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Changes of effective gene dispersal distances by pollen and seeds across successive life stages in a tropical tree

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Pollen and seed dispersal are the two key processes in which plant genes move in space, mostly mediated by animal dispersal vectors in tropical forests. Due to the movement patterns of pollinators and seed dispersers and subsequent complex spatial patterns in the mortality of offspring, we have little knowledge of how pollinators and seed dispersers affect effective gene dispersal distances across successive recruitment stages. Using six highly polymorphic microsatellite loci and parentage analyses, we quantified pollen dispersal, seed dispersal, and effective paternal and maternal gene dispersal distances from pollen- and seed-donors to offspring across four recruitment stages within a population of the monoecious tropical tree *Prunus africana* in western Kenya. In general, pollen-dispersal and paternal gene dispersal distances were much longer than seed-dispersal and maternal gene dispersal distances, with the long-distance within-population gene dispersal in *P. africana* being mostly mediated by pollinators. Seed dispersal, paternal and maternal gene dispersal distances increased significantly across recruitment stages, suggesting strong density- and distance-dependent mortality near the parent trees. Pollen dispersal distances also varied significantly, but inconsistently across recruitment stages. The mean dispersal distance was initially much (23-fold) farther for pollen than for seeds, yet the pollen-to-seed dispersal distance ratio diminished by an order of magnitude at later stages as maternal gene dispersal distances disproportionately increased. Our study elucidates the relative changes in the contribution of the two processes, pollen and seed dispersal, to effective gene dispersal across recruitment. Overall, complex sequential processes during recruitment contribute to the genetic make-up of tree populations. This highlights the importance of a multistage perspective for a comprehensive understanding of the impact of animal-mediated pollen and seed dispersal on small-scale spatial genetic patterns of long-lived tree species.

Pollen and seed dispersal are the two key processes in which plant genes move in space, with both pollen and seeds being transported by animals in most tropical tree species (Jordano 2000). Whereas effective seed dispersal and maternal gene dispersal reflect the same process (mother-to-offspring), paternal gene dispersal (father-to-offspring) is a two step process of pollen dispersal (father-to-mother) and seed dispersal (Fig. 1). The overall impact of pollen and seed dispersal on gene dispersal can be variable, depending on how different movement patterns are shaped by different requirements and traits of pollen and seed dispersal agents.

The distribution of pollinator and seed disperser movement distances is mostly leptokurtic with a majority of short distances combined with rare, long-distance dispersal events (Bittencourt and Sebbenn 2007, Nathan et al. 2008). Due to their high mobility, vertebrate seed-dispersers may sustain long-distance gene dispersal (Lenz et al. 2011). However, even small pollinators are capable of transferring pollen across substantial distances, sometimes across tens of kilometers (Dick et al. 2003). In fact, studies comparing the contribution of pollen and seed with the help of historical gene dispersal estimates (reviewed by Petit et al. 2005), have shown that pollen gene dispersal usually exceeds seed-mediated gene dispersal. Still, the number of studies comparing the range of animal pollination and seed dispersal to contemporary gene dispersal in tropical tree populations is still low (Ashley 2010). However, existing studies report animal-mediated pollen gene dispersal to equal (Hardesty et al. 2006) or even exceed animal-mediated seed gene dispersal (Ndiade-Bourobou et al. 2010, Sebbenn et al. 2011).

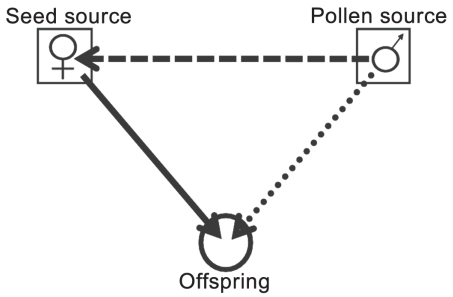

Figure 1. Schematic representation of dispersal and effective gene dispersal distances in plants. Seed dispersal (solid line) describes net displacement from mother tree to offspring; pollen dispersal (dashed line) describes net displacement from father to mother tree. Effective maternal gene dispersal is identical to seed dispersal (solid line), whereas effective paternal gene dispersal (dotted line) describes net displacement from father to offspring.

The initial spatial pattern of gene dispersal, generated by pollen and seed dispersal, is often profoundly altered during successive stages of recruitment (Schupp 1995, Schupp et al. 2010). Starting from the genetic template created in the seed rain, mortality due to seed predation or seedling herbivory, pathogen attack or competition, as well as the impact of environmental heterogeneity and microhabitat conditions lead to the thinning of the juvenile population. This is especially true in the tropics, where the influence of mortality factors acting on offspring can be complex (Hammond and Brown 1998). There are several scenarios describing the patterns of post-dispersal offspring mortality, depicting a decrease, an increase or an even probability of offspring establishment as a function of distance to the parent tree (McCanny 1985, Nathan and Casagrandi 2004). The most well-known and well-studied pattern is described by the Janzen–Connell model (hereafter J-C; Janzen 1970, Connell 1971), which postulates that density-dependent conspecific competition as well as density- and distance-dependent factors may cause a disproportionately high mortality close to the parent tree. If these effects operate across subsequent recruitment stages, the mean distance of offspring to the source tree is expected to increase during recruitment (Barot et al. 1999). This, in turn, could increase effective seed-mediated gene dispersal distances, and possibly pollen-mediated gene dispersal as well, implying that the spatial genetic make-up of plants is dynamically modulated during recruitment. Yet, combined investigations of pollen-mediated and seed-mediated gene dispersal across multiple successive recruitment stages are still lacking. To date, only a few studies have examined contemporary gene dispersal patterns across more than one stage (Isagi et al. 2007, Bittencourt and Sebbenn 2007, Steinitz et al. 2011) – and only one study examined directly estimated gene dispersal distances with respect to both the father and the mother tree (Hardesty et al. 2006), yet this study focused on only one stage. The lack of knowledge about the changes in pollen-mediated and seed-mediated gene dispersal across multiple successive recruitment stages hinders our understanding of the processes shaping local spatial genetic patterns in plants.

We examined dispersal and effective gene dispersal distances through pollen and seeds across four differently-aged life stages within a population of the tropical monoecious tree *Prunus africana* in Kakamega Forest, Kenya. *Prunus africana* is pollinated by small insects, e.g. hymenoptera and diptera (Hall et al. 2000). Seeds are dispersed by both large- and small-bodied birds as well as by different monkey species (Farwig et al. 2006). Using highly polymorphic microsatellite loci, we conducted parentage analyses for offspring at four early recruitment stages (seed stage to late sapling stage). For all offspring with an assigned father and mother tree we directly assessed pollen and seed dispersal distances and effective paternal (pollen-mediated) and maternal (= seed-mediated) gene dispersal distances. First, we aimed to compare pollen and seed dispersal distances and distributions. Lacking data on movement patterns of pollinators and seed dispersers, our null hypothesis postulates that these highly mobile animal species move comparable distances during pollen or seed dispersal events, hence no significant difference is expected between pollen and seed dispersal distances. Second, we assessed how distances of pollen dispersal (father to mother), seed dispersal (mother to offspring), and paternal gene dispersal (father to offspring) change across subsequent recruitment stages (Fig. 1). Effective (beyond the primary movement at the pollen or seed stage) gene dispersal distances from the father (= paternal gene flow) and especially from the mother tree (= maternal gene dispersal) were expected to increase across life stages due to general J-C effects. Flowering phenology, flowering intensity and pollen production may be variable between years (Rocha and Aguilar 2001). This may affect the behavior of pollinators, as pollinator flight distances depend on the density of flowering trees (Ghazoul 2005). Consequently, as our life stages represent cohorts from different recruitment years, pollen dispersal distances were expected to vary significantly across recruitment stages as well, but without showing a particular trend.

Material and methods

Study species

Prunus africana is an evergreen monoecious tree species. Its range expands across east Africa, Madagascar and the Comores (Hall et al. 2000). It is listed on CITES appendix II due to bark exploitation for medicinal purposes.

Even though some individual trees flower and fruit throughout the year, the flowering season of the species usually corresponds to September–October. Fruiting occurs 2–3 months after flowering (Berens unpubl.). However, individual trees flower and fruit only at two to three year time intervals (Hall et al. 2000).

Prunus africana has small white hermaphroditic protogynous flowers which are mainly pollinated by small insects (hymenoptera, diptera). The species is mainly outcrossing with the potential for self-fertilization (Hall et al. 2000). *Prunus africana* seeds (mean ± 1 SD: length: 8.1 ± 0.7 mm, width: 6.1 ± 0.4 mm, height: 5.5 ± 0.5 mm, mass: 0.15 ± 0.05 g, n = 30; Farwig et al. 2006) are dispersed by differently-sized bird and monkey species. The five most abundant seed dispersers were the common bulbul *Pycnonotus barbatus* (34–49.5 g, Del Hoyo et al. 2005),

violet-backed starling *Cinnyricinclus leucogaster* (33–56 g, Del Hoyo et al. 2009), yellow-whiskered greenbul *Andropadus latirostris* (23–35 g, Del Hoyo et al. 2005), blue monkey *Cercopithecus mitis* (3500–5000 g, Kingdon 1997) and blackcap *Sylvia atricapilla* (8.5–31 g, Del Hoyo et al. 2006, Farwig et al. 2006). While bird species mostly swallow fruits or peck on them, monkeys have been observed to drop a large proportion of fruits and seeds (Farwig et al. 2006). The seed embryo is covered by a woody endocarp of maternal origin. Seedlings germinate in the rainy season of the fruiting year without seed dormancy. The woody maternal endocarp stays attached to the germinating seedlings for about one month, which allows for identifying the mother tree of newly established seedlings.

Study site

Our study area was Kakamega Forest in western Kenya (Fig. 2). The mid-altitudinal rainforest (1500–1700 m a.s.l., KIFCON 1994) comprises a total area of 13 000 ha (Lung 2004). Mean daily temperatures range from 10.6 to 27.7°C (Tsingalia 1990). Average yearly precipitation is about 2000 mm year^{-1} (Forest Department records, Isecheno Forest Station, 1982 to 2005). The study area within Kakamega Forest is a 120 ha forest peninsula in the northwest of the main forest block, extending into the agricultural matrix (0°21′34.1″N, 34°51′31.0″E; Fig. 2). It comprises primary and secondary forest and is situated in a well-protected area with little human disturbance.

Sampling of plant material

We sampled recruits of four life stages in 2006 and 2008, i.e. seeds, young, middle-aged and old seedlings. Seedlings were between 0 to >3 years old, and resulted from different recruitment years.

In 2005, we established 22 parallel transects crossing the study area from the eastern to the western forest edge, covering a total area of ca 87 ha (Fig. 2). Transects were between 694 and 1225 m long, totaling ca 20 000 m, and were separated by 40 m. The entire study area was exhaustively searched for *P. africana* trees. In total, 261 *P. africana* trees (dbh > 10 cm) were detected in the entire area containing the transects as well as an adjacent 100 m northern and southern buffer zone. GPS coordinates of each tree were taken. Leaf material of all trees was collected between January and May 2006.

In the same year, 86 sampling plots were randomly established along transects (Fig. 2B). GPS coordinates of each plot were taken. Each plot consisted of four seed traps (0.7 × 0.7 m) made of a wire frame covered with mesh (Fig. 2C). Propagules were collected from the seed traps on a weekly basis between January and April 2006 during a fruiting peak of *P. africana*. They were assigned to fruits

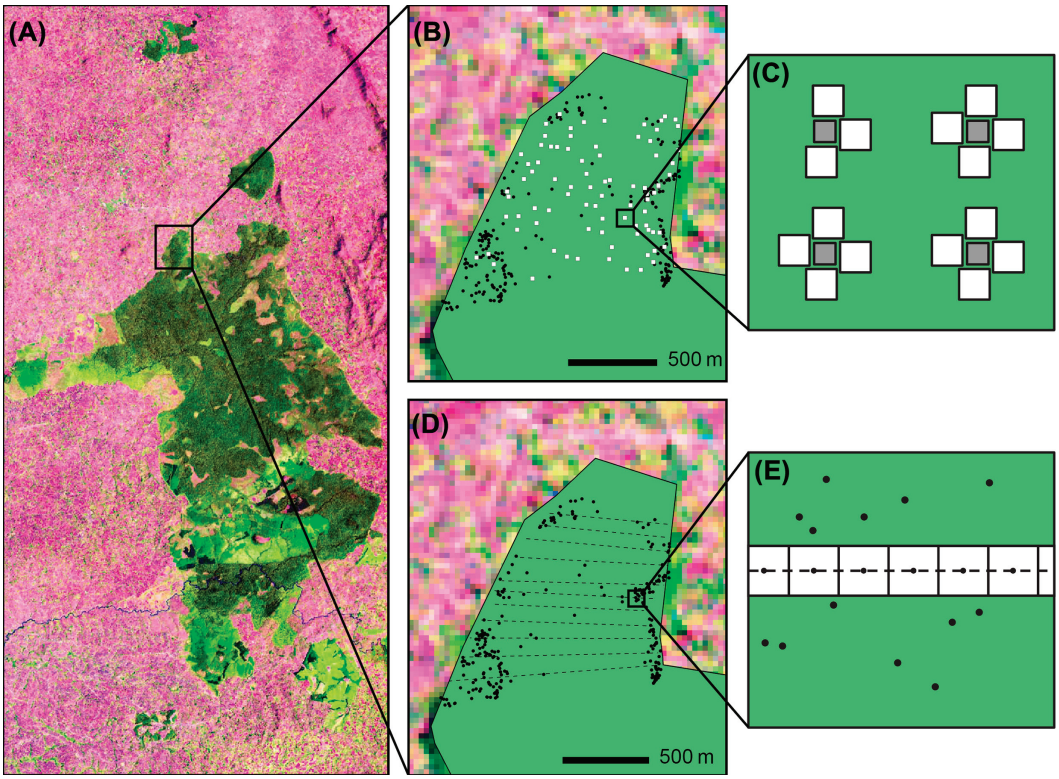

Figure 2. (A) Satellite image showing Kakamega Forest with forested area (green) and farmland (pink) and the location of the study area within the forest; (B) spatial distribution of adult *P. africana* trees (n = 261, black dots) and of sampling plots (n = 86, white squares) for sampling of fruits and seeds and young seedlings; (C) each sampling plot consisted of four seed traps (0.5 m^2, hatched squares) and 15 seedling sub-plots (1 m^2, white squares); (D) spatial distribution of adult *P. africana* trees (black dots) and of eleven transects (dashed lines) for sampling of middle-aged and old seedlings; (E) arrangement of virtual plots (white squares) for sampling of middle-aged and old seedlings. Landsat image courtesy of G. Schaab.

(with pulp) or seeds (without pulp). The fruit pulp was manually removed for storage and all samples were dried with silica gel. A number of 8544 propagules (5785 fruits, 2759 seeds) were counted in the traps. We aimed at having similar sample sizes (ca 300 individuals) for each life stage, evenly distributed across the study site. We therefore genotyped a sample of 311 propagules (selecting a random subset of propagules from traps where propagules were abundant and sampling all propagules from traps with only few propagules). Frequency distributions of fruit and seed dispersal distances did not differ significantly (χ^2-test of homogeneity: p-values > 0.05); therefore, data of fruits and seeds were pooled (seeds hereafter) for further analyses.

Current year seedlings (young seedlings hereafter) were collected at the same 86 sampling plots between March and May 2006. Fifteen 1-m² sub-plots were placed within each plot (Fig. 2C) and newly emerged seedlings were counted and collected on a biweekly basis. Leaf samples and woody endocarp attached to the roots were dried with silica gel. A sample of 309 of the 48 991 seedlings was used for genetic analyses (again, sampling a subset at sub-plots with high seedling abundance and sampling all seedlings at sub-plots with few individuals).

Older seedling stages were sampled between January and February 2008 along every other of the 22 transects crossing the study area (Fig. 2D). The area covered along transects was divided into virtual 10×10 m sampling plots with the help of ArcGIS 9.3.1 (ESRI; Fig. 2E), covering a total sampling area of ten ha. The centroid of each plot was used as its geographic position. We recorded each *P. africana* seedling encountered in these virtual plots. A leaf sample of each individual was taken, and the seedling age was determined by an experienced field assistant visually and manually inspecting the degree of lignification of the stem axis. A sample of 298 of the 861 seedlings up to three years of age (middle-aged seedlings hereafter) and 301 of the 368 seedlings older than three years (old seedlings hereafter) was taken for genetic analyses. Old seedlings comprised seedlings and saplings with an age of four to ten years. Thus, both middle-aged seedlings and old seedlings represent several distinct cohorts and integrate across several flowering and fruiting periods.

For seeds and young seedlings, both biparental and maternal tissue was used for genetic analyses. Biparental tissue was taken from the seed embryo for seeds and from leaf samples for young seedlings. Genotyping of maternal endocarp tissue of seeds and young seedlings enables a discrimination of the pollen donor and the seed source for both stages. For middle-aged seedlings and old seedlings, biparental tissue, taken from leaf samples, was genotyped.

Molecular analyses

All plant material (leaves of adult trees, young seedlings, middle-aged seedlings, old seedlings, embryo tissue of seeds, endocarp material of seeds and young seedlings) was ground to fine powder with a mixer mill. DNA was extracted following the protocol described by Wang et al. (1993). The standard protocol was modified by using 40 µl of NaOH and 95 µl of Tris buffer for each sample. Samples were genotyped at six microsatellite loci: UDP97-403 (Cipriani et al. 1999), P12A02 (Sosinski et al. 2000), BPPCT-002 (Dirlewanger et al. 2002), UDP 96-005 and UDP98-410 (Schueler et al. 2003) and EMPaS06 (Vaughan and Russell 2004) developed for *Prunus persica* and *P. avium*. Primers were fluorescent 5′-end-labeled (6-FAM, NED, HEX; Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction protocols followed Farwig et al. (2008), with annealing temperatures ranging from 54 to 62°C and number of cycles ranging from 44 to 46. For loci UDP97-403 and EMPaS06, a final extension step of 60°C for 30 min was added. Samples were genotyped on ABI 3730 and 3130xl capillary sequencers. Allele scoring was performed using GeneMapper 4.0. For all loci, base shifts between scorings of the different sequencers were calibrated by running a number of samples on both instruments. All samples except that of one adult tree were successfully amplified. The mean allele scoring error was $12.8 \pm 4.7\%$ SD (for 14% of repeated samples). Loci were highly polymorphic with a mean number of 20.0 ± 2.6 SD alleles per locus (range: 3.4–29.2).

Parentage analyses

Null allele frequencies were obtained using the software CERVUS 3.0.3 (Kalinowski et al. 2007). The mean null allele frequency was 0.07 across loci and life stages and ranged from -0.06 to 0.26. The null allele frequency at locus BPPCT-002 was slightly higher than 0.2 for middle-aged and old seedlings and, thus, was considered to be problematic for parentage analyses (Dakin and Avise 2004). Therefore, for middle-aged and old seedlings, homozygote offspring at this locus were coded as heterozygotes with one allele set to missing data in order to avoid wrong parentage exclusion. The maternal endocarp material of seeds and young seedlings reliably amplified at only four of the six loci (loci UDP97-403, P12A02, UDP 96-005, UDP98-410), and direct assignment of the seed source through identity analyses was not possible. Therefore, we used the biparental embryo or leaf material to assign two parents to each offspring with parentage analysis in a first step and, as a second step, we compared the endocarp genotype of the offspring with the parental genotypes to distinguish between pollen and seed source. A discrimination of pollen and seed source with the help of the endocarp genotype was possible for 40% (30/76) and 21% (27/131) of assignments for seeds and young seedlings, respectively. The seed source was the nearer of the two parental trees in 97% (29/30) and 93% (25/27) of all cases. We furthermore tested the three scenarios that a) the mother, unambiguously assigned with the endocarp material, is considered to be the seed source; b) the nearer of the two assigned parental trees is the seed source and c) the farther of the two parents is considered to be the seed source. Scenarios a) and c) had significantly different distance distributions (Kolmogorov–Smirnov (KS) tests: all p-values < 0.001), while distributions for a) and b) were not significantly different (KS tests: all p-values > 0.05). Therefore, the nearest parental tree was considered to be the seed source for all stages in all analyses. For those offspring where no parent pair was assigned, a paternity analysis was conducted to see if at least one parent could be found inside the study area.

Pollen or seed source were not distinguishable here and these analyses were only used to calculate overall gene immigration rates. Parentage and paternity analyses were done in FAMOZ (Gerber et al. 2003) which is suitable for parentage analyses in non-isolated stands. Cumulative parent pair exclusion probabilities for all loci exceeded 99.0%. FAMOZ conducts maximum-likelihood assignments based on log of the odds (LOD) scores, i.e. the likelihood of a parent pair of an offspring relative to the likelihood of an arbitrary parent couple (Marshall et al. 1998, Gerber et al. 2000). The LOD threshold for parentage assignment was obtained from two simulation procedures ('inside' and 'outside' the stand). For simulations 'inside' the stand, offspring is generated from random samples of specific gametes of parents inside the stand; simulations 'outside' the stand generate offspring from randomly generated gametes according to allele frequency data of the population. The LOD threshold is set at the intersection of the two LOD distributions. All parent pairs with a LOD score higher than the threshold are assigned. The parameter set used for parentage analyses, comprising the simulation and calculation error rate as well as a departure from Hardy–Weinberg equilibrium, is applied to simulated data in order to obtain statistical confidence about the decisions made in parentage analyses. Simulating 10 000 offspring, we tested varying parameter sets, starting from an error rate of 0.001. Eventually, an error rate of 0.001 was chosen as the parameter set as it yielded less than 20% α-error and the highest rate of correct parent pair choices in the parentage simulation.

Assessment of pollen dispersal, seed dispersal and gene dispersal distances

For all offspring with assigned parentage (i.e. those where both parents could be assigned within the study site), pollen and seed dispersal, as well as effective paternal gene dispersal distances were derived from x- and y-coordinates of adult *P. africana* trees and sampling plots (i.e. sampling plots in Fig. 2B, C or virtual sampling plots in Fig. 2D–E). Even though assignment rates are low especially for older stages, using offspring with assigned parentage allows us to get a conservative estimate of gene dispersal distances within our study site. Pollen dispersal distance is the distance between the pollen and seed source trees. Seed dispersal (= maternal gene dispersal) is the distance between the seed source tree and the sampling plot and paternal gene dispersal is the distance between the pollen donor and the sampling plot (Fig. 1).

Accounting for sampling biases

Sampling and genotyping of only a fraction of all offspring counted at each plot as well as the spatial arrangement of trees and sampling plots introduces bias to the frequency distributions of dispersal distances. To account for these biases we applied two types of weighting factors. First, we took into account the sampling bias caused by genotyping a variable proportion of offspring. If, for example, a low proportion of offspring in close proximity to the mother tree and a high proportion at farther distances are sampled, seed dispersal distances will be overestimated. In the following, seed traps on plots, sub-plots on plots (used to count and collect young seedlings) and virtual plots (used to count and collect middle-aged and old seedlings) are all referred to as 'plots'. To account for the sampling bias caused by genotyping a variable proportion of offspring, each offspring assigned parentage was weighted by the proportion of the total number of offspring that were genotyped at each plot according to weighting factor w_1:

$$w_1 = \frac{n_{total}}{n_{sample}} \quad (1)$$

where n_{total} is the total number of offspring counted per plot and n_{sample} is the sample size used for genetic analyses. If, for example, 35 propagules were recorded at a plot in total and 15 thereof were genotyped, each propagule assigned parentage of this plot got the weight 35/15 = 2.33 for all analyses.

Second, we considered the spatial position of plots and trees to remove the spatial arrangement bias. The spatial arrangement of sampling plots relative to the source trees can also bias the estimation of dispersal events (Robledo-Arnuncio and García 2007). The frequency of dispersal events in distance classes with higher numbers of mother-plot pairs will be overestimated. The same holds for pollen dispersal, where the spatial arrangement of trees affects the estimation of pollen dispersal distances. If, for example, the number of potential pollen source trees is high in close vicinity of the mother tree, the probability of observing short dispersal distances by chance alone is high and pollen dispersal distances will be underestimated. To account for the spatial arrangement bias of trees for assessing pollen dispersal distances, we defined arbitrary distance intervals of 40 m around each tree that acted as a seed source. For each of these seed source trees, the number of potential pollen donors, i.e. each adult *Prunus* tree, in each distance interval was determined. Each offspring of this tree was weighted with weighting factor w_2:

$$w_2 = \frac{1}{n_{trees}} \quad (2)$$

with n_{trees} being the number of *P. africana* trees in the respective distance interval. If, for example, the pollen dispersal distance for one offspring was 70 m, it was weighted by the number of trees within a distance interval of 40 to 80 m from the seed source tree. With five trees occurring in this distance interval, for instance, the offspring got the weight 1/5 = 0.2. Consequently, with few trees occurring in this distance interval, the probability for the observed dispersal event to occur by chance alone was low. Thus, the offspring got a higher weight in the calculation of pollen dispersal distance distributions.

To account for the spatial arrangement bias in seed dispersal/paternal gene dispersal distances we also worked with discrete distance intervals of 40 m. For each tree that acted as a seed/pollen source we determined the total area of plots (separately for seed traps on plots, sub-plots in plots and virtual plots) in each distance interval. Then, each

offspring of this tree was weighted with weighting factor w_3 according to the total plot area in this distance interval:

$$w_3 = \frac{1}{area_{total}} \quad (3)$$

where $area_{total}$ is the total plot area in the respective distance interval. If, for example, the seed dispersal distance for one offspring was 70 m, it was weighted by the total area covered by plots within a distance interval of 40 to 80 m from the seed source tree. With a total area of 10 m^2 of seed traps within this distance interval, a genotyped seed got the weight 1/10 = 0.1. Consequently, with a smaller plot area occurring in this distance interval, the probability for the observed dispersal event to occur by chance alone was lower. Thus, the offspring got a higher weight in the calculation of dispersal distance distributions.

For all analyses, dispersal and gene dispersal distances of an offspring were weighted for sampling bias and spatial arrangement bias. To do this, each offspring was weighted by the product of the two weighting factors, that is, by the product of w_1 and w_2 for pollen dispersal distances and w_1 and w_3 for seed dispersal distances and paternal gene dispersal distances.

Statistical analyses

To compare pollen and seed dispersal as well as maternal and paternal gene dispersal distances in our study system, frequency distributions and means of pollen and seed dispersal distances as well as of paternal gene dispersal distances were compared with Kolmogorov–Smirnov tests and Welch-tests for each life stage. Weighting of samples for the analyses inflates type I errors. Therefore, we followed a conservative approach for all statistical analyses and determined significance applying D-values (KS-test) and degrees of freedom (Welch-test) of the original sample size.

For a comparison of distances across life stages, pollen and seed dispersal as well as paternal gene dispersal distances were compared among offspring categories with KS-tests and Welch-tests. Again, samples were weighted for both sampling and spatial arrangement bias (by the product of weighting factors w_1 and w_2 for pollen and w_1 and w_3 for seed dispersal and paternal gene dispersal). Statistical analyses were performed using R 2.15.2 (R Development Core Team).

Results

Parentage assignment rates were 24% for seeds (n = 76/311), 42% for young seedlings (n = 131/309), and 12% for middle-aged (n = 36/298) and old seedlings (n = 36/301), respectively. Adding paternity assignments, i.e. assignments of a single parent within the study area, assignment rates of at least one parent within the study site added up to 68% for seeds (n = 212/311), 85% for young seedlings (n = 364/309), 45% for middle-aged (n = 136/298), and 65% for old seedlings (n = 195/301). Thus, gene immigration rates were 54% for seeds, 36% for young seedlings, 66% for middle-aged and 61% for old seedlings.

Mean ± SE (standard error) pollen dispersal distances were 114 ± 25 m (seeds), 123 ± 19 m (young seedlings), 325 ± 25 m (middle-aged seedlings) and 175 ± 48 m (old seedlings), and mean ± SE seed dispersal (= maternal gene dispersal; Fig. 1) distances 5 ± 3 m (seeds), 15 ± 6 m (young seedlings), 73 ± 8 m (middle-aged seedlings) and 84 ± 11 m (old seedlings). Mean paternal gene dispersal distances ranged between 124 ± 26 m (seeds), 168 ± 23 m (young seedlings), 290 ± 64 m (middle-aged seedlings) and 289 ± 77 m (old seedlings) (Table 1). Mean pollen dispersal distances were significantly longer than seed dispersal distances for all stages (Welch-tests, all p-values < 0.0125, after Bonferroni correction,Table 1). Pollen and seed dispersal distance distributions also differed significantly (KS-tests, all p-values < 0.0125), with the exception of the stage old seedlings (KS-test, p-value > 0.0125, after Bonferroni correction, Table 1). Paternal gene dispersal also occurred over significantly longer distances than maternal gene dispersal in each life-stage (Table 1). Maternal and paternal gene dispersal distance distributions always differed significantly (Table 1).

The shape of frequency distributions of pollen dispersal distances differed among recruitment stages only for the transition between young and middle-aged seedlings (KS-test, p-value < 0.0167 after Bonferroni correction; all other

Table 1. Comparison of distributions of pollen and seed dispersal distances in m (upper table) and of effective paternal and maternal gene dispersal distances in m (lower table) for four recruitment stages of the tree *P. africana*. Pollen dispersal describes net displacement from father to mother tree, seed dispersal net displacement from mother tree to offspring; effective maternal gene dispersal is identical to seed dispersal, effective paternal gene dispersal describes net displacement from father to offspring. Presented are weighted means with standard errors (in brackets), range, as well as D-values of two-sample Kolmogorov–Smirnov (KS) tests and t and DF-values of Welch two-sample tests. Significance (after Bonferroni correction) is indicated with *, n.s. = non-significant. Significant values (p-level < 0.0125) are bold.

Stage	Sample size	Pollen/paternal mean	Pollen/paternal range	Seed/maternal mean	Seed/maternal range	KS-test D	Welch-test T	Welch-test DF
Dispersal								
seeds	76	114 (25)	0–1308	5 (3)	0–698	**0.63***	**626.03***	89.14
young seedlings	131	123 (19)	0–1193	15 (6)	0–1058	**0.60***	**1809.52***	185.8
middle-aged seedlings	36	325 (55)	0–1128	73 (8)	10–489	**0.53***	**209.90***	41.55
old seedlings	36	175 (48)	0–1172	84 (11)	1–655	0.36n.s.	**39.84***	45.47
Effective gene dispersal								
seeds	76	124 (26)	0–1091	5 (3)	0–698	**0.85***	**923.57***	90.41
young seedlings	131	168 (23)	0–1136	15 (6)	0–1058	**0.79***	**874.22***	186.98
middle-aged seedlings	36	290 (64)	11–1200	73 (8)	10–489	**0.57***	**17.78***	41.04
old seedlings	36	289 (77)	2–1376	84 (11)	1–655	**0.39***	**9.30***	44.35

comparisons not significant) whereas mean pollen dispersal distances were significantly different among all successive stages (Welch-tests, all p-values < 0.0167, Fig. 3A, Table 2, Supplementary material Appendix 1 Fig. A1).

The shape of frequency distributions for seed dispersal (= maternal gene dispersal) distances differed significantly between young and middle-aged seedlings (KS-test, p-values < 0.0167), while no difference in distributions was found for the seeds to young seedlings- and middle-aged to old seedlings-transitions (KS-tests, p-values > 0.0167) (Table 2). Further, mean seed dispersal distances continuously and substantially increased across successive stages from 5 m to 84 m (Welch-tests, all p-values < 0.0167 except middle-aged to old seedlings-transition; Fig. 3C, Supplementary material Appendix 1 Fig. A2).

Concerning paternal gene dispersal distances, frequency distributions and means also varied significantly between successive life stages, with increasing mean distances from 124 m (seeds) to 290 m and 289 m for middle-aged seedlings and old seedlings, respectively (KS-tests and Welch-tests, all p-values < 0.0167, except middle-aged seedlings to old seedlings transition, Fig. 3B, Table 2, Supplementary material Appendix 1 Fig. A3).

At the initial seed rain stage, pollen dispersal distances exceeded seed dispersal distances by a factor of 22.8. However, across subsequent recruitment stages, the pollen to seed dispersal distance ratio dropped over 8.2 (young seedlings) and 4.5 (middle-aged seedlings) to 2.1 (old seedlings). The same was true for the paternal to maternal gene dispersal-ratio, which dropped from 24.8 (seeds) over 11.2 (young seedlings) and 6.4 (middle-aged seedlings) to 3.4 (old seedlings).

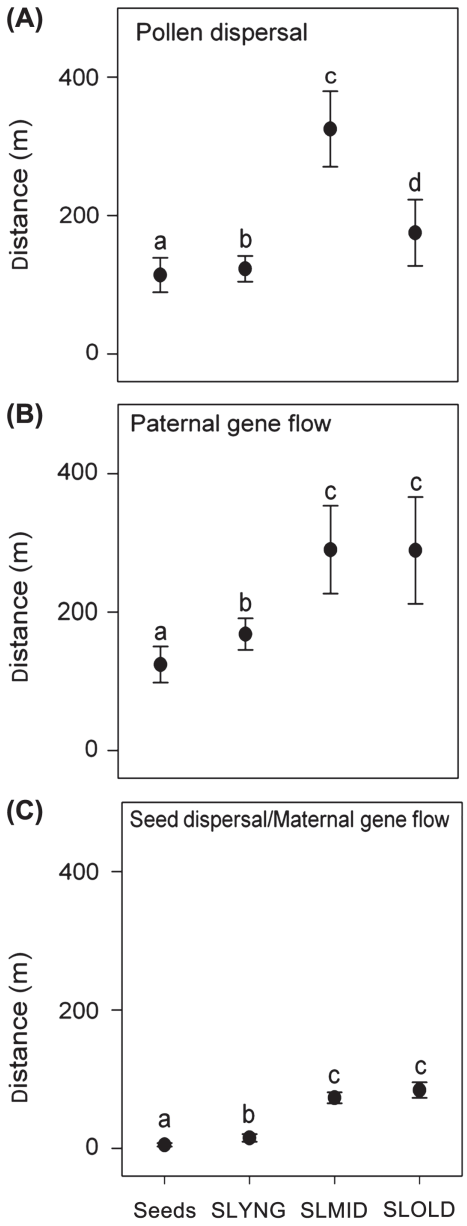

Figure 3. Mean ± 1 SE distances of (A) pollen dispersal, (B) effective paternal gene dispersal and (C) seed dispersal (= effective maternal gene dispersal) for four life stages of the tree *P. africana*. Different small letters indicate significant differences between life stages. SLYNG = young seedlings, SLMID = middle-aged, SLOLD = old seedlings.

Discussion

Our results show that the presumably insect-mediated pollen dispersal distances were much longer than the predominantly bird- and monkey-mediated seed dispersal distances in *P. africana*. Paternal gene dispersal distances were also much longer than maternal gene dispersal distances. Gene dispersal distances changed considerably across subsequent stages: as expected, maternal gene dispersal distances increased across successive stages; a similar increase also occurred in paternal gene dispersal distances. Consequently, the initial >22-fold differences between pollen and seed dispersal distance and between paternal and maternal gene dispersal distances declined to <4-fold differences in older recruitment stages.

Using maternal endocarp tissue allowed us to discriminate between mother and father tree at least for a subset of our samples. To our knowledge, only one study so far has been able to successfully apply this method for seedlings (Isagi et al. 2007). Using maternal tissue gave us the unique opportunity to test the widely applied assumption that the parent tree closer to the offspring is the seed source also for seedlings (Godoy and Jordano 2001). Applying this method can markedly increase the quality of analyses of effective gene dispersal across recruitment stages in future studies (Isagi et al. 2007). Nonetheless, our approach has some limitations. The practical inability to accurately age older seedlings or to account for the possible death of parents of those seedlings lead to an incomplete reconstruction of the recent history of all potential parents of the older seedlings. Further, a considerable proportion of the offspring seems to be sired by trees outside of our study area. Even though this restrains us from drawing conclusions about long-distance gene dispersal, the study area we exhaustively sampled is sufficiently large enough to draw valid conclusions about pollen and seed dispersal and gene dispersal within our population.

Pollen dispersal and paternal gene dispersal distances were always considerably longer than seed dispersal distances.

Table 2. Tests for differences in dispersal distance distributions and means of pollen dispersal, seed dispersal/maternal gene dispersal distances, and paternal gene dispersal distances between four successive life stages in the tree *P. africana*. Presented are D-values of two-sample Kolmogorov–Smirnov tests for comparisons of distributions, as well as t-values of Welch two-sample tests for comparisons of means. Significance (after Bonferroni correction, p-level < 0.0167) is indicated with *, n.s. = non-significant. Significant values are bold.

	Seeds – young seedlings		Young – middle-aged seedlings		Middle-aged – old seedlings	
	D	t	D	t	D	t
Pollen dispersal distances	0.13$^{n.s.}$	**46.13***	**0.40***	**359.9***	0.33$^{n.s.}$	**132.84***
Seed dispersal/maternal gene dispersal distances	0.18$^{n.s.}$	**321.49***	**0.80***	**20.30***	0.23$^{n.s.}$	0.59$^{n.s.}$
Paternal gene dispersal distances	0.19$^{n.s.}$	**204.66***	**0.33***	**8.73***	0.32$^{n.s.}$	0.13$^{n.s.}$

This is in line with earlier studies that showed that gene dispersal by pollen can be spatially more extensive than gene dispersal by seeds (Petit et al. 2005, Ndiade-Bourobou et al. 2010, Sebbenn et al. 2011). As a matter of course, species that are mainly outcrossing show long pollen dispersal distances that are determined by the proximity to the nearest flowering neighbor tree. Nevertheless, insect pollinators can also sustain gene dispersal across surprisingly long distances (Dick et al. 2003). In contrast, seed dispersal distances in our study system were remarkably short. This may result from a large proportion of seeds dropped under the crown due to the behavior of monkeys and perhaps other vertebrate seed dispersers. However, even considering only the seeds dispersed beyond the crown of the mother tree, the mean dispersal distances were still short (30 ± 5 m SE), and shorter than most seed dispersal distances found for other animal-dispersed species (Hardesty et al. 2006, Jordano et al. 2007, Spiegel and Nathan 2007). Birds and monkeys, which are the main seed dispersers in our study system, are highly mobile and can usually transport seeds over several km (Jordano et al. 2007, Lenz et al. 2011). However, inferring seed dispersal patterns only from the identity of the presumed dispersal agents can be misleading (Nathan et al. 2008, Damschen et al. 2008) and often cannot replace detailed analyses of the movements of both dispersal vectors and dispersed seeds (Spiegel and Nathan 2007, Nathan et al. 2008, Damschen et al. 2008); fruit and seed handling behavior, e.g. high rates of regurgitation of large seeds, as well as the spatial arrangement of roost locations, might result in short-distance dispersal even for highly mobile animals.

Despite the restricted gene dispersal patterns we documented within our study population, we recorded very high levels (36–66%) of gene immigration from the adjacent forest. The level of gene immigration found at the seed and fruit stage in *P. africana* is far higher than that found in *P. mahaleb* (18.5%, García et al. 2007). This is presumably due to our non-isolated study area bordering a large continuous forest. Moreover, pollinator and seed disperser communities differ among temperate and tropical ecosystems which may result in different gene dispersal distances observed for these two *Prunus* species. Both pollination and seed dispersal have the potential to facilitate gene immigration, and thus can counteract within-stand processes (Burczyk et al. 2004, Jordano et al. 2007). Regarding the limited within-stand seed dispersal distances, we suggest that in our study system, gene immigration occurs mostly due to long-distance pollination events.

Pollen and seed dispersal/maternal gene dispersal distances as well as paternal gene dispersal distances differed significantly among all successive recruitment stages. The seedlings we analyzed for the older stages represent multiple cohorts. Flowering and fruiting phenology differs among years, resulting in large inter-annual variance in the spatial distribution of resources, flowering and fruiting intensity, pollen and seed production and potentially in variable movement patterns of pollinators and seed dispersers (Ghazoul 2005, Pereira et al. 2010). This in turn leads to a variation in the basic dispersal distances among years, which may be a main source of the variation of distances among recruitment stages. In particular the observed variability in pollen dispersal distances may at least be partially explained by this inter-annual variability.

Furthermore, despite the dependence of *P. africana* on animal-mediated seed dispersal, some of the propagules encountered in seed traps could have been gravity-dispersed from nearby trees. The general tendency for higher germination probability of gut-passed seeds (Traveset and Verdu 2002) is expected, all else being equal, to yield longer average dispersal distances at the seedling stage. Even though we lack data on such a general gut passage effect on germination in the study species, a potential positive effect observed in other Rosaceae species (Yagihashi et al. 1998) could at least partially explain the observed increasing recruitment distances from the seed stage to seedling stages.

Moreover, changes in gene dispersal distances among stages may be driven by microhabitat conditions (e.g. soil nutrients or light environment) which strongly affect the survival to older recruitment stages (Schupp 1995, Willson and Traveset 2000). However, if the above-mentioned factors were the main driver of gene dispersal variation, distances would be assumed to vary randomly, which was the case for pollen dispersal. In fact, both paternal and maternal gene dispersal distances continuously and substantially increased across successive recruitment stages (Table 2). Hence, we conclude that the increasing effective gene dispersal distances are a result of density- and distance-dependent JC-like mortality near the adult trees, due to conspecific competition, predation and/or herbivory (Steinitz et al. 2011). The zone of influence of conspecific adults on survival may extend out to several crown radii (Swamy et al. 2011), explaining the large-scale effects of distance and density-dependent mortality found at older recruitment stages (73–84 m). The short seed dispersal distances found in our study result in the clumping of juveniles in close proximity of the parent trees, thereby setting

the template for this strong distance- and density-dependent mortality. However, the specific underlying mechanisms are still unknown. Thus, a combination of genetic, demographic and ecological data could strengthen our inferences and would be a promising approach for future studies.

The relative changes in effective pollen dispersal, seed dispersal, and paternal gene dispersal distances across recruitment stages elucidate the mechanisms affecting the contribution of the two processes, pollen and seed dispersal, to effective gene dispersal. Across subsequent recruitment stages, the pollen to seed dispersal distance ratio dropped from 22.8 (seeds) to 2.1 (old seedlings), and the paternal to maternal gene dispersal distance ratio dropped from 24.8 (seeds) to 3.4 (old seedlings). This implies that during successive recruitment stages, offspring location is shifted away from the mother trees at greater rates than from the father trees. The paternal gene dispersal distances are in the order of several hundred meters, with a mean distance of >100 m already at the initial seed stage. In comparison, the maternal gene dispersal distances were initially in the order of 5 m extending to nearly 100 m at later stages. This leads to a strong shift in the relative contributions of pollen and seeds to overall effective gene dispersal in the course of recruitment. The long-term consequences of such a rearrangement of successive gene dispersal patterns for local genetic population structure have yet to be evaluated. Nevertheless, we predict that the restricted vertebrate-mediated seed dispersal distances will give rise to genetic clustering despite the long pollen dispersal distances; furthermore, we predict that the initially strong small-scale genetic structure dilutes at later stages of recruitment.

Conclusions

Our study reports pollen and seed dispersal as well as effective gene dispersal distances across four recruitment stages within a population of the tree *P. africana*. Studying effective gene dispersal across recruitment stages is important to understand and predict gene dispersal patterns and genetic structuring of animal-pollinated and dispersed trees. Our study has important implications for basic concepts in plant population biology and genetics. Our results question common simplifications such as inferring dispersal distances from the identity of the dispersal vectors (e.g. the common assumption that vertebrates lead to far gene dispersal). To our knowledge, this study is the first to compare the contribution of both pollen and seed dispersal to effective gene dispersal across multiple recruitment stages. Our findings revealed strong shifts in the contribution of the two processes to effective gene dispersal across recruitment. In conclusion, to obtain reliable estimates of how pollen and seed dispersal jointly shape the spatial genetic structure of plant populations, analyses across multiple life stages are indispensable.

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Supplementary material (available as Appendix oik-00515 at <www.oikosoffice.lu.se/appendix>). Appendix 1.