

Genetic evidence for a Janzen–Connell recruitment pattern in reproductive offspring of *Pinus halepensis* trees

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Abstract

Effective seed dispersal, combining both dispersal and postdispersal (establishment) processes, determines population dynamics and colonization ability in plants. According to the Janzen–Connell (JC) model, high mortality near the mother plant shifts the offspring establishment distribution farther away from the mother plant relative to the seed dispersal distribution. Yet, extending this prediction to the distribution of mature (reproductive) offspring remains a challenge for long-living plants. To address this challenge, we selected an isolated natural Aleppo pine (*Pinus halepensis*) population in Mt. Pithulim (Israel), which expanded from five ancestor trees in the beginning of the 20th century into ~2000 trees today. Using nine microsatellite markers, we assigned parents to trees established during the early stages of population expansion. To elucidate the effect of the distance from the mother plant on postdispersal survival, we compared the effective seed dispersal kernel, based on the distribution of mother–offspring distances, with the seed dispersal kernel, based on simulations of a mechanistic wind dispersal model. We found that the mode of the effective dispersal kernel is shifted farther away than the mode of the seed dispersal kernel, reflecting increased survival with increasing distance from the mother plant. The parentage analysis demonstrated a highly skewed reproductive success and a strong directionality in effective dispersal corresponding to the wind regime. We thus provide compelling evidence that JC effects act also on offspring that become reproductive and persist as adults for many decades, a key requirement in assessing the role of postdispersal processes in shaping population and community dynamics.

Keywords: distance dependence, effective seed dispersal, Janzen–Connell, parentage analysis, *Pinus halepensis*, spread

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Introduction

Seed dispersal, the movement of seeds away from the parent plant, is the dominant process determining local population dynamics and enabling colonization of plants in new habitats. The seed dispersal pattern serves as a template for subsequent processes that shape plant population dynamics such as competition,

predation and parasitism (Howe & Smallwood 1982; Augspurger 1983; Howe *et al.* 1985). Postdispersal mechanisms can lead to several fundamental types of plant recruitment patterns (Nathan & Casagrandi 2004; Mari *et al.* 2008). One of the mechanisms that can produce an establishment pattern different from the seed dispersal pattern is portrayed by the Janzen–Connell (JC) model (Janzen 1970; Connell 1971; Clark & Clark 1984; Schupp 1992). To explain the observed high tree diversity in tropical forests, these authors proposed that distance-dependent mechanisms such as seed predation

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or seedling herbivory are expected to result in a monotonic increase in survival probability with increasing distance from the mother plant. As, for most plants, the probability of seed arrival decreases with distance from the mother plant, the JC model predicts a hump-shaped recruitment pattern (multiplying seed arrival and survival probabilities) with a mode at a longer distance from the parent tree relative to the mode of the seed dispersal kernel (the frequency distribution of dispersal). The resulting spacing pattern between conspecific trees facilitates species coexistence and thereby high species diversity. The JC prediction for the recruitment pattern has been tested empirically in many studies (reviewed by Hyatt *et al.* 2003; see also Mari *et al.* 2008). These studies analysed the spatial pattern of survival of seed and seedlings away from the mother tree but did not deal with later life stages. The mechanisms acting on the transition to later life stages are especially important for trees and other long-living perennial plants. As effective seed dispersal must include the reproductive maturation stage, the JC prediction for tree recruitment pattern has not been fully demonstrated until now.

The effective seed dispersal pattern, the pattern that also takes into consideration subsequent postdispersal processes leading to reproductive individuals, is central for different levels of organization from the population level to the community level (Nathan & Muller-Landau 2000) and has important implications for species distributions and for the genetic structure within and between populations (Broquet & Petit 2009). Quantification of effective seed dispersal is crucial for predicting the spread rates of invasive plants (Trakhtenbrot *et al.* 2005) and the ability of plants to respond to environmental changes (Thuiller *et al.* 2008; Nathan *et al.* 2011).

Plants with long life expectancy, such as trees, pose special challenges for quantifying effective seed dispersal and reproductive success. Following reproductive maturation in trees requires long-term monitoring beyond the duration of most studies. Trees have several life stages that differ in their interaction with the environment, e.g. for the same plant, the habitat suitability for germination of seeds may be different from that for seedling survival (Schupp 1995; Schupp *et al.* 2010). The distance-dependent survival pattern changes along early life stages in tropical tree species (Augsburger 1984; Condit *et al.* 1994; but see Peters 2003). Hamrick *et al.* (1993) suggested that the loss of genetic structure in larger size categories relative to seedlings and saplings in two wind-dispersed tropical trees was a result of thinning. Thus, spatial effects on survival may act differently at different life stages and extrapolation of these effects from one life stage to another is problematic. Yet, it is important to note that all quantitative

examples of effective seed dispersal of trees have been limited to early life stages (Schupp *et al.* 2010).

Standard methods for measuring seed dispersal (e.g. seed traps) suffer from critical limitations in representing the effective seed dispersal pattern. An important factor that may influence the effective dispersal kernel is anisotropic movement of the dispersal vector. Anisotropic kernels that take into consideration nonuniform distribution of wind speed and direction can provide a more realistic description of wind-assisted dispersal (Skarpaas & Shea 2007). Anisotropic movement of the vector may have implications for species migration (Higgins *et al.* 2003) and for biogeographical patterns of species composition (Muñoz *et al.* 2004). Even though understanding the factors determining the distribution of dispersal directions is highly important, quantifying the realized dispersal directions is limited by the ability to identify the seed source. Reproductive success is an important component of the effective seed dispersal (Schupp *et al.* 2010). The realized reproductive success is measured by the number of new adults produced by an individual (Meagher & Thompson 1987). Owing to the variance in survival for different life stages, the realized reproductive success is not necessarily correlated with fecundity or reproductive effort (Meagher & Thompson 1987). Thus, quantifying realized reproductive success for long-living species is challenging; it is especially important in the light of the high variance in reproductive success found between individuals (e.g. Aldrich & Hamrick 1998; González-Martínez *et al.* 2006).

Development of polymorphic markers and statistical analysis tools has improved our ability to infer family relationships between individuals (Jones & Ardren 2003; Jones *et al.* 2010). Parentage analysis can be a powerful tool for quantifying reproductive success and effective seed dispersal (Meagher & Thompson 1987) including long-distance dispersal events, the most difficult component of the dispersal kernel to measure (Cain *et al.* 2000; Nathan *et al.* 2003). It is particularly suitable for measuring dispersal in populations that have a large variance in reproductive success among individuals (Jones & Ardren 2003). Accurate assignment of offspring to mother trees by parentage analysis also enables quantifying the realized distribution of dispersal directions.

We suggest a general framework for exploring post-dispersal processes in long-living plants without the need for long-term survival monitoring. This approach consist of four major stages: (i) obtaining detailed historical information on the spatial and temporal distribution of individuals in the population under study for a required time frame; (ii) quantifying the distribution of effective seed dispersal distances by assigning offspring

to mother plants in the population; (iii) quantifying the predicted distribution of seed dispersal distances for the relevant time frame; and (iv) extracting the spatial recruitment pattern from previous stages. In this study, we implement this approach on a case study of Aleppo pine population. We aim to quantify the effective seed dispersal kernel during early expansion stages of a natural population using parentage analysis. Combining the effective seed dispersal kernel with a seed dispersal kernel derived from a mechanistic wind dispersal model will enable the estimation of the survival kernel as a function of distance from the seed source. We hypothesize that the net effect of postdispersal processes on offspring survival varies in time and space, thereby shaping the effective seed dispersal kernel. Based on the JC hypothesis, we predict a monotonic increase in offspring survival probability with distance away from the seed source, resulting in a shift in the mode of the effective seed dispersal kernel, farther away from the seed source than the mode of the seed dispersal kernel.

In this study, we also aim to quantify the realized distribution of the effective seed dispersal directions based on mother–offspring assignments. We hypothesize that in Aleppo pine, a wind-dispersed tree, the mother–offspring directions will reflect the dominant wind conditions during the seed dispersal period in the study area. Another objective is to measure the realized reproductive success based on mother/father–offspring assignments. The genetic contributions as a mother and as a father to adult offspring will be compared between individuals. To meet these objectives, we have chosen an isolated natural Aleppo pine population that has unique genetic characteristics relative to the neighbouring conspecific populations (Steinitz 2010). The genetic distinctness of this population allows the relatively easy identification of long-distance dispersal events from outside the population. The population has a very thorough historical documentation from aerial photographs and dendrochronological measurements that contribute to our ability to infer family relationships between the trees.

Methods

Study species

The Aleppo pine (*Pinus halepensis* Miller) is a self-compatible monoecious conifer with wind-dispersed seeds and pollen. It is the most common pine in the Mediterranean Basin (Barbéro *et al.* 1998), distributed mostly in the western part of the Mediterranean Basin but also has scattered populations in the eastern part (Critchfield & Little 1966), including several populations in Israel (Schiller & Waisel 1989; Ashkenazi 2004). Large-scale

afforestation, mostly from nonlocal seed sources, during the last decades (Ashkenazi 2004; Lavi *et al.* 2005; Osem *et al.* 2008; Perevolotsky & Sheffer 2009) has turned the Aleppo pine into one of the dominant trees in Israel (Liphschitz & Biger 2001).

Study site and field sampling

The study site is located in Mt. Pithulim within the Nachal Soreq Nature Reserve in the Judean Hills, Israel (31°45'N, 35°04'E). Mean annual rainfall at the site is 600 mm, and mean monthly temperature ranges from 12 °C (January) to 25 °C (August). Lithology includes limestone, dolomite, marl and chalks with mostly shallow light rendzina soil (Troupin *et al.* 2006). The vegetation in the study site is a mixture of maquis (scrubland) and batha (shrubland) dominated by *Quercus calliprinos*, *Arbutus andrachne*, *Pistacia lentiscus*, *Ceratonia siliqua*, *Sarcopoterium spinosum* and *Calycotome villosa*.

This site is occupied by a presumably natural Aleppo pine population that has expanded from five individuals in the early 20th century to thousands of individuals today. Grazing, presumably by livestock herds, was intense in the site until ~1948, and there is no evidence of any planting, cuttings or fires (Troupin *et al.* 2006). The closest old natural Aleppo pine population is located ~5.5 km to the northwest of Mt. Pithulim. Some Aleppo pine stands were planted at a distance of over 1.5 km from the Mt. Pithulim population as early as 1926, with a total of ~42 hectares within a radius of 2 km from the edge of the natural stand. However, massive planting in the surrounding area did not begin before ~1955. The five ancestor trees were probably not planted; however, it is unclear whether they are remnants of a larger population or the initial colonizers.

A 60-ha plot (750 m × 800 m) containing the initial five individuals within Mt. Pithulim was defined as the study area (Fig. 1). All individuals within the 60-ha plot with DBH (Diameter at Breast Height) >5 cm were mapped with 1-m accuracy using differential Global Positioning System (HiPer GD; Topcon) and a total station (TC1610; Leica geosystems). All Aleppo pine individuals that were established in early stages of population expansion were identified using aerial photographs of the study site taken in approximately 10-year intervals (1944–2003), and their age was determined by dendrochronology (Troupin *et al.* 2006). Apart from the five ancestor trees, no recruitment was identified before 1944 in the study area. The parentage analysis was limited to the 122 trees established during the 1944–1957 period when the five ancestor trees were the only potential parents within the plot. After 1957, 13 years after the first new recruitment, newly

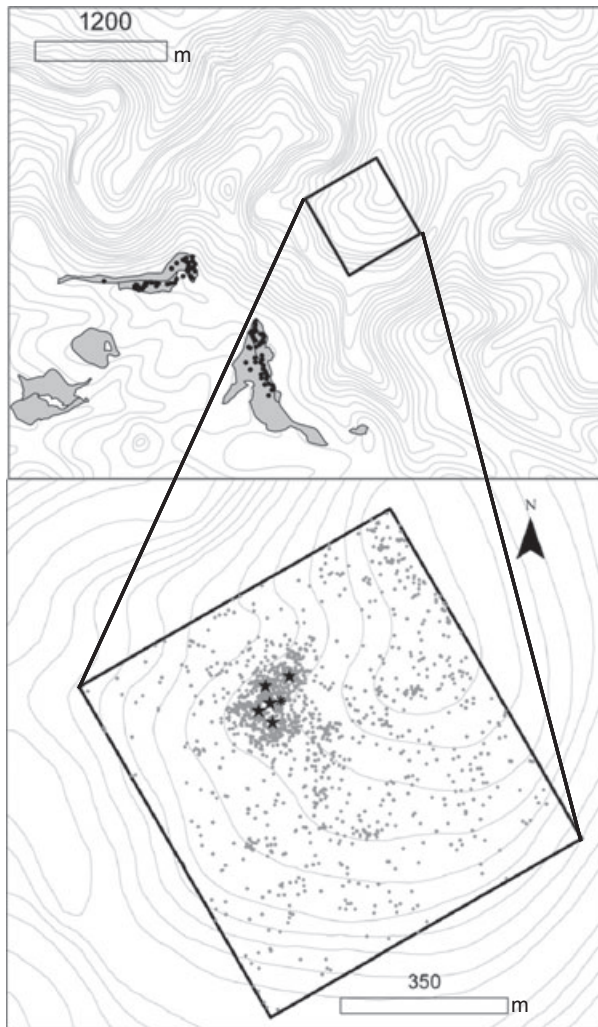


Fig. 1 Spatial distribution of the five ancestor trees established before 1917 (stars) and the 1573 reproductive trees with DBH > 5 cm (grey circles) within the 60-ha plot. The meteorological tower with a sonic anemometer is indicated by a black asterisk. The area of the surrounding stands that were planted before 1944 is marked in grey. The 80 sampled planted trees (planting year ~1926) are marked by black circles.

established offspring could become parents. The limit was determined based on the expected duration of ~10 years until substantial reproductive maturation takes place, with an additional 2–3 years of pollination and cone maturation in Aleppo pine (Thanos & Daskalakou 2000). The aerial photographs of the study area were carefully examined to ensure that no other potential parent was alive within the 60-ha plot during the period of the parentage analysis (1944–1957).

Tissue collection and DNA extraction

Needles were collected from each of the 122 trees established during the 1944–1957 period and were stored at

–80 °C. Genomic DNA was extracted from about 100 mg of needle tissue using an *i*-genomic Plant DNA Extraction Mini Kit (INTRON, Korea) according to the manufacturer's instructions. Using the same method, DNA was extracted from haploid megagametophytes of seeds that were collected from four of the five ancestor trees in Mt. Pithulim before their recent death. For each individual genotype, we used at least eight megagametophytes that were carefully separated from the embryo to avoid contamination by DNA from the pollen donor. DNA from the fifth ancestor tree was extracted separately from needles and megagametophytes. The two extraction methods yielded the same results.

Needles were also collected from a sample of 80 trees from the four closest stands that were planted in ~1926 at a distance of 1.5–2 km southwest of the core of the study area at Mt Pithulim.

The five ancestor trees, the 122 potential offspring and the sample of 80 planted trees were genotyped using eight nuclear and one chloroplast microsatellite markers (Table 1). As in other conifers, the chloroplast is inherited through the pollen (Mogensen 1996) enabling the identification of father/mother in a parent pair. Two of the nuclear markers (Table 1) developed by G. G. Vendramin for *Pinus pinea* were successfully transferred to *P. halepensis*.

PCR was carried out using Primus 96 Advanced Gradient thermal cycler (Peqlab, Erlangen, Germany) in a total volume of 15 µL. The reaction mix contained 20 ng of template DNA, 0.2 µM of each primer (the forward primer was fluorescently labelled with HEX, VIC, FAM, NED, PET dyes) and 7.5 µL Taq Mix Purple (Lamda Biotech, St. Louis, MO, USA) giving a total concentration of 10 mM KCL, 20 mM Tris HCL (pH 9.0), 16 mM (NH₄)₂SO₄, 0.1% Triton X-100, 1.5 mM MgCl₂, 200 µM of each dNTP and 0.45 units Taq DNA polymerase. The concentration of MgCl₂ in the reaction mix and PCR conditions were adjusted for each marker as specified in Table 1.

The multiplex PCR products were separated using an ABI 3730 genetic analyser (Applied Biosystems). Manual scoring of PCR product sizes with reference to a 500-Liz standard (Applied Biosystems) was made using GeneMapper Software version 4.0 (Applied Biosystems). Replicate scoring was carried out by two persons, and typing of the data was checked thoroughly to minimize genotyping error (Pompanon *et al.* 2005).

Data analysis

For assigning parents to individuals, we used the parentage analysis software FAMOZ (Gerber *et al.* 2000), a software that was chosen as it can deal with codominant

Table 1 The nuclear and chloroplast microsatellite markers used for the genetic analyses with their PCR conditions including the annealing temperature and MgCl₂ concentration

Marker name	Accession nos	Nuclear (N)/ Chloroplast (CP)	Reference	PCR programme*	T _a (Annealing temperature °C)	MgCl ₂ (mM)	No. of alleles	Allele size
PtTX3116	AY304036	N	González-Martínez <i>et al.</i> (2004)	3	50	4.5	2	116–122
B4F08	CR377943	N	Guevara <i>et al.</i> (2005)	3	50	1.5	5	178–210
ITPH4516	AJ012087	N	Keys <i>et al.</i> (2000)	3	59	2.5	12	136–178
PHAF01	AF195535	N	Keys <i>et al.</i> (2000)	4	56	2.5	2	205–209
PHAF02	AF195536	N	Keys <i>et al.</i> (2000)	2	60 → 50	2.5	2	156–158
PHAF10	AF195543	N	Keys <i>et al.</i> (2000)	3	50	4.5	2	131–137
pEST8	JF803635	N	This study†	3	54	3.5	4	192–204
pEST2669	JF803641	N	This study‡	3	50	2.5	4	145–151
Pt71936		CP	Vendramin <i>et al.</i> (1996)	1	55	2.5	5	145–149

*The following conditions were used in each PCR programme: Programme 1: 95 °C for 5 min, 25 amplification cycles (95 °C for 60 s, 55 °C for 60 s, 72 °C for 60 s) and a final extension step of 8 min at 72 °C; Programme 2: 95 °C for 5 min, 10 touch-down cycles (94 °C for 30 s, 60 °C (–1 °C per cycle) for 30 s, 72 °C for 60 s), 30 amplification cycles (94 °C for 30 s, 50 °C for 30 s, 72 °C for 60 s) and a final extension step of 8 min at 72 °C; Programme 3: 94 °C for 4 min, 35 amplification cycles (94 °C for 30 s, T_a for 30 s, 72 °C for 45 s) and a final extension step of 10 min at 72 °C; Programme 4: 95 °C for 5 min, 35 amplification cycles (94 °C for 90 s, 56 °C for 90 s, 72 °C for 90 s) and a final extension step of 10 min at 72 °C.

†F sequence (5'–3'): AGAAGTTGCCAGAGGTTCCA; R sequence (5'–3'): TGTCTACCGTACGCAGCAG; Repeat: (AT)₁₀.

‡F sequence (5'–3'): ATTGCTTCTGAAAGGGCATC; R sequence (5'–3'): TCCCTTGGCACCATGTAAAT; Repeat: (TA)₁₉.

and cytoplasmic markers in the same analysis (Jones *et al.* 2010). Single parent and parent pair exclusion probabilities were calculated according to Jamieson & Taylor (1997). The allele frequencies used for the parentage analysis were based on the parent and offspring in Mt. Pithulim, as well as the sample of 80 planted trees.

The 'Logarithm of the odds ratio' (LOD) scores for parent–offspring relationships (single parent and parent pair) (Gerber *et al.* 2000) were calculated for each offspring established in 1944–1957 considering the five ancestor trees as potential parents. For the LOD calculation, we used a mistyping error and consequentially a simulation error of 0.001 (Gerber *et al.* 2000). The thresholds for statistical significance of LOD scores were estimated following the simulation method described in Gerber *et al.* (2000). Using a categorical allocation approach (Jones & Ardren 2003), we assigned parentage to parent pairs or single parents (when it was impossible to identify a couple) with the highest LOD score above the estimated thresholds. Cryptic gene flow, determined by false assignments to local parents of gametes sired by outside parents (Devlin & Ellstrand 1990), and the performance of the parentage analysis were assessed using a simulation-based test in FAMOZ. Two sets of offspring were simulated with a simulation error of 0.001: 100 000 offspring produced from the five ancestor parents and 100 000 offspring produced from allele frequencies. Parentage assignment for the two sets was based on the estimated thresholds.

For estimating effective seed dispersal, we endeavoured to determine the seed source for each offspring that had an identified pair of parents based on the parentage analysis. We considered an assignment of offspring to a pair of parents as an ambiguous assignment if it was less than two times more likely than any other pair (i.e. the difference to the next likely LOD score was <0.69). Offspring with an ambiguous assignment was excluded from further analysis. The seed source was distinguished from the pollen donor based on the chloroplast marker identity as the chloroplast is paternally inherited. We used distances between the offspring and its inferred seed source to quantify the effective seed dispersal kernel. The effective seed dispersal distribution was inferred from the positions of established individuals and represents the recruitment pattern, that is, the transition from adult to the next generation adult (Nathan & Muller-Landau 2000; Piotti *et al.* 2009). The nonambiguous parent pair assignments were also used for determining effective pollen dispersal distances.

To fit an effective seed dispersal kernel, we used the WALD kernel, a closed-form simplification of a mechanistic model for seed dispersal by wind (Katul *et al.* 2005). The WALD kernel is a fat-tailed distribution that has provided a good fit for seed dispersal data sets from various wind-dispersed plant species (Katul *et al.* 2005; Skarpaas & Shea 2007), including Aleppo pine (Schurr *et al.* 2008). For a one-dimensional kernel, we used Katul *et al.* (2005, equation 5b):

$$f(r) = \left[\frac{p}{2\pi r^3} \right]^{1/2} \exp \left[-\frac{p(r-u)^2}{2u^2 r} \right]$$

where r is the dispersal distance, p is the shape parameter, and u is the scale parameter. WALD parameters correspond to basic measurable mechanistic determinants of seed dispersal by wind (see Katul *et al.* 2005). The WALD kernel was fitted to the distribution of offspring-seed source distances obtained by the parentage analysis between 0 and 600 m with 5-m intervals.

To infer the survival kernel (the transition probability for a seed to become a reproductive adult) from the effective dispersal kernel, the seed dispersal kernel is required. We used the mechanistic seed dispersal model WINDISPER-L (Nathan *et al.* 2001, 2002) to estimate the seed dispersal kernel from the five ancestor trees. This model was selected because it uses a logarithmic wind profile reflecting the wind regime typical of the open landscape condition to which the ancestor trees were exposed. The parameters of the model include seed terminal velocity, height of seed release, wind direction, horizontal wind speed, friction velocity, displacement height, roughness length and vertical wind speed. The terminal velocity was estimated for Aleppo pine seeds by analysing video photographs of falling seeds (Nathan *et al.* 1996). The height of seed release was estimated by field measurements of adult trees in the study site and in another Aleppo pine population in Mt. Carmel (Nathan *et al.* 2001). Wind measurements were obtained from a meteorological station placed in an open landscape within our study site (Nathan *et al.* 2002), resembling the conditions experienced by the five ancestor trees. The seed dispersal kernel was calculated as the probability of seed arrival at distances of 0–600 m per 1 m. The probability of survival was then calculated by dividing the effective dispersal kernel by the seed dispersal kernel.

The azimuth from an offspring to its inferred seed source was calculated based on their coordinates. The distribution of offspring-seed source angles was compared with the typical distribution of wind directions. Wind direction measurements were obtained by sonic anemometer at 8 Hz averaged over half-hour intervals during the main seed dispersal season (24 March–24 June 2008). Measurements were taken above the canopy (at 17 m height) to better reflect the open landscape wind conditions in the early stages of the population establishment (Fig. 1). Circular histograms and calculation of the circular mean for wind directions and angles from offspring to parents were produced using the software Oriana version 2.02e (Anglesey, Wales).

Female and male reproductive success was compared between the five ancestor trees by calculating the

number of offspring mothered and fathered by each potential parent. A X^2 test was conducted to test for uneven reproductive contribution. Spearman correlation was used to test for correlation between the mother and the father reproductive contribution.

Results

Parentage analysis

A total of 33 alleles were detected in the eight nuclear markers and five alleles in the chloroplast marker. Three ancestor trees shared one chloroplast haplotype, and the other two ancestor trees shared a second chloroplast haplotype. The number of alleles per marker ranged between 2 and 12 (Table 1). The genetic variability found in the set of nuclear markers combined with the chloroplast marker gave a high exclusion probability (0.902 for single parent and 0.995 for parent pair). The threshold for determining parentage was based on simulating 100 000 offspring genotypes with both parents among the five ancestor trees of the population and 100 000 offspring genotypes generated based on population allele frequency. LOD threshold values of 2.0 for determining the true parent and 3.2 for determining the true parent pair were selected based on the intersection of the distribution of the LOD scores for the two sets of simulated offspring (Gerber *et al.* 2000). The simulated data were used to evaluate the potential performance of the parentage analysis. Estimated probabilities of correct classifications were 85.7% and 71.6% for the single parent and parent pair assignments, respectively.

The test of parentage analysis revealed that 96 of the 122 trees that germinated in the study area before 1957 had two parents among the five ancestors, 18 had only one parent among the five ancestors, and eight had no parents among the five ancestors. The 18 trees (15%) that had only one parent among the five ancestor trees (Fig. 1) are assumed to be the result of pollen gene flow from outside the study area in accordance with Dow & Ashley (1996). In total, 210 ($96 \times 2 + 18$) gametes were produced by the five ancestors and 34 ($8 \times 2 + 18$) gametes were produced by other sources, determining an immigration rate of 13.9% by pollen and seeds combined. A level of 0.139 immigration per 13 years translates to an annual immigration rate of 0.01 (Smouse *et al.* 2007). Simulation tests indicated that the cryptic gene flow was slightly negative (–1.2%), which can be interpreted as not different from zero, thus confirming that the gene flow was not underestimated. The type II error was 0.24, indicating that gene flow is probably lower than measured. Of the 96 trees that had two parents among the five ancestors, 85 had nonambiguous

assignments (difference in LOD score to the next likely parent pair higher than a threshold of 0.69) and were used for further analyses. Of these 85 trees (two parents among the five ancestors) and 18 trees (one parent among the five ancestors), 57% (59 of 103) were the result of selfing events.

Estimating effective seed dispersal kernel

For 75 of the 85 trees with two parents among the five ancestors (nonambiguous assignment), we were able to accurately determine the identity of the pollen donor and the identity of the seed source (Fig. 2). This was possible when the pair of parents differed in their chloroplast haplotypes or when a selfing event occurred.

Identification of the seed sources enabled the quantification of the effective seed dispersal kernel. The range of distances between mother trees and their offspring was 5.2–492.2 m with a mean of 41.6 m, a median of 28.0 m and a standard deviation of 63.4 m. The fitted

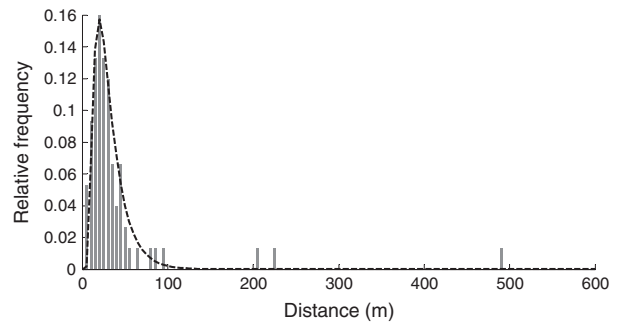


Fig. 3 Relative frequency in 5-m interval of effective seed dispersal distances within the 60-ha plot for the 75 trees with identified seed source. The dashed line represents the fitted WALD kernel ($R^2 = 0.95$).

WALD kernel (Katul *et al.* 2005) showed a good agreement with the distribution of the effective dispersal distances (Fig. 3) with a shape parameter (p) = 95.84 m and a scale parameter (u) = 31.76 m ($R^2 = 0.95$).

Estimating survival kernel

The survival kernel was inferred from the effective dispersal kernel obtained by the parentage analysis (Fig. 3) and the seed dispersal kernel that was based on WINDISPER-L simulations (Nathan *et al.* 2002). The survival kernel was expressed by the probability of effective dispersal divided by the probability of seed arrival. The probability of seed arrival, probability of

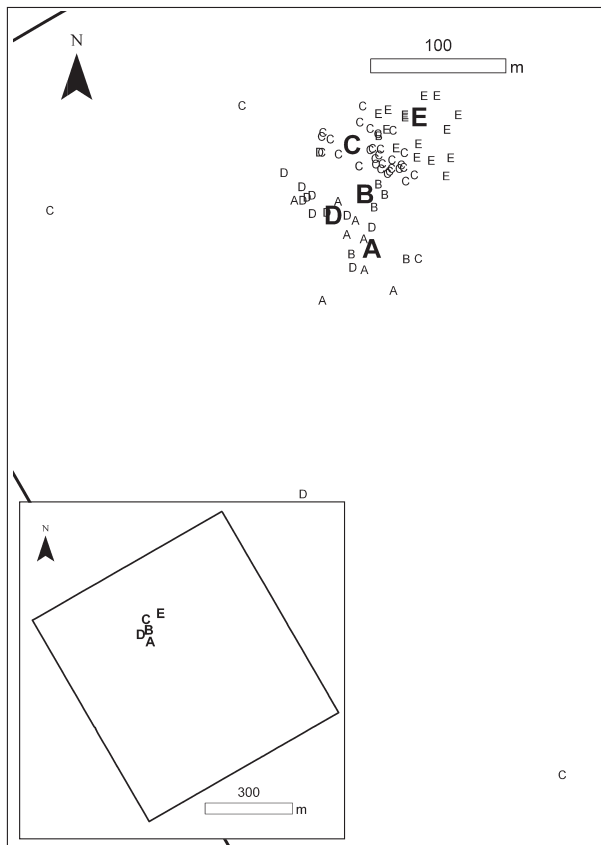


Fig. 2 The distribution of 75 trees with identified seed source that germinated in 1944–1957 in Mt. Pithulim. The big letters represent the five ancestor trees that germinated before 1917. The corresponding small letters represent the offspring of each seed source. The insert shows the entire 60-ha study plot.

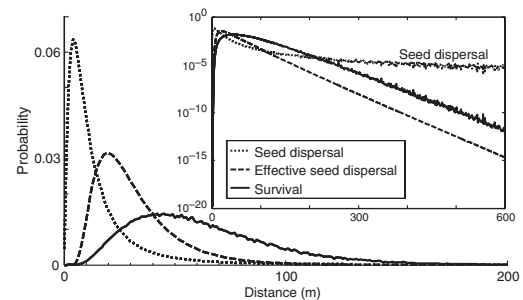


Fig. 4 The seed dispersal kernel, or the probability of seed arrival (dotted line), the probability of effective seed dispersal (dashed line) and the inferred probability of survival (solid line) as a function of distance from the seed source. The seed dispersal kernel is based on 1 000 000 random seeds simulated by WINDISPER-L (Nathan *et al.* 2002). The effective seed dispersal kernel is a WALD kernel (Katul *et al.* 2005) that was fitted to the data on offspring-seed source distances obtained by the parentage analysis (Fig. 3). The probability of survival is the effective dispersal kernel divided by the seed dispersal kernel. The insert depicts the three kernels in a semi-log figure to better visualize the differences at the tail part of the distribution.

establishment and the inferred probability of survival as a function of distance from the seed source are depicted in Fig. 4. In accordance with the main prediction of the JC hypothesis, the mode of the effective dispersal kernel (20 m) shifted farther away from the parent plant relative to the mode of the seed dispersal kernel (4 m). The probability of survival increased as a function of distance from the seed source in the first dozens of metres, with a peak at 45 m. However, a pattern unpredicted under J-C hypothesis was observed at farther distances: survival decays gradually with distance reflecting the fatter tail of the seed dispersal kernel relative to the effective dispersal kernel (Fig. 4).

Wind regime and effective seed dispersal directions

As predicted, the distribution of angles from offspring to identified mother as revealed in the parentage analysis was in accordance with the distribution of wind directions (Fig. 5). The circular mean was 322.7° for effective seed dispersal angles and 320.5° for wind directions. Seed source was located in two main directions from the offspring $\sim 300^\circ$ and $\sim 120^\circ$. These directions form the main axis of wind directions in the study area. This wind axis has a clear signature on the genetic structure visible in the assignment of offspring to each ancestor tree (Fig. 2).

Reproductive success

The parentage analysis revealed an uneven reproductive success among individuals. The ancestor trees were mothers to 6–34 individuals and fathers to 0–37 individuals (Table 2). The mother and father contribution to offspring was highly biased among the five ancestor trees ($X^2 = 33.3$, $df = 4$, $P < 0.001$ and $X^2 = 56.4$, $df = 4$, $P < 0.001$, respectively). Interestingly, individuals with a low selfing rate also had low reproductive success (Table 2). The reproductive contributions as a mother and as a father were positively correlated (Spearman $R = 0.9$, two-tailed $P = 0.037$), but not significantly correlated when selfing was excluded.

Discussion

A spatial reconstruction of the genealogy of an Aleppo pine population in its early stages of establishment was accomplished in this research owing to several important factors. The detailed spatial historical information on the Mt. Pithulim study site including a series of early aerial photographs and dendrochronological measurements overlaid on a high-resolution map of trees enabled an accurate detection of the potential parents

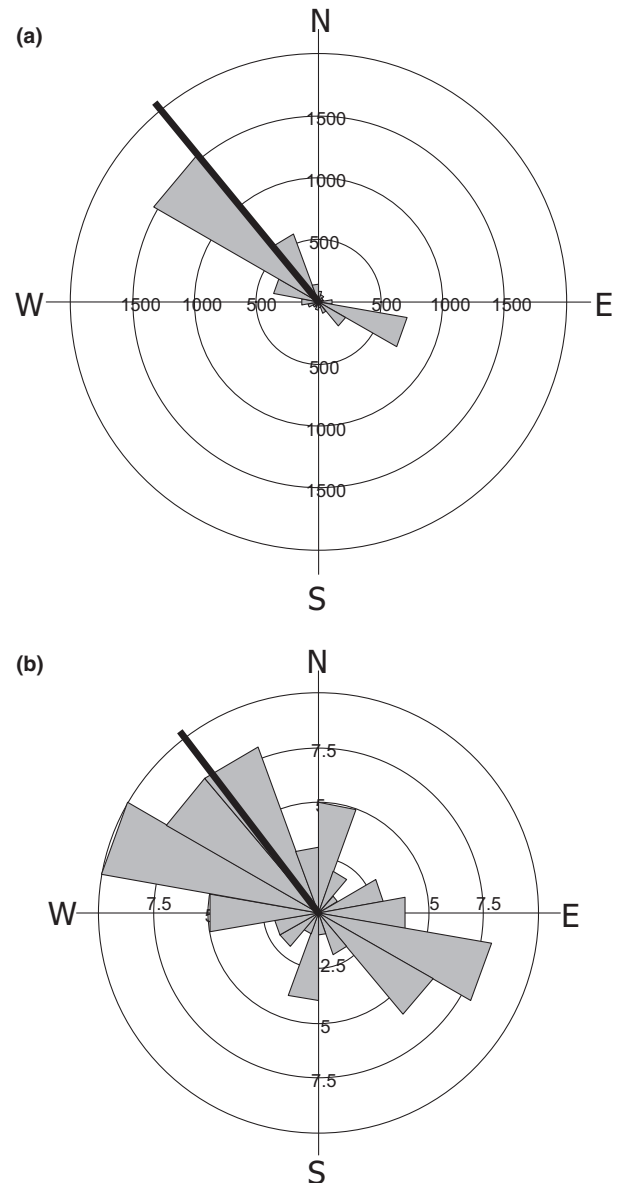


Fig. 5 Distribution of wind directions in the main dispersal season (a) and the angles from offspring to identified mother as revealed in the parentage analysis (b). The bold line represents the circular mean (320.5° for a and 322.7° for b).

and relevant offspring. The genetic distinctness of the population contributed to the ability to exclude nonlocal parents.

The majority of studies on wind dispersal have focused on seed dispersal without consideration of postdispersal survival (e.g. Greene *et al.* 2004; Soons *et al.* 2004; Clark *et al.* 2005; Katul *et al.* 2005). In this study, we measured the effective seed dispersal that encompasses postdispersal processes as well. The effective seed dispersal pattern quantified in this research demonstrates the importance of postdispersal processes

Table 2 The distribution of offspring among the five ancestor parent trees (location is given in Fig. 2). The numbers of offspring mothered and fathered by each ancestor tree are given (the number in parentheses represents these numbers when selfing is excluded) along with the total gametic contribution to the offspring and the selfing rate (proportion of selfed offspring out of the total number of offspring mothered by the ancestor tree)

Ancestor tree name	Seed source (selfing excluded)	Pollen source (selfing excluded)	Gametic contribution	Selfing rate
A	8 (5)	4 (1)	16	0.375
B	6 (6)	0 (0)	14	0
C	34 (1)	37 (4)	72	0.971
D	12 (2)	19 (9)	34	0.833
E	15 (2)	15 (2)	32	0.867

in structuring spatial patterns in plant populations. Specifically, we found strong JC effect in the early stages of population expansion, as reflected in the positive distance-dependent survival at short distances from the seed source.

Based on a meta-analysis of empirical studies that examined early life stages of plants (seeds and seedlings), Hyatt *et al.* (2003) concluded that the JC prediction on the recruitment pattern is generally not supported. However, when their data were analysed separately for seedlings, the JC prediction received greater support and the measured effect was significant. This, together with our results that constitute the first test of the JC prediction for mature (reproductive) individuals, indicates that the distance-dependent JC mechanism becomes more important in later life stages of the tree. Further support for this conclusion comes from spatial analysis of Aleppo pine adults and saplings by Nathan *et al.* (2000). In that study, conducted on Mt. Carmel in Israel, the density of saplings was measured at different distance intervals from the closest adult tree. The maximum density for the 3-year-old saplings was at a 5–10 m distance interval, whereas the 4- and 5-year-old saplings peaked at the 10–15 m distance interval. When seed deposition levels were taken into account, the survival of 3-year-old saplings at low distance intervals was higher than that of the 4- and 5-year-old saplings. This finding indicates that the JC effect strengthens with age, producing a ‘spacing mechanism’ of the seed dispersal kernel mode away from the mother tree for older saplings. Our study suggests that this trend continues at later life stages as the effective seed dispersal kernel for adult trees peaked at even farther distances from the mother tree (mode at 20 m).

Several mechanisms for lower survival of seeds close to adult trees were shown in Aleppo pine. Higher seed predation rates were found close to adult Aleppo pine trees (Nathan & Ne’eman 2004). Germination rate and seedling growth of Aleppo pine were lower under the unfavourable light conditions below the canopy (Schiller 1979). The mechanism for the increased JC effect observed at later life stages in Aleppo pine is yet unknown, but a possible explanation for this pattern is increased competition with conspecific adult trees. This explanation differs from the classical JC mechanism for seed and seedling distribution, which is based on clustering of predators, parasites, pathogens and herbivores near the mother tree.

Our study showed that at farther distances from the mother tree, survival decays gradually with distance. This pattern can be explained by habitat suitability and spatial autocorrelation in environmental conditions (see Nathan 2006 and Nathan *et al.* 2011). Beyond a certain distance, JC effects are likely to be overridden by the opposing effects of spatial autocorrelation in abiotic conditions: farther away from the mother tree, environmental conditions tend to be less similar (Nekola & White 1999) and hence less suitable (Nathan *et al.* 2011). The initial, relatively clustered, distribution of the five ancestor trees of the population in Mt. Pithulim (Fig. 1) may reflect favourable conditions for Aleppo pine survival, not related to pine density, clustered in the middle of the stand. Thus, a gradient of lower survival chance reflects the change in environmental conditions and habitat suitability with increasing distances from the place of establishment of the mother trees. An example for decrease in survival with distance from the mother tree was shown in *Liriodendron tulipifera*, where the higher survival near adult trees was explained by microenvironmental requirements of juvenile trees (HilleRisLambers & Clark 2003).

Although the observed pattern of declining survival from a certain distance from the mother plant can be explained by basic physical and biological principles (Nathan *et al.* 2011), it is important to take into account our limited accuracy in quantifying the tail of the seed dispersal kernel. The tail of the seed dispersal kernel is based on relatively few dispersal events. Extrapolating the shape of the tail of the seed dispersal kernel based primarily on dispersal events at short ranges is problematic (Nathan *et al.* 2003). This, together with the high stochasticity associated with long-distance dispersal (Nathan 2006; Nathan *et al.* 2008), may lead to severe bias in the estimation of the kernel tail. In addition, WINDISPER-L, the mechanistic wind dispersal model, has a much better fit to the empirical seed trap data for short-distance dispersal (distance to the nearest adult tree <20 m) with $R^2 = 0.81–0.86$ than for the

longer dispersal distances (distance to the nearest adult tree >20 m) with $R^2 = 0.59\text{--}0.61$ (Nathan *et al.* 2002). This implies that the model validation for the tail is relatively weak and calls for caution in concluding on the survival pattern at farther distances from the mother tree.

In this study, we quantified the overall effect of spatial postdispersal processes by integrating a genetic-based parentage analysis and a mechanistic model of seed dispersal. This method enabled coping with the challenge of assessing the effective seed dispersal including the adult stage in a long-living species (Schupp *et al.* 2010) and disentangling the recruitment phase from the pattern of seed movement. Our approach confronts the realized seed dispersal distance with the seed dispersal to quantify postdispersal spatial patterns in survival. The method is not limited to seed dispersal by wind and can be implemented for plants with quantifiable distributions of seeds including plants with multiple dispersal vectors (Jordano *et al.* 2007; Spiegel & Nathan 2007) and with multimodal seed distributions (e.g. Russo *et al.* 2006; Jordano *et al.* 2007). Although comparison of the effective seed dispersal kernel with the seed dispersal kernel has great potential for understanding spatial survival patterns, such analyses are rare. Based on parentage analysis for the vertebrate-dispersed Neotropical tree *Simarouba amara*, average seedling recruitment distance was estimated to exceed 390 m (Hardesty *et al.* 2006), as compared with an average seed dispersal of 39 m estimated using inverse modelling (Muller-Landau 2001). González-Martínez *et al.* (2006) found that the average sapling recruitment distance of *Pinus pinaster* (~75 m) revealed by parentage analysis was greater than the average seed dispersal distance (12 m) estimated by inverse modelling. The discrepancies between the effective seed dispersal kernel and the seed dispersal kernel were explained by distance- and density-dependent mortality of seeds (González-Martínez *et al.* 2006; Hardesty *et al.* 2006). For the *S. amara*, Hardesty *et al.* (2006) suggested a complicated density effect on seedling recruitment involving an elevated chance of survival nearby non-parent adults.

The use of genetic data for analysis of seed dispersal directionality can give important insights into dispersal mechanisms. However, linking genetic patterns to behaviour of dispersal agents has received very little attention thus far. Jones *et al.* (2007) attributed a spatial change in local-scale genetic structure between cohorts of *Eucalyptus globulus* to the prevailing westerly winds. García *et al.* (2007) found two prevailing seed deposition angles in the vertebrate-dispersed tree *Prunus mahaleb* using maternity analysis. This directionality was explained by the spatial arrangement of microhabitats

influencing the movement behaviour of frugivores. The analysis of effective seed dispersal directions in the present study, using a direct gene flow method, revealed a strongly anisotropic dispersal pattern. The effective deposition of seeds is dominated by a clear northwest/southeast axis that corresponds to the two prevailing wind directions in the site during the dispersal season. This demonstrates that the wind regime can have important implications for the spread of Aleppo pines and for the spatial genetic structure of a wind-dispersed species.

The parentage analysis in this study enabled quantifying the reproductive success of individual trees. The trees in the research area showed marked differences in male and female reproductive success. Skewed distribution of reproductive success was revealed in previous parentage analyses for other tree species (e.g. Aldrich & Hamrick 1998; González-Martínez *et al.* 2006). Unbalanced reproductive success might lower the effective population size (Schnabel *et al.* 1998) and contribute to increased spatial genetic structure (Vekemans & Hardy 2004). The positive correlation of reproductive success with high selfing rate might suggest a limited supply of pollen; this is plausible given the small size of the isolated population in its early stages of expansion.

The observed selfing rate is extremely high relative to the typical selfing rates attributed to pines (Richardson 1998). However, it is consistent with the high selfing rates found in small isolated pine populations (Robledo-Arnuncio *et al.* 2004). The small number of possible parents in the present analysis requires caution in interpreting the observed variation in the reproductive success. However, our results indicate that the mating system might have an important role for the fitness of individuals and population dynamics.

This study demonstrates the importance of postdispersal processes in the effective dispersal of Aleppo pines. Aleppo pine has been introduced throughout the world and is considered as one of the most invasive pine species (Richardson *et al.* 2000). Populations of planted Aleppo pine expand into adjacent natural plant communities (Lavi *et al.* 2005). The strong postdispersal effect structuring spatial patterns in Aleppo pine trees observed in this research is important for the estimation of spread rate. Determining the spread rate based on seed dispersal alone without consideration of postdispersal survival is likely to produce biased estimates (Nathan *et al.* 2011). Incorporation of the effective seed dispersal kernel of Aleppo pine is essential for estimating the risk of spread from planted stands. The highly anisotropic recruitment pattern corresponding to the wind regime revealed by the parentage analysis has also important implications for predicting Aleppo pine spread.

The parentage analysis enabled the detection of long-distance seed dispersal events over hundreds of metres. The long-distance component of seed dispersal has especially important implications for conservation (Trakhtenbrot *et al.* 2005) and is highly relevant considering the invasiveness of Aleppo pine over the world (Richardson *et al.* 2000). The substantial differences between the seed dispersal kernel and the actual distribution of recruiting individuals found in this study emphasize the potential role of postdispersal processes in population and community dynamics of plants.

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Data accessibility

DNA sequences: GenBank accessions AY304036, CR377943, AJ012087, AF195535, AF195536, AF195543, JF803635, JF803641; Sample locations and microsatellite data: DRYAD entry doi: 10.5061/dryad.9f8j2.