ORIGINAL ARTICLE

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Limited seed dispersal shapes fine-scale spatial genetic structure in a Neotropical dioecious large-seeded palm

Sebastián Escobar[1,2] | Yves Vigouroux[3] | Jordan Karubian[4] | Leila Zekraoui[3] | Henrik Balslev[1] | Rommel Montúfar[5]

[1]Ecoinformatics and Biodiversity, Department of Biology, Aarhus University, Aarhus, Denmark

[2]Grupo de Investigación en Biodiversidad, Medio Ambiente, y Salud, Universidad de Las Américas, Quito, Ecuador

[3]Diversité, Adaptation, Développement des Plantes, Institut de Recherche pour le Développement, University of Montpellier, Montpellier, France

[4]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, USA

[5]Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

Correspondence
Sebastián Escobar, Grupo de Investigación en Biodiversidad, Medio Ambiente, y Salud, Universidad de Las Américas, Quito, Ecuador.
Email: sebastian.escobar.a@udla.edu.ec

Associate Editor: Ferry Slik

Handling Editor: Eric Fuchs

Abstract

Seed and pollen dispersal contribute to gene flow and shape the genetic patterns of plants over fine spatial scales. We inferred fine-scale spatial genetic structure (FSGS) and estimated realized dispersal distances in *Phytelephas aequatorialis*, a Neotropical dioecious large-seeded palm. We aimed to explore how seed and pollen dispersal shape this genetic pattern in a focal population. For this purpose, we genotyped 138 seedlings and 99 adults with 20 newly developed microsatellite markers. We tested if rodent-mediated seed dispersal has a stronger influence than insect-mediated pollen dispersal in shaping FSGS. We also tested if pollen dispersal was influenced by the density of male palms around mother palms in order to further explore this ecological process in large-seeded plants. Rodent-mediated dispersal of these large seeds occurred mostly over short distances (mean 34.76 ± 34.06 m) while pollen dispersal distances were two times higher (mean 67.91 ± 38.29 m). The spatial extent of FSGS up to 35 m and the fact that seed dispersal did not increase the distance at which male alleles disperse suggest that spatially limited seed dispersal is the main factor shaping FSGS and contributes only marginally to gene flow within the population. Pollen dispersal distances depended on the density of male palms, decreasing when individuals show a clumped distribution and increasing when they are scattered. Our results show that limited seed dispersal mediated by rodents shapes FSGS in *P. aequatorialis*, while more extensive pollen dispersal accounts for a larger contribution to gene flow and may maintain high genetic diversity.

Abstract in Spanish is available with online material.

KEYWORDS
Arecaceae, genetic diversity, microsatellite markers, *Phytelephas aequatorialis*, rodent dispersal, W Ecuador

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Henrik Balslev and Rommel Montúfar should be considered joint senior authors.

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1 | INTRODUCTION

Seed and pollen dispersal contribute to gene flow and shape genetic patterns over fine spatial scales (Browne et al., 2018; Degen & Sebbenn, 2014; Hardy et al., 2006; Vekemans & Hardy, 2004). Limited dispersal promotes the non-random spatial distribution of genotypes and the formation of fine-scale spatial genetic structure (FSGS) between individuals (Dick et al., 2008; Hardy et al., 2006; Vekemans & Hardy, 2004). FSGS produces neighborhoods of genetically related individuals where genetic drift and natural selection can reduce genetic diversity (Sork, 2016); nevertheless, some degree of FSGS is common in plant populations because of their intrinsic dispersal limitations (Vekemans & Hardy, 2004). FSGS in seedlings is commonly caused by limited seed dispersal because seeds tend to accumulate at the base of parental plants (dos Santos et al., 2018; Ellstrand, 2014; Hardy et al., 2004; Torres et al., 2019); however, limited pollen dispersal can also shape FSGS (Loiselle et al., 1995; Vekemans & Hardy, 2004). Limited dispersal also promotes the isolation of populations and the potential decrease in effective population size, which reduces genetic diversity while increasing inbreeding (Browne & Karubian, 2018; Saastamoinen et al., 2018; Young et al., 1996). In this context, estimating FSGS and genetic diversity along with seed and pollen dispersal patterns allows a better understanding of the evolutionary and ecological dynamics that drive wild plant populations (dos Santos et al., 2018; Ellstrand, 2014; Sork, 2016).

Rodents display idiosyncratic behaviors that influence seed dispersal and genetic dynamics in plant populations (Choo et al., 2012; Howe, 2016). For instance, rodents tend to remove seeds from the ground (secondary dispersal) and move them over short distances (~10 m) to microsites such as fallen logs where they predate or cache the seeds for future consumption (Campos et al., 2017; Dittel et al., 2015; Vander Wall et al., 2005). Small seeds are usually predated after removal while large seeds are more prone to be cached (Kuprewicz & García-Robledo, 2019), which increases seed survival because burial impedes seed predation by other rodents or insects (Forget et al., 1994; Jansen et al., 2012; Mittelman et al., 2020). Large seeds can eventually disperse over long distances (> 100 m) escaping high mortality areas near conspecifics by scatter-hoarding rodents such as the Central American agouti *Dasiprocta punctata* Gray (Hirsch et al., 2012; Jansen et al., 2012). These rodents repeatedly bury and re-cache single seeds under the soil or leaf litter in a scattered way (Gálvez et al., 2009), but only a small part of the scatter-hoarded seeds recruit as seedlings (Choo et al., 2012). As a consequence, limited dispersal of large seeds by rodents can form stands of clumped and closely related plants that show strong FSGS (Choo et al., 2012).

Although the role of limited seed dispersal by rodents on FSGS is known for large-seeded plants (Choo et al., 2012), the contribution of pollination in shaping fine-scale genetic patterns has not been explored in these type of plants. Pollen tends to move over longer distances than seeds, particularly when it is dispersed by insects (dos Santos et al., 2018; Ismail et al., 2012). As a consequence, seed dispersal is the main process that usually shapes FSGS even when seeds disperse several hundred meters (Browne et al., 2018). In the case of plants with limited seed dispersal and pollen dispersal mediated by insects, pollen dispersal may not be able to fully counteract the effect of seed dispersal and therefore would not contribute to shape FSGS. In addition, pollen dispersal is influenced by different ecological factors such as the spatial distribution of individuals (Degen & Sebbenn, 2014). It has been determined that pollen dispersal distances promoted by insects depend on the density of flowering plants, decreasing when plants display clumped distributions and increasing when individuals are scattered (Cristóbal-Pérez et al., 2020; Grant et al., 2019; Ismail et al., 2012). In this way, pollinators improve their foraging efficiency by moving over short distances and therefore promoting the mating of near neighbors (Degen & Sebbenn, 2014). Given that large-seeded plants can form stands of clumped individuals (Choo et al., 2012), pollen dispersal may occur over short distances when individuals are aggregated. However, it is unknown if it could also contribute to shaping FSGS as longer pollen dispersal distances would be expected when scattered individuals are present. Thus, studying pollen dispersal and how it affects FSGS would provide a better understanding on the dispersal ecology and fine-scale genetic patterns of plants with large seeds.

Estimating dispersal distances is key for understanding evolutionary and ecological processes in wild plant populations. Realized seed and pollen dispersal can be estimated using genetic parentage analyses between adult plants and established seedlings. Realized seed dispersal is measured by the spatial distance from a maternal plant to a seedling after seed germination and seedling establishment (Ismail et al., 2017), while realized pollen dispersal is measured by the spatial distance from a paternal plant (pollen donor) to a maternal plant after seed dispersal and seedling establishment (Cristóbal-Pérez et al., 2020). In this way, realized seed and pollen dispersal contribute to the temporal transmission of genetic diversity within plant populations (Ismail et al., 2017). In addition, realized paternal gene dispersal measures the spatial distance from a paternal plant to an established seedling as this is the final distance that male alleles will be dispersed through both pollen and seed dispersal (Sork et al., 2015). In plants with extensive seed dispersal, realized paternal gene dispersal would exceed realized pollen dispersal as the former measure is the combination of pollen and seed dispersal (Browne et al., 2018). Alternatively, realized paternal gene dispersal would not exceed realized pollen dispersal in plants with limited seed dispersal because seed movement would not disperse male alleles further than what pollen movement does. By comparing realized paternal gene dispersal and realized pollen dispersal, we can therefore determine the influence of seed and pollen dispersal in the formation of FSGS and gene flow (Browne et al., 2018).

Even though dioecious species represent around one-quarter of all tropical tree species (Cristóbal-Pérez et al., 2020 and references therein), fine-scale genetic patterns and dispersal have been rarely explored in dioecious tropical plants (Bittencourt & Sebbenn, 2007; Cristóbal-Pérez et al., 2020; Riba-Hernández et al., 2016; Ward et al., 2005). The spatial distribution of male and female individuals and sex ratio are expected to affect FSGS

patterns in dioecious species (Cristóbal-Pérez et al., 2020). Dioecious species can show significantly stronger FSGS than monoecious species as the former tend to present clumped distributions because of limited seed dispersal and more restricted pollen dispersal distances than the latter (Nazareno et al., 2013). Strong FSGS would be therefore expected in a dioecious large-seeded plant population with limited seed dispersal and pollen dispersal dependent on the spatial distribution of individuals. Unbiased estimates of dispersal for dioecious plants can be obtained through parentage analyses because potential parents can be explicitly assigned as mothers and fathers, contrary to monoecious species (Ashley, 2010). Therefore, studying dioecious tropical plants can provide new insights on FSGS and dispersal ecology.

Here, we explore fine-scale spatial genetic structure and how it is influenced by realized seed and pollen dispersal in a focal population of the dioecious, insect-pollinated, large-seeded palm *Phytelephas aequatorialis* Spruce. We focus on a large-seeded plant because its dispersal dynamics are more prone to be affected by habitat loss and changes in frugivore communities compared to small-seeded plants (Mittelman et al., 2020). Therefore, large-seeded plants should be considered conservation priorities in tropical regions where deforestation threatens frugivore communities and the plants they disperse (Cramer et al., 2007). To characterize the genetic patterns and dispersal dynamics of this species, we first generated 20 microsatellite markers specific for *P. aequatorialis*. Given that the seeds of *P. aequatorialis* are removed from the ground by rodents (Escobar et al., 2020) and potentially dispersed mostly over short distances, we expect that this process contributes less to gene flow than pollen dispersal and therefore shapes FSGS. We address this expectation by comparing the spatial extent of FSGS with realized seed and pollen dispersal distances, and by testing that distances of realized paternal gene dispersal distances do not exceed those of realized pollen dispersal. We further explore pollination dynamics, expecting that higher aggregations of individuals reduce the distances of realized pollen dispersal and vice versa. We check this expectation by testing if pollen dispersal distances are limited by the spatial aggregation of male palms around mother palms. With this study, we aim to provide evidence on the dispersal ecology of *P. aequatorialis* and large-seeded plants in general, whose dispersal dynamics are highly affected by changes in their ecosystem (Mittelman et al., 2020).

2 | METHODS

2.1 | Study species and site

Phytelephas aequatorialis is a dioecious palm endemic to W Ecuador, a region highly threatened by deforestation where less than 25% of natural forests remain intact (Sierra, 2013). The species is considered vulnerable because land conversion is a severe threat for its populations (Montúfar, 2018). In addition, *P. aequatorialis* has

economic importance because its seeds are commercialized as raw material for button and handicraft manufacturing, while leaves are locally used for thatching (Acosta-Solís, 1948). This palm has a solitary stem and can reach up to 25 m in height. Staminate (male) and pistillate (female) individuals present strong dimorphic inflorescences and usually several inflorescences at distinct stages of development are found on the same individual (Barfod, 1991). Staminate inflorescences are pendulous and up to 2 m long, whereas pistillate inflorescences are up to 0.7 m long (Barfod, 1991; Montúfar et al., 2013). Both type of inflorescences produce heat during their anthesis (thermogenesis; Pincebourde et al., 2016), which could attract insects from Coleoptera, Diptera, and Hymenoptera that pollinate the flowers (Auffray et al., In press; Ervik et al., 1999). Infructescences produce up to 25 fruits and 150 large seeds that in average are 5 cm long and weight 36 g (Brokamp et al., 2014). Within forests, *P. aequatorialis* has dense populations with 40–365 individuals/ha, and the sex ratio usually favors male individuals (Brokamp et al., 2014).

We performed field work during the dry season (June–July) of 2018 within the private reserve Lalo Loor at Manabí province. The reserve has 200 ha of preserved seasonally dry forest (0°5′12″S, 80°9′W) and it is located at ~100 m a.s.l. Mean temperature in the area is 25°C and fluctuates between 13–36°C. The humid season goes from January to April with 400 mm of rain/month, whereas dry season occurs between May and December with 20 mm of rain/month (Instituto Nacional de Meteorología e Hidrología, 2014, 2015, 2017). Medium- and large-sized rodents such as agoutis (*Dasyprocta punctata* Gray), pacas (*Cuniculus paca* Linnaeus), and spiny rats (*Proechimys semispinosus* Tomes) remove and disperse the large seeds of *P. aequatorialis* in the study area (Escobar et al., 2020).

2.2 | Sampling

We established a core plot of 150×150 m (2.25 ha) in an area where *P. aequatorialis* individuals are distributed heterogeneously (Figure 1), which is useful when testing the effect of male density on pollination distances. We geo-referenced 25 male adults, 17 female adults, and 138 seedlings within the plot, and silica-dried leaf tissue for DNA extraction. Young individuals shorter than 0.5 m in height or with a unique closed stipule were considered seedlings. In this way, we aimed to explore only recent dispersal events. Additionally, we extended 25 m to each side of the plot (total area 4 ha) where we sampled 36 male adults and 21 female adults to increase the number of potential parentals.

2.3 | Microsatellite generation and genotyping

Laboratory procedures were conducted at the IRD of Montpellier, France. Details on the development of microsatellite markers and on the genotyping of samples are provided as Appendix S1.
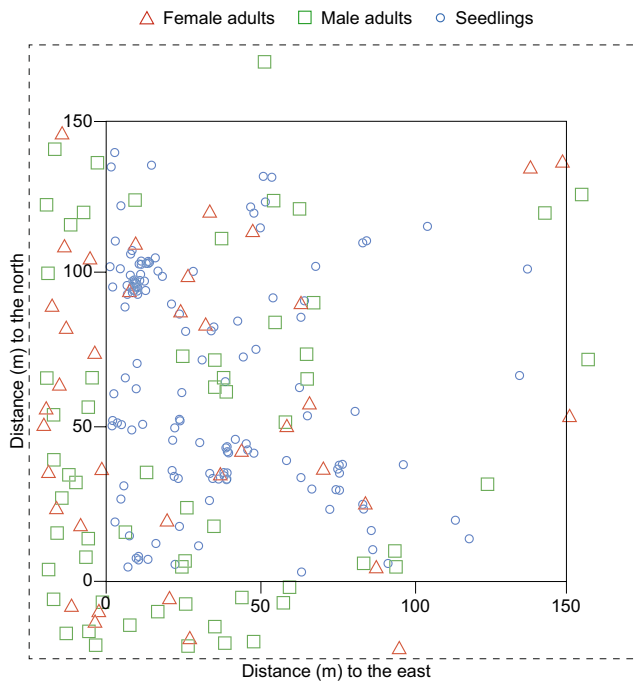
FIGURE 1 Distribution of *Phytelephas aequatorialis* in the studied area. Adults and seedlings were collected in the 150 x 150 m (2.25 ha) plot (core plot). Only adults were collected in the 25 m extensions to each side of the plot (extended plot; total area 4 ha).

2.4 | Statistical analyses

We first used CERVUS v.3.0.7 (Kalinowski et al., 2007) to test for Hardy–Weinberg deviations, null allele frequency, polymorphic information content, and non-exclusion probabilities for the first parent in the 20 loci (Table 1). All loci that deviated significantly ($p < .05$) from Hardy–Weinberg Equilibrium, that showed high frequency of null alleles (>0.05), or that did not show enough diversity to test for Hardy–Weinberg deviations were discarded following Choo et al. (2010).

2.4.1 | FSGS and genetic diversity

We tested for FSGS in seedlings and adults using spatial autocorrelation analyses in SPAGeDi v.1.5 (Hardy & Vekemans, 2002) using the reduced set of ten loci kept from the previous step (Table 1). Further details on FSGS analyses can be found in Appendix S1.

We used the R package *hierfstat* (Goudet, 2005) to obtain mean values of genetic diversity such as expected heterozygosity (H_s) and allelic richness rarefied per sample size, and inbreeding coefficients (F_{is}) for seedling and adult populations. We also obtained the number of private alleles between seedlings and adults with the package *poppr* (Kamvar et al., 2014).

2.4.2 | Parentage analysis

CERVUS was used to assign maternity and paternity to the seedlings with the adults as potential parents using the reduced set of ten loci.

Given the low rate of paternity assignment and therefore the estimation of pollen distances using ten loci (see Results), we also performed parentage analyses using the complete set of 20 loci. More details on parentage analyses are provided in Appendix S1.

2.4.3 | Dispersal distances

Dispersal distances were measured using the results from the parentage analysis with the reduced and the complete set of loci. We used the package *geosphere* implemented in R v.3.5 (R Development Core Team, 2019) to obtain pairwise Euclidean distances between pairs of individuals. Realized seed dispersal distances were measured from seedlings to the female palms inferred as their mothers (Ismail et al., 2017) while realized pollen dispersal distances were measured between male and female palms inferred as seedlings' parents (Cristóbal-Pérez et al., 2020). Realized paternal gene dispersal distances were measured between male palms and the seedlings assigned as their offspring (Browne et al., 2018). To test whether the distances of realized paternal gene dispersal exceed the distances of realized pollen dispersal, we conducted a one-sided Wilcoxon rank-sum test in R.

2.4.4 | Density-dependent pollen dispersal

To test whether pollen dispersal was dependent on the density of pollen donors, we performed Pearson correlations in R between realized pollen dispersal distances and the number of male palms around mother palms. We used pollen dispersal distances estimated from the parentage analyses done with both the reduced and the complete set of loci. The number of male individuals at 10, 20, 40, 60, and 80 m around each mother palm was counted. Different distances were used for this purpose because many female mother palms are located close to the borders of the plot, and male individuals lying outside the plot may not be included when using a fixed distance.

3 | RESULTS

3.1 | Sampling

We sampled and geo-referenced 138 seedlings and 99 adults within the study plot (Figure 1). From these, 41 adults were collected in the core plot (2.25 ha) and 58 additional individuals in the extended plot (total 4 ha). Sex ratio was male-biased, with 59 males and 40 females (1.47:1).

3.2 | FSGS and genetic diversity

We restricted this specific analysis to ten loci that were in Hardy–Weinberg Equilibrium ($p < .05$) and did not show an estimated

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TABLE 1 Characteristics of the 20 polymorphic microsatellite markers developed for *Phytelephas aequatorialis*. Diversity and frequency values were obtained from CERVUS. The ten loci that are in Hardy-Weinberg Equilibrium (ns) were used in all analyses, which was calculated from 138 seedlings and 99 adults.

Locus	Repeat motif	Primer sequences (5′–3′)	Size (bp)	Number of alleles	H_o	H_e	HWE	NAF	PIC	NE-1P
Pa01	(AG)15	F: AGCTGAAGAATTTGGAGACCC	95–109	7	0.39	0.43	ns	0.05	0.399	0.902
		R: GGAGGCACTGTAGAACATGGT								
Pa02	(AT)10	F: TGCGAAGGTTCAATAACAGCA	113–115	2	0.03	0.03	nd	0.12	0.033	0.999
		R: GCCCATTTCCAGCCCTACTA								
Pa03	(AG)11	F: TGAGTGCACATTCCAGGTCT	113–123	7	0.28	0.77	***	0.47	0.739	0.614
		R: ATGCCTATTGCCCATCTCGG								
Pa04	(AT)11	F: TGCTGTGGAAATTGGAGGATG	121–129	5	0.43	0.65	***	0.2	0.583	0.772
		R: GGTCATGATCAGTGCATGCG								
Pa05	(AAT)12	F: TTGGAAGGCTCTCTTTCGTCT	119–144	9	0.7	0.8	***	0.07	0.769	0.572
		R: TCCAAAGATTCCACCATAGCTT								
Pa06	(AG)10	F: GCCCACATACCTTCCAGTTCA	129–131	2	0.49	0.48	ns	0	0.366	0.883
		R: AGTCTTGGCCACCATCTTGG								
Pa07	(AT)14	F: TAATGCAAAGGAATCGCGGG	127–144	6	0.71	0.74	ns	0.02	0.689	0.681
		R: AGTTAATTACCCTTGGCAGGCA								
Pa08	(AG)13	F: GGTAGGTTCCTATCTGCAGTGT	133–158	5	0.16	0.16	nd	−0.02	0.148	0.988
		R: ACCTGATCCCACTTCCTATCAG								
Pa09	(AC)20	F:GGCTGGTGTAAATACAAGTCCC	150–179	15	0.55	0.79	***	0.17	0.763	0.578
		R: TAGTTGATGGGCGGAGACTG								
Pa10	(AT)17	F: ACCCTATGGTGCCGTAGAGA	147–171	14	0.46	0.77	***	0.25	0.749	0.588
		R: GCGTGTGCACAGTATTAGCA								
Pa11	(AG)13	F: TTCATTTGCTTCCACACCCT	157–184	11	0.72	0.79	ns	0.05	0.758	0.587
		R: TCGATGGGCTTAGATCAACCA								
Pa12	(AC)15	F: ACTAATGCAAACCCGACCGA	152–205	16	0.55	0.77	***	0.17	0.743	0.599
		R: TGAGAATAGGACCCTGGCCA								
Pa13	(AG)18	F: GTGCAACACCAGCTTCAGAA	175–202	7	0.67	0.67	ns	0	0.61	0.752
		R: AGGCACCGCTGTTTACTTCA								
Pa14	(AG)11	F: AACATGCATCGGCCGTATCT	189–215	8	0.79	0.73	ns	−0.04	0.686	0.68
		R: GCAGCCAAGTCGCATTATGG								
Pa15	(AT)15	F: CCAACTTGACCAGATGCAGC	170–218	7	0.59	0.57	ns	−0.02	0.478	0.836
		R: TTCTGGAGACCAACCACACA								
Pa16	(AC)12	F: CAACCACCTAAGACGGGTCA	205–225	10	0.82	0.8	ns	−0.02	0.772	0.564
		R: GTCCTTAGCTATCCCACGCA								
Pa17	(AT)12	F: TAGACAGCCACTTGCATCCC	207–224	6	0.43	0.55	*	0.12	0.448	0.85
		R: GTGCAGGAGATATTCGGGCA								
Pa18	(AG)12	F: GGTTGGCATCCAGAAAGGGA	220–227	8	0.49	0.57	***	0.08	0.531	0.818
		R: TGGAGGAATGACTGCTCGTG								
Pa19	(AG)20	F: GGGTGTGGCTCGTTTAATGG	220–287	12	0.65	0.65	ns	−0.01	0.617	0.746
		R: TGGTTAAGTTGCTAACGCTGT								
Pa20	(AAG)11	F: GTTGATGAGCTGTATGGCCC	223–241	7	0.67	0.67	ns	0	0.627	0.739
		R: CATCCGAGGTTGGTGGATGT								

Abbreviations: H_e, expected heterozygosity; H_o, observed heterozygosity; HWE, Hardy–Weinberg Equilibrium test; NAF, null allele frequency; nd, HWE not computed; NE-1P, non-exclusion probability to the first parent; ns, not significant; PIC, polymorphic information content.

***$p < .001$.; *$p < .05$.

frequency of null alleles higher than 5% (Table 1). We detected positive and significant FSGS up to 25 m for seedlings and up to 35 m for adults (Figure 2), indicating that near neighbors are more genetically similar than expected. The kinship coefficient (F_{ij}) decreased as the distance between individuals increased, suggesting that gene flow followed a pattern of isolation by distance. The strength of FSGS was significant ($p < .05$) in seedlings ($Sp = 0.01$) and adults ($Sp = 0.02$).

Expected heterozygosity (H_s), allelic richness, and the inbreeding coefficient (F_{is}) were slightly higher in adults than in seedlings (Table 2). Seedlings showed six private alleles with respect to adults, while adults showed 11 private alleles compared with seedlings.

3.3 | Parentage analysis

The parentage analysis in CERVUS under the 80% CI with the reduced set of ten loci assigned 89 seedlings to 31 of the 40 adult female palms, ranging from 1–11 seedlings assigned per maternal palm. Forty-five seedlings were assigned as offspring to 28 of the 59 adult male palms, with 1–4 seedlings assigned to each male palm. We could determine pollen dispersal distances only in seedlings that were assigned to both females and male palms. We determined 89 realized seed dispersal events from 138 potential events (64.5%), 24 events of realized pollen dispersal (17.4%), and 45 events of realized paternal gene dispersal (32.6%).

Using the complete set of 20 loci, CERVUS assigned 129 seedlings to 31 female palms (1–14 seedlings per palm) and 77 seedlings to 37 male palms (1–7 seedlings per palm) under the 80% CI. We determined 129 events of realized seed dispersal from 138 potential events (93.5%), 73 of realized pollen dispersal (52.9%), and 77 of realized paternal gene dispersal (55.8%).

3.4 | Realized seed, pollen, and paternal gene dispersal

Mean distances of realized seed, pollen, and paternal gene dispersal were similar using both the reduced set of ten loci and the complete set of 20 loci (Table 3). The mean distance of seed dispersal (34.34–34.76 m) was around half of the mean distance of pollen dispersal

(63.71–67.91 m). Between 26 and 31% of the seed dispersal events occurred 0–10 m away from mother palms, 60–64% occurred 0–30 m, and 5–7% exceeded 100 m (Figure 3a, d). Regarding pollen dispersal, 41–46% of the events occurred up to 60 m away from male palms and 18–21% occurred over 100 m (Figure 3b,e). Forty to 43% of paternal gene dispersal events occurred up to 60 m and 17–29% occurred longer than 100 m (Figure 3c,f). The distances of paternal gene dispersal did not exceed significantly the distances of pollen dispersal when using the reduced ($W = 624$, $p = .14$) nor the complete set of loci ($W = 2855$, $p = .43$).

3.5 | Density-dependent pollen dispersal

Using the reduced set of ten loci, distances of pollen dispersal decrease as the density of pollen donors increased only at 40 m around mother palms ($R^2 = 0.32$, $t = -3.24$, $df = 22$, $p = .003$; Figure 4c). However, correlations at 60 m ($R^2 = 0.15$, $t = -1.97$, $df = 22$, $p = .06$; Figure 4d) and 80 m ($R^2 = 0.13$, $t = -1.82$, $df = 22$, $p = .08$; Figure 4e) around mother palms were close to statistical significance. With the complete set of 20 loci, pollen dispersal distances were associated with the density of pollen donors at 60 m ($R^2 = 0.098$, $t = -2.78$, $df = 22$, $p = .006$; Figure 4i) and 80 m around mother palms ($R^2 = 0.086$, $t = -2.59$, $df = 71$, $p = .01$; Figure 4j). The correlation at 40 m around mother palms was also close to statistical significance ($R^2 = 0.04$, $t = -1.81$, $df = 71$, $p = .07$; Figure 4h).

4 | DISCUSSION

4.1 | Limited seed dispersal shapes FSGS

The significant FSGS detected up to 35 m in the population was closer to the mean distance of realized seed dispersal (34.34–34.76 m) than to the main distance of realized pollen dispersal (63.71–67.91 m). This is a first indication that the genetic associations between individuals were predominantly shaped by limited seed dispersal rather than by pollen dispersal. In agreement, the similitude between the distances of realized pollen dispersal and realized paternal gene dispersal shows that seed dispersal does not increase significantly the
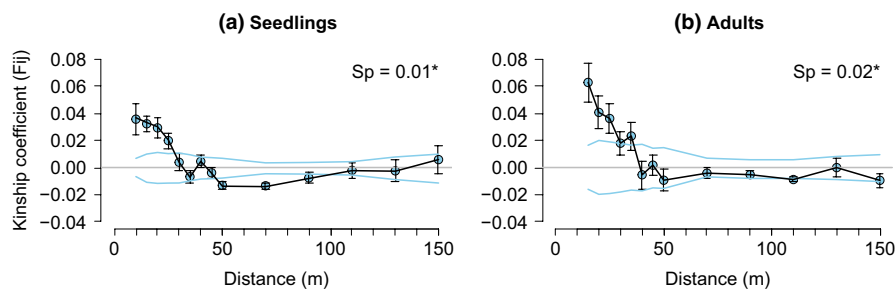

FIGURE 2 Fine-scale spatial genetic structure (FSGS) of (a) seedlings and (b) adults in *Phytelephas aequatorialis* obtained in SPAGeDi. Error bars were estimated from jackknifing across ten loci. Gray lines show the expected values under null models while sky blue lines show the upper and lower 95% confidence intervals. *Sp* statistic represents the strength of FSGS. * $p < .05$.

TABLE 2 Summary of the genetic diversity levels found in the distinct cohorts of *Phytelephas aequatorialis*. Values were obtained using the R packages *hierfstat* and *poppr*. Mean values and standard deviations across ten loci are shown for expected heterozygosity (H_s), allelic richness rarefied per sample size, and inbreeding coefficients (F_{is}). N = number of individuals.

Cohort	N	H_s	Allelic richness	F_{is}	Private alleles
Seedlings	138	0.65 ± 0.13	6.30 ± 2.07	−0.02 ± 0.04	6
Adults	99	0.66 ± 0.12	7.08 ± 2.59	0.04 ± 0.08	11

TABLE 3 Realized dispersal distances measured using the results of two independent parentage analyses performed in CERVUS. Mean values and standard deviations were obtained across ten (reduced set) and 20 loci (complete set)

Type of dispersal	Mean distance (m)	Min distance (m)	Max distance (m)
Seed (10 loci)	34.34 ± 35.34	1.04	152.47
Seed (20 loci)	34.76 ± 34.06	1.04	168.38
Pollen (10 loci)	63.71 ± 40.79	6.76	141.51
Pollen (20 loci)	67.91 ± 38.89	4.67	179.03
Paternal gene (10 loci)	75.03 ± 39.99	8.24	179.75
Paternal gene (20 loci)	67.72 + 33.31	6.59	151.02

distance at which male gametes are dispersed. Thus, limited seed dispersal contributes less to gene flow than pollen dispersal and is the process that strengthens FSGS in the large-seeded palm *P. aequatorialis*. A similar relation between dispersal and FSGS was observed in the Neotropical palm *Oenocarpus bataua* (Browne et al., 2018). Seed dispersal distances in *O. bataua* are larger than in *P. aequatorialis* and can extend up to several hundred meters but they are not as extensive as pollen dispersal distances which can surpass 1000 m (Browne et al., 2018). In this sense, seed dispersal seems to promote FSGS despite its extension as long as pollen dispersal occurs over longer distances.

FSGS estimates indicate that neighborhoods of genetically related individuals are spatially restricted in *P. aequatorialis*. FSGS was more spatially restricted in seedlings, suggesting that demographic thinning effects such as density- or distance-dependent mortality could reduce the spatial extent of FSGS from seedlings to adults (Sebbenn et al., 2011; Zhou & Chen, 2010). In spite of this, the strength of FSGS (*Sp*; Appendix S1) was unexpectedly higher in adults than in seedlings, which may have occurred because of overlapping generations in the adult cohort, past population bottlenecks, or recent enhanced dispersal capacity (Batista Leite et al., 2014; Jones et al., 2007). However, we are unable to determine the specific mechanism acting in the population. In agreement with our findings, plants with clumped distributions produced by limited seed dispersal present FSGS between near neighbors in several dioecious (Hardesty et al., 2005; Zhou & Chen, 2010) and monoecious plants (Choo et al., 2012; Dardengo et al., 2016; Giombini et al., 2017; Sebbenn et al., 2011). Due to the similitude between dispersal distances and the spatial extension at which FSGS occurs, FSGS has been used to estimate historical gene flow as it represents the

evolutionary forces that have acted on the populations over generations (Ellstrand, 2014; Sork, 2016). Thus, the extension of FSGS in *P. aequatorialis* and in other plants with potential limited seed dispersal could be used as an approximation of their actual patterns of seed dispersal.

Contrary to our expectations, the dioecious habit of *P. aequatorialis* did not promote stronger FSGS compared with monoecious species as previously observed in *Ficus* and other plant genera (Nazareno et al., 2013). The monoecious large-seeded palm *A. phalerata* presents significant FSGS up to 30 m and the strength of FSGS in adults (*Sp* = 0.02) was also similar to that in *P. aequatorialis*, which were attributed to limited seed dispersal by rodents (Choo et al., 2012). Further, the seedlings of *P. aequatorialis* showed weaker FSGS (*Sp* = 0.01) than the seedlings of *A. phalerata* (*Sp* = 0.03), suggesting that seed dispersal could be slightly less restricted in *P. aequatorialis*. In agreement, up to 7% of seed dispersal events in *P. aequatorialis* occurred over 100 m compared with ~6% of events in *A. phalerata* (Choo et al., 2012). This direct comparison between two Neotropical large-seeded palms shows that mating system (monoecious vs. dioecious) may not be an accurate predictor of FSGS strength. Instead, it adds evidence to the idea that seed dispersal patterns promoted by the behavior of animal seed dispersers are the main processes shaping FSGS in large-seeded plants.

4.2 | Seed dispersal by rodents

Rodents are effective secondary dispersers of large seeds (Vander Wall et al., 2005), which they remove from the ground and usually disperse close to parental plants (Choo et al., 2012; Dittel et al., 2015). So, it was not surprising that realized seed dispersal performed by rodents in *P. aequatorialis* (Escobar et al., 2020) occurred mainly over short distances, which may have promoted a clumped distribution of seedlings. Nevertheless, many seedlings were dispersed further perhaps by scatter-hoarding rodents that can retrieve cached seeds from the ground and increase dispersal distances through time (Jansen et al., 2012). The reduced and the complete set of loci yielded the same patterns of realized seed dispersal, providing confidence on the results shown here. Similar patterns of seed dispersal by rodents were observed in the monoecious large-seeded palm *Attalea phalerata*, in which 73% of the seed dispersal events were estimated to occur within 30 m (Choo et al., 2012). Thus, rodents seem to promote similar patterns of seed dispersal in large-seeded palms, occurring mostly locally but not limited to the surroundings of the parental plants.
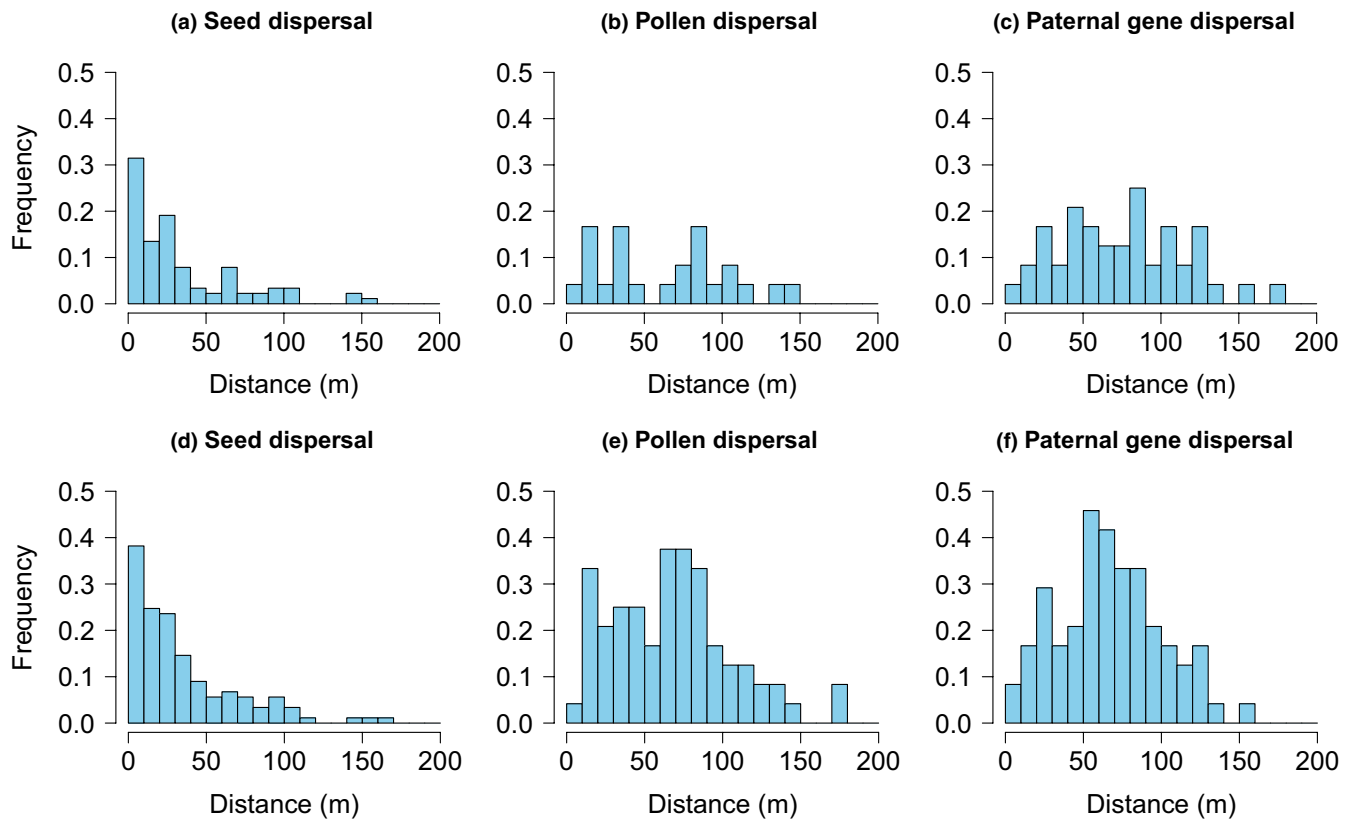
FIGURE 3 Frequency distributions of realized seed, pollen, and paternal gene dispersal distances in *Phytelephas aequatorialis* estimated with ten (a, b, c) and 20 loci (d, e, f). Dispersal distances were estimated from genetic maternity and paternity analyses in CERVUS.

Although seed dispersal tended to be limited, dispersal over long distances (>100 m) also occurred but with a low frequency (5–7%). Between six and 35.5% of seedlings in the plot were immigrants depending on the set of loci used (without accounting for erroneous assignments obtained from the 80% CI), suggesting that seed dispersal over long distances may occur at higher frequency than estimated here. Long-distance seed dispersal was most probably performed by agoutis, which are prolific scatter-hoarders of large seeds (Kuprewicz & García-Robledo, 2019). For instance, a single large seed of the palm *Astrocaryum standleyanum* can be buried and re-cached up to 36 times by agoutis, being dispersed up to 280 m away from the maternal plant (Jansen et al., 2012). Realized seed dispersal in the palm *A. phalerata*, which includes agoutis as one of their seed dispersers, was also estimated to occur up to 150 m (Choo et al., 2012). These findings coupled to our own show that long-distance dispersal is not uncommon for Neotropical large-seeded palms in the presence of effective dispersers such as agoutis. Neotropical large-seeded plants have been considered dispersal anachronisms because their seeds show adaptations for dispersal by the now extinct Pleistocene megafauna (Guimarães et al., 2008; Janzen & Martin, 1982). However, long-distance seed dispersal promoted by rodents suggests that large-seeded plants may have never solely relied on megafauna for their dispersal but rather have a long history of dispersal by rodents (Jansen et al., 2012). It remains to be explored whether scatter-hoarding rodents can connect isolated populations of large-seeded plants through effective seed dispersal over long distances.

4.3 | Density-dependent pollen dispersal

Pollen dispersal distances in *P. aequatorialis* were larger than those of seed dispersal, supporting our expectation. Similar patterns of pollen dispersal were observed using the reduced and the complete set of loci; nevertheless, the patterns obtained with the complete set are more reliable because of higher paternity assignment. Pollen dispersal distances were explained by the spatial distribution of male individuals around mother palms when radius larger than 40 m were used. Pollen was dispersed over shorter distances when male individuals were clumped but also over longer distances when they were more scattered. Pollen dispersal may have been performed by beetles, which are the main visitors of *P. aequatorislis* flowers (Auffray et al., In press) and can occasionally fly distances of up to 1 km (Young, 1986). In this way, beetles could enable pollination between spatially scattered plants. A decrease in pollination distances due to an increase in the density of pollen donors around mother plants has been observed in other dioecious (Born et al., 2008; Grant et al., 2019; Hardy et al., 2006) and monoecious (Castilla et al., 2017; Duminil et al., 2016; Hardy et al., 2006; Ottewell et al., 2012) insect-pollinated tropical plants, indicating that this pattern is not influenced by mating system (monoecious vs. dioecious). Pollen dispersal patterns similar to those observed in *P.*
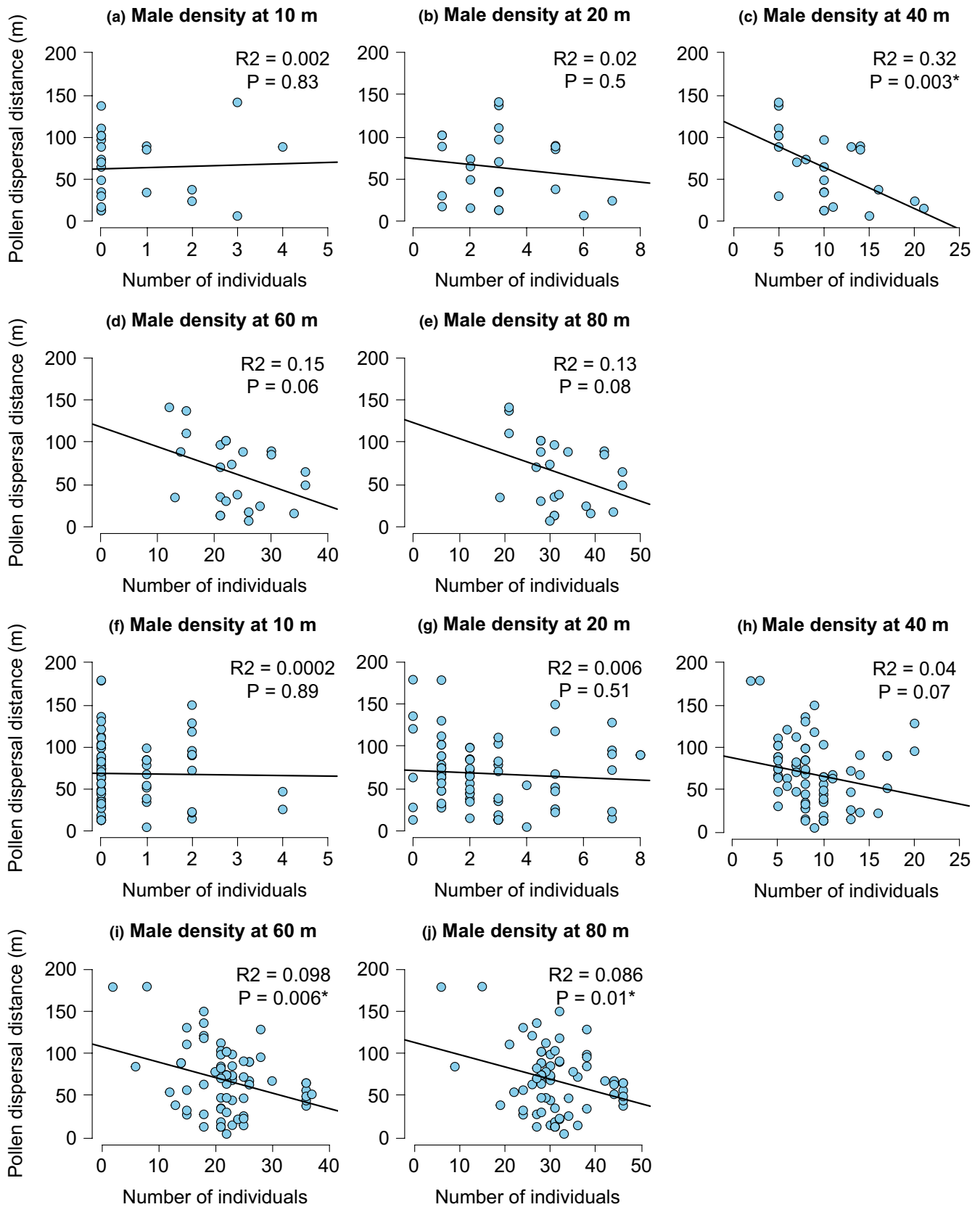
FIGURE 4 Influence of male palms around mother palms on realized pollen dispersal distances in *Phytelephas aequatorialis* tested with Pearson correlations. Male density was obtained within a circle of 10, 20, 40, 60, and 80 m of radius centered on mother palms using ten (a, b, c, d, e) and 20 loci (f, g, h, i, j).

aequatorialis may be common for other insect-pollinated large-seeded plants.

Pollen dispersal in turn may be responsible for maintaining genetic diversity levels and keeping inbreeding low in the studied population by promoting the mating of unrelated individuals (Charlesworth, 2003; Takeuchi & Diway, 2021). We detected high levels of genetic diversity in terms of expected heterocigozity (H_s) in seedlings and adults, similar to other large-seeded (Giombini et al., 2017; Ibanes et al., 2015) and medium-seeded Neotropical palms (Browne et al., 2015; Browne & Karubian, 2018; Gaiotto et al., 2003; Ottewell et al., 2012). Therefore, the population studied could be considered "healthy" because expected heterozygosity (H_s) is often correlated with fitness (Nutt et al., 2016). The low inbreeding coefficients detected, which are expected for dioecious species (Grant et al., 2019), suggest that mating mostly occurred between unrelated individuals because of extensive pollen dispersal that surpassed the spatial extent of FSGS. The relative low assignment of seedlings to male palms in the plot as their fathers and the private alleles found in seedlings suggest extensive gene flow from outside the plot through pollen dispersal (Dardengo et al., 2016). Even though we extended our plot 25m to each side where we only sampled adults, it was not enough to include most of the seedlings' fathers because of extensive distances of pollen dispersal. It is possible that pollen is dispersed over much larger distances than what our study design allowed us to infer, being able to connect isolated populations and increasing genetic diversity by bringing new alleles to the population.

5 | CONCLUSIONS

Our findings show that dispersal in the studied population is sufficient to maintain genetic diversity and counteract inbreeding. Seed dispersal by rodents is limited and promotes restricted FSGS in *P. aequatorialis* while pollen dispersal is more extensive and does not influence this fine-scale genetic pattern. Although pollination occurs over longer distances in general, its dispersal distances can decrease when individuals show a clumped distribution without affecting overall FSGS. Nevertheless, pollen dispersal could be maintaining high genetic diversity in the population because of the introduction of new alleles from outside the plot (Burczyk et al., 2004). In contrast, other species with limited seed and pollen dispersal show low levels of genetic diversity ($H_s < 0.4$) in adults and young cohorts (Grant et al., 2019). Our results on FSGS show that for ex situ conservation of *P. aequatorialis*, seeds should be collected from palms separated by at least 35m to reduce genetic similarity that could lead to reduced genetic diversity. We also developed a set of 20 SSR markers specific for *P. aequatorialis* that can be used for genetic diversity and parentage analyses. As a whole, our results provide evidence on the dispersal ecology of *P. aequatorialis* where limited seed dispersal shapes FSGS while more extensive pollen dispersal may contribute to maintaining high genetic diversity.

AUTHOR CONTRIBUTION

S. E. involved in conceptualization, formal analysis, funding acquisition, investigation, and writing—original draft; Y. V. involved in investigation, resources, and writing—review and editing; J. K. involved in methodology, validation, and writing—review and editing; L. Z. involved in investigation and writing—review and editing; H. B. involved in conceptualization, funding acquisition, and writing—review and editing; R. M. involved in conceptualization, funding acquisition, and writing—review and editing.

CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.ttdz08m1k (Escobar et al., 2022).

ORCID

Sebastián Escobar https://orcid.org/0000-0002-4918-7967
Rommel Montúfar https://orcid.org/0000-0001-5309-4889

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SUPPORTING INFORMATION

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