

Contemporary pollen and seed dispersal in a *Prunus mahaleb* population: patterns in distance and direction

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Abstract

Pollination and seed dispersal determine the spatial pattern of gene flow in plant populations and, for those species relying on pollinators and frugivores as dispersal vectors, animal activity plays a key role in determining this spatial pattern. For these plant species, reported dispersal patterns are dominated by short-distance movements with a significant amount of immigration. However, the contribution of seed and pollen to the overall contemporary gene immigration is still poorly documented for most plant populations. In this study we investigated pollination and seed dispersal at two spatial scales in a local population of *Prunus mahaleb* (L.), a species pollinated by insects and dispersed by frugivorous vertebrates. First, we dissected the relative contribution of pollen and seed dispersal to gene immigration from other parts of the metapopulation. We found high levels of gene immigration (18.50%), due to frequent long distance seed dispersal events. Second, we assessed the distance and directionality for pollen and seed dispersal events within the local population. Pollen and seed movement patterns were non-random, with skewed distance distributions: pollen tended to move up to 548 m along an axis approaching the N–S direction, and seeds were dispersed up to 990 m, frequently along the SW and SE axes. Animal-mediated dispersal contributed significantly towards gene immigration into the local population and had a markedly nonrandom pattern within the local population. Our data suggest that animals can impose distinct spatial signatures in contemporary gene flow, with the potential to induce significant genetic structure at a local level.

Keywords: dispersal direction, dispersal distance, gene immigration, microsatellites, pollination, seed dispersal

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Introduction

Pollination and seed dispersal are two critical stages in plant recruitment cycles, frequently involving the interaction with one or more animal species that provide dispersal services for pollen or seeds (Howe & Westley 1988). Thus, the feeding and post-feeding movements of pollinators and animal frugivores influence the distance, direction, and magnitude of pollen and seed dispersal, which ultimately determine the spatial pattern of gene movement within and among plant populations (Nathan & Muller-Landau 2000). Pollinators and frugivores can remove large amounts of pollen and fruits, a fraction of which may be deposited several hundreds of meters away from the source tree

(Howe 1986; Harder & Barret 1996; Nason *et al.* 1998; Schnabel *et al.* 1998; Godoy & Jordano 2001; Hardesty *et al.* 2006), thereby connecting fragmented populations and favouring the colonization of new patches (Cain *et al.* 2000; Tewksbury *et al.* 2002). In spite of having demographic and genetic consequences for plant populations, the relative contribution of contemporary pollen and seed dispersal to overall gene immigration is still poorly reported.

Ideally, we should be able to characterize both pollen and seed dispersal movements (the processes) in order to understand their role in shaping the adult spatial distribution and genetic structure in plant populations (the patterns) (Bossart & Prowell 1998). Dispersal usually involves an array of different vectors (biotic and abiotic) that distribute pollen and seeds nonrandomly over all available target sites, either receptive flowers (pollination) or deposition

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sites in the landscape (seed dispersal). When animals are the dispersal vectors, the distribution of propagules is aggregated and heterogeneous due to selective feeding movements (Fragoso 1997; Nason *et al.* 1998; Wenny & Levey 1998; Jordano & Schupp 2000; Schupp *et al.* 2002; Russo & Auspurger 2004). The source of the dispersed propagules is usually unknown due to the difficulty of tracking pollen and seed movement (Cain *et al.* 2000). However, this information is highly relevant since it would provide reliable estimates on dispersal distance and directionally required to assess the role of dispersal processes in shaping the recruitment patterns and genetic structure in plant populations. Moreover, the ability to identify pollen and seed sources enables the dissection of the relative contribution of both gene vectors to a local population, an issue that has so far only been addressed by indirect estimates (Ennos 1994). Both aspects of contemporary pollen and seed dispersal are required to fully understand the ecological outcomes for plant species of plant–animal interactions.

The combination of genetic markers yielding individual multilocus genotypes with the spatial locations of both adult trees and dispersed propagules (either seed or pollen) provides a spatially-explicit framework for the study of seed and pollen dispersal and their long-lasting consequences in plant populations (Sork *et al.* 1999; Jordano & Godoy 2002). Assignment methods allow local pollen and seed dispersal events to be tracked in terms of distance and directionality, while also enabling the identification of pollen and seed immigrants — i.e. those propagules collected in the study area but produced in another population — thus inferring the relative contribution of seed and pollen to overall gene immigration. Here, we used highly variable molecular markers (simple sequence repeats; SSRs) to identify the source tree for a sample of dispersed seeds, by genotyping the woody seed endocarps (a tissue of maternal origin) and comparing them with all adult trees within the population (see also Ziegenhagen *et al.* 2003; Grivet *et al.* 2005; Jones *et al.* 2005). The percentage of immigrant seeds can thus be estimated as the percentage of seeds with no matching genotype within the population, when the sampling is exhaustive (see Godoy & Jordano 2001 for details). Our general goal was to estimate seed immigration in our study population and combine this estimate with previous results for pollen dispersal (García *et al.* 2005) in order to dissect the potential contribution of pollen and seed dispersal to total gene immigration. Second, we described and compared the seed and pollen dispersal patterns within the population in terms of distance and directionality from the source plants. If dispersal patterns of pollen and seeds essentially capture the foraging patterns of pollinators and frugivores, we expected a strong nonrandom and spatially-aggregated pattern of dispersal (Jordano & Schupp 2000; García-Castaño 2001).

Materials and methods

Study species

The study species is *Prunus mahaleb* (L.), a gynodioecious rosaceous tree. In any given population there are individuals with hermaphrodite flowers (functional hermaphrodites) and others with androsterile flowers (functional females; Jordano 1993). In the southern Iberian Peninsula flowers are produced between mid-May and mid-June at high elevations (over 1300 m). Bees (Hymenoptera: Andrenidae, Apidae) and flies (Diptera: Calliphoridae, Syrphidae) are the main pollen vectors (Jordano 1993). Between late July and mid-August, fleshy fruits are produced and consumed by frugivorous birds, such as blackbirds (*Turdus merula*), mistle thrushes (*Turdus viscivorus*), blackcaps (*Sylvia atricapilla*), and black redstarts (*Phoenichurus ochruros*) (Jordano & Schupp 2000). Jordano (1995) reported seed dispersal by lizards (*Lacerta lepida*) and Herrera (1989) and J. L. García-Castaño (personal communication) reported seeds being dispersed by red foxes (*Vulpes vulpes*), badgers (*Meles meles*) and stone martens (*Martes foina*). See Jordano & Schupp (2000) and García *et al.* (2005) for additional details.

Study site and sampling design

The study population is located in Nava de las Correhuelas (Parque Natural de Sierra de Cazorla, Segura y las Villas, Jaén province, Spain, 37–59°N, 2–54°W) at 1615 m elevation. Populations of *P. mahaleb* in this region are typically isolated and small (< 100 trees). Vegetation is dominated by grasslands with scattered patches of deciduous vegetation, gravely soil or rock outcrops covered by shrubs or small isolated trees. The rocky slopes are dominated by open pine forest (*Pinus nigra* ssp. *salzmannii*). Our study population consists of a total of 104 hermaphrodite and 92 female trees distributed as shown in Fig. 1. The next nearest *P. mahaleb* population is found at 1.5 km, and eight more are found within 20 km.

We analysed two distinct seed samples, one for the study of pollen dispersal patterns (see García *et al.* 2005) and the other for seed dispersal. To study pollen dispersal, we used 200 fruits harvested when ripe, before the start of consumption by frugivores, from the canopy of 20 mother trees previously used to characterize the mating system of *Prunus mahaleb* (L.) (for details on sampling design see García *et al.* 2005). To study seed dispersal we collected all dispersed seeds ($N = 914$) from seed-traps located at 613 sampling points all over the study area, each consisting of two seed-traps randomly located within a microhabitat type (for further details see García-Castaño 2001). We genotyped a random subsample of the dispersed seeds collected in the seed traps ($N = 549$). As some amplifications



Fig. 1 Map of the study population showing the spatial location of all adult reproductive *Prunus mahaleb* trees (white dots). The area is highly heterogeneous, dominated by a pine forest (*Pinus nigra* ssp. *salzmannii*) at the edge of the *P. mahaleb* population, a central open grassland area, and rocky soils (white areas).

failed for several markers, we finally successfully obtained the multilocus genotype for at least eight markers for 465 endocarps.

Microsatellite genotyping

To obtain direct estimates of seed and pollen dispersal distances we obtained the genotype at 10 microsatellite loci for all adult trees within the population, along with the multilocus genotype of the endocarp of dispersed seeds and of the embryos extracted from the canopy-harvested seeds. The study population was previously genotyped and mapped by Godoy & Jordano (2001), and a few trees were added to this previous sample by García *et al.* (2005). Briefly, endocarps were opened and separated from the embryo tissue and immersed in liquid nitrogen before being ground in a ball-mill (Retsch Mixer Mill MM 200). DNA was extracted following Cheung *et al.* (1993) and finally resuspended in 80 μ L of TLE buffer (10 mM Tris-HCl,

0.1 mM EDTA). Five μ L extract was used as template for the polymerase chain reaction (PCR). Amplified fragments were analysed using an ABI 310 Genetic Analyser (Applied Biosystems). The genotyping of adult trees was performed similarly, from leaf tissue. For further details on genotyping protocols see Godoy & Jordano (2001). Embryos from fruits harvested directly from the mother tree were carefully separated from the endocarp and the seed coat. DNA was extracted from all embryos using a SIGMA GenElute™ Plant Genomics DNA Kit, with 5–10 μ L used as a template for PCR in a final volume of 20 μ L. Amplified fragments were analysed using an ABI 310 capillary electrophoresis system (Applied Biosystem). For full details on genotyping protocols for embryos see García *et al.* (2005).

Data analysis

Pollen immigration and paternity analysis. In a previous study we used paternity analysis to describe the mating system of this *P. mahaleb* population, by assigning a most-likely father to each pair of mother–offspring (paternity analysis) (for a detailed analysis of the procedure, see García *et al.* 2005). By doing so, we estimated the apparent pollen immigration as the percentage of embryos not assigned to a candidate tree within the population (Devlin & Ellstrand 1990). We also used the set of unassigned (i.e. apparent gene flow) embryos along with the cryptic gene flow estimation to assess total (actual) pollen immigration. The cryptic pollen flow (CPF) is defined as the fraction of the offspring assigned to a putative father within the population, whereas the true father is outside the population (Devlin & Ellstrand 1990). We used CERVUS 2.0 for the paternity analysis (Marshall *et al.* 1998) following the criteria proposed by Slate *et al.* (2000) to define the scoring threshold, and FAMOZ software (Gerber *et al.* 2003) to estimate the pollen CGF. For the latter, we compared the estimate of actual pollen immigration provided in our previous paper (García *et al.* 2005) with the estimates for dispersed seeds. Additionally, we used the set of assigned embryos to characterize pollen dispersal patterns in terms of distance and directionality within the population.

Seed immigration and the assignment of the source tree for dispersed seeds. In order to identify the source tree for each dispersed seed (maternity analysis), we searched the matching genotypes between each endocarp and the candidate adult trees for a complete set of eight microsatellite markers out of 10 loci, as some loci were excluded due to a high frequency of failed amplifications. We used CERVUS 2.0 (Marshall *et al.* 1998) to identify the endocarp–source tree matching pairs, allowing zero mismatches. Given that all adult trees in the population yielded a different multilocus genotype, we assumed that endocarps with no matching adult genotype came from other populations, and we

considered the percentage of nonassigned endocarps as an estimate of apparent seed immigration (Godoy & Jordano 2001). We estimated the cryptic seed flow (CSF) that, in analogy to CPF, represents the proportion of seeds assigned to a local source tree that have been produced outside the population. Based on Harju & Nikkanen (1996) we estimated the sum of the all expected probabilities for the local maternal genotypes to be produced in a nearby population (i.e. the proportion of nondistinguishable gametes; NDG). Assuming Mendelian inheritance, the Hardy–Weinberg equilibrium, and no linkage disequilibrium, this can be estimated based on the allele frequencies of the eight nearest populations (for details on the nearby populations see Jordano & Godoy 2000). The actual seed flow is obtained by dividing the observed seed flow by NDG. Moreover, the simulations performed with CERVUS indicated that with genotyping error rates ranging between 0 and 0.1, an increment of 0.001 in the error rate increases the estimated seed immigration percentage in 2.5% (C. García, unpublished).

Intrapopulation dispersal distances and directions. The identification of the source tree for each pollen and seed dispersal event allowed the estimation of pollen and seed dispersal distances and directionality (Godoy & Jordano 2001; García *et al.* 2005). The x, y coordinates for each adult tree and seed trap were recorded in a GIS database and used to calculate the distance and azimuth between each adult tree (i.e. intertree distance), each mother tree and their siring trees (i.e. pollen dispersal events), and between each dispersed seed and its source tree (i.e. seed dispersal events), with ARCVIEW 3.2 (Mitchell 1999). We tested for differences in pollen and seed dispersal distance distributions by applying a Kolmogorov–Smirnov nonparametric test. Additionally, we used ORIANA 2.0 (Kovach Computing Services, Wales, UK) to describe pollen and seed dispersal direction patterns. This software estimates the mean direction for both data sets. If we have n observations and θ_i is the direction of the $n = i$ observation, we can calculate the mean vector direction as:

$$\theta = \arctan(S/C)$$

and the mean length as

$$\bar{R} = \sqrt{S^2 + C^2/n}$$

where

$$S = \sum_{i=1}^n \sin \theta_i \quad \text{and} \quad C = \sum_{i=1}^n \cos \theta_i$$

ORIANA 2.0 also provides statistical tests to assess if pollen and seed dispersal direction patterns differ from a uniform distribution (Rayleigh Z-test), and to compare pollen and

seed dispersal direction distributions (Watson U^2 -test for two samples). We expected the direction distributions to differ for pollen and seed dispersal since they result from the activity of different dispersal agents. To evaluate the influence of the adult hermaphrodite trees (pollen sources) arrangement in shaping pollen dispersal directions we estimated the potential pollen (PP) dispersal angle distribution in the population (angles between each sampled mother tree and each hermaphrodite in the population) and compared them with the actual pollen (AP) dispersal angle distribution (angle between each sampled mother tree and its siring hermaphrodite). Similarly, we compared the potential seed (PS) dispersal angle distribution (angle between each adult tree and each seed trap) and the actual seed (AS) dispersal angle distribution to evaluate the influence of the seed traps arrangement imposed by the sampling design on seed dispersal angle distribution. Both comparisons were performed by applying a Watson U^2 -test for two samples (Zar 1999).

Results

Among-population dispersal

Observed seed immigration was more than twice the documented pollen immigration within the study site (García *et al.* 2005). While we found 20.34% of the dispersed seeds were derived from other populations, the previously reported percentage of actual pollen immigration showed that only 9.5% of the embryos were fathered by trees located outside the population. By relaxing the assumption of correct genotyping in allowing for one mismatch, we still obtained a high rate of seed immigration (18.61%) – i.e. a conservative estimate between 19 and 20% of seed immigration. The proportion of nondistinguishable gametes was extremely low, NDG = 10^{-7} , and therefore the actual seed gene flow should be almost equivalent to the above reported percentage of seed immigration. In terms of gene immigration, this means that 189 gametes carried by seeds ($465 \text{ seeds} \times 2 \text{ gametes} \times 0.2034$) and 19.5 gametes carried by pollen ($197 \text{ embryos} \times 1 \text{ gametes} \times 0.0950$) came from other populations. Thus, 208.5 of the analysed gametes from a total of 1127 (seeds: 465×2 + pollen: 200×1) were immigrant, which represents 18.50% of the total gamete pool.

Within-population dispersal

Overall, pollen tended to move over shorter distances than seeds within the population, with a range of dispersal from 0 m (for self pollinations in hermaphroditic trees) to 548.0 m. On the other hand, frugivore-mediated seed dispersal distances ranged from 0 m (seeds collected beneath the source tree) to 990.2 m (Fig. 2). The frequency distributions of seed and pollen within-population dispersal

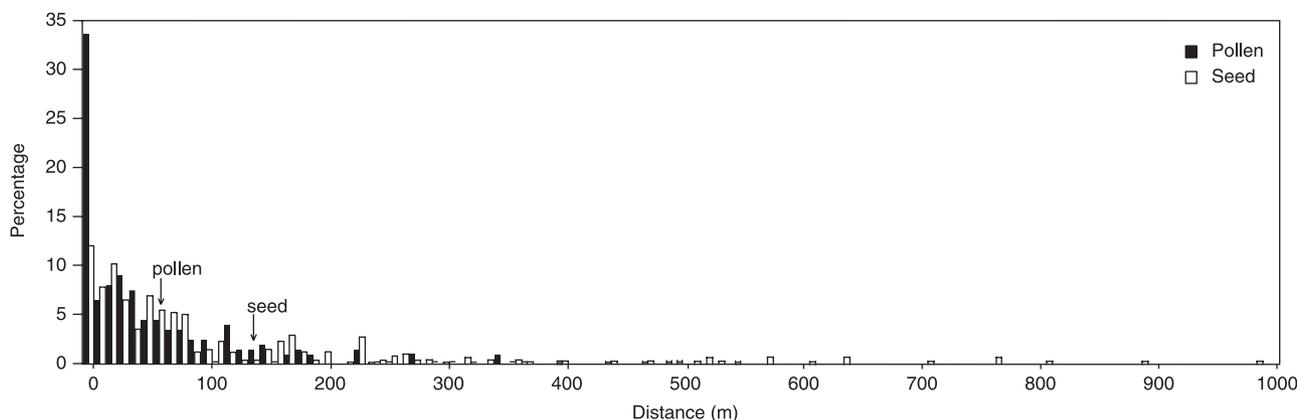


Fig. 2 Frequency distribution of the pollen (black bars) and seeds (white bars) dispersal distances in the study area. The high percentage of pollen dispersal events in the 0 m interval is due to selfing and the seed dispersal events in this interval mostly include seeds collected beneath the source tree canopy. The arrows indicate the median dispersal distance value.

distances differed significantly (Kolmogorov–Smirnov test, $Z = 5.71$, $P < 0.01$). The pollen dispersal distances had a median value of 62.9 m, with 22.5 m and 129.3 m as the first and third quartile, respectively. The median seed dispersal distance was 145.2 m, with 54.1 m and 267.3 m as first and third quartile, respectively (Fig. 2).

Potential and actual pollen dispersal angle distributions (PP and AP) did not differ ($U^2 = 0.155$, $df_1 = 100$, $df_2 = 2099$, $P > 0.05$) (Fig. 3a–b), while potential and actual seed dispersal angle distributions (PS and AS) differed significantly ($U^2 = 4.26$, $df_1 = 368$, $df_2 = 120\ 343$, $P < 0.01$) (Fig. 3c–d). All circular histograms shown in Fig. 3 depicting the potential and actual angle distribution for pollen and seed differed from the uniform distribution ($U^2 = 11.46$, $P < 0.01$ for PP; $U^2 = 0.50$, $P < 0.01$ for AP; $U^2 = 190.25$, $P < 0.01$ for PS; and $U^2 = 0.43$, $P < 0.01$ for AS). Actual pollen and seed dispersal directionality also differed ($U^2 = 0.595$, $d.f. = 362, 100$, $P < 0.001$), with an estimated mean vector $\theta = 352.76^\circ$ and $R = 0.187$ for pollen, and $\theta = 174.30^\circ$ and $R = 0.225$ for seeds (Fig. 3b, d). Thus, on average pollen moved with a marked N–S directional component, but with wide variance from the NW and NE (Fig. 3b), whereas most of the dispersed seeds followed the SE–SW directions (Fig. 3d).

Discussion

Plant–animal mutualisms shape seed and pollen dispersal patterns in heterogeneous landscapes, having pervasive consequences on the genetic diversity, spatial distribution and recruitment patterns of plants (Nason *et al.* 1998; Schnabel *et al.* 1998; Hamilton 1999; Jordano & Schupp 2000; Oddou-Muratorio *et al.* 2001; Westcott *et al.* 2005). Our results indicate that pollinators and frugivores generate extensive gene migration into the study population, despite its relative geographical isolation: 18.50% of the observed gametes came from another population. This level of gene immigra-

tion might change quantitatively (amount) or qualitatively (population source) among years, but it is clear that a significant amount of gene migration connects our study population with nearby populations located between 1.5 km and up to 20 km. The extensive gene flow observed is mainly due to a high percentage of seed immigration, which was double the pollen immigration in our population. This result is congruent with concurrent field work on the seed dispersal ecology of *P. mahaleb* in the same study population, showing that frugivores consistently remove a high fraction of the available fruit crop allowing for wide-ranging dispersal (Jordano 1995; Jordano & Schupp 2000; García-Castaño 2001). In contrast, other studies have reported pollen reaching longer distances than seeds, especially in outcrossed-anemophilous species with no special devices for seed dispersal, or with highly restricted seed dispersal around the mother tree by scatter-hoarding vertebrates (e.g. *Quercus*, *Fagus*, *Pinus*, etc.) (Ennos 1994; Nason *et al.* 1998). Recent evidence on vertebrate-dispersed species suggests that long-distance movements occur consistently and can account for a significant number of dispersal events, a pattern likely when large-bodied frugivores disperse the seeds (Fragoso 1997; Holbrook *et al.* 2002; Schupp *et al.* 2002; Bacles *et al.* 2004; Westcott *et al.* 2005). Pollinators are also able to fly long distances (Handel 1983) but, in the case of *P. mahaleb*, the patchy distribution of the adult trees coupled with their nonoverlapping phenology appears to restrict pollen immigration in the study population (García *et al.* 2005). The present study, along with recent publications dealing with fleshy-fruited species (Aldrich *et al.* 1998; Oddou-Muratorio *et al.* 2001; Bacles *et al.* 2004; Levey *et al.* 2005; Sezen *et al.* 2005), highlights the role that frugivorous vertebrates play in the interchange of individuals and genes among fragmented patches or populations.

As pointed out by Hamrick & Nason (1996), the interpretation of spatial genetic patterns and their variation

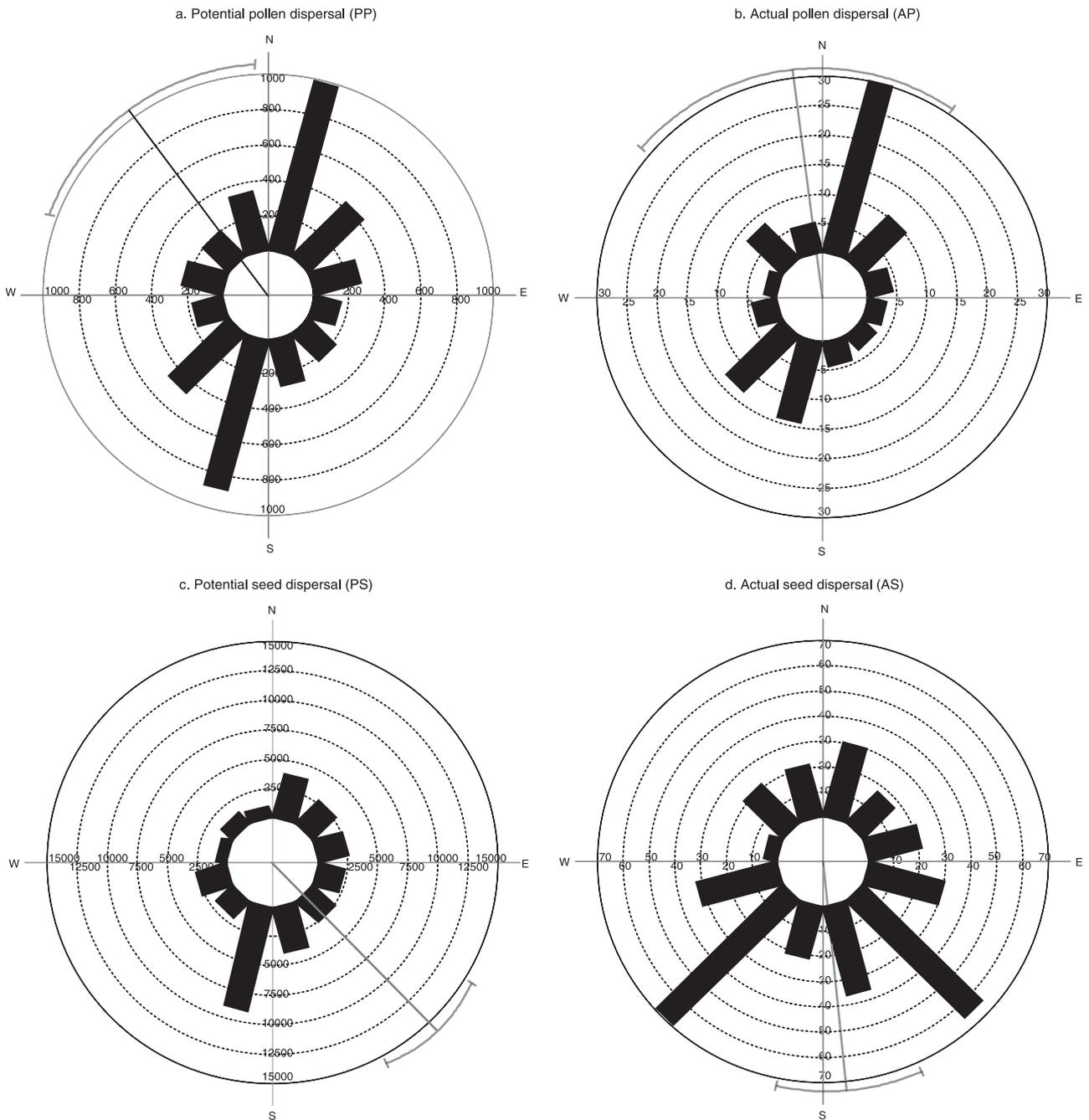


Fig. 3 Circular histograms of the directions distribution for the potential pollen dispersal events (a, PP), actual pollen dispersal events (b, AP), potential seed dispersal events (c, PS), and actual seed dispersal events (d, AS). The mean direction and the associated standard error are indicated as an inner solid line direction and an external bar and the figures on the axis are the sample size (N). Note that the large difference on sample size between histograms containing potential dispersal events (a, c) and actual dispersal events (b, d) is due to the formers include all potential mother–father pairs and tree–seed trap pairs, respectively, whereas the latter include only the observed data.

requires the consideration of both pollen and seed dispersal biology. Despite the evidence for not-so-infrequent long-distance dispersal events (the tail of the dispersal curve), our data revealed highly skewed pollen and seed dispersal distance distributions, with almost 30% of the

seeds and 47% of the pollen (23% excluding self-pollination events) being dispersed within a 30 m radius from the tree source. Additionally, a previous study reported frequent selfing in the same *P. mahaleb* population (García *et al.* 2005). These figures suggest that both seed and pollen dispersal

are locally restricted, increasing in the long term the probability of the spatial clustering of related individuals (Heywood 1991), and therefore the emergence of spatial genetic structure at a fine scale. Moreover, by extending the study of dispersal from one dimension (distance) to two dimensions (directionality), our analysis shows that dispersal events are highly directional. This result suggests that the observed nonrandom and restricted pollen and seed dispersal patterns assisted by animals in heterogeneous landscapes set the initial conditions to generate a heterogeneous genetic spatial structure at a fine scale.

As in previous studies, we found a close relationship between disperser movements and the seed and pollen shadow patterns relative to the sources (e.g. Chase *et al.* 1996; Nason *et al.* 1998; Wenny & Levey 1998; Jordano & Schupp 2000; Jordano & Godoy 2002; Schupp *et al.* 2002; Tewksbury *et al.* 2002). Pollen tended to move preferentially along a N–S direction following the spatial location of the adult trees, as confirmed by the lack of significant difference between the potential and actual pollen dispersal angles. Pollinators tended to fly from one *P. mahaleb* to the next one or to a nearby nonconspecific tree or shrub with an overlapping blooming, frequently located following the main N–S directional axis of tree arrangement. On the other hand, frugivores tended to forage, tracking the location of *P. mahaleb* trees and using short flights to nearby perches or, less frequently, longer flights to pine trees (Jordano & Schupp 2000). These landscape effects on behaviour would explain that frugivores tend to frequently contribute dispersal events following the SE and SW directions. The dominance of the S vs. the N directions in the seed dispersal movements can also be caused by a higher frequency of open rocky patches and a lower availability of the more preferred, covered microhabitats in the Northern area of the population. Open substrates are rarely visited by frugivorous birds; only the black redstart (*Phoenicurus ochruros*) and mammals contribute to the scarce seed rain reaching open microhabitats (Jordano & Schupp 2000; García-Castaño 2001). These results indicate that the combination of a complex landscape and the nonrandom behaviour of pollinators and frugivores can modulate distance effects by imposing directionalities and nonrandom paths relative to the flowering and fruiting trees (Herrera & Jordan 1981). Therefore, our data suggest that animal vectors influence the template of propagules that starts the recruitment cycle, and that ultimately might shape the spatial demographic and genetic structure of the plant population.

Additional fieldwork involving long-term studies of marked individuals is required to assess the contribution of dispersal and postdispersal processes to recruitment patterns (Schupp & Fuentes 1995; Aldrich *et al.* 1998; Kalisz *et al.* 2001). Direct data will elucidate the role of dispersal vectors due to their recurrent and nonrandom movements. However, we still lack a precise understanding of the

differential survival prospects for immigrant progeny compared to *in situ*-dispersed propagules, and of the relative contribution of postdispersal mortality factors and local drift or selection modifying allele frequencies (Gram & Sork 2001). The usefulness of the direct approach for dissecting pollen and seed dispersal contribution to overall gene flow and describing dispersal patterns is that it provides a first step towards the elucidation of the lasting consequences of plant–animal mutualisms in heterogeneous plant populations.

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