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The key role of the largest extant Neotropical frugivore (*Tapirus terrestris*) in promoting admixture of plant genotypes across the landscape

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ABSTRACT

The historical and contemporary loss of large-bodied frugivores has disrupted many plant-disperser mutualisms, with potentially profound consequences for plants. Although several aspects of seed dispersal by megafrugivores have already been examined, the role of these species in promoting seed-mediated gene flow has remained unexplored. We evaluated the role of the Amazonian tapir (*Tapirus terrestris*), the largest Neotropical frugivore, in shaping plant genetic structure through seed-mediated gene flow. We used microsatellites to analyze the genetic patterns of *Syagrus romanzoffiana* seedlings recruited in tapir latrines and around conspecific adult palms, the two sites where seeds and seedlings are most frequently found in this species. While the genetic diversity of seedlings was rather similar in both sites, the kinship structure was substantially weaker in latrines. Most seedlings recruited around adult palms were half- or full-sibs originating from those adults. In contrast, seedlings recruited in latrines came from several (>5, on average) contributing mothers other than the nearest adult (95%) and were mostly non-sibs (72%). Kinship patterns indicated that tapir-mediated dispersal promotes the admixture of genotypes across space. Also, our results suggested that genetic diversity and the number of contributing mothers in latrines increase with the number of fruiting adults visited by tapirs before defecating and with the accumulation of feces over time. We provide evidence of the relevance of tapirs in mobilizing maternal progenies (and genotypes) across the landscape and recruiting clusters of unrelated seedlings. This study suggests a key role for plant–megafrugivore interactions in seed-mediated gene flow and emphasizes the importance of preserving such mutualisms.

Abstract in Spanish is available with online material.

Key words: defaunation; Iguazú National Park; kinship analysis; megafauna; plant-disperser mutualisms; seed dispersal; seed-mediated gene flow; *Syagrus romanzoffiana*.

Plant-disperser mutualisms have profound consequences for plants at the ecological (Howe 1989, Wenny 2001), population genetics (Loveless & Hamrick 1984, Karubian *et al.* 2010), and evolutionary levels (Bascompte & Jordano 2007, Galetti *et al.* 2013). Seed dispersers, along with pollinators, mediate the occurrence of gene flow in many plants (Jordano 2010). The frugivore-generated seed rains determine the primary distribution of maternal progenies across the landscape (Jordano *et al.* 2007, García *et al.* 2009, García & Grivet 2011), thus shaping the patterns of gene flow and genetic structure of animal-dispersed plants (Loveless & Hamrick 1984, Jordano 2010).

Though not exclusively, plant-disperser mutualisms are extremely widespread across tropical and subtropical regions (Howe & Smallwood 1982). Contemporary anthropogenic disturbance in such regions has resulted in the loss of many large frugivores, disrupting numerous dispersal mutualisms (Stoner *et al.* 2007, Markl *et al.* 2012, Harrison *et al.* 2013, Kurten 2013, Dirzo *et al.* 2014, Ripple *et al.* 2015). Also, the past extinction of most Neotropical 'megaherbivores' at the end of the Pleistocene (Lessa & Fariña 1996) deprived many plants of their main dispersers (Janzen & Martin 1982, Guimarães *et al.* 2008, Hansen & Galetti 2009). For instance, several large-seeded Neotropical palms show dispersal syndromes of extinct megafauna (*e.g.*, Janzen & Martin 1982, Guimarães *et al.* 2008) or currently rely on tapirs, the largest frugivores in the Neotropics, for long-distance dispersal (*e.g.*, Fragoso & Huffman 2000, Giombini *et al.* 2009).

Previous research on dispersal mutualisms involving megafrugivores (*e.g.*, elephants, rhinoceroses, tapirs, and some species of extinct megafauna) has been focused mainly on their consequences for plant regeneration, ecological networks, and/or dispersal syndromes (*e.g.*, Janzen & Martin 1982, Dinerstein & Wemmer 1988, Fragoso *et al.* 2003, Donatti *et al.* 2007, Guimarães *et al.* 2008, Campos-Arceiz & Blake 2011, Bueno *et al.* 2013, Vidal *et al.* 2013, Pires *et al.* 2014). The potentially key role of megafrugivores in promoting seed-mediated gene flow in plants, although previously mentioned (Collevatti *et al.* 2003, Guimarães *et al.* 2008, Hansen & Galetti 2009, Barcelos *et al.* 2013, Vidal *et al.* 2013, Pires *et al.* 2014), has not yet been investigated.

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Relevant information on the ecological processes driving gene movement via seeds (and pollen) at the contemporary time scale can be obtained by direct methods based on the estimation of genealogical relationships (Meagher & Thompson 1987, Ouborg *et al.* 1999, Godoy & Jordano 2001, Sork & Smouse 2006, Ashley 2010, Hamrick & Trapnell 2011). The analysis of kinship among seeds or recruits in dispersal foci (*e.g.*, forest gaps, perching or resting sites, leks, latrines), where seeds arrive disproportionately due to non-random deposition by certain dispersal agents (Fragoso 1997, Wenny 2001, Grivet *et al.* 2005, Karubian *et al.* 2010, Bravo 2012), offers an invaluable opportunity to linking seed dispersal to its consequences on plant genetic structure. The foraging patterns, stereotyped behaviors, and range of displacements of dispersal agents are reflected in the degree of maternal genetic correlation of deposited seeds and may produce distinctive patterns of kinship structure in the seed rain (Grivet *et al.* 2005, Karubian *et al.* 2010, Scofield *et al.* 2010, 2011, García & Grivet 2011). Hence, different levels of seed-mediated gene flow may be predicted based on dispersal distances and the amount of genetic variation in the seeds carried by dispersers (Schuster & Mitton 1991, Jordano *et al.* 2007, Torimaru *et al.* 2007, García *et al.* 2009, Grivet *et al.* 2009, Karubian *et al.* 2010). Also, genotype diversity and/or relatedness among seeds or seedlings in deposition sites may significantly affect their survival. For instance, mortality due to pathogens and intraspecific competition might be higher for groups of homogeneous genotypes (Schmitt & Antonovics 1986, Crawford & Whitney 2010). Alternatively, kin selection might favor the survival of related (genetically similar) individuals (Schuster & Mitton 1991). Overall, the effectiveness of seed dispersal may result from the joint effects of ecological factors (*e.g.*, density-dependent mortality and microhabitat quality) and the genetic microenvironment of the seed pool in deposition sites.

The Amazonian or lowland tapir, *Tapirus terrestris* Linnaeus, weighs up to 300 kg and plays a prominent role in the seed dispersal of a wide variety of plant species across South American forests (Fragoso & Huffman 2000, Barcelos *et al.* 2013, Bueno *et al.* 2013). This large-bodied frugivore may consume fruits of more than 300 taxonomically diverse plant species, including large-seeded ones, often providing a non-deleterious handling during digestion (Fragoso & Huffman 2000, Barcelos *et al.* 2013). Tapirs defecate recurrently at the same sites (*i.e.*, latrines), where seed and seedling clumps are usually produced (Fragoso & Huffman 2000, Sica *et al.* 2014). In the Atlantic Forest of South America, the Amazonian tapir heavily consumes fruits of the palm *Syagrus romanzoffiana* (Chamisso) Glassman (Olmos *et al.* 1999, Galetti *et al.* 2001, Giombini *et al.* 2009), promoting a high recruitment of seedlings and juveniles in its latrines (Giombini *et al.* 2009, Sica *et al.* 2014; see Fig. S1). A higher level of seed-mediated gene flow should be entailed if recruited seedlings come from several distant maternal trees rather than from a single (or few) nearby one(s).

In the present work, our main purpose was to understand the role of Amazonian tapirs, a seriously threatened megafrugivore (Taber *et al.* 2008), in shaping the genetic structure of plants through seed-mediated gene flow and the potential genetic consequences of their extinction. Based on its physical and behavioral traits, we hypothesized that seedlings recruited from tapir-generated seed rain represent a heterogeneous admixture of maternal progenies. Therefore, we compared the genetic patterns of palm seedlings recruited in tapir latrines with those of seedlings recruited around conspecific adult palms. We specifically tested whether: (1) the levels of genetic diversity in *S. romanzoffiana* seedlings are higher in tapir latrines than around conspecific adults, (2) the seedlings recruited around adult palms and in latrines may be assigned as the offspring of the nearest (putative maternal) adult, (3) the seedlings recruited in latrines are less related, and come from a higher diversity of contributing mothers, than those around adult palms, and (4) the genetic diversity and the number of contributing mothers are positively correlated with the number of feces in latrines.

METHODS

Study area and species.—This study was conducted within Iguazú National Park (25°32′–45′ S, 54°09′–33′ W; Fig. S2), a 58.600-ha protected area in the Northeast of Argentina (Misiones province). The area represents the southern limit of the Upper Paraná Atlantic Forest ecoregion, which is an inland extension of the coastal Atlantic Forest of South America (Di Bitetti *et al.* 2003). The region has a wet subtropical climate, with mean annual temperatures of 16–22°C, averaging 15°C in winter and 25°C in summer. Rainfall occurs throughout the year, averaging 1200–2000 mm/yr.

Syagrus romanzoffiana (Cham.) Glassman is widely distributed in the southeast of South America (Henderson *et al.* 1995), being the most common palm in the Upper Paraná Atlantic Forest. In the study area, adults are normally found at densities of 10–20 individuals/ha, showing a fairly random spatial distribution, with no apparent strong aggregations. Despite potential variation in local densities at small scales, this seems to be the general pattern of spatial distribution across the entire eco-region (Galetti *et al.* 1992, Olmos *et al.* 1999, Keuroghlian & Eaton 2009). Adults typically reach 10–20 m in height. Infructescences commonly contain 500–1000 fruits. The ripe fruit is a 2–3 cm ovoid drupe containing a single seed covered by a hard 3-mm-thick lignified endocarp. The sugar-rich pericarp consists of an orange flesh pulp. Several medium to large mammals, besides tapirs, and some large birds feed on this fruit. Reproductive phenology is quite asynchronous, with extended fruiting periods almost all year round (Keuroghlian & Eaton 2008, Genini *et al.* 2009, Freire *et al.* 2013). *Syagrus romanzoffiana* drupes represent a main component of the total fruit biomass most of the year, thus providing a staple food resource for the frugivore assemblage, especially when other fruits are scarce (Keuroghlian & Eaton 2008, Genini *et al.* 2009).

Sample collection.—We collected leaf tissue from a maximum of 16 early-stage individuals recruited under the crown of eight conspecific adult palms and in eight tapir latrines (Fig. S2; geographical coordinates deposited at Zenodo, DOI: 10.5281/zenodo.44364). Those sites usually contain higher densities of *S. romanzoffiana* seeds and seedlings than random sites (Giombini *et al.* 2009, Sica *et al.* 2014). Except for adults, all individuals sampled in this study (hereafter referred as 'seedlings' indistinctively) corresponded either to seedlings presumably younger than 1 yr old and still depending on seed resources or to saplings (early juveniles) of more than 1 yr old, with wider (but still not pinnate) leaves. In each sampling site, we established a 3-m-radius circular plot centered on the adult palm stem ('under-adult plots') or on an arbitrary center point in latrines ('latrine plots'). In some under-adult plots, we found fewer than 16 seedlings (Table 1). We also collected leaf tissue from the nearest conspecific adult to be tested as a candidate mother. The distances from sampling plots to the nearest adult are provided in Table 1. We estimated the number of individual feces in each latrine plot (Table 1) from the number of single dung piles and/or seedling patches (Fig. S1).

DNA EXTRACTION AND MICROSATELLITE MARKERS.—We extracted DNA from 5 to 35 mg leaf tissue using the NucleoSpin 96 Plant II kit (Macherey-Nagel, Düren, Germany). Nine previously characterized microsatellites (Table S1; Barat *et al.* 2012) were amplified by PCR, as described in the original manuscript (http://tomato.bio.trinity.edu/manuscripts/12-6/mer-12-0111.pdf), for all individuals analyzed in the present study ($N = 259$; genotypic data deposited at Zenodo, DOI: 10.5281/zenodo.44364). Further details of these microsatellites and genotyping procedures are provided in Appendix S1.

GENETIC DATA ANALYSES.—Genetic diversity was measured as gene diversity (*i.e.*, the expected heterozygosity under random mating) and allelic richness (*i.e.*, the number of different alleles), using SPAGeDi 1.4 (Hardy & Vekemans 2002). Gene diversity was calculated according to the unbiased estimator of Nei (1978), and allelic richness was corrected to standardized (rarefacted) sample sizes (*i.e.*, equal number of individuals and genes). Both genetic diversity estimates were statistically compared between latrine and under-adult plots by the non-parametric Wilcoxon matched-pairs test, pairing treatments (latrine vs. under-adult) by locus, so that differences among loci were controlled. The relationship of both estimates with the number of feces in latrine plots was evaluated by a Spearman rank-order correlation. Statistical tests were conducted with STATISTICA 6.0 (StatSoft & Inc 2001). As the relationship with the number of feces was also evaluated for maternal richness and diversity, as described below, the significances were then corrected for multiple testing (four tests). To this end, we obtained the adjusted *P*-values by sequential Bonferroni (P_{SB}) and Benjamini & Hochberg ($P_{B\&H}$) procedures using SGoF+ software (Carvajal-Rodriguez & de Uña-Alvarez 2011).

TABLE 1. *Summary of sample collection of* Syagrus romanzoffiana *seedlings recruited under the crown of conspecific adults and in tapir latrines within Iguazú National Park (northeast of Argentina).* N*: number of genotyped seedlings;* N_T*: total number of seedlings found within sampling plots;* d*: distance, in meters, to the first* (d_1) *and second* (d_2) *nearest adults.*

Under-adult plots	$N = N_T$	d_2 ($d_1 \leq 3$ m)	Latrine plots	N (N_T)	d_1	Number of feces
U-a.1	14	33	Lat.1	16 (27)	17	1
U-a.2	14	6	Lat.2	16 (27)	53	3
U-a.3	14	21	Lat.3	16 (120)	12	1
U-a.4	16	27.5	Lat.4	16 (183)	10	6
U-a.5	16	57	Lat.5	16 (27)	20	1
U-a.6	16	19.5	Lat.6	16 (144)	17	6
U-a.7	10	21	Lat.7	16 (52)	16	4
U-a.8	16	12	Lat.8	16 (84)	9	3

We estimated the proportion of seedlings within sampling plots not mothered by the nearest adult by three maternity exclusion methods: (1) full-pedigree maximum likelihood (hereafter 'ML') reconstruction (Wang 2004, Wang & Santure 2009), performed with COLONY v.2.0.5.0 (Jones & Wang 2010); (2) ML estimation of pairwise relationships for seedling-candidate mother dyads (Wagner *et al.* 2006), implemented with ML-RELATE (Kalinowski *et al.* 2006); and (3) crude exclusion analysis (Wang 2010), carried out with WHICHPARENTS 1.0 (Hedgecock & Eichert 1999) and MicroErrorAnalyzer (Wang 2010). The former has been shown to generally outperform the others, as it exploits more information from the data (Wang 2012). However, we used the three approaches to check for consistency in the relative amounts of excluded (dispersed) seedlings. Technical details of these three alternative methods are provided in Appendix S2. For all the maternity and kinship analyses that required 'reference allele frequencies' (Blouin 2003, Hardy 2003), we used those estimated from 819 individuals sampled across Iguazú National Park and a broader area in the north of Misiones province (Appendix S1; Table S2).

The kinship structure of seedlings was characterized both in terms of kinship coefficients and the assignment to relationship categories (Blouin 2003). SPAGeDi 1.4 (Hardy & Vekemans 2002) was used to estimate the average kinship coefficients of seedlings within and among under-adult and latrine plots and to compare them against values obtained by randomization of under-adult and latrine samples, respectively. We used the kinship estimator (ρ_{ij}) proposed in Loiselle *et al.* (1995), which is generally the most accurate and does not suffer biases in the presence of low-frequency alleles (Vekemans & Hardy 2004, see SPAGeDi 1.4 user′s manual). The average kinship values and 95% confidence intervals were estimated with a jackknife resampling procedure over loci, excluding one locus at a time. Averages and 95% confidence intervals for randomized samples were obtained by 1000 permutations of individuals (genotypes) among plots. In addition, to directly compare the average kinship values with those expected according to the relationship categories inferred from sibship reconstruction (see below), we adjusted kinship values following Hardy (2003). To this end, the reference kinship value of unrelated individuals was obtained from the average value among under-adult or latrine plots. Lastly, the proportion of full-sibs, half-sibs, and unrelated (non-sibs) seedlings in each plot was estimated from the full pedigrees reconstructed with COLONY. The average proportion of unrelated seedlings was compared between under-adult and latrine plots with a one-sided Mann–Whitney *U*-test using STATISTICA 6.0.

Maternal genetic correlations were estimated both in terms of the absolute number of different mothers or 'maternal richness' (N_m) and the effective number of mothers or 'maternal diversity' (N_{em}), which is informative about the number and evenness of maternal contributions. The procedures to infer maternal genotypes and estimate N_m and N_{em} are detailed in Appendix S3. We compared N_m and N_{em} between under-adult and latrine plots with a Mann–Whitney *U*-test. We also evaluated if N_m and N_{em} were positively correlated with the number of feces deposited in latrine plots by a Spearman rank-order correlation, adjusting significance for multiple testing as described above.

Finally, we tested for spatial dependence or autocorrelation for all the variables measured as described in Appendix S4.

RESULTS

GENETIC DIVERSITY.—The overall (multilocus) allelic richness was slightly higher for seedlings in latrine than under-adult plots, but the difference was not consistent across loci ($Z_{\mathrm{Wilcoxon}} = 1.36$, $P = 0.173$; Table 2). Likewise, gene diversity values exhibited no significant differences ($Z_{\mathrm{Wilcoxon}} = 1.01$, $P = 0.314$; Table 2). We found significant (or marginally significant) positive correlations between the number of feces in latrine plots and both the total (or mean) number of alleles ($R_{\mathrm{Spearman}} = 0.79$, $P_{\mathrm{SB}} = 0.056$ and $P_{\mathrm{B\&H}} = 0.037$) and gene diversity ($R_{\mathrm{Spearman}} = 0.86$, $P_{\mathrm{SB}} = 0.022$ and $P_{\mathrm{B\&H}} = 0.022$).

MATERNITY EXCLUSION.—Both ML-based methods and the crude exclusion method based on at least one mismatch showed that the vast majority (83–95%) of seedlings in latrine plots did not represent the offspring of the nearest adult (Fig. 1). The full-pedigree ML method excluded 94.5% ± 3.2 SE of seedlings (Fig. 1). This percentage remained high (85.2% ± 3.9 SE) even when setting the prior probability of the nearest adult being the mother of an offspring to 0.95. Even the most conservative method (crude exclusion based on at least two mismatches) excluded almost 50 percent of seedlings in latrines.

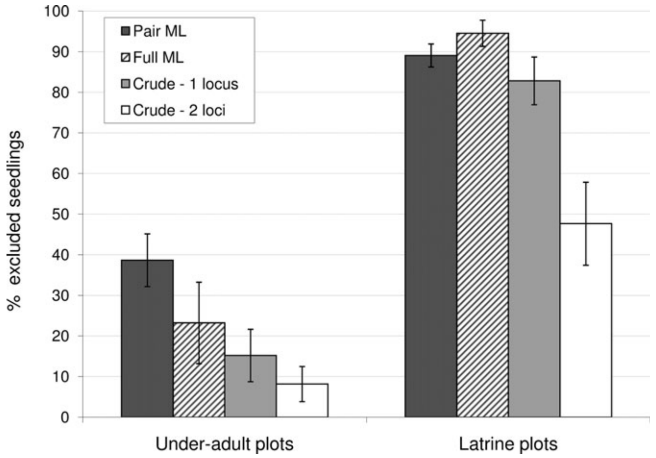

FIGURE 1. Percentages of maternity exclusion, with respect to the nearest conspecific adult, for *Syagrus romanzoffiana* seedlings recruited under the crown of conspecific adults and in tapir latrines within Iguazú National Park (northeast of Argentina). Pair ML: pairwise maximum likelihood method (implemented with ML-RELATE); Full ML: Full-pedigree maximum likelihood method (implemented with COLONY); Crude 1 locus and Crude 2 loci: crude exclusion methods based on at least one and two mismatching loci, respectively (implemented with WHICHPARENTS). Error bars indicate standard errors ($N = 8$).

The full-pedigree ML method excluded 23.2% ± 10.0 SE of seedlings in under-adult plots (Fig. 1). Consistently, estimates ranged between 8 and 39 percent among the remaining exclusion methods (Fig. 1). The pairwise ML method yielded the highest value (38.6% ± 6.5 SE), which is likely an overestimation due to the misclassification of several parent–offspring dyads as full-sibs.

KINSHIP STRUCTURE AMONG SEEDLINGS.—The average kinship of seedlings in under-adult plots ($\rho = 0.129 \pm 0.034$) was significantly higher than in latrine plots ($\rho = 0.034 \pm 0.016$ SE) (Fig. 2A). The pedigree-adjusted kinship value in under-adult plots (ρ^{P}=0.122) was in agreement with the expected value for a pedigree mostly consisting of half-sibs (ρ^{HS}=0.125), as inferred by full-ML sibship reconstruction (Fig. 2B). Likewise, the lower pedigree-adjusted kinship value in latrine plots (ρ^{P}=0.021) consistently reflected the prevalence of pairs of unrelated seedlings (Fig. 2B). The mean percentage of pairs of unrelated seedlings was significantly higher in latrine plots (72.4% ± 3.07 SE) than

TABLE 2. *Summary statistics of genetic diversity of* Syagrus romanzoffiana *seedlings recruited under the crown of conspecific adults and in tapir latrines within Iguazú National Park (northeast of Argentina).* A*: Mean allelic richness rarefied to minimum sample sizes of 16 genes,* H_e*: Mean gene diversity.*

	mBgCIR091	CNZ04	mBgCIR053	CAC2	Bg02-10	EE54	CNZ26	CNZ44	CNZ50	Overall mean
Under-adult plots	A = 4.08	3.34	4.27	2.13	4.30	2.00	2.95	1.93	2.87	3.10
	H_e = 0.68	0.57	0.62	0.28	0.70	0.42	0.32	0.19	0.44	0.47
Latrine plots	A = 4.24	3.23	5.10	1.86	4.31	2.00	3.19	1.93	3.28	3.24
	H_e = 0.71	0.59	0.70	0.11	0.70	0.49	0.38	0.16	0.52	0.48

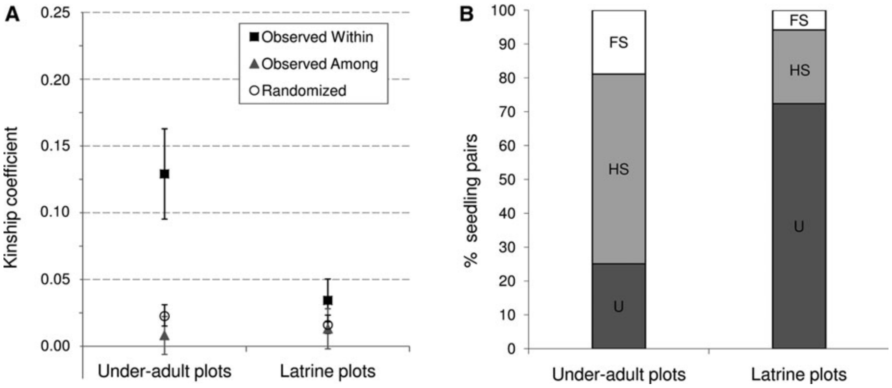

FIGURE 2. Kinship structure of *Syagrus romanzoffiana* seedlings recruited under the crown of conspecific adults and in tapir latrines within Iguazú National Park (northeast of Argentina). (A) Average kinship coefficients of seedlings (±95% confidence limits) observed within-and among-sampling plots and after randomizing individuals (genotypes) among plots. (B) Relationship categories among seedlings within plots (FS: full-sibs; HS: half-sibs; U: unrelated seedlings).

in under-adult plots (25.1% ± 9.79 SE) ($U_{\text{Mann–Whitney}} = 5.0$, one-sided $P = 0.001$; Fig. 2B).

The difference between the kinship values observed within plots and those expected under the absence of kinship structure (*i.e.*, kinship values obtained after permuting individuals among plots) was markedly smaller for latrine than for under-adult plots, as indicated by overlapping and non-overlapping confidence intervals, respectively (Fig. 2A). Yet, we found a small but non-negligible proportion of full- and half-sibs (27.6% ± 3.07 SE) in latrine plots (Fig. 2B). The average kinship among plots was higher (and also closer to the randomized value) for latrine than for under-adult plots ($\rho_{\text{among-plots}} = 0.0132 \pm 0.0075$ SE vs. 0.0082 ± 0.0071 SE, respectively), although such difference cannot be declared significant as indicated by overlapping confidence intervals (Fig. 2A). Even the two distant latrine clusters (20 km apart; Fig. S2) showed lower genetic differentiation (i.e., higher average kinship) than much less distant (0.4–2.9 km) under-adult plots ($\rho_{\text{among-plots}} = 0.011$ vs. 0.0082, respectively). Overall, these results point to a weaker genetic structure among seedlings recruited in latrines.

MATERNAL RICHNESS AND DIVERSITY.—The mean numbers of contributing mothers for samples of 10 and 16 seedlings were $N_m(\text{n} = 10) = 4.80 \pm 0.42$ SE and $N_m(\text{n} = 16) = 5.75 \pm 0.65$ SE in latrine plots, and $N_m(\text{n} = 10) = 1.92 \pm 0.32$ SE and $N_m(\text{n} = 16) = 2.15 \pm 0.42$ SE in under-adult plots (Figs. 3 and S3). The differences in maternal richness were significant for both sample sizes ($U_{\text{Mann–Whitney}} = 1.0$ and 3.0, respectively, one-sided $P < 0.001$ in both cases). The effective number of contributing mothers was also significantly higher in latrine ($N_{em} = 5.27 \pm 0.78$ SE) than in under-adult plots ($N_{em} = 1.52 \pm 0.25$ SE) ($U_{\text{Mann–Whitney}} = 3.0$, one-sided $P < 0.001$; Fig. 3). A positive correlation was found between the number of feces deposited in latrine plots and the number of contributing mothers

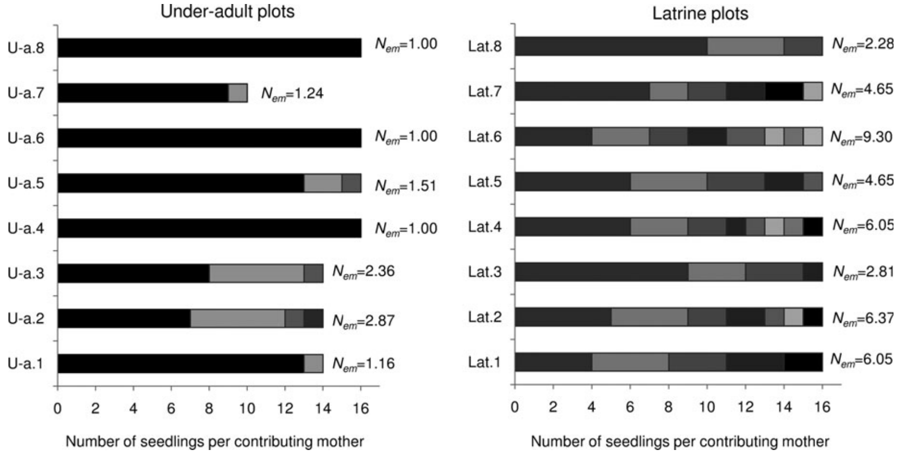

FIGURE 3. Diversity of maternal origins of *Syagrus romanzoffiana* seedlings recruited under the crown of conspecific adults and in tapir latrines within Iguazú National Park (northeast of Argentina). N_{em}: effective number of contributing mothers. The number of seedlings mothered (putatively) by the nearest conspecific adult in each sampling plot is represented by a black portion.

($R_{\text{Spearman}} = 0.75$), which remained significant or marginally significant after adjustments for multiple testing ($P_{\text{B\&H}} = 0.043$ and $P_{\text{SB}} = 0.064$). The correlation was also positive but not significant for the relationship between the number of feces in latrine plots and the effective number of mothers ($R_{\text{Spearman}} = 0.46$, $P = 0.256$).

SPATIAL DEPENDENCE OF THE VARIABLES MEASURED.—Autocorrelation coefficients were not significant for any variable or distance, as indicated by confidence intervals containing the expected value under random permutation of plot locations (Fig. S4). Accordingly, entire correlograms were not significant (Bonferroni-corrected P-values > 0.05). Finally, we found no significant decline of pairwise kinships among plots with distance in either under-adult or latrine plots ($b = 0.0018$, $P = 0.522$ and $b = -0.0004$, $P = 0.060$, respectively; Fig. S5). Overall, spatial analysis indicated no evidence of strong spatial dependence on the variables measured.

DISCUSSION

BEHAVIORAL PATTERNS OF DISPERSERS AND GENETIC PATTERNS OF RECRUITED SEEDLINGS.—The heterogeneity of maternal origins in tapir latrines is very likely promoted by foraging across several fruiting adults. Even in those latrines in which only a single defecation was evident (three cases; Table 1), we found four or five contributing mothers (Fig. 3). In addition, the number of mothers represented in each latrine, along with genetic diversity, may be enhanced by the accumulation of individual feces due to repeated defecation, as suggested by the significant (or marginally significant) positive correlations between the corresponding variables and the number of deposited feces. Overall, these results show that the genetic diversity of seedlings recruited in latrines and the number of maternal sources increase with both the number of adults visited by tapirs previous to each defecation and the accumulation of feces by recurrent defecation. Whether latrines are produced by a single or several individuals is an unresolved issue that also deserves attention.

A rough estimate of the number of *S. romanzoffiana* fruiting adults available for tapirs may be obtained from literature data. Assuming modest to low values of tapir's home range (100 ha; Noss *et al.* 2003, Tobler 2008, Medici 2010), *S. romanzoffiana* adult density (10 individuals/ha; Galetti *et al.* 1992, Olmos *et al.* 1999), and percentage of individuals bearing mature fruits (at least 2% for most of the year; Genini *et al.* 2009), 20 fruiting adults would be available under normal situations. This suggests that the number of contributing mothers observed in latrine plots would not result from a restriction in the number of (potentially) available fruiting adults, at least for most of the year. In addition, because of recurrent defecation, maternal diversity in latrines may reflect the cumulative effect of different fruiting events (Fig. S1 E, F). Yet, the kinship structure of seedlings in latrines might be affected by a rather complex relation between the spatial patterns of plant fruiting phenology and tapir foraging behavior.

Several plant-disperser systems have been shown to yield highly maternally correlated seed pools in dispersal foci (Furnier *et al.* 1987, Schuster & Mitton 1991, Grivet *et al.* 2005, 2009, Torimaru *et al.* 2007, Scofield *et al.* 2010). In contrast, systems in which dispersal foci contain weakly structured seed pools of diverse maternal origins have been less documented (*e.g.*, Karubian *et al.* 2010). Our results indicate that tapir-mediated dispersal yields weakly structured seed pools of assorted maternal origins in *S. romanzoffiana* and that similar patterns may be expected for other plants dispersed by tapirs. Moreover, as long as the seed rain produced by many megafrugivores is typically characterized by long-distance dispersal of numerous viable seeds deposited in clumps (Janzen & Martin 1982, Dinerstein & Wemmer 1988, Howe 1989, Fragoso 1997, Campos-Arceiz & Blake 2011), our results could be qualitatively extended to other such systems.

Despite the slightly higher values observed in latrine plots, the overall gene diversity and allelic richness of seedlings did not differ significantly between the two types of sampling plots. The prevalence of half-sibs around adult palms (Fig. 2B) suggests a high outcrossing rate, as is common in Neotropical palms (Nazareno & Dos Reis 2012, and references therein), may be responsible for the relatively high genetic diversity in those seedlings. In addition, the presence of some dispersed seedlings (*ca* 23%), as detected by maternity analysis, may also explain the occurrence of several different alleles around adults. Similar results revealing that seeds or seedlings recruited around adults of vertebrate-dispersed plants do not only represent the seed shadows of the nearest adult have been previously reported (Hardesty *et al.* 2006, Wang *et al.* 2007, Grivet *et al.* 2009). In our study system, such pattern very likely reflects the activity of frugivores other than tapirs, such as large birds (*e.g.*, guans and toucans), feeding across fruiting palms, and dropping seeds beneath their crowns.

INSIGHTS ON THE ROLE OF TAPIRS IN SHAPING PLANT GENETIC STRUCTURE.—Due to density-dependent mortality, no more than one adult plant is normally yielded per each fecal clump deposited by large frugivores (Howe 1989). Consequently, the contagious spatial pattern of recruited seedlings is considerably dissipated for those reaching adulthood. Yet, several individuals may be recruited in the vicinity, from nearby fecal clumps and/or by secondary short-distance dispersal from feces, thus retaining patchiness at some larger spatial scale (Howe 1989, Fragoso 1997, Fragoso *et al.* 2003). Hence, clump dispersal might lead to spatial structuring in family groups and local inbreeding if those neighboring individuals were genetically related and subsequently interbreed (Loveless & Hamrick 1984, Howe 1989). Instead, we showed that each fecal clump mainly produces unrelated seedlings and nearby feces usually contribute with additional alleles and maternal genotypes. Therefore, neighboring adults recruited by tapir-mediated dispersal, even if spatially aggregated due to clump-deposition of seeds (*e.g.*, Fragoso *et al.* 2003), will probably be genetically unrelated.

A further assessment of tapir's contribution to the admixture of genotypes could, in principle, be achieved by comparing the kinship structure of seedlings in latrines with those in random sites, beyond the immediate vicinity of adult trees, which would reflect the dispersal process by the assemblage of remaining dispersers. Unfortunately, the relatively low density of *S. romanzoffiana* seedlings in random sites (Giombini *et al.* 2009, Sica *et al.* 2014) precludes the estimation of kinship structure from a reasonable number of seedlings randomly sampled in 3-m-radius (or similar dimension) plots. Despite this limitation, our results show that seedlings in tapir latrines contain weakly structured gene pools (Fig. 2A) and strongly suggest that tapir-mediated seed dispersal substantially promotes the admixture of genotypes across space. Based on this evidence, we might predict that such dispersal mutualism should tend to counteract the development of spatial genetic structure within plant populations (Loveless & Hamrick 1984, Vekemans & Hardy 2004, Karubian *et al.* 2010). An analysis of the spatial genetic structure of adult plants would be required to support this prediction.

The limited sample sizes and clustered pattern of our sampling scheme may represent potential limitations to the generality of our conclusions, and hence some cautiousness should be taken on this regard. However, we would not expect our results to be largely biased. While the genetic patterns of seedlings in latrines varied quantitatively among sampling plots, the essential features (*e.g.*, a high proportion of unrelated individuals, several contributing mothers other than the nearest adult) were notably invariant (Table S3). It seems likely that the variation captured by sampling at eight different latrines was sufficiently representative, at least qualitatively, and we do not expect the variables measured to show such a strong dependence on tapir individuals to significantly affect those results. For instance, if we assume that each of the two distant latrine clusters (Fig. S2) belonged to a single tapir, a rough inspection of the results indicates that all the variables vary more widely among latrines in the same cluster than between clusters (Table S3). Consistently, as already mentioned, we found no significant spatial autocorrelation for all the variables measured.

THE SALIENT DISPERSAL SERVICE BY THE LARGEST NEOTROPICAL FRUGIVORES AND CONSERVATION CONCERNS.—Among the variety of frugivores that typically characterizes the diffuse plant-disperser interactions, long-distance seed dispersal and its associated gene flow are frequently restricted to a small subset of large-bodied vertebrates (Jordano *et al.* 2007, Nathan *et al.* 2008). Accordingly, the array of potential effective dispersers of *S. romanzoffiana* is much more restricted than the whole frugivore assemblage that feeds on its fruits. For instance, many vertebrates do not ingest these large seeds and hence only provide short-distance dispersal (*e.g.*, opossums, monkeys, brocket deers), or they may even act as seed predators (*e.g.*, squirrels, agoutis). Other frugivorous mammals (*e.g.*, coatis, foxes) and large birds (*e.g.*, toucans, guans) may be capable of dispersing *S. romanzoffiana* seeds at relatively long distances by endozoochory. Yet, these animals transport considerably smaller quantities of large seeds and presumably have shorter gut retention times and smaller home ranges than tapirs. In addition, while the use of *S. romanzoffiana* by tapirs appears to be quite sustained along the year (Galetti *et al.* 2001, Giombini *et al.* 2009, Keuroghlian & Eaton 2009), other potential dispersers probably consume these fruits more sporadically or during more limited periods.

A direct estimation of seed dispersal distances by tapirs using molecular markers is highly challenging. Nonetheless, by virtue of the extensive home ranges of Amazonian tapirs (several hundred to thousand hectares; Noss *et al.* 2003, Tobler 2008, Medici 2010) and straight-line displacements (daily averaging 5 km and eventually more than 20 km; Fragoso *et al.* 2003, Noss *et al.* 2003, Tobler 2008), coupled with long gut retention times (>48 h; Olmos *et al.* 1999, Clauss *et al.* 2010), average dispersal distances are probably in the order of several hundred to several thousand meters. Accordingly, dispersal distances by the Malayan tapir, *Tapirus indicus*, have been estimated in 1 km on average and up to more than 3 km, based on field telemetry (Campos-Arceiz *et al.* 2012). Thus, combined with the evidence of their likely contribution to plant recruitment (Fragoso & Huffman 2000, Fragoso *et al.* 2003, Sica *et al.* 2014), our findings support the idea that Amazonian tapirs are currently one of the key frugivores contributing disproportionately to long-distance seed-mediated gene flow in many Neotropical plants. Furthermore, they might be the only species capable of providing this service to some large-seeded plants formerly dispersed by extinct megafrugivores (Guimarães *et al.* 2008, Pires *et al.* 2014). The potential evolutionary or functional extinction of tapirs could result in the disruption of an important component of long-distance gene flow in many plants.

Long-distance dispersal drives many ecological and evolutionary large-scale phenomena, such as genetic connectivity, population spread, and colonization, and is therefore of critical conservation concern (Nathan *et al.* 2008, McConkey *et al.* 2012). Seed dispersal mediated by large vertebrates is likely a relevant process affecting the vegetation structure in heterogeneous landscapes (Fragoso *et al.* 2003, McConkey *et al.* 2012, Corlett 2013). As demonstrated experimentally, plant colonization success of novel environments may be greater when the group of founder propagules is composed of more variable genotypes (Crawford & Whitney 2010). In the light of our results, we could hypothesize that through the long-distance dispersal of genotypically diverse seed clumps, tapirs (and other megafrugivores) might facilitate timely successful plant colonization and range shifts in response to changing environmental conditions. In that case, granting the possibility that tapirs move across heterogeneous habitats, although challenging in non-protected areas (Medici 2010), might be largely beneficial for plant responses to environmental change.

Recent research has highlighted the importance of dispersal services provided by megafrugivores in different regions (Donatti *et al.* 2007, Guimarães *et al.* 2008, Hansen & Galetti 2009, Campos-Arceiz & Blake 2011, Bueno *et al.* 2013, Corlett 2013, Vidal *et al.* 2013). In particular, tapir-mediated dispersal has been shown to promote recruitment and shape the spatial distribution of recruited individuals in Neotropical plants (Fragoso 1997, Fragoso *et al.* 2003, Giombini *et al.* 2009, Sica *et al.* 2014). Here, we provide evidence of the relevance of tapirs in mobilizing maternal progenies across the landscape and recruiting clusters of unrelated neighboring seedlings. Therefore, this study strongly suggests a key role for plant–megafrugivore interactions in seed-mediated gene flow and emphasizes the importance of preserving such mutualisms.

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SUPPORTING INFORMATION

Additional Supporting Information may be found with online material:

APPENDIX S1. Characterization of the microsatellite markers and genotyping procedures.

APPENDIX S2. Description of the three alternative approaches used for maternity exclusion analysis.

APPENDIX S3. Procedures used to infer maternal genotypes and estimate maternal richness and diversity.

APPENDIX S4. Data analysis conducted for assessing the spatial dependence of the variables measured.

TABLE S1. *Characteristics of the genetic variability of the nine microsatellites used in the present study.*

TABLE S2. *Reference allele frequencies for the nine microsatellites used, estimated from 819 individuals of Syagrus romanzoffiana sampled in the north of Misiones province, Argentina.*

TABLE S3. *Values of all variables measured in single latrine plots, and average and range values for the two distinct geographic clusters.*

FIGURE S1. Images of *Syagrus romanzoffiana* seed and/or seedling pools deposited in latrines by Amazonian tapirs (*Tapirus terrestris*) within Iguazú National Park.

FIGURE S2. Geographic locations of Iguazú National Park (INP) and sampling plots. Under-adult and tapir latrine sampling plots are indicated by black triangles and crosses, respectively.

FIGURE S3. Richness of maternal origins of *Syagrus romanzoffiana* seedlings recruited under the crown of conspecific adults and in tapir latrines within Iguazú National Park, as a function of the number of genotyped seedlings.

FIGURE S4. Estimated correlograms for the variables measured, in terms of the Moran's *I* autocorrelation coefficient corresponding to under-adult and tapir latrine plots.

FIGURE S5. Pairwise kinship coefficients of *Syagrus romanzoffiana* seedlings among under-adult and tapir latrine plots as a function of distance between plots.

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