersal limited.

2 | MATERIALS AND METHODS

2.1 | Study species and area

Oenocarpus bataua (Arecaceae) is an abundant canopy palm tree that is broadly distributed across the Neotropics (Henderson, Galeano, & Bernal, 1995; ter Steege et al., 2013). In a continuous forest site in Ecuador, *O. bataua* is highly outcrossing with low rates of selfing, with an average of 10 pollen donors and 5.4 effective pollen donors (N_{ep}) per maternal family group (i.e., infructescence) and a mean effective pollination neighbourhood of 18.5 ha, based on sampling 20 progeny from each of 16 maternal trees (Ottewell, Grey, Castillo, & Karubian, 2012). Pollen is dispersed by small insects, including beetles (Curculionidae) and bees (Meliponinae) (Núñez-Avellaneda & Rojas-Robles, 2008) that are capable of moving pollen large distances in continuous forest (303 m mean, 1,263 m maximum, Ottewell et al., 2012). *Oenocarpus bataua* produces large, lipid-rich fruits containing a single, large seed (range: 33.4–45.9 mm in length, 17.9–28.2 in width) (Browne et al., 2015) that are presented on large infructescences that last 4–8 weeks. In our study area, the major primary seed dispersers include the long-wattled umbrellabird (*Cephalopterus penduliger*), toucans (*Ramphastos* spp.), squirrels (*Sciurius* spp.), with occasional primary dispersal by kinkajous (*Potos flavus*), small rodents and oilbirds (*Steatornis caripensis*, J. Karubian and L. Browne, unpublished data). Umbrellabirds, which are rare outside of pristine habitat (BirdLife International 2016; Walter et al., 2017), are capable of long-distance seed dispersal, with maximum estimated dispersal distances of >1 km in continuous forest in our study area (Karubian & Durães, 2014; Karubian et al., 2012). Umbrellabirds generate genetically heterogeneous seed pools at traditional display sites known as leks Karubian, Sork, Roorda, Durães, & Smith, 2010; Scofield, Smouse, Karubian, & Sork, 2012). Toucans are also capable of long-distance seed dispersal (Holbrook & Loiselle, 2007), but are more resilient to habitat loss and fragmentation and are found in the majority of forest fragments in our study area (Walter et al., 2017). Major secondary dispersal agents include the Central American agouti (*Dasyprocta punctata*), lowland paca (*Cuniculus paca*) and various smaller rodents (J. Karubian and L. Browne, unpublished data). The lifespan of *O. bataua* is likely >100 years, with 50–80 years spent in a nonreproductive stemless stage (Isaza et al., 2016).

Fieldwork took place in and around the Mache-Chindul Ecological Reserve, Esmeraldas province, northwest Ecuador (Figure 1). The area contains humid Chocó rainforest that has been heavily deforested in the last half-century due to population growth and agricultural expansion (Carrasco, Berg, Litz, Cook, & Karubian, 2013; Dodson & Gentry, 1993). The current landscape consists of patches of small, isolated forest fragments contained within a matrix primarily composed of cattle pasture, cacao (*Theobroma cacao*) and African palm (*Elaeis guineensis*). *Oenocarpus bataua* is occasionally left as a remnant tree in pastures interspersed between forest patches. We used Bilsa Biological Station (BBS), a 3,500 ha privately owned reserve and one of the largest remaining tracts of continuous forest in the area, as a point of reference to compare to the genetic
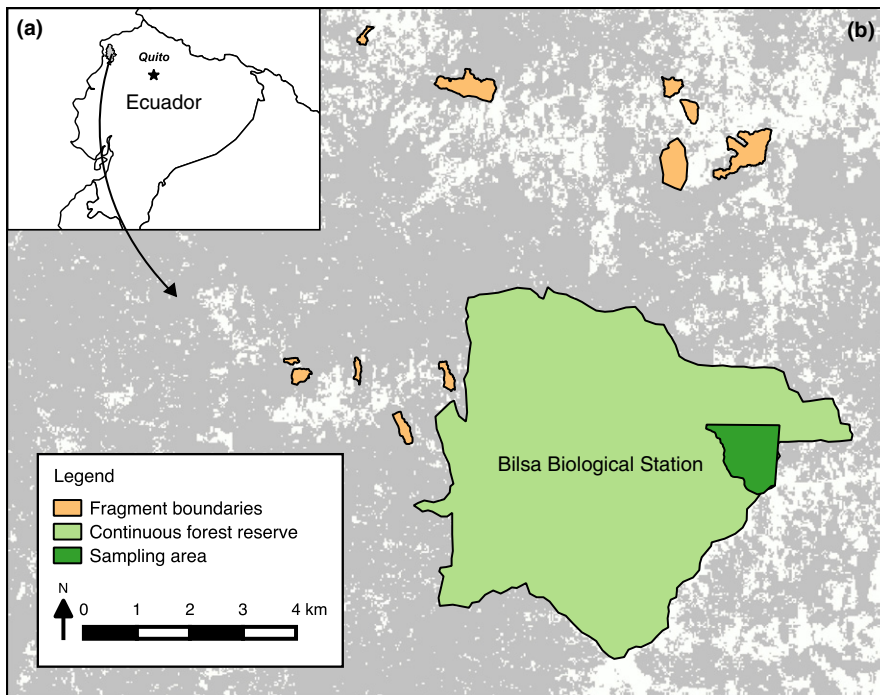
FIGURE 1 Map of study area showing (a) location of study area in the Mache-Chindul Ecological Reserve (grey outline) on the northwest coast of Ecuador and (b) the 11 forest fragments (orange outline), the continuous forest reserve Bilsa Biological Station (green) and the area within Bilsa (dark green) sampled for *Oenocarpus bataua* seedlings in this study. Background shading shows areas classified as containing at least 95% forest cover within each 30 × 30 m cell

variation of seedlings in continuous forest to that of forest fragments (Figure 1). We defined a fragment as a patch of forest that is completely surrounded by nonforest habitat (e.g., pasture or cropland) and is spatially isolated to some degree from nearby forest, which we confirmed by walking the borders of each sampled fragment. We sampled a total of 11 privately owned fragments that ranged in size from 2.4 to 46.4 ha (Figure 1, Table S1, Supporting Information). The average spatial distance between fragments was 5,354 m (range: 321–9,438 m, Figure 1), and the average distance between sampled fragments and the nearest border of BBS was 2,895 m (range: 312–5,298 m).

To test how the degree of habitat loss and fragmentation was related to patterns of genetic variation, we calculated the proportion of forest cover in a 2 km radius surrounding the centre of each fragment following the methods of Browne and Karubian (2016). We expected genetic diversity of male and/or female gametes to be lower in fragments with less surrounding forest cover if habitat loss and fragmentation strongly impacted pollen and/or seed dispersal, respectively. The amount of forest cover surrounding each fragment is increasingly viewed as an appropriate explanatory variable in fragmentation studies (Fahrig, 2013; Jackson & Fahrig, 2016; Melo, Sponchiado, Cáceres, & Fahrig, 2017) because it provides information on the available habitat in the matrix surrounding each forest fragment that other variables (e.g., patch area and degree of isolation) do not. Tree cover in the matrix surrounding forest patches can play an important role in providing habitat for a variety of taxa (Mendenhall, Sekercioglu, Brenes, Ehrlich, & Daily, 2011; Mendenhall, Shields-Estrada, Krishnaswami, & Daily, 2016), including avian seed dispersal agents of *O. bataua* in our study area (Walter et al., 2017). We used a 2 km radius for these analyses because population responses in fragmented landscapes may be predicted by the

amount of habitat in a "local landscape" at a scale approximately 4–9× the median movement distances of the organism (Fahrig, 2013; Jackson & Fahrig, 2012), and a 2-km threshold falls within that range for pollen and seed dispersal of *O. bataua* in our study area (Karubian et al., 2012; Ottewell et al., 2012). We found qualitatively similar results for other radii sizes (Figure S1, Supporting Information). To estimate surrounding forest cover of each fragment, we used the Global Forest Change data set (Hansen et al., 2013) and produced a map of forest cover in our study area in 2014 (the most recent data set available) at a 30 × 30 m resolution by converting grid cells that had ≥95% canopy cover in the year 2000 to a binary forest/nonforest classification and then converting cells that lost forest (defined as stand-replacement disturbance or the complete removal of tree cover canopy, Hansen et al., 2013) between 2000 and 2014 to nonforest (Figure 1, Browne & Karubian, 2016). More detailed maps of land cover classification (e.g., land use or crop types in matrix habitat) are not currently available for this area. Therefore, the proportion of forest cover surrounding each fragment provides the best available estimate of suitable habitat for *O. bataua* itself and its pollen and seed dispersers.

2.2 | Genetic sampling and genotyping

To sample *O. bataua* in forest fragments, we searched each fragment for established seedlings that had been dispersed at least 10 m from the nearest *O. bataua* adult. We removed a small piece of the biparentally inherited leaf tissue and a portion of the outer seed coat (maternal tissue) from each sampled seedling for genetic analysis. We aimed to collect both leaf and seed tissue for at least 20 seedlings per fragment, although in two fragments, we only encountered 14 and 17 seedlings (Table S1, Supporting Information). Because *O.*

bataua adult and seedlings tend to have locally patchy abundances, we opportunistically searched as much of each fragment as possible for suitable seedlings in lieu of using a strict plot-based sampling approach. In addition, because we required both leaf and seed tissue from each seedling, our sampling was not an exhaustive sample of seedlings in each fragment—seedlings were not included in this study if they did not have an attached seed, which naturally detaches with age. We collected seedlings that were found both in close proximity to each other (<10 m) and at farther spatial distances (max pairwise distance within each fragment in Table S1, Supporting Information) to obtain suitable sample sizes across a range of distance intervals for fine-scale spatial autocorrelation analysis (Figure S2, Supporting Information). The average pairwise distance between samples was >50 m in most fragments (Table S1, Supporting Information). The total area within each fragment that encompassed seedlings genotyped in this study, which depended on how large of an area was available for sampling within each fragment and whether a suitable number of samples was encountered, ranged from 0.3 to 5.3 ha (Table S1, Supporting Information).

Samples collected in continuous forest correspond to the "Away" seedlings presented and analysed in Browne et al. (in press) and in this study are used as a point of comparison to seedlings in forest fragments. Seedlings in continuous forest, similar those collected in fragments, were located at least 10 m away from the nearest adult *O. bataua*. Seedlings in continuous forest were originally sampled in 10-m-diameter circular plots that contained >8 established *O. bataua* seedlings with seeds still attached. Differences in both area sampled and sample sizes within each plot could cause biases when comparing forest fragments to continuous forest for genetic diversity parameters that require plot-level sampling. To minimize these biases, we post hoc combined spatially proximate seedlings in continuous forest into larger "plots," such that the total area sampled in continuous forest was divided into 10 different plots, with each plot being similar to plots sampled in forest fragments in terms of area and number of seedlings sampled (Table S1, Supporting Information). This post hoc adjustment did not affect the spatial genetic structure analyses as those are based on individual-level spatial locations rather than plots.

We collected samples in fragments between January and September 2015, and samples in continuous forest were collected in 2007. Because we are using continuous forest as a reference of a relatively unimpacted area, which would be the case regardless of year of sampling, we do not expect the temporal difference in sampling between continuous forest and fragmented forest to cause major bias in our data. All sampled seedlings had a seed still attached to the base of the seedling, and because *O. bataua* seeds stay attached to seedlings for <2 years and habitat loss and fragmentation in the study area began 30–40 years ago, we can be confident that each sampled seedling was of similar age and established post-fragmentation.

We derived the haploid male and female gametic genotypes of each seedling using the diploid leaf and seed genotypes of each seedling and a modified TwoGener gametic extraction (Smouse et al., 2001). In ambiguous cases where both leaf and seed tissue were

heterozygous at the same alleles and male vs. female contribution could not be assigned definitively (Smouse et al., 2001), we use partial gametic assignment where each allele was assigned a 50% probability of coming from either the paternal or maternal tree, which is a conservative approach that does not make assumptions about allele frequencies in pollen pools. These ambiguous cases represented ̤10% of locus–individual combinations, and qualitative results did not differ when these ambiguous cases were removed from our data set. The R code used for gametic extraction is available on Github (https://github.com/lukembrowne/gametic-extraction; https://doi.org/10.5281/zenodo.1274538).

After collection, we stored leaf and seed tissue in paper envelopes under dry conditions in silica gel until DNA extraction. We extracted DNA following the Qiagen DNeasy protocol, and PCR amplified a total of nine microsatellite loci following the protocol of Ottewell et al. (2012). Marker diversity and quality for seedlings in fragments are available in Table S2, Supporting Information. We found no evidence of null alleles, but we removed one locus due missing data in seed tissue (Table S2, Supporting Information), leaving an array of eight loci for analysis. Genotypes of seedlings at these same eight loci in continuous forest were taken from Browne et al. (in press). For many seedlings, the DNA extracted from the seed tissue did not amplify, and we were not able to obtain usable genotypes. We culled samples that did not have at least three loci genotyped at both leaf and seed tissue. Final sample sizes included $n = 156$ seedlings across 11 fragments (Table S1, Supporting Information, range: 9–18 per fragment) and $n = 176$ seedlings across 10 plots in continuous forest (7–24 per plot).

2.3 | Genetic diversity

To estimate genetic diversity, we use calculated gene diversity Hs (Nei, 1987) and allelic richness Ar, rarefied to $n = 3$ gene copies (the minimum number of gene copies with complete genotyping at all loci) using the R package "hierfstat" (Goudet, 2005). We estimated diversity for the separate male and female gametic contributions to allelic diversity and compared these to the diversity of diploid leaf tissue for both seedlings in forest fragments and continuous forest. We expected the diploid leaf tissue to show intermediate levels of diversity compared to male and female gametes as the high diversity contributed by either female or male gametes would be reduced in diploid tissue if gametic genetic diversity of the other sex was low (Sork et al., 2015). We did not compare diversity of male gametes, female gametes, and diploid seedlings within continuous forest in this study; this comparison is provided in Browne et al. (in press).

We also translated traditional ecological diversity estimates of α, β and γ to their genetic counterparts following the framework established by Scofield et al. (2012) and modified to estimate allelic diversity by Sork et al. (2015). A benefit of using this approach is that these diversity estimates provide a coherent framework for estimating patterns of genetic diversity across scales (e.g., within-patch, between-patch and across all patches) and can be directly compared to estimates of species diversity for comparison of the effects of

habitat loss and fragmentation on species and genetic diversity. The measure of α-diversity, which here represents the effective number of alleles per locus per sampled patch, where a patch represents either a sampled fragment or plot within continuous forest, is estimated as the reciprocal of r_{gg}, the unbiased estimate of the probability of drawing identical alleles for a locus in a sampled patch (Scofield et al., 2012; Sork et al., 2015). We estimated β-diversity, or the effective number of genetically nonoverlapping patches (Scofield et al., 2012; Sork et al., 2015), to assess the degree of allelic turnover across sampled patches. As another metric of allelic divergence across patches, we estimated δ, which when $\delta = 1$ represents no overlap of allele frequencies across patches and when $\delta = 0$ represents complete sharing of allele frequencies across patches. We estimated allelic diversity at the scale of our entire study area with γ, which is the effective number of alleles per locus across the entire group of seedlings without regard to patch.

All diversity estimates were averaged across the eight loci and calculated separately for seedlings in forest fragments and continuous forest. We also present scaled diversity metrics (between 0 and 1) to allow comparison to other studies (Sork et al., 2015). We tested for statistical differences between the α and γ diversity metrics of paternal gametes, maternal gametes and diploid leaf tissue using a nonparametric analogue of Bartlett's variance heterogeneity test (Scofield et al., 2012; Sork et al., 2015) with 9,999 bootstraps implemented in the DispersalDiversity R package (Scofield, 2015). We tested for differences in Hs and Ar within fragments among paternal gametes, maternal gametes and diploid leaf tissue using a nonparametric Friedman test, performing post hoc tests using the Nemenyi post hoc method in the PMCMR package in R (Pohlert, 2014). We compared Hs and Ar between forest fragments and continuous forest for paternal gametes, maternal gametes and diploid leaf tissue using a nonparametric Kruskal–Wallis rank sum test. We tested for correlations (Pearson's R) between α, Hs and Ar of paternal gametes, maternal gametes and diploid leaf tissue and proportion forest cover surrounding each fragment (i.e., a metric of habitat loss and fragmentation) using a one-sided significance test that diversity and forest cover surrounding each fragment were positively correlated. Unless otherwise noted, all statistical tests were conducted in R 3.4.1 (R Core Team, 2017).

2.4 | Parental correlations and effective parental numbers

To estimate parental correlations and number of effective parents of established seedlings in each fragment and established seedlings in each plot in continuous forest, we used the parental structure analysis (PSA) developed by Robledo-Arnuncio et al. (2012; see also Grivet et al., 2009). This method uses the maternal seed genotypes and biparentally inherited leaf tissue to estimate parental correlations within patches: Q_w^p, correlation of paternity within patches—the probability that two seedlings drawn at random from the same patch share the same father, Q_w^m, correlation of maternity within patches—the probability that two seedlings drawn at random from the same

patch share the same mother, Q_w^{mp}, cross-parental correlation within patches—the probability that two seedlings drawn at random from the same patch have a cross-parental match. We also estimated parental correlations among patches: Q_b^p, correlation of paternity among patches—the probability that two seedlings drawn at random from two different patches share the same father, Q_b^m, correlation of maternity among patches—the probability that two seedlings drawn at random from two different patches share the same mother, Q_b^{mp}, cross-parental correlation among patches—the probability that two seedlings drawn at random from two different patches have a cross-parental match. The parental correlations within patches allow us to estimate the effective number of fathers ($N_{ep} = 1/Q_w^p$), effective number of mothers ($N_{em} = 1/Q_w^m$) and effective number of parents ($N_e = 4/(Q_w^p + Q_w^m + 2Q_w^{mp})$; Robledo-Arnuncio et al., 2012). We estimated standard errors of parental correlations and effective parental sizes by bootstrap resampling over individuals within patches ($n = 999$). We did not apply a "threshold-distance" correction for seedlings in fragments because there was no strong decay in among-patch parental correlations with spatial distance (Robledo-Arnuncio et al., 2012). Note that negative values of parental correlations are possible because the PSA is based on kinship coefficients, which themselves can take negative values if individuals are less related than the average in the sample (Robledo-Arnuncio et al., 2012). We tested for a correlation (Pearson's R) between Q_w^p, Q_w^m and Q_w^{mp} and forest cover surrounding each fragment using a one-sided significance test that parental correlations and forest cover surrounding each fragment were negatively correlated. We tested for differences in Q_w^p and Q_w^m within forest fragments using a paired Wilcoxon signed rank test. Finally, we tested for differences in Q_w^p, Q_w^m and Q_w^{mp} between forest fragments and continuous forest using nonparametric Mann–Whitney test with the hypothesis that parental correlations were higher in forest fragments.

2.5 | Fine-scale and landscape-scale spatial genetic structure

We tested for spatial genetic structure at both fine (i.e., within fragments or within continuous forest) and landscape (i.e., among fragments) spatial. We estimated fine-scale spatial genetic structure using the kinship coefficient F_{ij} of Loiselle, Sork, Nason, and Graham (1995) in the program SPAGEDI version1.5 (Hardy & Vekemans, 2002). Following the recommendations of Hardy and Vekemans (2002), we choose distance intervals to ideally include a minimum of 100 pairwise comparisons, >50% of individuals and a coefficient of variation of participation ≤1.0. We chose five distance intervals: 0–10, 10–25, 25–50, 50–75, 75–100 m. We restricted analyses to a maximum distance of 100 m because previous studies of O. bataua (Browne et al., 2015) and preliminary analyses showed that most variation in fine-scale spatial genetic structure for this species occurs at distances <100 m (Table S1, Supporting Information). Including all pairwise comparisons with no distance restriction did not change qualitative results. We pooled samples across fragments to increase precision (i.e., reduce the size of confidence intervals) on spatial

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autocorrelation estimates by increasing the overall number of pairwise comparisons at each distance interval, but seedlings were only compared to other seedlings within their respective fragment and reference allele frequencies were calculated separately for each fragment. In addition, separate reference allele frequencies were calculated for seedlings in continuous forest. We assessed statistical significance at each distance interval based on the 95% confidence interval of the null distribution of permuting individuals among locations $n = 9,999$ times.

We estimated the strength of fine-scale spatial genetic structure using the Sp statistic of Vekemans and Hardy (2004), which is calculated as $-b_{Flog}/(1 - F_1)$, where b_{Flog} is the mean slope of the regression coefficient of F_{ij} on a log distance scale and F_1 is the mean estimate of the kinship coefficient of the first distance class. The Sp is generally robust to the choice of distance intervals and allows comparisons of fine-scale spatial genetic structure across studies (Vekemans & Hardy, 2004). We tested for statistical differences in Sp across paternal gametic genotypes, maternal gametic genotypes and diploid leaf genotypes with paired t tests, using locus as the pairing factor. Similarly, we tested for differences in Sp between paternal gametic genotypes, maternal gametic genotypes and diploid leaf genotypes in forest fragments compared to continuous forest with a paired t test. At the landscape scale (i.e., among fragments), we estimated genetic structure and differentiation of male and female gametic genotypes and diploid leaf genotypes using the D_{est} (Jost, 2008) calculated in GENALEX version 6.5 (Peakall & Smouse, 2012) with statistical significance assessed via permutation ($n = 999$)

and standard errors estimated by jackknifing over loci. We also calculated other metrics of population structure F_{st} and G_{st} (Meirmans & Hedrick, 2011) in a similar manner for comparison purposes. To test how genetic differentiation (pairwise D_{est}) was related to log-transformed geographic distance and differences in forest cover among fragments, we used multiple regression on distance matrices (Legendre, Lapointe, & Casgrain, 1994; Lichstein, 2007). We assessed significance via permutation ($n = 999$) as implemented in the "ecodist" R package (Goslee & Urban, 2007).

3 | RESULTS

3.1 | Genetic diversity

Within-patch diversity of male gametes, female gametes and diploid leaf tissue were all lower in forest fragments than in continuous forest (Table 1), but these differences were only statistically significant for gene diversity (Hs) of male gametes (Kruskal–Wallis $\chi^2 = 4.46$, $p = 0.035$) and rarefied allelic richness (Ar) of male gametes ($\chi^2 = 4.17$, $p = 0.041$) and female gametes ($\chi^2 = 3.88$, $p = 0.048$). In forest fragments, Hs and Ar were significantly higher for male gametes compared to female gametes ($p < 0.001$ for both pairwise comparisons with post hoc Friedman test), and Hr and Ar of female gametes were significantly lower than diploid leaf tissue ($p < 0.008$ for both). Hs and Ar were not different between male gametes and diploid leaf tissue ($p > 0.54$ for both). We found similar patterns for α-diversity (Table 1).

TABLE 1 Genetic diversity for male gametes, female gametes and diploid leaf tissue of seedlings of the palm *Oenocarpus bataua* sampled from 11 forest fragments and a nearby continuous forest reserve in northwest Ecuador

	Forest fragments			Continuous forest		
	Male gametes	Female gametes	Diploid seedlings	Male gametes	Female gametes	Diploid seedlings
Within patches						
Hs	**0.61 ± 0.06**[a]	0.43 ± 0.09[b]	0.60 ± 0.06[a]	0.67 ± 0.06	0.49 ± 0.09	0.62 ± 0.06
Ar	**2.06 ± 0.13**[a]	**1.71 ± 0.16**[b]	2.03 ± 0.12[a]	2.17 ± 0.11	1.84 ± 0.18	2.07 ± 0.12
α	2.66[a]	1.81[b]	2.48[a]	3.10	2.07	2.56
α'	0.67	0.48	0.61	0.71	0.54	0.62
Total across patches						
γ	2.90[a]	2.32[b]	2.79[a]	3.08	2.26	2.66
γ'	0.66	0.57	0.64	0.68	0.56	0.63
Among patches						
β	1.09	1.28	1.12	0.99	1.09	1.04
β'	0.10	0.25	0.13	−0.01	0.10	0.04
δ	0.13	0.31	0.16	−0.03	0.10	0.04

Notes. Shown are gene diversity (Hs) ± 1 standard deviation and allelic richness (Ar) ± 1 standard deviation rarefied to $n = 3$ gene copies, within-patch α-diversity, total diversity across patches (γ), scaled versions of α and γ diversity (α', γ') along with turnover of alleles among patches (β and β') and average pairwise divergence (δ). Bold values show statistically significant differences in within-patch or total diversity comparing forest fragments to continuous forest for either male gametes, female gametes or diploid seedlings. We tested for statistical differences between the α-and γ-diversity metrics of paternal gametes, maternal gametes and diploid leaf tissue using a nonparametric analogue of Bartlett's variance heterogeneity test (Scofield et al., 2012; Sork et al., 2015) with 9,999 bootstraps implemented in the DispersalDiversity R package (Scofield, 2015). Superscripts indicate statistically significant differences comparing male gametes, female gametes and diploid seedlings within only forest fragments. Statistical tests were not conducted for scaled or among patch diversity metrics.

Fragments surrounded by more forest had higher levels of Hs and Ar for female gametes (Figure 2b,e, Hs: $R = 0.68$, $t = 2.80$, $df = 9$, $p = 0.010$; Ar: $R = 0.68$, $t = 2.80$, $df = 9$, $p = 0.010$). α-diversity for female gametes showed similar patterns (Pearson's $R = 0.61$, $t = 2.29$, $df = 9$, $p = 0.024$, Figure 2h). The α-diversity of diploid leaf tissue increased significantly with increasing forest cover (Figure 2i, $R = 0.60$, $t = 2.23$, $df = 9$, $p = 0.027$), but was not significantly related to Hs or Ar in diploid leaf tissue (Figure 2c,f, Hs: $R = 0.48$, $t = 1.62$, $df = 9$, $p = 0.069$; Ar: $R = 0.51$, $t = 1.79$, $df = 9$, $p = 0.053$). We found no statistically significant relationship between surrounding forest cover and male gametes in terms of Hs (Figure 2a, $R = 0.32$, $t = 1.00$, $df = 9$, $p = 0.171$), Ar (Figure 2d,

$R = 0.29$, $t = 0.92$, $df = 9$, $p = 0.190$), or α-diversity (Figure 2g, $R = 0.35$, $t = 1.14$, $df = 9$, $p = 0.143$).

Total diversity (γ) was not significantly different between forest fragments and continuous forest for either male gametes, female gametes or diploid leaf tissue ($p > 0.05$, Table 1). Similar to within-patch diversity, total diversity across fragments (γ) of male gametes was higher than female gametes ($p = 0.011$), but not significantly different than diploid leaf tissue ($p = 0.551$), while γ-diversity of female gametes was significantly lower than in diploid leaf tissue ($p = 0.010$, Table 1). Allelic turnover among fragments (β) was highest for female gametes, lowest for male gametes and intermediate for diploid seedling leaf tissue; a similar
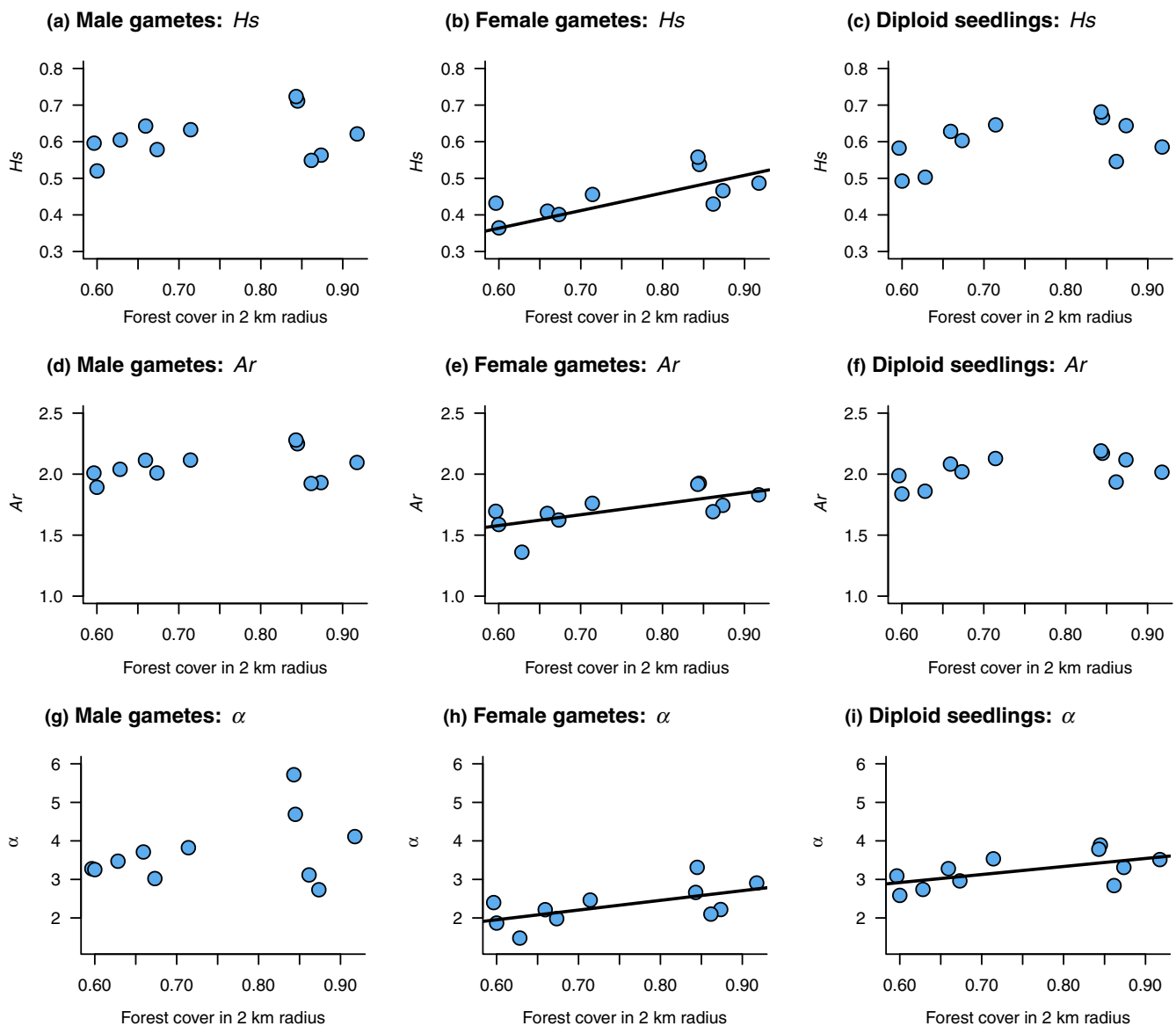

FIGURE 2 Relationship between proportion of forest cover in a 2 km radius around each of 11 forest fragments and the within-fragment gene diversity (Hs, a–c), allelic richness (Ar, d–f) and α-diversity (g–i) of male gametes, female gametes and diploid leaf tissue of *Oenocarpus bataua* seedlings in northwest Ecuador. Solid black lines show line of best fit of a linear regression in cases where there was a statistically significant ($p < 0.05$) correlation between forest cover and diversity

pattern emerged for the metric of allelic divergence (δ) among fragments (Table 1).

3.2 | Parental correlations and effective parental size

Comparing forest fragments to continuous forest, the effective number of fathers (N_{ep}) was 81% lower in forest fragments, the effective number of mothers (N_{em}) was 55% lower and the overall number of effective parents (N_e) was 71% lower than in continuous forest (Table 2). The probability of two seedlings sharing the same mother were higher (Q_w^m, Mann–Whitney $U = 97$, $p = 0.001$) and the probability of two seedlings sharing the same father were higher (Q_w^p, Mann–Whitney $U = 93$, $p = 0.003$) in forest fragments than in continuous forest, but there was no difference in cross-parental correlations (Q_w^{mp}, Mann–Whitney $U = 75$, $p = 0.086$, Table 2).

Within fragments, the probability of two seedlings sharing the same mother (Q_w^m) was nearly four times that of sharing the same father (Q_w^p, Table 2, $V = 0$, $p < 0.001$), yielding an effective number of fathers (N_{ep}) per fragment approximately 4× higher than the effective number of mothers (N_{em}) per fragment (Table 2). The overall effective number of parents (N_e) per fragment was intermediate between the effective number of fathers and mothers. Cross-parental correlations within fragments (Q_w^{mp}) were low (Table 2). The probability of two seedlings sharing the same mother (Q_w^m) within fragments decreased with increasing levels of surrounding forest cover (Pearson's $R = -0.69$, $t = -2.88$, $df = 9$, $p = 0.009$, Figure 3b), but there was no significant relationship between forest cover and

paternal correlations (Q_w^p, $R = -0.12$, $t = -0.37$, $df = 9$, $p = 0.360$, Figure 3a) or cross-parental correlations (Q_w^{mp}, $R = -0.19$, $t = -0.59$, $df = 9$, $p = 0.285$, Figure 3c). Between fragments, paternal correlations, maternal correlations and cross-parental correlations were all close to 0 (Table 2).

3.3 | Fine-scale and landscape-scale spatial genetic structure

Compared to continuous forest, forest fragments had stronger spatial genetic structure for female gametes ($Sp = 0.161 \pm 0.016$, mean $\pm$ standard error in forest fragments, vs. 0.037 ± 0.004 in continuous forest, $t = 2.82$, $df = 7$, $p = 0.013$, Figure 4, Table S3, Supporting Information), male gametes ($Sp = 0.041 \pm 0.004$ vs. 0.004 ± 0.001, $t = 3.22$, $df = 7$, $p = 0.007$) and diploid leaf tissue ($Sp = 0.026 \pm 0.001$ vs. 0.011 ± 0.001, $t = 4.82$, $df = 7$, $p < 0.001$, Figure 4, Table S3, Supporting Information). For male gametes, kinship estimates did not differ significantly from $F_{ij} = 0$ at any distance interval in either forest fragments or continuous forest (Figure 4, Table S3, Supporting Information). For female gametes, kinship was higher at short-distance intervals (<10 m) and lower at longer distance intervals (25–75 m) in forest fragments compared to continuous forest (Figure 4, Table S3, Supporting Information). Similarly, for diploid leaf tissue, kinship was higher at small distance intervals (<10 m) in forest fragments than in continuous forest and lower at longer distance intervals (25–75 m). For all groups, there was no significant patterns of relatedness (e.g., $F_{ij} = 0$) by the 100 m distance interval (Figure 4, Table S3, Supporting Information). Within fragments, female gametes showed the strongest patterns of fine-scale spatial genetic structure, which was significantly higher than in male gametes ($t = -2.55$, $df = 7$, $p = 0.019$) or diploid leaf tissue ($t = 3.08$, $df = 7$, $p = 0.009$, Figure 4, Table S3, Supporting Information); Sp did not differ between male gametes and diploid leaf tissue ($t = 1.31$, $df = 7$, $p = 0.884$).

Landscape-scale genetic structure among fragments was highest for maternal gametes ($D_{est} = 0.256 \pm 0.074$, jackknifed mean $\pm$ SE), lowest for paternal gametes (0.121 ± 0.033) and intermediate for diploid leaf tissue of seedlings (0.132 ± 0.037) and was significant for all groups ($p < 0.001$ for all). F_{st} and G_{st} showed similar patterns, except for F_{st} of diploid leaf tissue being slightly lower than that of male gametes rather than intermediate between male and female gametes (Table S4, Supporting Information). Pairwise D_{est} among fragments was not significantly associated with log-transformed distance or differences in surrounding forest cover for either maternal gametes, paternal gametes or diploid leaf tissue ($p > 0.05$ for all).

TABLE 2 Mean estimates $\pm$ standard error (SE) of within-patch paternal (Q_w^p), maternal (Q_w^m) and cross-parental (Q_w^{mp}) correlations; between-patch paternal (Q_b^p), maternal (Q_b^m) and cross-parental (Q_b^{mp}) correlations; and effective number of fathers (N_{ep}), mothers (N_{em}) and total parents (N_e) of *Oenocarpus bataua* seedlings sampled across 11 forest fragments and a nearby continuous forest reserve in northwest Ecuador

	Forest fragments	Continuous forest
Within patch		
Q_w^p	0.104 ± 0.033^a	0.019 ± 0.034^b
Q_w^m	0.403 ± 0.042^a	0.144 ± 0.024^b
Q_w^{mp}	0.044 ± 0.026^a	0.006 ± 0.020^a
Between patch		
Q_b^p	-0.008 ± 0.003	0.001 ± 0.003
Q_b^m	-0.037 ± 0.004	-0.015 ± 0.002
Q_b^{mp}	-0.020 ± 0.006	-0.007 ± 0.005
Effective number of parents		
N_{ep}	9.61 ± 0.64	52.24 ± 1.71
N_{em}	2.48 ± 0.21	6.95 ± 0.59
N_e	6.72 ± 0.56	22.80 ± 1.85

Notes. Statistically significant differences in within-patch parental correlations between forest fragments and continuous forest are shown with superscripts.

4 | DISCUSSION

For most plants, the sequential processes of pollen and seed dispersal shape the diversity and spatial structure of genetic variation. Habitat loss and fragmentation have the potential to differentially disrupt these dispersal processes, but most studies either focus on
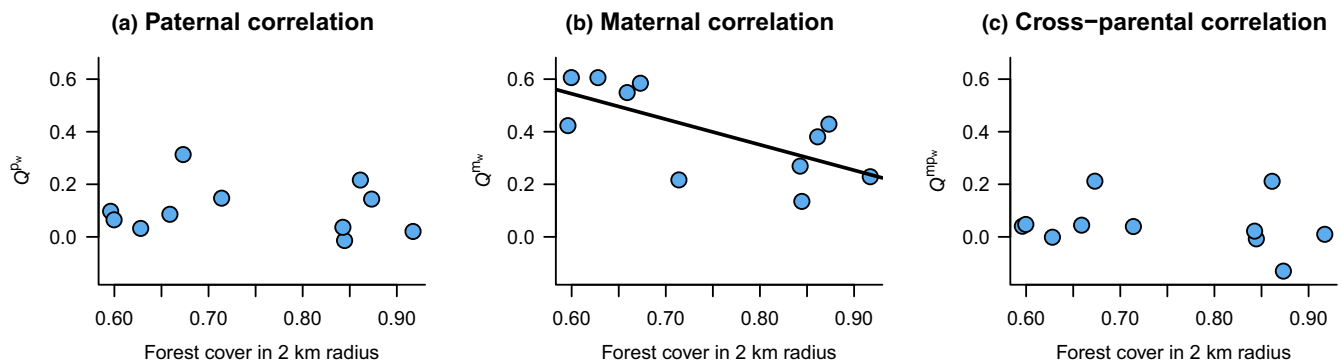
FIGURE 3 Relationship between proportion of forest cover in a 2 km radius around each of 11 forest fragments and within-fragment (a) paternal correlation, (b) maternal correlation and (c) cross-parental correlation for seedlings of *Oenocarpus bataua* in northwest Ecuador. Solid black lines show line of best fit from a linear regression where there was a statistically significant correlation ($p < 0.05$) between forest cover and maternal correlation

the combined effect of both processes on genetic variation, or one process individually, rather than comparing them directly within the same set of individuals. Here, we provide the first direct comparisons of the relative contributions of male gametes from pollen sources and female gametes from seed sources to genetic diversity and structure of established seedlings in a fragmented tropical landscape. Together, our results support the hypothesis that habitat loss and fragmentation restrict seed dispersal, which in turn leads to a reduction genetic diversity, effective parental sizes and strong spatial genetic structure. We also find evidence that pollen dispersal is likely negatively affected by habitat loss and fragmentation in this system, although the relatively high genetic diversity contributed by male gametes may buffer populations against negative genetic consequences associated with anthropogenic perturbations. These insights into the ways in which these two dispersal processes are impacted by habitat loss and fragmentation can aid in the prediction and management of evolutionary changes to plant populations in human-modified landscapes.

The parental structure and spatial genetic structure analyses provide compelling evidence that seed dispersal is disrupted within forest fragments in this system. Compared to nearby continuous forest, seedlings in fragments showed stronger patterns of fine-scale spatial genetic structure and were almost twice as likely to share the same mother. In addition, allelic richness of female gametes was lower in forest fragments than in continuous forest. A restriction in seed dispersal is expected to lead to increased strength of fine-scale spatial genetic structure and high maternal correlations in patches of seedlings because siblings will be aggregated, even if pollen dispersal remains extensive (Dick, Hardy, Jones, & Petit, 2008; Wang et al., 2011). Alternatively, differences in the densities of adult trees between forest fragments and continuous forest could produce similar patterns, as a result of nonoverlapping seed shadows at low adult densities (Hamrick, Murawski, & Nason, 1993) or a reduction in seed dispersal distance at high adult densities (Carlo & Morales, 2008). Although we were unable to directly measure adult density in the current study, previous work in our study area shows that densities of adult *O. bataua* in fragments may be higher (Browne et al., 2015) or lower (Browne & Karubian, 2016) than in continuous forest, and identifying the degree to which

variation in adult densities may contribute to the patterns we observed is a priority for future work. In addition, seed dispersal between fragments may be impacted, reflected by the high levels of genetic differentiation and allelic turnover in female gametes among forest fragments compared to male gametes and continuous forest. Altogether, these results are consistent with the hypothesis that seed dispersal in this system is vulnerable to disruption by habitat loss and fragmentation as seen in other animal-dispersed plants (Fontúrbel et al., 2015; McConkey et al., 2012).

For example, in fragments of Atlantic rainforest in Brazil, extirpation of large-bodied seed dispersers of the palm *Euterpe edulis* has led to rapid changes in allele frequencies among fragments (Carvalho, Galetti, Colevatti, & Jordano, 2016) and phenotypic changes in seed size (Galetti et al., 2013). In defaunated forest fragments in Argentina where the Amazonian tapir (*Tapirus terrestris*) is absent, fine-scale spatial genetic structure of the palm *Syagrus romanzoffiana* is stronger than in nearby continuous forest, likely due to a lack of seed dispersal by tapirs (Giombini et al., 2017). While we were not able to directly measure the impacts of habitat loss on either seed or pollen disperser populations in the current study, previous surveys from the same study area found that umbrellabirds were locally absent from the majority of fragments (>85%) sampled in the area (Walter et al., 2017), indicating that their functional role as an important seed disperser for *O. bataua* (Karubian et al., 2010, 2012) in fragmented landscapes is likely compromised, potentially leading to the observed increase in spatial genetic structure. Toucans were present in the majority of fragments (Walter et al., 2017); however, it remains unknown the degree to which toucans are able to compensate for the local extirpation of umbrellabirds in forest and maintain seed dispersal services either within or among forest fragments for *O. bataua*. More refined tracking of individual disperser species across this fragmented landscape remains a priority for future work.

We also found evidence that the amount of genetic diversity contributed by pollen in this system may be impacted by habitat loss and fragmentation. Compared to continuous forest, male gametes in forest fragments had significantly lower gene diversity (Hs) and allelic richness (Ar), stronger fine-scale spatial genetic structure and an 80%

(a) Male gametes

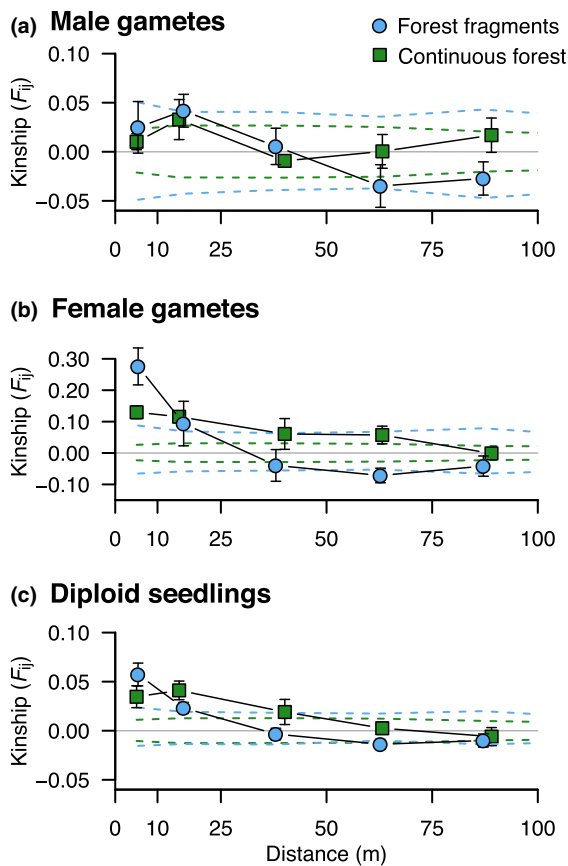## (b) Female gametes

(c) Diploid seedlings

FIGURE 4 Spatial autocorrelation analysis of genetic kinship in (a) male gametes, (b) female gametes and (c) diploid leaf tissue of *Oenocarpus bataua* seedlings sampled in 11 forest fragments (blue circles) and continuous forest (green rectangles) in northwest Ecuador. Dashed blue and green lines show the 95% CI range of the null hypothesis that $F_{ij} = 0$ for seedlings in forest fragments and continuous forest, respectively. The genetic kinship coefficient (F_{ij}) ± 1 standard error is plotted against distance classes

a reduction in genetic diversity and increased genetic structure of male gametes. Finally, the pollinators of *O. bataua*, which are mainly small beetles and bees (Núñez-Avellaneda & Rojas-Robles, 2008), could be directly impacted by habitat loss and fragmentation, although little is known about their natural history and susceptibility to habitat loss and fragmentation in this system. Whether or not the foraging behaviour of these pollinators shift in response to habitat loss and fragmentation in other systems (Aldrich & Hamrick, 1998) or are capable of maintaining long-distance dispersal despite habitat loss and fragmentation remains unknown.

By examining the relationship between genetic diversity and the amount of forest cover surrounding each forest fragment, we were able to assess how the amount of habitat in the "local landscape" surrounding each fragment predicts patterns of genetic diversity. We discovered that within-fragment allelic diversity of female gametes across all measured metrics and α-diversity of diploid seedlings were higher in fragments with more surrounding forest cover, along with a reduced probability of two seedlings sharing the same mother (Q_w^m). This mirrors a pattern found in species α-diversity of large-bodied avian seed dispersers in the same study area (Walter et al., 2017). Fragments with higher amounts of surrounding forest cover had higher species richness of large-bodied avian frugivores, suggesting that the total amount of forest cover surrounding each fragment is in some cases a useful metric for predicting patterns of biodiversity both within species and across taxa (Fahrig, 2013; Melo et al., 2017). It should be noted that the amount of forest cover surrounding a fragment is not a perfect proxy for either fragment size or degree of fragment isolation, as it incorporates information across these two metrics. In our study, smaller fragments in terms of area tended to have higher amounts of surrounding forest cover (Figure 1), although this relationship was not statistically significant (Pearson's $R = -0.52$, $t = 1.83$, $df = 9$, $p = 0.101$). However, this does reveal that measuring fragments only in terms of their area may fail to capture important information about the amount of overall habitat available in the local landscape, which as shown in this current study can serve as a useful metric for predicting genetic diversity.

A key contribution of this study is the use of gametic assays to obtain a previously unavailable perspective on how habitat loss and fragmentation influence patterns of male and female gametic genetic diversity and structure in established seedlings. While a previous study (Browne et al., 2015) found a similar increase in spatial genetic structure of *O. bataua* seedlings in forest fragments compared to continuous forest, it was unknown whether this observed increase was due to an increase in the genetic structure of male gametes, female gametes or both. The gametic assays in this study provide a process-oriented perspective that revealed the increased spatial genetic structure in forest fragments is most likely due to restricted movement of female gametes, which showed much higher levels of genetic kinship at short-distance intervals in forest fragments compared to continuous forest. In addition, the gametic assays revealed that female gametic diversity contributed by seeds is a major bottleneck constraining overall levels of genetic diversity in forest fragments, while male gametic diversity from pollen serves to contribute most of the genetic diversity to seedlings. These results support the conclusions of

reduction in the effective number of fathers. However, within fragments, male gametes from pollen contributed significantly higher amounts of genetic diversity to established seedlings compared to female gametes from seeds, highlighting the important role that pollen plays in maintaining levels of genetic diversity within forest fragments. It remains unclear the degree to which pollen movement itself was directly impacted by habitat loss and fragmentation, as a reduction in the genetic diversity of male gametes could arise from interactions between pollen and seed dispersal. For example, a reduction in seed dispersal can lead to reduced representation of pollen donors because seed dispersal impacts the final location and diversity of male gametes (Grivet et al., 2009; Browne et al., in press; Sork et al., 2015). This effect could potentially mask a scenario where pollen dispersal is maintained or enhanced in fragmented landscapes (Dick, 2001, 2003), leading to an overall decline in the genetic diversity contributed by male gametes despite potentially extensive pollen dispersal. Alternatively, if the spatial proximity and/or genetic relatedness of pollen donors in forest fragments are increased, this may also lead to

other studies using gametic assays on wind-pollinated, animal-dispersed plants (Parejo-Farnés et al., 2017; Sork et al., 2015) and provide a strong example of the important role of seed dispersal in determining patterns of genetic diversity and structure in fragmented landscapes. Additional studies that use gametic assays on fragmented plant populations are needed to determine whether the patterns observed in this study are pervasive across species and contexts.

Several limitations of this study should be highlighted to help guide future endeavours aiming to understand patterns of gametic diversity in fragmented landscapes. Because long-term exposure of seeds to field conditions degrades DNA, it can be a serious challenge to obtain high-quality DNA from seeds attached to recruited seedlings for use in gametic assays (Smouse, Sork, Scofield, & Grivet, 2012). In the context of this study, we were unable to extract usable DNA from the seed tissue of many samples we collected in the field (Table S1, Supporting Information), which limited sample sizes per fragment and also the number of genetic markers used. As a result, the relatively low samples sizes may have resulted in high variance of genetic diversity estimates and in part explain the decreased power to detect differences in diversity within and across forest fragments and plots in continuous forest. To address this, future studies should collect many more samples than expected to be required for statistical analysis, assuming a high failure rate of DNA extraction of weathered maternal seed tissue, especially if working in tropical forests where decomposition is rapid. Attention should also be paid in future studies to make sure the spatial configuration of sampling is as comparable as possible across sites to not confound differences in spatial sampling with the effect of interest. In this study, we placed seedlings in continuous forest into sampling groups post hoc to match our sampling in forest fragments, which may have influenced our results, though this post hoc grouping only slightly changed the estimated diversity metrics compared to a similar analysis done with the original sampling groupings in continuous forest and full genetic marker panel (Browne et al., in press). In addition, this study in particular may be influenced by the fact that our representative sample of continuous forest is located at the edge of the forest reserve, bounded on one side by a road and pasture (Figure 1), which may act as a barrier to either pollen or seed dispersal in this direction, and could potentially explain the lack of difference in overall or female gametic diversity between forest fragments and continuous forest. At last, as a shared limitation to most studies in natural landscapes, we are not able to separate the distinct effects of habitat loss and habitat fragmentation (Fahrig, 2003), as both of these processes are operating concurrently in our study area. Thus, it is important to highlight that we are observing the joint effects of habitat loss and fragmentation and not attempting to disentangle their separate consequences.

As habitat loss and fragmentation continue to increase in the tropics (Hansen et al., 2013), understanding how the conversion of formerly continuous landscapes to isolated patches impacts the genetic diversity and structure of plant populations is key to predicting and managing these biodiverse habitats. Genetic diversity is necessary to ensure the adaptive potential of isolated populations as they face changing selective pressures from habitat loss and fragmentation and other drivers of global change (Jump & Peñuelas,

2005). This study shows how decomposing the relative contributions of male gametes from pollen and female gametes from seed to genetic diversity and structure in fragmented landscapes can be used to assess the vulnerability of the linked, but distinctive, pollen and seed dispersal processes to habitat loss and fragmentation and how modifications to these processes may impact evolutionary potential and viability of plant populations. We provide support for the idea that seed dispersal processes are especially vulnerable to the negative consequences of habitat loss and fragmentation, suggesting that preserving these processes in fragmented landscapes should be a key goal of conservationists and managers. We found that the genetic diversity contributed by seed dispersal processes within forest fragments are associated with the degree of surrounding forest cover, placing a premium on retaining and restoring as much intact habitat as possible in these landscapes to avoid or offset negative genetic consequences of habitat loss and fragmentation for plants.

DATA ACCESSIBILITY

Sampling locations and microsatellite genotypes are publicly available through Figshare: (https://doi.org/10.6084/m9.figshare.5142172), and R code for gametic extraction is available on Github (https://github.com/lukembrowne/gametic-extraction; https://doi.org/10.5281/zenodo.1274538).

AUTHOR CONTRIBUTIONS

L.B. and J.K. designed the research and wrote the manuscript. L.B. performed the research and analysed the data.

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