



Genetic diversity of dispersed seeds is highly variable among leks of the long-wattled umbrellabird

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ARTICLE INFO

Keywords:

Alpha, beta, delta and gamma diversity
Allelic diversity
Gene flow
Heterozygosity
Central place foraging
Seed dispersal
Ecuador

ABSTRACT

Frugivorous animals frequently generate clumped distributions of seeds away from source trees, but genetic consequences of this phenomenon remain poorly resolved. Seed dispersal of the palm *Oenocarpus bataua* by long-wattled umbrellabirds *Cephalopterus penduliger* generates high seed densities in leks (i.e., multi-male display sites), providing a suitable venue to investigate how dispersal by this frugivore may influence seed source diversity and genetic structure at local and landscape levels. We found moderate levels of maternal seed source diversity in primary seed rain across five leks in northwest Ecuador (unweighted mean alpha diversity $\alpha = 9.52$, weighted mean $\alpha_r = 3.52$), with considerable variation among leks (α_r range: 1.81–24.55). Qualitatively similar findings were obtained for allelic diversity and heterozygosity. Higher densities of *O. bataua* adults around leks were associated with higher values of α_r and heterozygosity (non-significant trends) and allelic diversity (significant correlation). Seed source overlap between different leks was not common but did occur at low frequency, providing evidence for long-distance seed dispersal by umbrellabirds into leks. Our findings are consistent with the idea that seed pool diversity within leks may be shaped by the interaction between density of local trees, which can vary considerably between leks, and umbrellabird foraging ecology, particularly a lack of territorial defense of fruiting trees. Taken as a whole, this work adds to our growing appreciation of the ways resource distribution and associated frugivore foraging behaviors mechanistically shape seed dispersal outcomes and the distribution of plant genotypes across the landscape.

1. Introduction

Seed dispersal and resulting patterns of seed deposition are of particular interest to ecologists and evolutionary biologists because they influence seed survival (Howe and Smallwood, 1982; Howe and Miriti, 2004), and shape patterns of genetic structure and diversity within and among plant populations (Sork and Smouse, 2006; Garcia and Grivet, 2011). Animal-mediated seed dispersal has received considerable attention because the majority of plant species in many habitat types, especially tropical rainforests, depend upon frugivores for dispersal. Frugivore movement is typically non-random, which in turn is likely to have important consequences for seed deposition (Karubian and Durães, 2009). For example, many frugivores repeatedly use the same locations for behaviors such as sleeping, displaying, or defecating, and deposit high densities of seeds at these sites (Schupp et al. 2002, 2010). While the demographic and ecological consequences of non-random seed deposition have received extensive attention (e.g., Wenny and

Levey, 1998; Russo and Augspurger, 2004), the genetic consequences remain unclear, and have been identified as a priority for future study (Garcia and Grivet, 2011).

The mixture of seed sources at deposition sites is of interest because some of the ecological benefits of dispersal, such as avoidance of localized pathogens or sibling competition, depend on the diversity of genotypes locally and across the site (Karubian et al., 2016; Browne and Karubian, 2016). This mixture has evolutionary implications as well, because spatial patterns of genetic variation are a direct consequence of how dispersal vectors move seeds. At the local scale, the diversity of maternal source trees that contribute to clumped patches of seeds drives patterns of local genetic diversity and genetic structure among recruiting individuals at these sites (Bialozyt et al., 2014). At larger spatial scales, the degree of overlap in maternal seed sources between isolated deposition sites shapes landscape-scale genetic structure, including the degree of population differentiation (F_{ST}) and isolation by distance (Sork and Smouse, 2006). Identifying the degree of maternal

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seed source sharing within and between deposition sites, and how this may relate to more traditional measures of genetic structure at these spatial scales, therefore advances our mechanistic understanding of the genetic consequences of animal-mediated seed dispersal.

When a patch of dispersed seeds can be attributed to a specific frugivore, molecular analyses can be employed to gain insights into the impact of that vector on seed source diversity (e.g., Grivet et al., 2005; Jordano et al., 2007). Studies employing this approach have documented considerable variation in maternal seed source diversity (α_r , Scofield et al., 2012) at deposition sites, both within and among dispersal mutualisms. For example, α_r among seedlings of the palm *Oenocarpus bataua* dispersed by the long-wattled umbrellabird *Cephalopterus penduliger* (hereafter umbrellabird) was several times higher than among acorns of the oak *Quercus lobata* dispersed by acorn woodpeckers (Scofield et al., 2012). Also, within a single system, α_r varied one order of magnitude among *O. bataua* seeds encountered beneath different individual sleeping trees of the white-bellied spider monkey *Ateles belzebuth* (Karubian et al., 2015). This variation both within and among systems is likely due to differences in the foraging ecologies of the focal dispersal agents, which in turn may be mediated by factors such as the spatial distribution of fruiting trees. For example, differences in the degree of territoriality exhibited by a frugivore, or number of adult trees in proximity to a deposition site, may individually or collectively impact α_r , along with other metrics of genetic diversity (e.g., expected heterozygosity and allelic richness). At present, however, ways in which these factors interact to impact dispersal outcomes remains unclear.

To better understand these relationships, we quantified genetic diversity and maternal source diversity and overlap among freshly dispersed *O. bataua* seeds collected from five umbrellabird leks in northwest Ecuador. Umbrellabird males consume large numbers of *O. bataua* fruits and disperse most of the seeds they consume into leks (Karubian et al., 2012), providing a suitable system to investigate how foraging ecology of a known dispersal agent impacts dispersal outcomes. Observational studies have indicated that both male and female umbrellabirds show relatively high fidelity to a lek; males hold long-term display territories at a single lek and females typically only visit a single lek during a breeding season (Karubian and Durães, 2014). However, satellite tracking of umbrellabird movements has also documented large foraging ranges and occasional movements of ‘floater’ males between leks (Karubian et al., 2012; Karubian and Durães, 2014). A previous study revealed high seed source diversity at a single lek in this system (Karubian et al., 2010), but it is unclear whether consistently high seed source diversity is observed across leks, or whether α_r varies between leks in response to external factors that may include the density of adult *O. bataua* trees surrounding leks. Further, given the observed foraging and dispersal behavior of umbrellabirds, the degree to which seed source sharing amongst leks occurs is also a parameter of interest, given its consequences for landscape scale genetic processes, such as the distribution of genetic diversity within and between leks.

Thus, given the above observations we predicted that: (1) maternal seed source diversity represented within leks will be high and will be correlated with density of adult trees surrounding the lek; (2) due to the fidelity of umbrellabirds to individual leks, each lek would contain distinct subsets of maternal seed sources but a low level of overlap may occur as a consequence of movement between leks by floater males or females, or because foraging ranges of territorial males from different leks overlap; (3) measures of population genetic diversity (e.g., allelic richness, heterozygosity within leks) will be correlated with maternal seed source diversity at a local scale; and (4) given the low level of anticipated overlap in seed sources represented in leks, that genetic differentiation amongst lek sites will be moderate to high. By testing these predictions and relating our findings to other systems, this study provides insights into the complex relationships between frugivore foraging ecology, resource distribution, and seed dispersal outcomes.

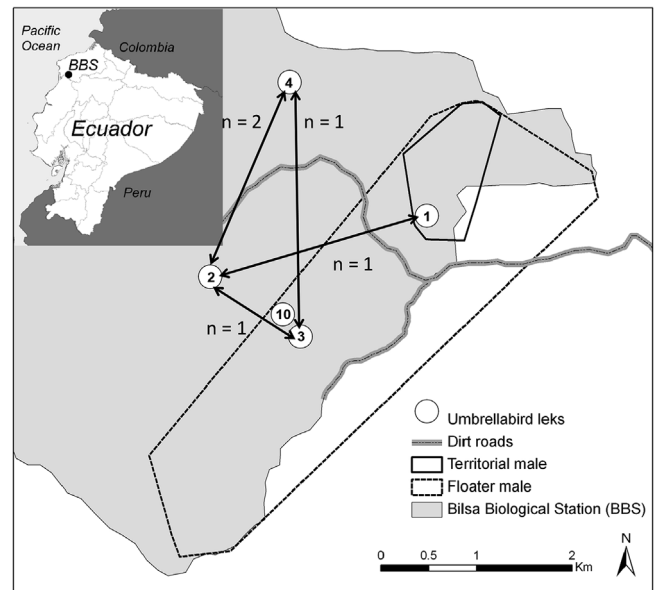


Fig. 1. Map of the study area depicting the position of Bilsa Biological Station (BBS) in northwest Ecuador and the location of five long-wattled umbrellabird leks within BBS. Arrows indicate cases of seed source sharing between leks, with the number of shared maternal seed sources between each pair of leks provided adjacent to the arrow and lek number within each circular marker. Shown also for reference are the home ranges of a territorial male and a floater male gathered over a two-month tracking period using GPS-based tracking device.

2. Materials and methods

2.1. Study species and location

The canopy palm *Oenocarpus bataua* is widely distributed throughout Neotropical rain forest on both sides of the Andes cordillera in South America, and is one of the most common trees in the Amazon (Henderson et al., 1995; ter Steege et al., 2013). It is a long-lived, slow-growing monoecious species that produces inflorescences of thousands of small inconspicuous flowers. Fertilized flowers develop large-seeded, lipid-rich fruits (up to 2000 fruits per infructescence, with seeds ~40 mm length, 22 mm diameter surrounded by a thin aril) that are available for 4–8 weeks (Browne et al., 2015). Across *O. bataua*'s range, fruits are consumed by a range of large-bodied vertebrates including birds, primates and humans (Goulding and Smith, 2007). Our study focussed on the relationship of *O. bataua* and a principal dispersal agent in the humid Chocó rainforest in northwest Ecuador, the long-wattled umbrellabird *Cephalopterus penduliger*, at Bilsa Biological Station (hereafter BBS; 79°45' W, 0°22' N; 330–730 m elevation; 3.4 m rain/yr), a 3500-ha forest reserve surrounded by an increasingly agricultural landscape (Fig. 1). Because *O. bataua* seeds dispersed into umbrellabird lek sites can be reliably attributed to umbrellabird dispersal (see Karubian et al., 2010), we can directly assess the impacts of bird foraging behavior on plant genetic outcomes.

Umbrellabirds are large (c. 550 g) frugivorous birds endemic to Chocó rainforests of Ecuador and Colombia (Snow, 1982) and ‘Vulnerable to Extinction’ because of habitat loss (BirdLife International, 2000). Umbrellabirds swallow whole *O. bataua* fruits and regurgitate intact, viable seeds. At BBS, umbrellabirds are the most commonly observed species consuming *O. bataua* fruits, and *O. bataua* are among the most common seeds recovered from seed traps placed in leks (J. Karubian, unpublished data). Most adult male umbrellabirds hold fixed display territories on a single lek but have large (ca. 50 ha; Karubian et al., 2012), overlapping foraging ranges away from the lek. Territorial males bring ~50% of the *O. bataua* seeds they ingest while foraging back to the lek, yielding high seed and seedling densities at these sites (Karubian et al., 2012). At a single lek, source diversity among

established seedlings was higher than in control sites outside that lek (Karubian et al., 2010; Scofield et al., 2012). At least some males are ‘floaters’ that move between several leks and are therefore capable of dispersing seeds between leks; females may also move between leks and thus are also capable of dispersing seeds between leks (Karubian et al., 2012; Karubian and Durães, 2014). Seed source overlap between leks might also occur because of overlapping foraging ranges of males or females associated with neighboring leks. The current study focuses on five umbrellabird leks that we have monitored since 2005, with an average pairwise distance of 1766 m (range = 198–2671 m) between leks (Fig. 1). All five leks were approximately 1 ha in size, with an estimated 6–10 territorial males in attendance (J. Karubian, unpublished data), and present habitat that is qualitatively similar to other leks in the area and to control sites outside leks (J. Karubian, unpublished data; Karubian et al., 2016). We mapped all adult (reproductive) *O. bataua* within a 200 m radius of the centre of each lek to assess the density of potential source trees around each lek.

2.2. Sample collection and genetic analysis

We collected dispersed *O. bataua* seeds (ungerminated) from umbrellabird leks from 2007 to 2012. Three of the leks we studied (leks 1, 2 and 4) have been continuously active from at least 2003–2016. Lek 3 was active from at least 2003 until 2009, at which point activity ceased and Lek 10 was concurrently established, presumably by the birds that had been in Lek 3 (see below). Seeds were collected at monthly intervals from all leks using a series of 1×1 m seed traps suspended 1 m above the ground. Each lek had 16 seed traps that were arrayed in a similar design, with the exception of lek 1, which had 52 seed traps; in all five leks, seed traps covered an area of approximately 0.75 ha. Each trap was > 10 m from the nearest adult *O. bataua* tree, and typically placed beneath perches where umbrellabirds had been observed displaying, although the impact of this may have been limited because males may use a given display perch for only a few weeks. In particular, the low seed arrival rates for Lek 10 ($n = 8$ total seeds), suggest that these traps were placed in areas that were not heavily used by umbrellabirds. Samples for genetic analysis were obtained by removing the fibrous outer covering (pericarp) of each seed, drying it, and storing it for subsequent use in DNA extractions.

Genomic DNA was extracted from pericarp material by grinding with tungsten beads and using the Qiagen 96 Plant kit (Qiagen, California USA) following the manufacturer's instructions. We amplified seeds for 9 microsatellite loci (Ob 03, 06, 07, 10, 12, 16, 19, 22, 23) using PCR and genotyping conditions described in Ottewell et al. (2012). The combined 9-locus probability of identity, calculated in GenAlEx version 6.5 (Peakall and Smouse, 2012), was $1.99\text{e-}08$ and the expected number of individuals with the same multilocus genotype was $3.52\text{e-}06$. Thus, the loci we used were sufficiently variable to allow a high rate of discrimination between individuals in each case.

2.3. Data analysis

Because the pericarp is derived solely from maternal tissue, as opposed to the diploid embryo genotype, which contains both maternal and paternal genetic material (Godoy and Jordano, 2001), we were able to unambiguously differentiate source trees for each of the seeds. To estimate the number of maternal genotypes represented in our sampling sites we firstly checked for the possibility of genotype mismatches between pericarp samples resulting from PCR amplification errors, such as allelic dropout, using the R routine checkNullAlleles, available from <https://github.com/douglasgscfield/popgen>. Genotypes were manually adjusted to match where seeds were identical except for a mismatch at a single locus that could be explained by allelic dropout (i.e. were homozygous for either of the alleles at a heterozygous locus; 4/177 seeds). Overall, we had very low rates of missing data (mean = 1.1% across loci). We similarly allowed matches between genotypes when up to two loci had missing data (i.e. genotypes present at 7/9 loci) (e.g. Paetkau, 2003). We used the multilocus matches function in GenAlEx to group and count matching genotypes.

We characterized seed source diversity of primary seed rain within each lek, using the lek as the unit of analysis, ensuring a minimum of $n = 8$ seeds per lek. We quantified seed source diversity using the formulas in Scofield et al. (2012) to estimate *alpha diversity* (α_r , the effective number of maternal seed sources represented in each lek; *beta diversity* (β , degree of overlap between leks, defined as gamma diversity/alpha diversity); *delta diversity* (δ , the degree of differentiation in seed sources between leks, ranging from 0 for complete overlap to 1 for no overlap); and *gamma diversity* (γ , the effective number of maternal seed sources contributing to the entire collection of seeds across leks). These estimators are equivalent to those used to characterize species diversity (Whittaker, 1960, 1972), except in this case they estimate the diversity of maternal seed sources represented within and between seedling pools. Analyses were implemented in the R routine pmiDiversity.R available at <https://github.com/douglasgscfield/dispersal>. In all cases, we used the unbiased probability of maternal identity statistic, q_{gg}^* , as it is robust to small sample sizes. Sample-size weighted alpha diversity (α_r) is calculated by taking the reciprocal of this value, and is equivalent to the effective number of maternal seed sources N_{em} from Grivet et al. (2005), with an adjustment to avoid biases associated with small sample sizes (see Scofield et al., 2012). To further ensure our results were robust to differences in sample sizes among leks, we performed a randomized sub-sampling routine to equalize sampling across leks. Matching the number of seeds in Lek 10, which had the lowest number across leks (Table 1), we randomly chose 8 seeds in each of Leks 1, 2, 3, and 4 and calculated metrics of seed source diversity. We repeated this process 1000 times and present mean and SD values of seed source diversity estimates among this set of randomized sub-samples (Table 1). The sub-sampling routine was written in R (R Core Development Team, 2015).

We assessed the correlation between seed source and genetic diversity metrics (α_r , expected heterozygosity H_E , allelic richness A_R ;

Table 1

Summary of maternal seed source diversity in primary seed rain of the Neotropical palm *Oenocarpus bataua* across five leks of the long-wattled umbrellabird *Cephalopterus penduliger* in northwest Ecuador. Adult density = number of *O. bataua* adults per hectare (ha) counted within a 200 m radius of lek centre, n_{seeds} = total number of seeds sampled per lek, $n_{sources}$ = number of source maternal trees identified per lek, q_{gg}^* = correlation of maternal genotypes within leks using an unbiased estimator, α_r = effective number of mothers. In parentheses, mean and standard deviation of each metric from random subsampling in each lek to $n = 8$ seeds (see text).

	Lek 1	Lek 2	Lek 3	Lek 4	Lek 10
Adult density	4.78	2.70	1.51	0.56	1.59
n_{seeds}	77	19	38	35	8
$n_{sources}$	31	14	16	5	3
	(6.56 ± 0.98)	(7.06 ± 0.76)	(4.9 ± 1.19)	(2.77 ± 0.89)	
q_{gg}^*	0.061	0.041	0.217	0.548	0.552
	(0.095 ± 0.052)	(0.07 ± 0.031)	(0.246 ± 0.136)	(0.556 ± 0.189)	
α_r	16.4	24.6	4.6	1.8	1.8
	(13.69 ± 7.26)	(17.13 ± 7.35)	(5.73 ± 4.16)	(2.04 ± 0.82)	

below) in each lek and adult *O. bataua* density (i.e., number of individuals in a 200 m radius surrounding each lek) with Spearman's rank correlation, ρ , using a one-sided test that adult density and diversity metrics were positively correlated. We then adjusted resulting P values for multiple testing ($n = 3$ tests) using a Bonferroni correction, where the P values were multiplied by the number of tests. We investigated the accumulation of γ diversity in each lek using gammaAccum.R (available at <https://github.com/douglasgscfield/dispersal>). The fact that Lek 3 and Lek 10 were not active concurrently (see above) may have impacted α_r , β , and δ diversity, though the expected direction of this potential effect is not clear. The lower amount of temporal sampling effort in Lek 10 may reduce α_r at that site, though small sample sizes precluded us from sub-sampling to equalize the amount of temporal sampling effort across leks. Also, on one hand, it may be that β and δ diversity values reported here are biased downward because it would not be possible for seeds from a single fruiting event to reach both leks, yet on the other hand values reported here may be biased upward because presumably the same set of males was present at both leks, and therefore likely to visit the same trees during different fruiting events that may have occurred during the 5 year seed collection period.

We calculated standard population genetic parameters in GenALEX, including the number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e) and Wright's inbreeding coefficient (F_{is}). We used rarefaction to estimate allelic richness (A_R) standardised for sample size using HP-Rare (Kalinowski, 2005). We estimated all pairwise genetic differentiation (F_{ST} , D_{est}) between lek sites using GenALEX. The significance of the observed F_{ST} and D_{est} values was assessed using permutation testing (999 permutations). We evaluated the correlation between F_{ST} and D_{est} genetic distances and the δ divergence matrix using Mantel tests in R. Further, we tested for an isolation by distance effect using Mantel tests of linearized genetic distance ($F_{ST}/1-F_{ST}$) or δ divergence and geographic distance between lek sites (in kilometres) in GenALEX.

3. Results

3.1. Local scale: within leks

We detected a sample-size weighted average of 3.52 maternal seed sources (α_r) per lek among ungerminated seeds arriving at seed traps; the mean unweighted α value was 9.83. There was considerable variation in α_r across leks (range: 1.8–24.6; Table 1), and a non-parametric test for homogeneity of variance showed that this variation in seed source diversity among leks was highly significant ($T_\alpha = 6.019$, $P = 0.0001$). Closer examination reveals two leks (1 and 2) with relatively high values of α_r (> 16.39), while the three remaining leks had low values (< 4.60). Randomly subsampling seeds to equalize sampling across leks led to qualitatively similar results (Table 1).

Mean density of adult *O. bataua* trees in a 200 m radius around each lek was also highly variable, with a mean \pm S.D. of 2.23 ± 1.62 individuals/ha, and a range of 0.56–4.78 individuals/ha. There was a strong positive correlation between α_r and adult tree density among the five leks (Spearman's $\rho = 0.72$, $P = 0.258$, Fig. 2), though this was not statistically significant.

Genetic diversity as measured by expected heterozygosity (H_e) averaged 0.56 (\pm SD 0.04) across all five leks, with moderate variation among leks (0.34–0.69; Table 2). Similarly, allelic richness (A_R) was variable across leks, ranging from 2.7 to 4.2 (mean 3.65 ± 0.36). As with maternal seed source diversity, leks 1 and 2 contained higher levels of allelic richness (A_R) and H_e than did the remaining leks (Table 2). Both expected heterozygosity and allelic richness showed strong positive correlations with surrounding adult *O. bataua* density across leks (Spearman's $\rho = 0.70$, $P = 0.350$, Spearman's $\rho = 1.0$, $P = 0.025$, respectively; Fig. 2), though the correlation with expected heterozygosity was not statistically significant. Expected heterozygosity and allelic richness also showed strong positive correlations with α_r ,

(Spearman's $\rho = 0.87$, $P = 0.027$, Spearman's $\rho = 0.72$, $P = 0.087$).

3.2. Landscape scale: among leks

From 177 seeds, we identified 69 unique maternal seed sources across leks. We detected five total cases of seed source sharing between leks: two maternal genotypes were shared between leks 2 and 4; one between leks 2 and 3; one between leks 3 and 4; and one between lek 1 and 2 (Fig. 1). These five cases involved a total of three maternal source trees, one of which was represented in three leks. β diversity was 6.26, consistent with relatively low levels of seed source sharing between leks. Similarly, values of δ divergence, a measure of pairwise differentiation in seed sources between leks (i.e., the opposite of overlap), ranged from moderate to high (0.578–1.00; mean = 0.954; Fig. 3a). Cumulative γ diversity across the study area was 22.02, and rarefaction analysis showed that with the addition of each lek site the accumulation of γ increased in a more or less linear fashion (Fig. 3b), also consistent with modest levels of overlap between leks.

Low to modest overlap in seed source sharing between leks was also reflected in a significant degree of genetic differentiation between leks. Across the five leks mean pairwise F_{ST} was 0.170 ($P = 0.001$). There was no significant correlation between our measures of genetic differentiation (F_{ST} , D_{est}) and δ divergence ($r = -0.050$, $P = 0.613$; $r = 0.009$, $P = 0.528$; respectively). There was a weak and non-significant positive trend between linearized genetic (F_{ST} , D_{est}) and geographic distance between leks ('isolation-by-distance'; $r = 0.273$, $P = 0.280$; $r = 0.286$, $P = 0.268$; respectively, Fig. 4), but no correlation between δ divergence and geographic distance ($r = -0.020$, $P = 0.626$).

4. Discussion

This study shows that seed source diversity among dispersed, ungerminated seeds of the canopy palm *Oenocarpus bataua* varies substantially across display sites (i.e., leks) of the long-wattled umbrella-bird *Cephalopterus penduliger*. Of the five leks included in the study, two exhibited relatively high diversity while three exhibited consistently low diversity. There was high correlation of measures of seed source diversity with traditional measures of genetic diversity, such as allelic richness and heterozygosity, which followed a qualitatively similar pattern characterized by high variation among leks. Despite the small number of leks we sampled, there was a statistically significant correlation between density of adult *O. bataua* trees surrounding each lek and allelic richness, and strong positive, but non-significant, trends with α_r and heterozygosity. At the landscape scale, overall there was a low level of overlap in seed sources represented in each lek, though we did detect several instances of seed source sharing amongst leks, which were separated by an average of approximately 1.8 km. These findings can help extend our understanding of how disperser foraging ecology and resource distribution may interact to influence patterns of genetic structure of plant species at local and landscape scales, and provide a useful point of comparison for similar studies of other dispersal mutualisms.

This study adds to a small but growing body of work that links patches of dispersed seeds to a specific frugivore species and then seeks to make inferences about the impact of that frugivore on seed dispersal outcomes. In addition to our work on umbrellabirds, we are aware of three other studies that use this approach in combination with directly comparable diversity statistics (see also Jordano et al., 2007). The first of these examines seed source diversity beneath sleeping trees of the white-bellied spider monkey from Amazonian Ecuador. These sleeping trees are similar to umbrellabird leks in that they also receive high densities of dispersed *O. bataua* seeds, and pairwise distances between deposition sites are comparable across systems (1.77 km for leks vs 1.16 km for sleeping trees; Karubian et al., 2015). Mean sample-size

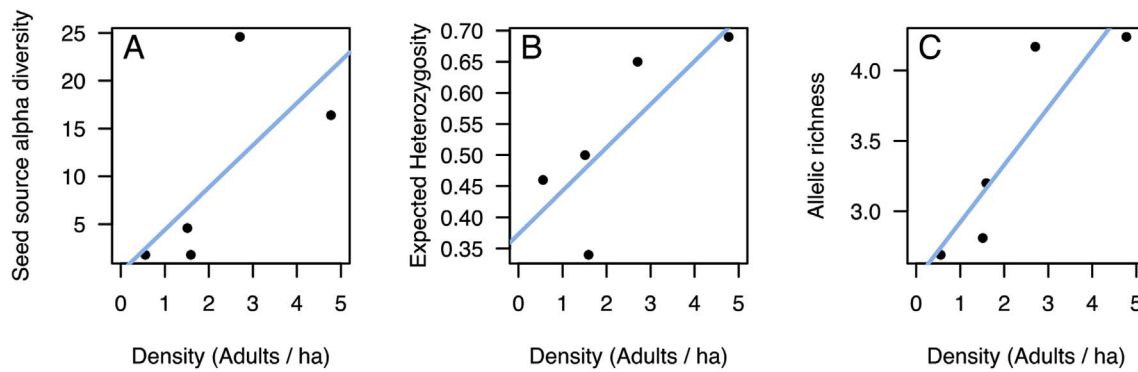


Fig. 2. Correlations between density of adult *Oenocarpus bataua* palm trees within a 200 m radius of five umbrellabird leks included in the current study, and line of best fit. (A) Maternal seed source alpha diversity (α_r), (B) expected heterozygosity (H_E), and (C) allelic richness rarefied to $n = 8$ seeds (A_R) in each lek.

weighted α diversity value across the five Umbrellabird leks was $\alpha_r = 3.52$ (range 1.8–24.6). Spider monkey sleeping trees are characterized by similar average maternal seed source diversity ($\alpha_r = 3.74$) along with substantial variation between sites (1.8–10.1). In California, acorn woodpeckers *Melanerpes formicivorus* generated low α_r values of the oak *Quercus lobata* at granaries ($\alpha_r = 2.15$), with variation in α_r between granaries ranging from 1.0 to 6.6 (Scofield et al., 2012; see also Thompson et al., 2014). Also in South America, tapirs *Tapiris terrestris* generate moderate seed source diversity of seedlings of another palm species, *Syagrus romanzoffiana*, at traditional latrines ($N_{em} \approx \alpha_r = 5.27$), with variation in α_r between latrines ranging from 2.3 to 9.3 (Giombini et al., 2016).

Even from this small number of studies, it is clear that substantial variation in seed source diversity exists within and between systems. The underlying causes of this variation remain poorly resolved. In the umbrellabird - *O. bataua* system, we found that the two leks with high diversity of seed sources and highest allelic diversity had the highest concentrations of adult trees within 200 m radius whereas leks with low seed source diversity tended to have low densities of surrounding trees. Correlations between density of trees surrounding leks with α_r and H_E were strong but not statistically significant, likely due to low statistical power associated with the relatively low number of leks included in this study. The statistically significant correlation between adult density and allelic richness provides stronger evidence of a positive relationship between adult tree density and genetic diversity across leks. While tree density *per se* may sometimes be a simple predictor of seed genetic diversity, for tree species that rely on a mobile dispersal vector, foraging behavior and the distribution of concurrently fruiting trees may also be expected to play a significant role in modifying seed dispersal outcomes. For example, the oak trees that acorn woodpeckers forage upon in California occur at densities comparable to those of *O. bataua* around the highest diversity leks we studied, but levels of maternal seed source diversity (α_r) at granaries are low because acorn woodpeckers actively defend relatively small foraging territories containing only a few adult oak trees (Thompson et al., 2014). In contrast to oaks, *O.*

bataua exhibits asynchronous phenology, in that at least some individuals have fruit in every month of the year, with population-wide peaks in fruit production occurring approximately every four years (J. Karubian, unpublished data; Rojas-Robles and Stiles, 2009). These relatively extended periods of low density fruit availability, in combination with the facts that the identity of fruiting trees vary from month-to-month and that long-wattled umbrellabird males are non-territorial away from the lek and therefore free to feed from all fruiting trees within their foraging ranges, are all likely to promote diversity within leks. However, we did find a positive relationship between tree density and genetic diversity within leks, suggesting that, holding all else equal, higher local densities of fruiting plants within a given dispersal agent's foraging range will be associated with higher seed source and genetic diversity at deposition sites (see also Thompson et al., 2014).

The average α_r value of 3.52 that we obtained for the five leks included in the current study on dispersed seeds is substantially lower than the α_r value of 25.9 reported from established seedlings in the same system by Scofield et al. (2012; see also Karubian et al., 2010). Scofield et al. (2012) estimate of α_r was based on sampling of seedlings from Lek 1; seed trap samples from the same lek in the current study had a α_r value of 16.4, considerably higher than the average across all five leks. The difference between α_r for seeds and seedlings is possibly due to the different spatial and temporal scales of sampling in each study (1 m² seed traps for seeds vs. 10 m diameter plots for seedlings; a five-year period for seeds vs. a two-month period for seedlings). However, Browne and Karubian (2016) have also demonstrated in an experimental study that a form of negative frequency dependence in this system leads to selection against more common genotypes in the recruitment of seedlings, which may also account for the higher α_r observed in seedlings in this lek. More broadly, an important insight from this study is that not all leks are equal in terms of seed source diversity. As a consequence, because our earlier studies (Scofield et al., 2012; Karubian et al., 2010) were conducted in a lek that contains relatively high levels of diversity, the major differences we detected in these studies between leks and 'control' plots outside of leks might not always

Table 2

Genetic diversity summary of *Oenocarpus bataua* seeds deposited in seed traps in *Cephalopterus penduliger* leks. Number of seeds genotyped (N), number of alleles (N_a), allelic richness (A_R) standardised for sample size $n = 8$, observed heterozygosity (H_O) and expected heterozygosity (H_E). Superscripts represent significant differences amongst groups assessed using Tukey HSD tests.

Lek	N	N_a	A_R	H_O	H_E
Lek 1	77	5.40 ± 0.98 ^a	4.24 ± 0.54 ^a	0.71 ± 0.06 ^{ns}	0.69 ± 0.05 ^a
Lek 2	19	4.60 ± 0.81 ^a	4.17 ± 0.68 ^a	0.65 ± 0.07 ^{ns}	0.65 ± 0.07 ^a
Lek 3	38	4.00 ± 0.32 ^{a,b}	2.81 ± 0.30 ^{a,b}	0.58 ± 0.13 ^{ns}	0.50 ± 0.06 ^{a,b}
Lek 4	35	3.80 ± 0.58 ^b	2.69 ± 0.24 ^b	0.61 ± 0.19 ^{ns}	0.46 ± 0.05 ^{a,b}
Lek 10	8	3.20 ± 0.58 ^b	3.20 ± 0.58 ^{a,b}	0.35 ± 0.17 ^{ns}	0.34 ± 0.09 ^b
Mean across leks	35.4	4.63 ± 0.35	3.65 ± 0.36	0.59 ± 0.05	0.56 ± 0.04
Cumulative Total	177	5.60 ± 0.87	-	0.64 ± 0.03	0.69 ± 0.04

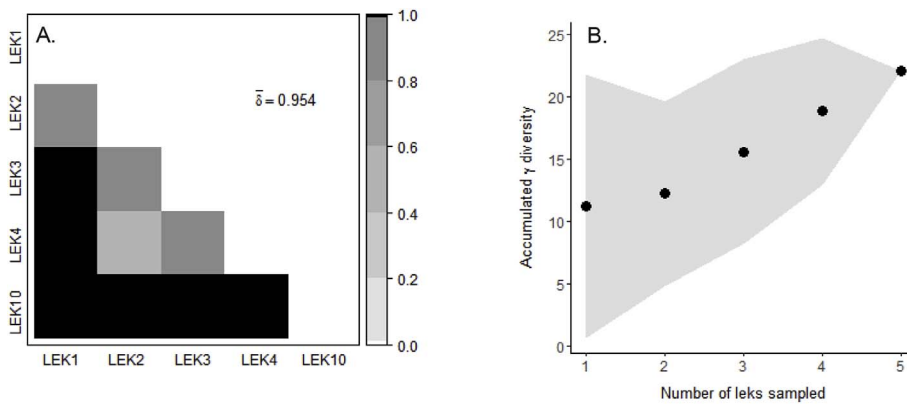


Fig. 3. Patterns of seed source diversity observed among seeds of the palm tree *Oenocarpus bataua* in lek sites of the long-wattled umbrellabird in northwest Ecuador. (A) Pairwise divergence in seed-source diversity (δ) between umbrellabird leks, with darker shades indicating higher levels of divergence. (B) Accumulation of gamma diversity (γ) with increasing numbers of leks sampled and 95% confidence interval (grey envelope).

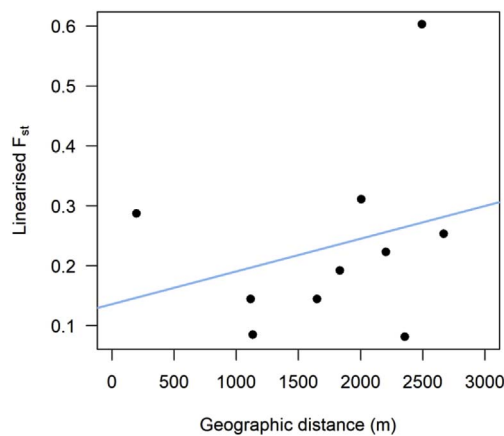


Fig. 4. Pairwise genetic differentiation, F_{ST} , and geographic distance in metres, and line of best fit between umbrellabird lek sites at Bilsa Biological Station. All pairwise comparisons were significant at $P = 0.001$ determined by permutation testing.

exist.

At the landscape scale, we recorded four cases in which two leks shared seeds from at least one common source tree (40% of 10 possible comparisons). These included three maternal seed sources (4.3% of the 69 maternal trees identified in this study), with one maternal seed source detected in three leks. In contrast, there was no evidence for overlap in seed sources among spider monkey sleeping trees among 10 possible comparisons, and only five instances in which acorn woodpecker granaries shared seeds from at least one common source tree among 136 comparisons (3.7%); information on the degree of seed source overlap between tapir latrines is not provided (Giombini et al., 2016). Thus, although seed source sharing among umbrellabird leks cannot be considered common, it occurs relatively frequently compared to other systems for which equivalent data is available, consistent with relatively high rates of long-distance seed dispersal. Umbrellabird foraging ecology, and in particular the behaviors of 'territorial' males, 'floater' males, and females, can help to explain this pattern. 'Territorial' males hold fixed territories on a single lek, but may contribute to seed source sharing between leks if foraging ranges of males from different leks overlap, causing them to feed from the same tree and carry its seeds to their respective leks. Home range size estimates from radio- and GPS-tracking (Fig. 1; Karubian et al., 2012; Karubian and Durães, 2014) suggest that this is possible. Similarly, female home ranges may also include two or more leks (Karubian et al., 2012), such that females are capable of dispersing seeds from the same tree to different leks. In contrast, 'floater' males move between leks on a regular basis rather than maintaining a fixed territory at a single lek. This behavior is exhibited by both juvenile and adult male umbrellabirds, and 10% (3/30) of the individuals we have tracked to date (Karubian and Durães, 2014). Floater males are capable of moving several kilometres per day, and

their home ranges may contain several leks (Fig. 1; Karubian et al., 2012; Karubian and Durães, 2014), so it is possible that floater males may contribute disproportionately to long-distance dispersal events in this system, but more research is needed to fully resolve the role of each strategy type.

The frequency of long-distance dispersal events that give rise to seed source overlap between umbrellabird leks are likely to have substantive consequences for seed survival and the distribution of genotypes across the landscape in this system (Nathan, 2006). Despite observing a low level of gene flow amongst leks (five instances of seed source sharing amongst leks), there was a relatively high level of differentiation in allele frequencies, as measured by F_{ST} , amongst leks (mean $F_{ST} = 0.17$). This again supports the observation that umbrellabirds are primarily foraging from the subset of trees associated with each lek, and highlights the importance of each lek as a contributor to overall (γ) genetic diversity (Scofield et al., 2012). Because γ diversity accumulates in a more or less linear manner as new leks are added, the loss of any one of these lek sites would have a meaningful reduction in overall diversity of *O. bataua* seed sources represented in umbrellabird leks. Long-wattled umbrellabirds are considered 'Vulnerable to Extinction' (BirdLife International, 2000), further underscoring their importance as seed dispersal agents and their role in ecosystem function.

The current study simultaneously presents results on diversity of seed sources and more traditional population genetic indices. The degree to which maternal seed source diversity translates into allelic diversity is of interest because allelic diversity may proximately mediate survival and population structure (Sork et al., 2015). Other studies have shown that seed source and genetic diversity metrics corroborate reasonably well at both local and landscape scales (e.g., Karubian et al., 2015); our study suggests that there may be a further correlative link between adult tree density surrounding leks, and the allelic and seed source diversity found in umbrellabird leks, though larger sample sizes are needed to evaluate this relationship in a more statistically robust manner. We also find that genetic divergence amongst seed pools represented in leks (δ divergence) and F_{ST} follow qualitatively similar patterns, highlighting the intricate link between frugivore foraging behavior, seed deposition patterns and population genetic outcomes. Whilst we observe these patterns in seed deposition sites, further research is required to determine whether these patterns persist or how they may be modified by a range of other ecological and micro-evolutionary forces during transition to different plant life-stages, from seedling to juvenile to mature adult. Further, in this study, we focused on the observable seed deposition pattern created by umbrellabird dispersal to lek display sites, yielding high *O. bataua* seed and seedling densities; umbrellabirds may also contribute to more diffuse dispersal processes (50% of seed ingested are not dispersed to the lek), along with other large-bodied avian and mammalian frugivores (e.g. toucans, agoutis) present at the study site, contributing to the overall cumulative genetic structuring observed for *O. bataua* at a landscape scale. As in

many other tropical forest systems, defaunation at our study site, particularly of large mammalian seed dispersers, is likely to have altered plant dispersal patterns with ecological and evolutionary consequences (Galetti and Dirzo, 2013; Terborgh, 2013). Despite similarities in the observed outcomes of destination-based seed dispersal by spider monkeys (Karubian et al., 2015), tapirs (Giombini et al., 2016) and umbrellabirds (this study), it is unclear whether large-bodied avian frugivores, in the absence of large mammalian seed dispersers, are providing equivalent quality and quantity of seed dispersal service to *O. bataua*.

4.1. Conclusions

Our findings are consistent with the idea that differences in seed source diversity among lek sites of long-wattled umbrellabirds may be shaped by the interaction between this species' foraging ecology and the underlying distribution of source trees surrounding these deposition sites. We present correlative evidence that a high density of fruiting trees in close proximity to deposition sites may be associated with high genetic and maternal seed source diversity at that site, whereas a low number of trees around a site may be associated with lower diversity. Along with resource distribution, these patterns are likely to be related to umbrellabird foraging ecology, and in particular a lack of territoriality away from the lek (i.e., fruiting trees are not defended). The same spatial distribution of trees could have resulted in different dispersal outcomes if dispersed by a species with a distinctive foraging ecology; for example, a strongly territorial species that restricted access to trees within territories would be expected to generate lower levels of diversity. The nexus between foraging ecology, resource distribution, and dispersal outcomes continues to represent a core challenge for studies of plant-animal dispersal mutualisms. The current, correlative study lays the groundwork for future research that experimentally manipulates resource distributions to test predictions, and that investigates a broader range of frugivores with distinctive foraging ecologies or forms of social organization.

Statement of authorship

JK designed the study; LB, JK, JO and DC collected field samples and data; KO performed genetic and statistical analyses with contribution from LB; KO and JK wrote the paper and all authors provided input on data interpretation and revisions.

Acknowledgments

We thank D. Scofield, V. Sork and P. Smouse for helpful conversation, diversity analysis advice and access to R code. M. Newman and T. Roorda assisted in the laboratory, J. Cabrera assisted in the field, R. D. Ribeiro produced the map, and Z. Diaz-Martin, C. Dick, P. Smouse and two anonymous reviewers provided feedback on earlier drafts of the manuscript. Funding was provided by the National Science Foundation (# 1548548); National Geographic Society (CRE 9006-11); Disney Conservation Fund; Conservation, Food & Health Foundation; and Tulane University. This research was conducted with all applicable permits from the Ecuadorian Ministry of the Environment. We thank the staff at Bilsa Biological Station and Jatun Sacha Foundation for their support with our long-term study of forest dynamics at their research station.

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