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Fire-induced population reduction and landscape opening increases gene flow via pollen dispersal in *Pinus halepensis*

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Abstract

Population reduction and disturbances may alter dispersal, mating patterns and gene flow. Rather than taking the common approach of comparing different populations or sites, here we studied gene flow via wind-mediated effective pollen dispersal on the same plant individuals before and after a fire-induced population drop, in a natural stand of *Pinus halepensis*. The fire killed 96% of the pine trees in the stand and cleared the vegetation in the area. Thirteen trees survived in two groups separated by ~80 m, and seven of these trees had serotinous (closed) prefire cones that did not open despite the fire. We analysed pollen from closed pre and postfire cones using microsatellites. The two groups of surviving trees were highly genetically differentiated, and the pollen they produced also showed strong among-group differentiation and very high kinship both before and after the fire, indicating limited and very local pollen dispersal. The pollen not produced by the survivors also showed significant prefire spatial genetic structure and high kinship, indicating mainly within-population origin and limited gene flow from outside, but became spatially homogeneous with random kinship after the fire. We suggest that postfire gene flow via wind-mediated pollen dispersal increased by two putative mechanisms: (i) a drastic reduction in local pollen production due to population thinning, effectively increasing pollen immigration through reduced dilution effect; (ii) an increase in wind speeds in the vegetation-free postfire landscape. This research shows that dispersal can alleviate negative genetic effects of population size reduction and that disturbances might enhance gene flow, rather than reduce it.

Keywords: dilution effect, disturbance, fire, gene flow, pollen wind dispersal, population reduction

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Introduction

Dispersal of individual organisms, gametes or propagules, is the movement process ultimately responsible for gene flow across the landscape, within and between populations (Slatkin 1987; Adams 1992; Hamrick & Nason 1996). In plants, pollen and seed dispersal distances determine the scale and extent of gene flow and are among the most important factors in determining the population spatial genetic structure (Loveless & Hamrick 1984; Hamrick & Nason 1996; Troupin *et al.* 2006), especially at small spatial scales (Vekemans & Hardy 2004). Seed and pollen dispersal are influenced by the mechanisms and vectors enabling their movement, such as the movement behaviour of animals and the velocity and turbulence of the wind (Howe & Smallwood 1982; Loveless & Hamrick 1984; Nathan *et al.* 2008).

Habitat disturbances could alter gene flow, mating patterns and genetic population structure by opening the landscape, reducing population size and increasing distances between newly created population fragments. Classic population genetics theory predicts that such changes reduce gene flow, increase genetic divergence between small and fragmented populations and decrease genetic variation within them, altogether

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negatively impacting individual fitness and population viability (Ellstrand & Elam 1993; Lowe *et al.* 2005; Bacles & Jump 2011). However, the direction of change in gene flow and ensuing genetic effects may be opposite to these theoretical expectations, when the potential scale of dispersal exceeds the scale of isolation of the disturbed population, overcoming fragmentation and reduction in effective population size (Kramer *et al.* 2008; Bacles & Jump 2011). For instance, landscape opening can affect gene flow patterns by modifying the behaviour or physical properties of biotic or abiotic dispersal vectors, respectively, potentially increasing or decreasing dispersal depending on the specific vectors (Chen *et al.* 1993; Kearns *et al.* 1998), while population reduction can remove local genetic sources and effectively increase the proportion of immigrant alleles (Sork & Smouse 2006). Moreover, even if predisturbance and postdisturbance gene flow are comparable, genetic patterns could be altered because the incoming gene flow is less or more diverse, the number of donors is lower or higher (Sork & Smouse 2006), or the genetic composition entirely different than the predisturbance one (Iwaizumi *et al.* 2010).

Pollen dispersal is often considered as having the greatest chances of alleviating negative genetic effects of fragmentation and population reduction, especially in wind pollination when the small, lightweight pollen could potentially travel great distances (Hamrick 2004; Petit & Hampe 2006; Robledo-Arnuncio 2011). Although empirical results from plants in fragmented landscapes show a rather mixed picture (Lowe *et al.* 2005; Aguilar *et al.* 2008; Kramer *et al.* 2008), a growing body of evidence from both animal- and wind-pollinated trees suggests that spatially isolated trees and tree population fragments can receive extensive pollen gene flow and are not necessarily genetically isolated (Schuster & Mitton 2000; Hamrick 2004; Bittencourt & Sebbenn 2007; Bacles & Jump 2011; Robledo-Arnuncio 2011). To further contrast the expected adverse genetic effects of population size reduction, a few, mainly agronomic, experimental studies suggest that increasing population size and/or density may in fact have a negative effect on pollen gene flow (Handel 1983; Rognli *et al.* 2000; Aylor *et al.* 2003). Several studies comparing forest stands with different densities have also negatively associated conspecific or total vegetation density with various measures of pollen wind dispersal (Adams 1992; El-Kassaby & Jaquish 1996; Dyer & Sork 2001; Millerón *et al.* 2012; Piotti *et al.* 2012). Unlike fragmentation, however, this line of investigation has not received much attention.

The common approach used for studying the genetic consequences of population reduction, fragmentation or disturbances in general, is either examining only postdisturbance populations, comparing sites differing in their time-since-disturbance, or comparing different predisturbance and postdisturbance populations in different sites. This 'space-for-time substitution' (or 'chronosequence') approach, perhaps irreplaceable for studying long-term consequences, can provide limited insight or can even be misleading (Johnson & Miyanishi 2008), as any difference that may arise among sites and populations could be due to different population and genetic histories, environmental conditions, dispersal vector patterns, distance to other pollen or seed sources or other unknown factors unrelated to the phenomenon in question. On the other hand, comparison to a site too close to the focal site might not be informative, as the disturbance may indirectly impact an area larger than the one it directly affects, for instance through changing the behaviour or properties of dispersal vectors.

Following the genetic dynamics of the same population or individuals in the same site before and after disturbance has very rarely been accomplished. Most studies compared different pre and postdisturbance life stages or did not investigate gene flow directly (i.e. at the pollen or seed level), making it hard to draw inferences on proximate, direct effects on gene flow (but see Cloutier *et al.* 2007).

The present study aimed to investigate how landscape opening and reduction in population size and density affect plant pollen-mediated gene flow. Our study case, a stand of *Pinus halepensis* Miller (Pinaceae), is the first to follow the *same* plant individuals in a natural population and directly investigate gene flow before and after a fire-induced population drop and landscape opening. To overcome the common problem of a lack of predisturbance genetic data, we used the few fire-surviving trees as natural 'pollen traps', capturing and storing pollen within serotinous cones that remained closed despite the fire and hold within them seeds that have been pollinated before the fire, providing a unique opportunity to 'look into the past' – into the pollen pool of the predisturbance population.

We hypothesized that, as the scale of pollen dispersal in this wind-pollinated tree species is potentially greater than the scale of isolation of the disturbed study stand, the large-scale vegetation clearing, local-scale population drop and drastic thinning of the neighbourhood of each surviving individual will increase gene flow via effective pollen dispersal. We depict the extent of gene flow using mating patterns (correlated matings) and spatial genetic structure of the pre- and postfire pollen pools, and break them down into components of identified local vs. unidentified putative local or immigrant pollen.

Materials and methods

Study species and area

Pinus halepensis (Aleppo pine) is a wind-pollinated, wind-dispersed tree and an obligate seeder that regenerates only from canopy-stored seed banks in serotinous cones that open under hot and dry weather or fire (Ne'eman *et al*. 2004).

The research was conducted in a ridge in the lower southwestern area of the Carmel range of Israel (Fig. 1a; 32°41′25″N, 34°58′12″E; altitude 110 m a.s.l). The focal *P. halepensis* stand of this study is the westernmost stand of trees on the ridge (Fig. 1a). The closest adult *P. halepensis* is located 270 m south of the stand, with a few more isolated trees within a radius of 400 m. The three largest stands in the close vicinity consist of 20–40 trees 400–1200 m from the focal stand (Fig. 1a), all natural (native). Farther away (>1.8 km), there are some large and continuous planted *P. halepensis* forests.

In October 1998, a large fire burnt through 517 hectares of scrub and *P. halepensis* stands including the focal stand (Fig. 1a). This relatively isolated, natural stand has been studied prior to the fire (e.g. Nathan *et al.* 2000) and knowledge of the population dynamics before and after the fire is fully available (Table 1; see also Appendix S1, Supporting Information). Only 13 of 363 *P. halepensis* trees survived the fire, a drastic, 96% drop in size and density (Table 1; Fig. 1b). The fire cleared all other vegetation in the stand, with only a few olive trees surviving in its western-most boundary. Ten years after the fire, the stand consisted of the 13 fire survivors and 30 saplings, some of which already reached maturity (Table 1), with considerable regrowth of the scrub vegetation.

The 13 surviving trees were located in two distinct north–south spatial groups, ~80 m apart, which were also vaguely apparent in the prefire population (Fig. 1b). This clustering was confirmed by the affinity propagation clustering algorithm (Frey & Dueck 2007) using negative squared Euclidean distances as similarities and their median as a common preference value.

Sample collection

Although no prefire genetic data were obtained in the previous studies, a few of the surviving trees had prefire serotinous cones that remained closed despite the fire until the current study. These serotinous cones contain seeds that have been pollinated before the 1998 fire and thus enable estimating the prefire pollen pool, assuming that the 13 surviving trees represent an unbiased sample of the prefire population. Thus, the prefire serotinous cones serve as a 'self-control' group to examine fire effects. In addition, a nearby stand in the first ridge south of the focal population, outside the 1998 fire boundaries (Fig. 1a), was initially chosen to serve as a more traditional 'space-for-time' control site. However, due to sample size limitations (see below), results from this external control group (Appendix S2, Supporting Information) should be interpreted with caution (see Discussion).

Closed cones were collected in 2010 from three cohorts representing different time frames of interest: prefire, early postfire and late postfire (Table 1). Cone ages were initially defined by colour (grey >= 4-year-old; brown = 3; green = 2) and by location on the branch and tree (the nearer to the trunk, and the lower the branch, the older). The exact age of all collected cones was verified at the laboratory by counting annual rings on the branch where the cone is attached (S. Lev-Yadun, personal communication): core samples were extracted, or the branch was sliced, under the cone and sanded, and annual rings were counted under stereo microscope.

The existence of prefire serotinous cones was the limiting factor in determining the choice of maternal trees. Of the 13 surviving trees in the focal population, seven had cones from all three cohorts, four in the northern and three in the southern spatial group (Fig. 1b; Table S1, Supporting Information). Old serotinous cones were much harder to find in the fire-free stand: only four trees had cones from all three cohorts. Six were eventually chosen as maternal trees in the fire-free stand (Table S1, Supporting Information).

In addition to cones, fresh needle samples were taken from the 13 surviving adult trees in the focal stand and from the six maternal trees in the fire-free stand, in order to obtain the adult genotypes.

Inferring the pollen genetic composition

The paternal nuclear genetic contribution to each seed was isolated by subtracting the megagametophyte haplotype from the diploid embryo genotype, as the megagametophyte tissue surrounding the embryo in the pine seed is maternally derived and its haploid nuclear genotype equals the maternal contribution to the embryo. The embryo chloroplast is paternally inherited.

Selected cones were opened after 12 h in 70 °C, 10% relative humidity in a climate chamber (CLIMACELL, MMM Medcenter Einrichtungen GmbH). Seeds were easily removed from the opened cones and placed in distilled water for 12 h (old cones) or 24 h (young cones), helping both in removing the hard seed coat and in determining whether the seed was ripe and full (sank in water) or empty (floated).

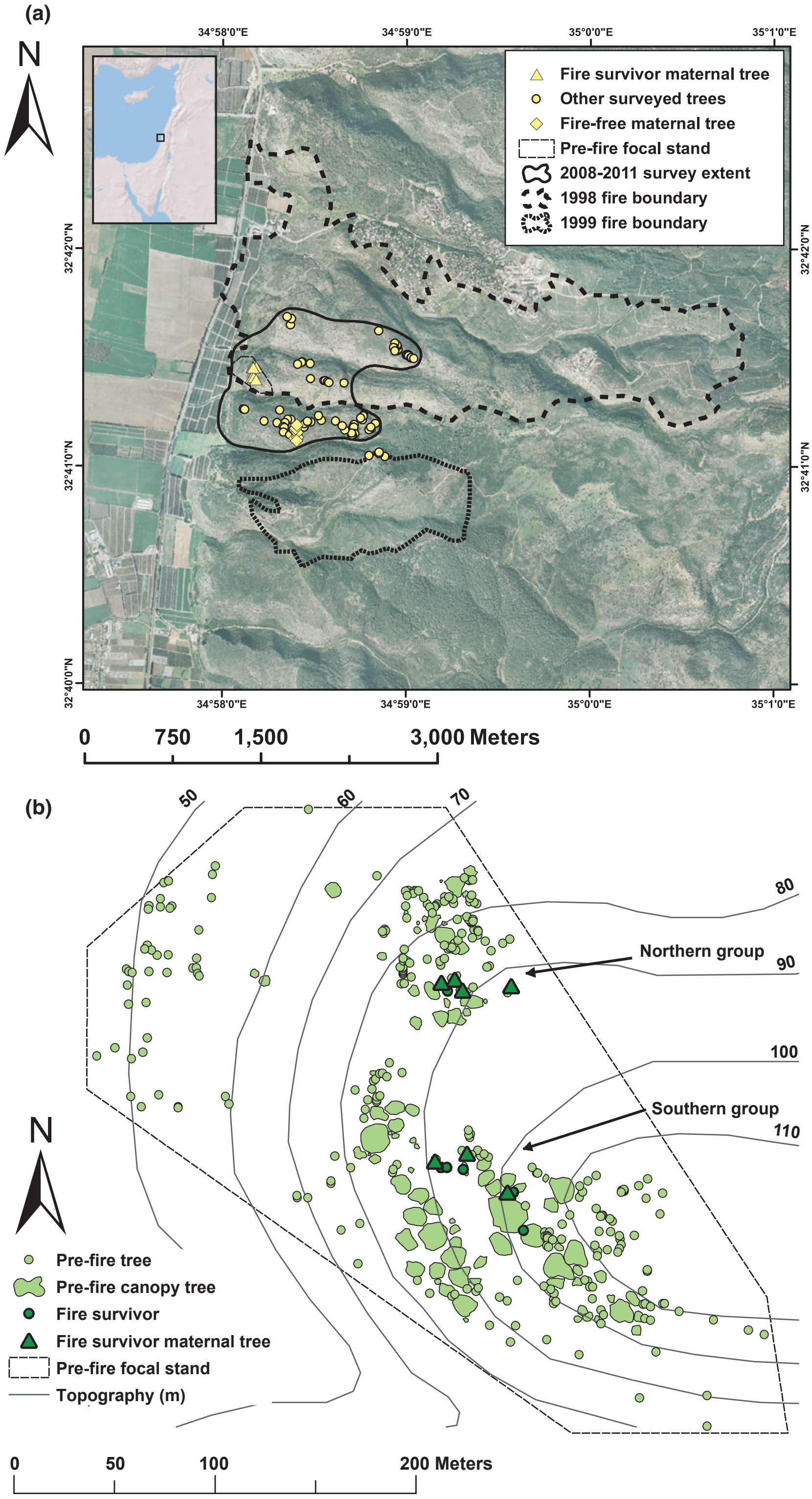

Fig. 1 (a) Map of the study area. Dotted areas mark the boundaries of the 1998 and 1999 fires (see Discussion) and of the prefire focal stand (see b). Sampled maternal trees are marked as triangles (focal stand) or diamonds (fire-free stand; outside the fire boundaries). Other adult *P. halepensis* trees found in the 2008–2011 survey (survey area marked in black polygon) are marked in circles. (b) A zoomed-in view of the focal stand from spring 1998, before the fire. Polygons denote the largest 95 canopy-forming trees. Circles mark the other 268 trees. The 13 fire-surviving trees are marked in dark: triangles indicate the seven maternal trees (same as in a) and circles indicate the six nonmaternal trees.

Embryos of randomly selected full seeds were delicately and cleanly separated from the surrounding megagametophyte under binoculars using disposable sterile scalpel blades, avoiding cross-contamination between the two. The embryo and megagametophyte were then placed in separate 2-mL Eppendorf tubes and stored at −80 °C until DNA extraction. Ten seeds per tree per cohort were taken in this manner for

Table 1 Cohort definitions, stand population dynamics and sample sizes. See Results for definitions of *identified* and *unidentified*

Cohort	Cone ages	Time of cone pollination (years after fire)	Number of *P. halepensis* trees in stand	Stand density (*P. halepensis* trees/ha)	Sample size			
					Cones	Total pollen	*Identified* pollen	*Unidentified* pollen
Prefire	>12	−2 to −8	363	132	10	69	25	44
Early postfire	9–11	1–3	13	6	13	70	37	33
Late postfire	2–4	8–10	43	18	14	70	42	28

subsequent DNA extraction. A pine cone gathers many pollen grains for days to weeks before fertilization (e.g. Greenwood 1986; Owens *et al.* 2001), so a cone is expected to represent more than one pollination event (we estimated, on average, at least 2.7 different fathers per five seeds of the same cone; data now shown). Nevertheless, except for few cases when cones or seeds were limited, two cones were selected and five seeds taken from each, in order to lessen the effects of possible dependency between the pollen within a cone. Final sample sizes are given in Table 1 with additional details in Table S1 (Supporting Information).

DNA extraction and amplification

Needles, megagametophytes and embryos were homogenized each in a 2-mL Eppendorf with 50 μL double-distilled water and four glass beads, in a Mini-Beadbeater-96 (BioSpec Products) run for 1:45 min. The Eppendorf was then directly used as starting material for total genomic DNA extraction using either G-spin IIp Plant Genomic DNA Extraction Kit (iNtRON Biotechnology) or DNeasy Plant Mini Kit (Qiagen). We used seven nuclear and three chloroplast microsatellite markers (details in Table S2, Supporting Information) following Steinitz *et al.* (2011).

The PCR products were joined in multiplexes and scored using an ABI 3730*xl* DNA Analyzer (Applied Biosystems). Manual scoring of PCR product sizes with reference to a 500-Liz standard (Applied Biosystems) was done using GENEMAPPER Software, version 4.0 (Applied Biosystems). Independent DNA extractions are impossible for seeds, but we always compared the PCR scores among the megagametophyte, embryo and maternal needles of each seed for validation and in order to verify no errors occurred in DNA extraction, scoring and sample assignment. Diploid embryos were also always checked against their respective megagametophytes to infer the paternal contribution to the embryo genotype as described above. Other than five failed allele amplifications in three embryos, no errors or mismatches were found. PCRs and scoring of ~15% of the samples were repeated for further validation.

Data analysis

All subsequent analyses were done on the *haploid* pollen data and not on the diploid seeds, apart from inbreeding coefficient calculations and analyses of the adult trees.

Standard genetic diversity indices (Tables S3 and S4, Supporting Information) were calculated in GENEALEX, version 6.4 (Peakall & Smouse 2006). F_{ST}, a measure of the genetic divergence among sample units and its confidence intervals were calculated in GDA, version 1.1 (Lewis & Zaykin 2001). We used POWSIM, version 4.1 (Ryman & Palm 2006), to evaluate the statistical power of our seven nuclear loci for detecting genetic differentiation with an expected $F_{ST} = 0.05$ ($N_e = 5000$, $t = 513$) and the allele frequencies of each cohort separately. Power was 0.99 using the same sample sizes and allele frequencies of each of the cohorts, decreasing to 0.77 in the extreme case of the smallest of our sample sizes.

The pairwise kinship coefficient (F_{ij}) estimator of Loiselle *et al.* (1995), calculated in SPAGEDi, version 1.3d (Hardy & Vekemans 2002), was used to measure F_S, the degree of genetic relatedness between individuals (pollen, i.e. paternal gametes) within maternal progeny arrays (cones) (Hardy *et al.* 2004). Calculating F_S is a way of inferring whether a mother is sampling nonrandom pollen, which in wind-pollinated plants could most likely be the result of spatially restricted pollen dispersal, a limited number of pollen donors, asynchronous male flowering and unequal male fecundity (Robledo-Arnuncio *et al.* 2004), hence an estimate of the degree of correlation between mating events. The multilocus correlation of paternity (r_p), which should be comparable with $2F_S$ (Hardy *et al.* 2004), was calculated using MLTR, version 3.4 (Ritland 2002). MLTR was run with all seeds together, cohort as grouping variable and megagametophyte specified, using 1000 bootstraps for variance estimation.

In order to assess selfing and to break down the F_{ST} and kinship results into components of identified (non-excluded) vs. unidentified (excluded) putative local or immigrant pollen, we carried out simple paternity exclusion by comparing the multilocus pollen haplotype to the multilocus genotypes of the 13 fire-surviving adults in the focal stand, using a Matlab (The MathWorks) code written for this purpose. If an allele in the pollen haplotype was absent from the adult genotype, that adult was excluded as a potential father for this seed. This straightforward paternity exclusion may be justified in this case, where there are very few candidate fathers in our defined stand boundaries and the multilocus probability of exclusion is high (see Results) (Jones & Ardren 2003). Furthermore, as the mother was always known for every seed sampled, the presence of null alleles, mutations and scoring errors that can cause the seed (or megagametophyte) to be incompatible with its mother is easily detected (Jones & Ardren 2003). The combined multilocus probability of paternity exclusion was calculated using FAMOZ (Gerber *et al.* 2003). Selfing rates were also obtained through the multilocus population outcrossing rate (t_m) using MLTR and compared with those estimated by simple paternity exclusion.

T-test and One-Way ANOVA with Bonferroni *post hoc* tests were conducted in Matlab.

Results

Among-cohort genetic diversity and differentiation

Standard genetic diversity indices of the pollen pool were very similar and not significantly different between the three cohorts (Table S3, Supporting Information) (One-Way ANOVA; $P > 0.90$ for all tests). Pairwise F_{ST} of the pollen pool between the three cohorts was very low and insignificant in all three comparisons ($0.002 < F_{ST(\text{among-cohorts})} < 0.007$, $P > 0.14$), indicating no pollen genetic differentiation among the cohorts. Thus, it seems the fire and population drop had no effect on the genetic make-up of the pollen pool fertilizing this stand as a whole, and no genetic bottleneck was observed in the sampled pollen.

Within-cohort, among-group genetic differentiation

As mentioned in Materials and Methods, the 13 surviving trees in the focal stand are found in two north–south spatial groups, ~80 m apart, which were also vaguely apparent before the fire (Fig. 1b). Despite this short distance, the two groups of 5 and 8 surviving trees are highly genetically differentiated ($F_{ST\,(\text{adults})} = 0.264$, $P = 0.002$), likely indicating a genetic structure that was already established in the prefire tree population, and not some result or artefact of the fire. The very high genetic differentiation is also kept in the subsample of seven maternal trees (four in the northern group, three in the southern) ($F_{ST(\text{maternal})} = 0.172$, $P = 0.014$).

The genetic differentiation of the surviving trees is also reflected in the pollen sampled by the maternal trees. F_{ST} analysis of the pollen between the north and south groups reveals high genetic differentiation in all three cohorts, with a nonsignificant yet relatively large decrease in F_{ST} in the early postfire cohort (Fig. 2a) ($F_{ST\,(\text{prefire, total})} = 0.094$, $P < 0.001$; $F_{ST(\text{early postfire, total})} = 0.053$, $P = 0.004$; $F_{ST(\text{late postfire, total})} = 0.118$, $P < 0.001$).

Pollen kinship within maternal sibships

The average pairwise kinship coefficient, or degree of genetic relatedness, within maternal sibships (i.e. between the paternal gametes sampled within each maternal tree, or F_S) was significantly higher than zero in the prefire cohort ($F_{S(\text{prefire, total})} = 0.105$, $P < 0.001$) (Fig. 3a), indicating highly correlated mating events and few different pollen donors per tree. In the early postfire cohort, pollen kinship decreased sharply and became not significantly different from zero ($F_{S(\text{early postfire, total})} = 0.021$, $P = 0.287$), indicating that the paternal gametes fertilizing the seeds within each maternal tree were a random sample from the general pollen pool (i.e. uncorrelated mating events). In the late postfire cohort, kinship increased to the same level as before the fire, again indicating highly significant nonrandom mating ($F_{S(\text{late postfire, total})} = 0.106$, $P < 0.001$) (Fig. 3a). The decrease in $F_{S(\text{early postfire, total})}$ is significant (One-Way ANOVA with Bonferroni *post hoc* test; $F_{2,934} = 5.47$, $P < 0.01$).

The multilocus correlation of paternity (r_p) was close to $2F_S$ as expected (Hardy *et al.* 2004) ($r_{p(\text{prefire, total})} = 0.194$ (95% CI: 0.051–0.337), $r_{p(\text{early postfire,}}$

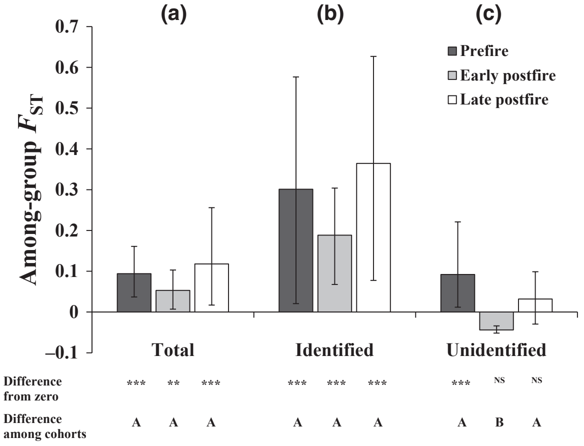

Fig. 2 (a) Pollen genetic differentiation (F_{ST}) among the north and south groups within each cohort. Errors bars are 95% CI. (b,c) As in (a), but for *identified* (b) and *unidentified* (c) pollen only. See Results for definitions of *identified* and *unidentified*. $^{NS}P > 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

$_{\text{total})}$ = 0.055 (0.024–0.086), $r_{\text{p(late postfire, total)}}$ = 0.212 (0.047–0.377), outcrossing fixed at 1 following de-Lucas *et al.* (2008). When outcrossing was estimated jointly with r_p, late postfire r_p changed to near zero ($r_{\text{p(late postfire, total)}}$ = −0.026 (−0.373–0.321), probably due to the much higher levels of selfing in this cohort (see below).

Paternity exclusion and separation of unidentified pollen

The combined paternity exclusion probability, including the three chloroplast loci as one locus with six alleles, was 0.901 for single unknown parent and 0.992 for unknown parent pair. Not surprisingly, for a substantial number of seeds (40–64%), the 13 fire survivors were excluded as potential fathers (*unidentified;* Fig. 4).

Levels of apparent selfing (i.e. the nonexcluded father is the maternal tree) were quite high and similar in the prefire and early postfire cohorts (26%), but increased sharply to 43% in the late postfire cohort (Fig. 4). The population-level multilocus estimates of outcrossing rate (t_m) obtained from MLTR are nearly identical to these results ($t_{\text{m(prefire)}}$ = 0.751, $t_{\text{m(early postfire)}}$ = 0.744, $t_{\text{m (late postfire)}}$ = 0.579). The inbreeding coefficient (Table S3, Supporting Information) was significant only in the early postfire cohort (1-sample t_6 = 2.47, P = 0.048).

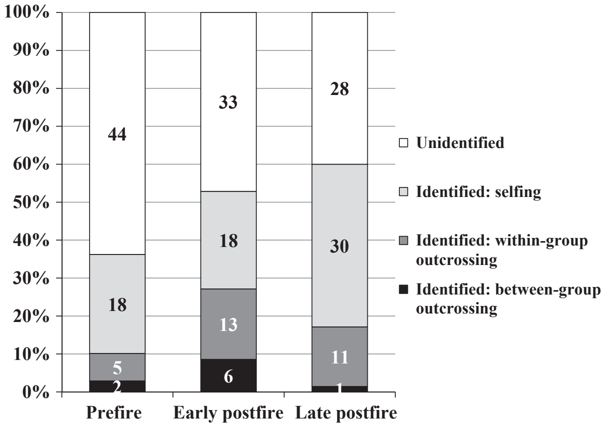

Fig. 4 Paternity exclusion analysis on the sampled pollen. Numbers within bars indicate number of cases in each category. Within- and between-group refers to the northern and southern spatial groups. See Results for definitions of *unidentified* and *identified*.

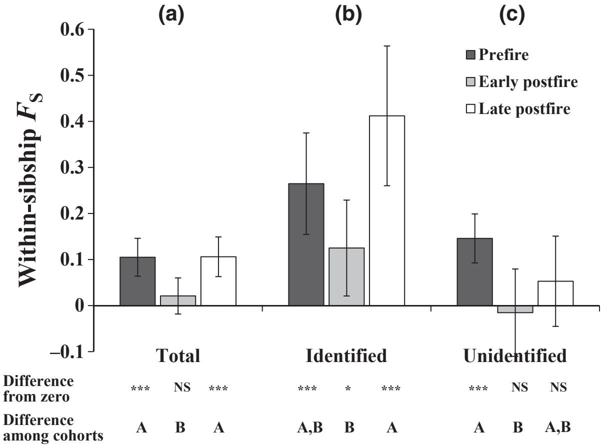

Fig. 3 (a) Average pollen kinship coefficient within maternal sibships (F_S) of each cohort. Error bars are 95% CI. (b, c) As in (a), but for *identified* (b) and *unidentified* (c) pollen only. See Results for definitions of *identified* and *unidentified*. $^{\text{NS}}P > 0.05$; $^{*}P < 0.05$; $^{***}P < 0.001$.

Genetic differentiation and kinship of the unidentified pollen

Because a substantial fraction of the sampled pollen (36–60%) likely originated from the surviving adults (the *identified* pollen; Fig. 4), and because there were very few *identified* cross-group pollination events (1–9%; Fig. 4), the strong spatial genetic structure of the surviving adults likely confounds the among-group pollen F_{ST} and the kinship results (Figs 2a and 3a), masking possible patterns of gene flow. We therefore separated the *identified* from the *unidentified* pollen and analysed them again, separately, for between-group F_{ST} and kinship within maternal sibships.

The *identified* pollen, not surprisingly and in accordance with its assignment to the highly structured surviving adults, showed very high levels of differentiation between the north and south spatial groups, just like the adults do (Fig. 2b; $F_{\text{ST(prefire, identified)}}$ = 0.301; $F_{\text{ST(early postfire, identified)}}$ = 0.188; $F_{\text{ST(late postfire, identified)}}$ = 0.364; $P < 0.001$ for all three). The decrease in the early postfire cohort reflects the increase in identified cross-group geneflow events (Fig. 4), and the increase in the late postfire cohort reflects the increase in apparent selfing (Fig. 4). Interestingly, however, the *unidentified* pollen (Fig. 2c) was also highly structured before the fire ($F_{\text{ST (prefire, unidentified)}}$ = 0.092, $P < 0.001$), much like the surviving adults and the *identified* pollen, but became completely undifferentiated in the early postfire cohort ($F_{\text{ST (early postfire, unidentified)}}$ = −0.044, P = 0.993) and remained so in the late postfire cohort ($F_{\text{ST(late postfire, unidentified)}}$ = 0.032, P = 0.156), unlike the adults of the population and the total and *identified* pollen (Fig. 2a,b). The decrease in $F_{\text{ST(early postfire, unidentified)}}$ is significant (calculated by comparing the distributions of 10000 bootstraps of each *unidentified* cohort, $P < 0.01$ after Bonferroni correction). It is important to note that the *unidentified*, undifferentiated pollen in the early postfire cohort must have come from outside the stand as it did not originate from the 13 fire-surviving adult trees. This indicates that distant and mixed pollen sources outside the study stand produce a homogeneous pollen pool, as reasonably expected from a fat-tailed dispersal kernel (Klein *et al.* 2006). Furthermore, the north–south differentiation shown before the fire by both the *identified* and the *unidentified* pollen suggests that they largely originated from the same local (i.e. within-population) sources that were themselves highly differentiated.

F_S portrayed a very similar picture to F_{ST}: the *identified* pollen (Fig. 3b) exhibited very high levels of genetic relatedness in all three cohorts ($F_{S(\text{prefire, identified})} = 0.265$, $P < 0.001$; $F_{S(\text{early postfire, identified})} = 0.125$, $P = 0.012$; $F_{S(\text{late postfire, identified})} = 0.412$, $P < 0.001$), whereas the *unidentified* pollen (Fig. 3c), which also exhibited very high F_S before the fire ($F_{S(\text{prefire, unidentified})} = 0.146$, $P < 0.001$), became no different than random after the fire ($F_{S(\text{early postfire, unidentified})} = -0.015$, $P = 0.658$; $F_{S(\text{late postfire, unidentified})} = 0.053$, $P = 0.310$) (Fig. 3c). The decrease in $F_{S(\text{early postfire, unidentified})}$ is significant (One-Way ANOVA with Bonferroni *post hoc* test; $F_{2,237} = 5.02$, $P < 0.01$). The increase in F_S of the total pollen in the late postfire cohort seen in Fig. 3a is therefore largely explained by the *identified* fraction, of which 71% is the result of selfing (Fig. 4), whereas the large *unidentified* fraction still shows much less relatedness than before the fire and not significantly different from zero (Fig. 3c).

Discussion

Disturbances, by reducing populations and opening gaps in the landscape, can typically be regarded as a mechanism turning large areas into barriers for dispersal. The traditional approach of comparing different disturbed and undisturbed sites could provide limited or even misleading insights, especially in population genetics. Here, in the first direct investigation of gene flow on the same plants before and after fire in a natural population, we have shown that landscape opening and population reduction can actually promote gene flow and population connectivity through enhanced pollen dispersal by wind. This generalization, however, likely requires the scale of dispersal to be greater than the scale of isolation of the disturbed population.

Gene flow via pollen in P. halepensis in the study stand

The similarity in genetic diversity and absence of genetic differentiation among the three cohorts of the entire sample of pollen indicate that overall, at a large scale, a pollen pool homogeneous across years flows through the study area. Yet, its spatial genetic structure and genetic relatedness among pollen donors seem to have been affected by the fire. The pattern of gene flow emerging from analysing effective pollen dispersal at three temporal snapshots – before a fire-induced population drop and landscape opening, immediately after the fire and after 8–10 years of postfire recovery – all in the same *P. halepensis* stand, is as follows.

The large, dense prefire population likely consisted of two genetically distinct spatial groups of trees at a north–south orientation. The pollen sampled by our seven surviving maternal trees (four in the northern group, three in the southern) was also highly differentiated, mirroring the spatial genetic structure of the surviving adults. As can be expected, a considerable fraction of prefire pollen was not produced by any of the surviving trees (pollen is *unidentified*); it too was highly differentiated, meaning that it most likely originated from unsampled local adults that did not survive the fire, rather than from some immigrant, global homogeneous pollen pool (see below). Furthermore, both *identified* and *unidentified* pollen showed a high degree of genetic relatedness between the male gametes sampled by each maternal tree, indicating highly correlated mating events and few different pollen donors.

The genetic differentiation and kinship results thus demonstrate that the majority of pollen fertilizing the seeds before the fire originated from trees not only within the population but also within each spatial group, with relatively few events of successful pollen dispersal between the groups despite the very short distance apart. Gene flow via pollen from other populations was probably a fairly rare event.

The fire killed 96% of the *P. halepensis* trees in the stand and cleared nearly all vegetation in its range. Yet, 5 and 8 trees survived in the northern and southern groups, respectively. During the first four postfire years, when these trees were the only local potential pollen donors, pollen movement was far less restricted than before the fire, and incoming gene flow greatly enhanced. We first infer this from the reduction in the among-group spatial genetic structure of the pollen sampled by our seven surviving maternal trees. This was especially prominent in the *unidentified* pollen, which became completely homogeneous and which, unlike the cohort before the fire, must have been immigrant as all surviving adults in the population have been sampled, faithfully representing the ‘global’ pollen pool. Second, the pollen sampled by each maternal tree was significantly less correlated than before the fire, showing a fivefold drop in genetic relatedness and becoming no different than random; again, the *unidentified* pollen showed the most drastic change, signifying the importance of immigration for increasing the number of diverse pollen donors and enriching the local pollen pool.

As the stand started to recover from the fire, its density and number of potential pollen-donor trees increase, and the vegetation around and within it began to grow and close again. Nevertheless, analysis of the *unidentified* pollen separately from the total pollen suggests that high gene flow relative to prefire conditions was still maintained. While the total pollen sampled by the seven maternal trees 8–10 years after the fire again showed significant genetic structure and relatedness within each maternal tree, both very similar to prefire levels, it was only because selfing greatly increased. Although the *unidentified* pollen had significantly different F_{ST} values at early and late postfire cohorts, both F_{ST} and F_S were not significantly different from zero at these cohorts for this subsample, suggesting that pollen immigration acted to increase genetic homogenization and reduce kinship also at the late postfire stage.

The noteworthy increase in selfing is expected after a 96% population drop, but it is more expected to occur immediately after the fire rather than in the late postfire cohort. A possible explanation is reduced male fecundity of the 13 surviving trees in the first few years after the fire due to stress and to fire damage to the lower parts of the trees where the male cones are mainly produced (Ne'eman *et al.* 2011).

An alternative explanation for the observed changes in pollen gene flow could be some interannual variation in wind speed and directionality that is unrelated to the fire, population size reduction and landscape opening. Although we do not have sufficiently long-term wind data from our study site, we note that each cohort does not represent one but several years (Table S1, Supporting Information), greatly lessening the likelihood for year-to-year variation in wind patterns or other random effects.

General mechanisms for enhanced postdisturbance gene flow

We suggest two major mechanisms for enhanced pollen dispersal after fire, related to the opening of the landscape and the reduction in population size and density, the major fire-induced ecological effects. These general mechanisms should be applicable for a wide range of disturbances and scenarios that generate these effects.

The first putative mechanism is an ecological one. Pollen wind-dispersal kernels are typically leptokurtic (Levin & Kerster 1974; Ellstrand 1992; Klein *et al.* 2006), implying that while pollen grains can and do travel great distances (Petit & Hampe 2006; Robledo-Arnuncio 2011), their number rapidly decreases as the distance from any one source increases. Upon reaching a dense conspecific patch, immigrant pollen will be vastly outnumbered by locally produced pollen (i.e. dilution effect), making successful capture highly unlikely. A reduction in population density or suppressed reproduction in a surviving postdisturbance population reduces the number of pollen-generating individuals and, therefore, the level of competition between immigrant and locally produced pollen for receptive female cones/flowers. Thus, even if pollen influx from neighbouring populations is unchanged, reduced dilution effect effectively increases incoming gene flow.

The second putative mechanism is a physical one. A postfire, vegetation-free landscape should facilitate long-distance pollen movement in wind-pollinated plants due to stronger winds (Raynor *et al.* 1974; Chen *et al.* 1993; Trakhtenbrot 2010; Nathan *et al.* 2011) and less pollen collision with and subsequent deposition on vegetative elements (Tauber 1967). Although additional factors such as turbulence (Real 1983; Di-Giovanni & Kevan 1991) and vertical plume broadening (Raynor *et al.* 1974) also play an important role, mechanistic models of wind-dispersed seeds show a positive association between wind speed and dispersal distance (Nathan *et al.* 2011), and longer seed dispersal distances in sparser canopies (Nathan & Katul 2005), both potentially valid also for pollen dispersal by wind (Levin & Kerster 1974; Nathan *et al.* 2011). Stronger winds within the sparse forest may also increase rebound and re-entrainment of deposited grains from vegetative elements back to the airborne pollen pool (Paw U & Braaten 1992).

It is difficult to disentangle these mechanisms, as an open physical structure (hence stronger winds) and a sparse population (less competition, hence weaker dilution effect) are tightly linked. The pollen dilution mechanism is more parsimonious because it does not require a change in the pollen dispersal kernel, only in the effective pollen kernel. The physical mechanism, however, argues for a significant change in the properties of the dispersal kernel caused by the postdisturbance environment. Keeping in mind its sample size limitations, the fire-free stand may offer potential insight hinting towards the importance of landscape opening (Appendix S2, Supporting Information). The kinship results (Fig. S1, Supporting Information) resemble those of the focal stand (Fig. 3a), even though the fire-free stand was outside the fire boundaries (Fig. 1a). While decreased competition exists only within the burnt population, stronger postfire winds can act at a larger scale, even beyond that encompassed by the fire. The sudden clearing of the nearby landscape from vegetation, including the bulk of the focal stand, may have caused a general increase in wind speeds across the area, increasing pollen dispersal towards the fire-free stand and decreasing kinship as trees sample pollen from more sources (Adams 1992; Ellstrand 1992). The fire-free stand was also very close to another fire that broke on December 1999 (Fig. 1a). The early postfire cohort of the fire-free stand could have therefore been indirectly affected by landscape opening from both fires, as most cones were 7–9 years old at the time they were collected (Table S1, Supporting Information). The similar kinship results between the two stands might be independent of the fire, yet random interannual variation unrelated to fire that affected both stands is unlikely to be the cause as each cohort represents several years, and there is little overlap between the early postfire cohorts of the two stands (Table S1, Supporting Information).

Conclusions

The genetic effects of population reduction and landscape opening found in our study case could be extended to the more general case and hold also for large continuous populations that lack spatial structure. As a forest grows and matures, it becomes dense, closed and less receptive to penetration of immigrant wind-dispersed pollen, due to a physical barrier imposed by the forest structure, reduced winds, and competition with great quantities of pollen produced and dispersed locally. Similarly, within-population wind-dispersal distances will also decline as the forest closes. Limited dispersal distances (of both pollen and seeds) in such conditions may result in an excess of mating between neighbouring individuals, an increase in nonrandom matings and eventually the build-up of spatial genetic structure (Hamrick & Nason 1996; Vekemans & Hardy 2004; Troupin *et al.* 2006), as each individual mates mainly with relatively few neighbours. As demonstrated at the pollen level in the *P. halepensis* stand in this study, enhanced postdisturbance gene flow increased random matings for each maternal individual and disrupted the strong genetic structure that exists between the two spatial groups.

These genetic effects, however, may have varied consequences (Ellstrand 1992). Enhanced gene flow homogenizes genetic variation between populations, limiting local adaptation. Genetic variation in the receiving population could be reduced if the immigrant genes come from less genetically diverse populations, as in the case of a natural population surrounded by conspecific plantations or agricultural fields. Furthermore, local variation unique to the predisturbance population may be lost if the postdisturbance population is 'swamped' by immigrant genes (Lenormand 2002). These implications are of special concern for contamination by genetically modified crops. For natural *P. halepensis* populations in Israel, which have been shown to be genetically distinct (Steinitz 2010), the greatest threat in this sense comes from pollen contamination by the abundant planted *P. halepensis* forests, which have originated from seed sources alien to Israel (Steinitz *et al.* 2012).

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R.N. conceived research, D.S. & R.N. designed research, D.S. performed research, analysed data and wrote the paper with contribution from R.N.

Data accessibility

DNA sequences: GenBank Accessions nos CR377943, AJ012087, AF195535, AF195543, JF803635, JF803641, AF195536; Sample locations and microsatellite data: DRYAD entry doi: 10.5061/dryad.28kh9.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Description of samples collected and used for genetic analysis.

Table S2 Description of microsatellite markers used in this study.

Table S3 Standard genetic diversity indices for the surviving trees and sampled pollen.

Table S4 As Table S3, fire-free stand.

Fig. S1 As Fig. 3a, fire-free stand.

Appendix S1 Stand population dynamics before and after the fire.

Appendix S2 Results of the fire-free stand.