MOLECULAR ECOLOGY

Molecular Ecology (2012) **21**, 300–313 doi: 10.1111/j.1365-294X.2011.05394.x

Effects of forest plantations on the genetic composition of conspecific native Aleppo pine populations

O. STEINITZ,* J. J. ROBLEDO-ARNUNCIO† and R. NATHAN*

**Movement Ecology Laboratory, Department of Ecology, Evolution, and Behavior, The Alexander Silberman Institute of Life Sciences, Edmond J. Safra Campus, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel, †Departamento de Ecología y Genética Forestal, Centro de Investigación Forestal (CIFOR) – INIA, Ctra. de la Coruña Km 7.5, 28040 Madrid, Spain*

Abstract

Afforestation is a common and widespread management practice throughout the world, yet its implications for the genetic diversity of native populations are still poorly understood. We examined the effect of Aleppo pine (*Pinus halepensis*) plantations on the genetic composition of nearby conspecific native populations. We focused on two native populations in Israel with different levels of isolation from the surrounding plantations and compared the genetic diversity of naturally established young trees within the native populations with that of local native adults, using nine nuclear microsatellite markers. We found that the genetic composition of the recruits was significantly different from that of local adults in both populations, with allelic frequency changes between generations that could not be ascribed to random drift, but rather to substantial gene flow from the surrounding planted Aleppo pine populations. The more isolated population experienced a lower gene-flow level (22%) than the less isolated population (49%). The genetic divergence between native populations at the adult-tree stage (F_{st} = 0.32) was more than twice as high as that of the young trees naturally established around native adults (F_{st} = 0.15). Our findings provide evidence for a rapid genetic homogenization process of native populations following the massive planting efforts in the last decades. These findings have important implications for forest management and nature conservation and constitute a warning sign for the risk of translocation of biota for local biodiversity.

Keywords: dispersal, gene flow, genetic homogenization, non-indigenous gene pool, *Pinus halepensis*, planting

Received 12 September 2011; revision received 1 November 2011; accepted 2 November 2011

Introduction

Biodiversity threats associated with interspecific hybridization between invasive and native species have been widely recognized, and their potential for extinction of native species has been demonstrated (Rhymer & Simberloff 1996; Mooney & Cleland 2001). Yet, the potential threats of genetic introgression from non-indigenous conspecifics into native populations have received much less attention (but see Laikre *et al.* 2006; Robledo-Arnuncio *et al.* 2009; Byrne *et al.* 2011). The growing capabilities for artificial massive translocation of plants and animals for agricultural, recreational and environmental purposes in the modern era calls for careful consideration of the genetic implications of spatial redistribution of biota. Plantations of forest trees are common in large areas of the world and their area is rapidly expanding (Paquette & Messier 2010). Seeds used for plantations are usually collected from a few seed trees that exhibit favourable phenotypes and are grown in nurseries (Larsen 1995; Perevolotsky & Sheffer 2009), bypassing the selection pressure on seed establishment. Moreover, plantations are often established in close proximity to native inter-fertile conspecific populations using seeds from non-local and sometimes distant sources (Perevolotsky & Sheffer 2009; Robledo-Arnuncio *et al.* 2009; Byrne *et al.* 2011).

Correspondence: Ofer Steinitz, Fax: 972 2 6584655; E-mail: ofer.steinitz@mail.huji.ac.il

Several problems may arise from afforestation with non-indigenous individuals. One of the possible detrimental consequences of this planting policy is artificial genetic homogenization, the increase in the genetic similarity between the introduced and native gene pools with time via gene flow (Olden *et al.* 2004). Especially in native populations of relatively small size, the human-mediated inflow of non-local genes may eventually swamp locally adapted genotypes. This process has rarely been assessed in natural ecosystems (but see Ayllon *et al.* 2006) and remains unexplored for many biological systems including forest trees.

Aleppo pine (*Pinus halepensis*) populations in Israel consist of native and planted stands enabling testing of the genetic consequences of planting. Aleppo pine is the most common pine in the Mediterranean Basin (Barbéro *et al.* 1998) with forests covering about 2.5 million hectares (Quézel 2000). Its natural distribution is mostly concentrated in the western part of the Mediterranean Basin but also includes scattered populations in the eastern part, with several populations in Israel (Schiller & Waisel 1989; Ashkenazi 2004). Palynological investigations and historical evidence suggest that Aleppo pine was a rare element in the vegetation of Israel in the last 10 000 years (Liphschitz & Biger 2001). Large-scale afforestation, especially during 1950–1970 (Osem *et al.* 2009), has turned Aleppo pine into a dominant tree species in Israel (Liphschitz & Biger 2001), covering as much as 40% of the forested area (Osem *et al.* 2008), including areas and habitats beyond the natural range of the species (Liphschitz & Biger 2001). Seed sources used for Aleppo pine plantations in Israel were mostly from non-local distant regions of the Mediterranean basin (Ashkenazi 2004; Lavi *et al.* 2005; Osem *et al.* 2008; Perevolotsky & Sheffer 2009). However, detailed historical documentation on the seed sources of plantations at the stand level is unavailable, and low genetic polymorphism did not allow differentiating genetically between potential natural sources and planted populations in previous studies (Grunwald *et al.* 1986; Madmony *et al.* 2007). Currently, there are no known genetic markers that provide clear-cut differentiation between Eastern and Western Mediterranean populations (Steinitz 2010). Another Mediterranean pine species that was planted over large areas in Israel is *Pinus brutia*. This species, a close relative of Aleppo pine, does not grow naturally in Israel. Unidirectional gene flow between male *P. halepensis* and female *P. brutia* was demonstrated by crossing trials (Panetsos 1975) and by the presence of *P. halepensis* chloroplast haplotypes in *P. brutia* seeds but not the reverse (Bucci *et al.* 1998).

The afforestation history of Aleppo pine in Israel, involving a rapid and massive planting of non-local trees in the vicinity of native populations, is a large-scale experiment that provides an opportunity for studying the effects of massive translocation of tree populations and subsequent gene flow on the genetic variation in native conspecifics. Our goal is to assess the effect of Aleppo pine plantations on the genetic composition of neighbouring native populations with differing levels of spatial isolation.

We hypothesize that gene flow from plantations into native populations can be substantial and predict some of its genetic consequences:

1. Allele frequencies among naturally established recruits in the native populations are expected to differ from those of the native adults, beyond random-drift expectations, because of genetic introgression from the plantations.
2. The genetic diversity of naturally established recruits in the native populations is expected to be higher than that of native adult trees, as a consequence of the introgression of plantation-specific alleles and the lower genetic diversity found in the native populations relative to the surrounding plantations (Steinitz 2010).
3. Inbreeding levels among young trees are expected to be lower than those among adult trees because of the mixing of the native populations with genetic material from planted populations.
4. Spatial proximity between native and planted populations is expected to enhance genetic introgression.
5. Given the high genetic similarity among planted populations and the relatively high genetic divergence among adult native populations (Steinitz 2010), a genetic homogenization process is expected to reduce the genetic differentiation between native populations, with lower among-population divergence at the offspring than at the adult-tree level.

Additionally, the large-scale analysis of young trees within native populations provides the opportunity to examine genetic introgression from *P. brutia* into the native Aleppo pine populations. We predict that interspecific introgression will be negligible as a result of the unidirectional gene-flow barrier (Bucci *et al.* 1998).

Methods

The studied species

Aleppo pine (*Pinus halepensis* Miller) is a monoecious wind-pollinated, wind-dispersed conifer. Pollination takes place in the spring, fertilization a year later and seed dispersal begins in the third year after pollination (Thanos & Daskalakou 2000). Aleppo pine produces large amounts of seeds annually (Nathan *et al.* 1999; Thanos & Daskalakou 2000), which are adapted for wind dispersal by their asymmetric samara morphology (a samara seed has an attached wing) that reduces the terminal velocity of the propagule (Nathan *et al.* 1996). As in most plants, gene flow is achieved through the movement of a gamete by pollen dispersal, or the movement of a zygote by seed dispersal.

Study sites and field sampling

We selected three native Aleppo pine populations in Israel with different levels of spatial isolation from nearby conspecific plantations: Mt. Pithulim (Judean hills), Mt. Sumak (Carmel) and Mt. Shfanim (Galilee), with minimum distances to plantations of 450, 1800 and 3600 m, respectively (Table 1, Fig. 1). In each of the native populations, needle samples were collected from all adult native trees that established before ~1950 within the study area, ensuring that they originated before the beginning of major planting in the surrounding areas. In Mt. Pithulim, the age limit was accurately determined based on dendrochronological measurements (age determination using growth ring counts; Troupin *et al.* 2006), whereas in Mt. Sumak and Mt. Shfanim, age limit was approximately estimated based on size, limiting the sample to trees with a diameter at breast height (DBH) >33 cm. The estimated number of standing reproductive trees during the field sampling period in 2008 was ~2000, ~500 and ~150 in Mt. Pithulim, Mt. Sumak and Mt. Shfanim, respectively (the number of sampled trees is given in Table 1).

Needle samples were also collected from a set of planted conspecific stands that were selected for genetic analyses based on their potential as sources for gene flow into the native population (Table 2, Fig. 1). In particular, current control maps of Keren Kayemeth LeIsrael (the forest authority of Israel) were used for the selection of stands that were planted before 1970 based on their proximity to the native population, area and density. We refer to each polygon of planted area as a population, although there is no available information on the seed source of these stands. In the planted populations, an effort was made to collect needles from adult trees evenly distributed across the stand keeping a minimum distance of 10 m between sampled trees.

Table 1 The studied native populations. Sample sizes of adult (all trees established before ~1950) and young trees (all trees established ~1990)

Population name	Latitude	Longitude	Number of trees sampled	
			Adults	Young
Mt. Pithulim	31°45′16	35°04′24	42	118
Mt. Sumak	32°39′39	35°01′58	35	19
Mt. Shfanim	32°58′00	35°23′25	27	16

Additionally, we collected needles from all the reproductive trees with DBH of 5–7 cm naturally established within the native population areas, referred to here as young trees (Table 1, Fig. 1). The size range was set for framing the analysis to an age-limited reproductive cohort. A strong allometric relation between age and perimeter of Aleppo pines between the ages of 6 and 24 (Noy-Meir 2003) enables a fair estimate of tree age. We converted the measured DBH to the highly correlated base diameter using a relevant equation in Schiller (1977, p. 15). Based on the equations in Noy-Meir (2003, p. 27), we estimated that the size criterion for sampling (DBH 5–7 cm) corresponds to a germination year around 1990, i.e. after the major surrounding plantations were established and had reached maturity. Higher recruitment in Mt. Pithulim yielded a larger sample size of young trees relative to the other two populations (Table 1). In addition, more effort was invested in sampling the surrounding planted stands (number of stands and sample size) in the less isolated population in Mt. Pithulim (Table 2). Thus, we expect to obtain a more accurate gene-flow rate estimate for the Mt. Pithulim population.

DNA extraction and genotyping

Sampled needles 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.

All sampled native adult trees, planted adult trees and young trees were genotyped using a common set of nine nuclear microsatellite markers (Table 3). PCR was carried out using a Primus 96 Advanced Gradient thermal cycler (Peqlab, Erlangen, Germany) in a total volume of 15 μL. The reaction mix contained 20 ng 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 $(NH_4)_2SO_4$, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.45 units Taq DNA polymerase. The concentration of $MgCl_2$ in the reaction mix was adjusted for each marker as specified in Table 3. The following conditions were used for PCR: 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 (T_a is reported in Table 3). A different PCR programme was used for the marker PHAF1: 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.

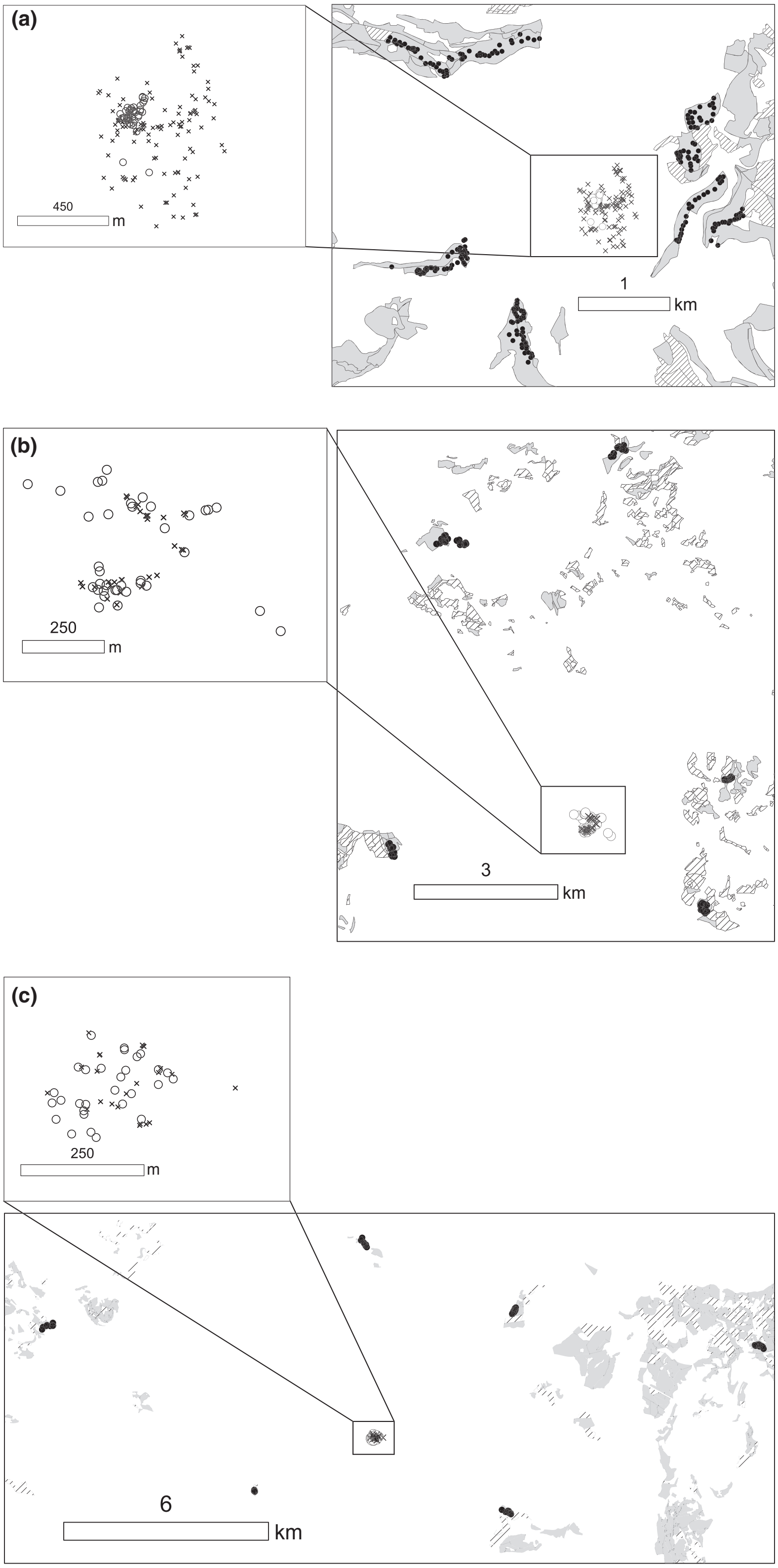

Fig. 1 Distribution of native adult and young trees and surrounding planted stands in Mt. Pithulim (a), Mt. Sumak (b) and Mt. Shfanim (c) study areas. Surrounding planted stands of *Pinus halepensis* and *Pinus brutia* are marked by grey and hatched polygons, respectively. Open circles and crosses indicate native adults and young trees, respectively. The sampled planted trees are indicated by black closed circles.

Table 2 Planted populations studied

Population name	Latitude	Longitude	Nearby native population	Distance to nearby native population (m)*	Number of sampled individuals	Planting year
Carmia west	32°42′54	35°00′02	Mt. Sumak	6210	11	<1948
Carmia east	32°42′54	35°00′15	Mt. Sumak	6120	10	<1948
Damun Junction	32°43′57	35°02′22	Mt. Sumak	7120	10	1952
Hirbet krach	32°40′13	35°03′50	Mt. Sumak	2620	10	1965
Elyakim caves	32°38′44	35°03′30	Mt. Sumak	2680	11	1968
Kerem Maharal	32°39′26	34°59′20	Mt. Sumak	3600	10	1960
Nes Harim north	31°44′58	35°03′27	Mt. Pithulim	1280	21	∼1926
Refaim turn	31°45′08	35°05′14	Mt. Pithulim	780	20	1964
Refaim Bridge west	31°45′32	35°05′02	Mt. Pithulim	600	17	1966
Ramat raziel	31°46′09	35°03′42	Mt. Pithulim	1580	25	1968
Ksalon	31°46′04	35°03′18	Mt. Pithulim	1890	27	1967
Bar Giora south	31°44′27	35°03′51	Mt. Pithulim	1160	21	∼1926
Bar Giora north	31°44′37	35°03′51	Mt. Pithulim	1010	20	∼1926
Nes Harim south	31°44′51	35°03′14	Mt. Pithulim	1470	22	∼1926
Tayasim southwest	31°45′46	35°05′06	Mt. Pithulim	860	20	1969
Har Giora north	31°45′16	35°05′05	Mt. Pithulim	450	15	1960
Har Hod	32°57′10	35°21′10	Mt. Shfanim	3600	6	1950
Hemdat Yamim	32°56′50	35°25′55	Mt. Shfanim	4000	10	1950
Birya	32°59′29	35°30′39	Mt. Shfanim	11050	7	1955
Safsufa	33°00′02	35°26′03	Mt. Shfanim	5150	5	<1948
Mt. Hiram	33°01′07	35°23′13	Mt. Shfanim	5320	10	1950
Kefar Veradim	32°59′49	35°17′16	Mt. Shfanim	9750	10	1964

*Edge-to-edge.

Table 3 Nuclear microsatellite markers used for the genetic analyses

Marker name	Accession nos	Reference	T_a (annealing temperature °C)	$MgCl_2$ # (mM)	# of alleles	Allele size (bp)
PtTX3107	AY304043	González-Martínez *et al.* (2004)	50	2.5	2	159–164
PtTX3116	AY304036	González-Martínez *et al.* (2004)	50	4.5	2	116–122
A5A12	CR354476	Guevara *et al.* (2005)	50	2.5	4	230–245
B4F08	CR377943	Guevara *et al.* (2005)	50	1.5	8	178–210
ITPH4516	AJ012087	Keys *et al.* (2000)	59	2.5	16	136–178
PHAF1	AF195535	Keys *et al.* (2000)	56	2.5	4	205–209
PHAF10	AF195543	Keys *et al.* (2000)	50	4.5	2	131–137
pEST8	JF803635	Steinitz *et al.* (2011)	54	3.5	5	192–204
pEST2669	JF803641	Steinitz *et al.* (2011)	50	2.5	4	145–151

The multiplex PCR products were separated using an ABI 3730 genetic analyzer (Applied Biosystems). Manual scoring of PCR product sizes with reference to a 500-Liz standard (Applied Biosystems) was carried out using GeneMapper Software version 4.0 (Applied Biosystems). A total of 47 alleles were detected in the nuclear markers. The number of alleles per marker ranged between 2 and 16 (Table 3). Linkage disequilibrium, tested with FSTAT v.2.9.3.2 (Goudet 2001), was not detected in any of the populations for any pair of nuclear markers except for the marker pair ITPH4516 and B4F08 in the Mt. Pithulim population. This pair was not found in linkage disequilibrium in any of the other 24 populations, and therefore the nuclear markers were considered to be unlinked.

Data analysis

The effect of plantations on the genetic divergence between native adult trees and naturally established offspring. Allele frequencies were calculated using GENALEX v.6.2 (Peakall & Smouse 2006). Because the planted stands showed low levels of genetic differentiation among each other, population allelic frequencies for the plantations of each of the three study areas were calculated by pooling trees from all sampled planted stands within the corresponding area (Steinitz 2010). To analyse the genetic differentiation between native adult trees and naturally established young trees, we calculated the multilocus Weir & Cockerham (1984) estimator of F_{st} (theta) and tested for significant differentiation with FSTAT v.2.9.3.2 (Goudet 2001).

To test whether allele frequency changes between native adult and young trees were caused by gene flow from nearby plantations, we used a sign test. First, from each marker, we chose the allele that showed the highest difference in frequency between native adults and the nearby planted populations. Second, we filtered out loci for which the maximum difference in allelic frequencies between the adults and the nearby planted populations was <5%. For these filtered loci, allelic frequency changes due to gene flow would be difficult to estimate accurately because of the low initial difference. Third, we counted the number of times that the allele's frequency shifted between the adult and the young trees towards the frequency of the planted population. This statistic was used in a sign test to determine whether the changes in allele frequency were related to the frequency in the nearby planted populations or represent pure chance with no significant role for gene flow. Because the markers were found to be unlinked, we treated each of them as an independent trial.

Estimation of gene-flow rates. To estimate levels of gene flow, we used the slope of the linear regression of the difference in frequency between young and adult trees vs. the difference in frequency between planted populations and adults for each allele in each of the nuclear markers. This measure is derived from the following equation adapted from equation (1) in Robledo-Arnuncio *et al.* (2009) for estimating introgression of chloroplast haplotypes:

$$q_{a,Y} = (1 - m)q_{a,N} + m\, q_{a,P},$$

where $q_{a,Y}$ is the frequency of allele a among young trees of the native population, $q_{a,N}$ is the frequency of allele a among adult trees of the native population, $q_{a,P}$ is the frequency of allele a in the surrounding plantations and m is the immigration rate, defined as the proportion of gene copies of the young trees of the native population that originated in the plantations. This equation relies on the (realistic) assumption that gene flow from the native population to the planted populations is negligible, as expected from the much larger populations of planted trees. It also assumes alleles are selectively neutral.

The effect of plantations on the genetic variance within and among native populations. To compare the genetic diversity, inbreeding level and genetic differentiation between adults and young trees, we used the software FSTAT v.2.9.3.2 (Goudet 2001). We calculated three genetic diversity indices for each population: allelic richness (accounting for sample size with the rarefaction method of El Mousadik & Petit 1996), observed heterozygosity (H_o) and unbiased gene diversity (H_s; Nei 1987). The same program was used to calculate inbreeding coefficients (F_{is}) (Weir & Cockerham 1984) and genetic differentiation between populations F_{st} (Weir & Cockerham 1984). We used the 'compare-groups' option in FSTAT to obtain significance levels for comparisons of genetic diversity indices, levels of inbreeding and levels of genetic differentiation among native populations measured at the adult-tree stage vs. at the young-tree stage.

Introgression of alleles from Pinus brutia. Four *P. brutia* planted adult trees (identified morphologically by right angle cones and by location in forest control maps) were analysed at four loci (PtTX3116, B4F08, PHAF10 and pEST2669) and used as a reference population for the detection of introgression of *P. brutia* alleles into native *P. halepensis* populations. A total of eight alleles were detected in this analysis, none of them shared with *P. halepensis*.

Results

Pairwise F_{st} analyses revealed that native populations both in Mt. Pithulim and in Mt. Shfanim were significantly different from their respective surrounding plantations (F_{st} = 0.07–0.13 and F_{st} = 0.41–0.55, respectively), while the native population at Mt. Sumak did not show a significant pairwise F_{st} with any of its neighbouring planted populations (F_{st} = 0.01–0.04). Because the high genetic similarity of the native population in Mt. Sumak with its surrounding planted population at the used loci prevents the detection of gene flow, we restricted further analyses to the Mt. Pithulim and Mt. Shfanim populations. The high F_{st} values between the native population in Mt. Shfanim and its surrounding planted populations reflect its relatively low polymorphism and its genetic uniqueness relative to any of the sampled native and planted populations (F_{st} = 0.36–0.55, with an average of 0.44).

Introgression of alleles from Pinus brutia

No *P. brutia*-specific alleles were detected among the sampled young trees from Mt. Pithulim, nor among sampled adults from the native or planted populations. Of the 1573 reproductive trees that established by means of natural regeneration within the 60-ha plot in

Mt. Pithulim, one individual tree was identified as a *P. brutia* based on morphology (this individual was not included in the genotyping sample), probably as a result of long-distance seed dispersal. No *P. brutia* trees were identified in the Mt. Sumak and in Mt. Shfanim study sites.

The effect of plantations on the genetic divergence between native adult trees and naturally established offspring

Allele frequency changes between the adults and the young trees that naturally established in the area of the native population were observed in both Mt. Pithulim and Mt. Shfanim populations. Changes included the presence of alleles at several loci among the young trees that were not detected among the adults (Table 4), as well as changes in the frequencies of alleles shared between cohorts (Fig. S1, Supporting information). Analysis of genetic differentiation revealed that young trees were significantly different from adults in both the Mt. Pithulim ($F_{st} = 0.029$, $P < 0.001$ based on 1000 permutations) and the Mt. Shfanim populations ($F_{st} = 0.063$, $P < 0.001$ based on 1000 permutations).

In one nuclear SSR in Mt. Pithulim (PHAF1) and in one nuclear SSR in Mt. Shfanim (PtTX3116), the largest allele frequency differences between native adults and nearby plantations were below 5% and were thus excluded from the gene-flow analysis. The remaining markers allowed for eight independent trials (test of change in allele frequency). In both the Mt. Pithulim and the Mt. Shfanim populations, the sign test showed that changes in allele frequencies were not random, but rather produced by gene flow from the surrounding plantations. In the Mt. Pithulim population, eight of eight cases showed a change in frequency towards the frequency of nearby plantations ($P = 0.004$) with an average absolute change of 0.12, while in the Mt. Shfanim population seven of eight cases showed a change in frequency towards the frequency of the nearby planted populations ($P = 0.031$) with an average absolute change of 0.09. The sign test remained significant also when the 5% threshold was eliminated, yielding nine independent trials (with difference in allele frequency between native adults and nearby plantations as low as 2.1%), in Mt. Pithulim and Mt. Shfanim populations ($P = 0.02$ and $P = 0.03$, respectively). The aforementioned results confirm the presence of gene flow from plantations into native populations in both Mt. Pithulim and Mt. Shfanim sites.

Level of gene flow into native populations with contrasting spatial isolation

The linear regression of the difference in allele frequencies between young and adult native trees against the difference in allele frequencies between planted trees and native adults is shown in Fig. 2. In both populations, a positive slope was observed, confirming substantial gene flow into the native populations from the surrounding plantations. The Mt. Shfanim population, which is more isolated from the planted populations,

Table 4 Introgression of new alleles into the native populations in Mt. Pithulim and Mt. Shfanim. For each population, the number of newly introduced alleles (alleles that were detected in the young trees but not in the adult population) and their frequency in the young trees are compared with the number of potential alleles for new introduction (alleles that were detected in nearby planted populations but not in the adult population) and their frequency in the planted population.

	Mt. Pithulim				Mt. Shfanim			
Nuclear markers	Number of newly introduced alleles	Number of potential alleles for new introduction	Frequency of newly introduced alleles	Frequency of potential alleles for new introduction	Number of newly introduced alleles	Number of potential alleles for new introduction	Frequency of newly introduced alleles	Frequency of potential alleles for new introduction
ITPH4516	5	5	0.114	0.193	3	6	0.156	0.750
PHAF10	0	0	0.000	0.000	1	1	0.063	0.435
B4F08	2	2	0.013	0.015	1	2	0.094	0.391
PHAF1	1	2	0.004	0.005	0	0	0.000	0.000
pEST2669	1	1	0.008	0.007	1	1	0.094	0.094
PtTX3116	0	0	0.000	0.000	0	1	0.000	0.034
pEST1489	0	1	0.000	0.012	0	0	0.000	0.000
A5A12	0	2	0.000	0.010	0	0	0.000	0.000
PtTX3107	0	0	0.000	0.000	1	1	0.031	0.133
Average	1	1.556	0.020	0.035	0.778	1.333	0.049	0.204

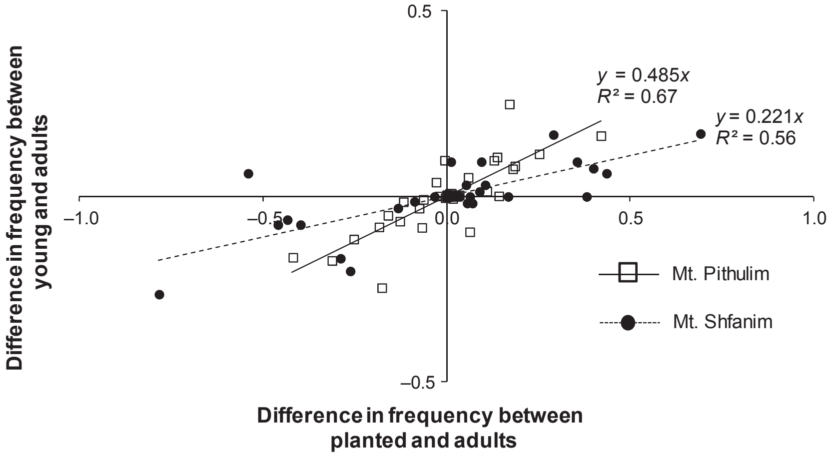

Fig. 2 Regression analysis for estimating gene-flow levels. The difference in frequency between young and native adult trees vs. the difference in frequency between planted populations and native adult trees for each nuclear microsatellite allele in Mt. Shfanim and in Mt. Pithulim. The slope of the linear regression (significantly different from zero, $P < 0.001$) yields an estimate of gene flow from the planted population into the native population.

had a shallower slope (0.221) than the Mt. Pithulim (0.485), indicating a twofold higher migration rate in the latter, consistent with our prediction of lower gene flow into more isolated populations.

The effect of plantations on the genetic variance within and among native populations

The pooled sample of naturally established young trees from all three native populations (Mt. Pithulim, Mt. Sumak and Mt. Shfanim) had higher levels of genetic diversity than the pooled native adult sample, for all three genetic diversity indices tested, although the difference between pooled samples was not significant (Table 5). Higher levels of genetic diversity in young trees compared with adults were detected also for each population separately, with the largest difference observed in the Mt. Shfanim population (Table S1, Supporting information). The increase in genetic diversity in the young can be the consequence of gene flow from the planted populations, which have higher genetic variation than native adults (Steinitz 2010).

Table 5 Comparison of genetic diversity, inbreeding and genetic differentiation between adult and young trees in the Mt. Pithulim, Mt. Sumak and Mt. Shfanim populations

Index	Adults	Young	*P*
Allelic richness	1.934	2.163	0.232
H_o (observed heterozygosity)	0.289	0.419	0.171
H_s (gene diversity)	0.335	0.432	0.189
F_{is} (inbreeding coefficient)	0.138	0.030	0.365
F_{st} (genetic differentiation)	0.319	0.146	0.239

Significance levels were determined based on 1000 permutations.

Levels of inbreeding were over four times higher among native adult trees than among young trees, considering the three native populations together, although this difference was not significant (Table 5). Higher levels of adult inbreeding were observed also in each separate population (Table S2, Supporting information). These results are consistent with our prediction that mating between planted and native individuals will generate a lower number of inbred offspring.

Finally, among-population genetic differentiation was twice as high for native adults than for naturally established young trees (although not significantly so, Table 5), in accordance with our prediction of the attenuating effect of plantations on genetic differentiation between native populations.

Discussion

Population translocation studies usually analyse genetic diversity in a spatial framework (e.g. comparing an introduced vs. a source population), but rarely make such an analysis in a temporal framework (i.e. comparing indices before and after introduction) because no data are available before the introduction event (Olden *et al.* 2004). The longevity of Aleppo pine and the detailed historical record available for the study sites, especially for Mt. Pithulim (Troupin *et al.* 2006), enabled us to compare the genetic composition of native stands before the massive planting with that observed many years later. Detecting the potential genetic effects of plantations on native populations requires knowing the genetic differences between introduced and native trees at the time the plantations were established, for which appropriate genetic markers are necessary. Sufficient genetic resolution is crucial for detecting the potential subtle genetic differences involved in the homogenization process (Olden *et al.* 2004). Previous attempts to analyse differences in genetic composition between planted and native Aleppo pine populations using eight chloroplast and three nuclear microsatellite markers failed as a result of low genetic resolution (Madmony *et al.* 2007).

The absence of *P. brutia* alleles among sampled young Aleppo pine trees is encouraging from a conservation perspective and is in accordance with the unidirectional gene flow from male *P. halepensis* to female *P. brutia* trees revealed in previous analyses (Bucci *et al.* 1998). However, the introgression of genetic material from *P. brutia* trees into *P. halepensis* in future generations through several stages of hybridization and a gradual reduction in the unidirectional reproductive barrier cannot be ruled out.

In the two native populations that were genetically different from the planted populations at the employed loci, a clear change in the genetic composition during ~40 years was revealed, including the appearance of alleles that were absent in the native populations. The allelic frequency changes in the natural regeneration can be attributed to extensive gene flow from surrounding Aleppo pine plantations. Schiller *et al.* (1997) found new isoenzyme alleles in a native population of the species in Mt. Carmel, which regenerated after a forest fire, and attributed the occurrence of these alleles to '*intra-specific hybridization with planted P. halepensis from West Mediterranean group*'. However, closer inspection of the 'newly introduced' alleles and other isoenzyme analyses revealed that these alleles were actually present in native populations of Mt. Carmel (Grunwald *et al.* 1986; Korol *et al.* 2002), and therefore the lack of marker resolution precluded conclusive inferences about the potential presence of introgression in that population.

Our gene-flow estimates for the two populations revealed that the more isolated population, at Mt. Shfanim, experiences lower gene introgression than the population at Mt. Pithulim. This result is consistent with a previous meta-analysis showing a negative effect of geographic distance to neighbouring populations on the immigration rate into small stands for different species of wind-pollinated trees (Bittencourt & Sebbenn 2007). Nevertheless, the high cumulative rate of gene flow, even in the highly isolated population in Mt. Shfanim, demonstrates the strong long-term genetic contamination from plantations. Such high immigration rates are not unusual for wind-pollinated tree species (Valbuena-Carabaña *et al.* 2005; Bittencourt & Sebbenn 2007; Robledo-Arnuncio *et al.* 2009, 2010; Kremer *et al.* in press), even into highly isolated stands, with an annual immigration rate of 0.043 into a *Pinus sylvestris* stand more than 30 km distant from any other population (Robledo-Arnuncio & Gil 2005) and an annual immigration rate of 0.065 into a *Pinus flexilis* stand more than 2 km distant from the closest population (Schuster & Mitton 2000). Yet, the effect of planting on the genetic composition revealed in this study is striking. In a period of ~40 years, substantial changes occurred in the genetic composition of the two studied populations. This rapid process occurred as a consequence of large initial allelic frequency differences between the planted and the native populations and high inter-population gene flow.

Beside geographical isolation, introgression levels may be influenced by other factors, such as the abundance (area and tree density) in source and recipient populations, the relative location of the populations (especially if dispersal is anisotropic; e.g. Steinitz *et al.* 2011) and variations in fecundity and flowering phenology. In our study system, relating these factors to gene-flow level from a specific plantation would be limited by the low genetic differentiation among planted populations.

Genetic changes brought by introgression included allele frequency changes and an ensuing loss of the genetic variation unique to the native populations. A conspicuous example of loss of unique genetic variation is demonstrated in the marker B4F08 in the Mt. Pithulim population (Fig. S1, Supporting information). In this marker, the frequency of allele 210 is reduced from 14.3% in the adult trees to 7.6% in the young trees. The rate of loss of this allele is especially rapid because its average frequency in the planted populations is as low as 0.6%. The genetic markers used represent a very small portion of the total genome, but the frequency of unique alleles in other loci, including functional loci, can be expected to have been reduced similarly as a result of immigration, with possible extinctions of low frequency alleles. It is important to note that changes in allele frequency between generations at any specific locus might reflect also local processes such as unequal contribution of individual adult trees to the next generation, drift and selection. However, the observed consistent trend across loci of allelic frequency convergence between native and surrounding planted populations demonstrates the role of introgression in shaping the genetic composition of Aleppo pine populations.

It would be most interesting to investigate to what extent the homogenization revealed by neutral markers can be generalized to genomic regions of adaptive significance. Changes in allele frequencies produced by incoming gene flow have potential maladaptive consequences for native populations. Because local adaptation is widespread in trees (Savolainen *et al.* 2007), migrants from plantations originating from distant regions can be expected to generate a migration load (outbreeding depression) in the recipient population, and even a displacement of the local gene pool when the level of immigration is very high (gene swamping; Lenormand 2002). Even small genetic divergence between native and non-indigenous populations may involve serious risk for the loss of adaptations for traits that are controlled by a large number of loci (Allendorf *et al.* 2001). Detecting migration load or gene swamping may be difficult, though, because local adaptation may only be expressed under critical periods of extreme environmental stress, such as a prolonged drought (Allendorf *et al.* 2001). Environmental fluctuations can be very important throughout the long life cycle of Aleppo pine (Nathan & Ne'eman 2000, 2004), so that stress tolerance traits may be detectable only in certain life stages.

The widespread distribution of Aleppo pine suggests that its populations may be differentially adapted to climatic conditions (Grivet *et al.* 2009). Because of ongoing climate change, populations growing at the rear-edge of species distribution ranges may be disproportionately important for the survival and evolution of biota (Hampe & Petit 2005). Populations of Aleppo pine in Israel form the southern limit of the species distribution (Melzack *et al.* 1981; Ashkenazi 2004), and they presumably have special adaptations to extreme environmental conditions, including severe drought. Aleppo pine populations from different regions do indeed differ in their water-use efficiency and in their response to drought conditions (Atzmon *et al.* 2004; Voltas *et al.* 2008; Schiller & Atzmon 2009). Expression of water-deficit stress across a drought gradient differed between two native populations of Aleppo pine in Israel (Sathyan *et al.* 2005).

More evidence of adaptation to local environmental conditions in Aleppo pine comes from its pests. While about 50% of all planted Aleppo pine stands in Israel show various degrees of damage by the bast scale (*Matsucoccus josephi*; Bonneh 2000), native Eastern Mediterranean provenances are less susceptible to damage by the scale than West Mediterranean ones (Mendel 2000). The decline in Aleppo pine planted stands at a relatively young age and their high sensitivity to attacks by the bast scale have been attributed to their more humid Western Mediterranean origin (Osem *et al.* 2008).

Independently of the risk of migration load and gene swamping posed by genetic introgression from non-local plantations, our results showed also increased genetic diversity and reduced inbreeding among naturally established offspring, relative to those of native adults. These changes are a logical outcome of the higher genetic diversity of the planted populations, and of cross-population mating. Using RAPD and isoenzyme markers, Korol *et al.* (2002) found that the genetic diversity in seeds from adults growing within native populations in Mt. Shfanim and Mt. Carmel was lower than that of seeds collected from trees planted and raised in an arid environment from seeds of the same native populations (plantations at Yatir). This result was attributed to heterozygote advantage in stressful environments. However, a more parsimonious explanation is that the increase in genetic diversity in F1 offspring is simply because of introgression of new alleles from the abundant and more diverse surrounding plantations. This explanation is supported by Korol *et al.* (2002)'s finding of new alleles among the F1 offspring that were absent in the adults from Mt. Shfanim, a result that was repeated in our microsatellite analyses.

Genetic introgression, by increasing the genotypic variance available for the selection and potentially reducing inbreeding depression, may produce a 'genetic rescue' effect in small populations (Lenormand 2002). It is, however, unclear whether these positive consequences of gene flow will prevail over the negative ones brought by outbreeding depression, especially after several generations of back-crosses (Lynch 1991; Fenster & Galloway 2010). Answering this question would require quantitative experiments to determine the relative magnitude of inbreeding and/or outbreeding depression in native Aleppo pine populations in Israel. The high selfing rate found in early stages of an Aleppo pine population expanding in the region (Steinitz *et al.* 2011), points to a relatively minor role of inbreeding depression, which would diminish the potential positive influence of genetic introgression.

An additional effect of extensive gene introgression from non-local plantations is the genetic homogenization of the different native populations present in the region. Genetic differentiation among native Aleppo pine populations was indeed lower at the young-tree stage than at the adult-tree stage, indicating a genetic homogenization process following the massive Aleppo pine plantations in the last decades. To the best of our knowledge, this is the first evidence of such process in plants. An analogous process of genetic homogenization was demonstrated in wild Atlantic salmon populations following stocking (Ayllon *et al.* 2006), where F_{st} between populations was lower after the peak in foreign stocking than it was before.

Genetic homogenization, by reducing the spatial and total variance of gene diversity, may decrease the potential for adaptation to environmental change and undermine the resilience of biological communities (Olden *et al.* 2004; Olden 2006) and should thus receive special attention in forest management. The planting strategy in many areas around the world involves the use of seeds from one or a few non-local sources (Richardson *et al.* 2007; Robledo-Arnuncio *et al.* 2009) and may pose the same threats to genetic variance suggested by this study. Ecological forestry promotes the perception of forest stands as integral elements of large ecological systems, rather than as independent management entities, calling for the maintenance of stand diversity, and not only within-stand diversity, in a community context (Perevolotsky & Sheffer 2009). To maintain genetic diversity, as an important component of biodiversity, forest management should focus on preserving the overall genetic diversity and natural evolutionary processes present in metapopulations, rather than on the genetic diversity within each separate subpopulation.

Overall, given the complex and potentially antagonistic effects of genetic introgression, any related policies and conservation management strategies should be adopted on a case-by-case basis (Allendorf *et al.* 2001). The evidence of genetic introgression from non-local plantations and the resulting genetic homogenization of native populations call for a cautious reconsideration of afforestation and forest management policies in Israel and elsewhere. Local genetic variation in Aleppo pine eastern distribution may be preserved by minimizing the use of non-local seed sources for future plantations. However, prevention of the genetic homogenization process in the presence of widespread plantations is a difficult task that would require drastic management measures, including the maintenance of large buffer zones to reduce genetic introgression rates or assisted regeneration measures. Given the small size and the scarcity of the native populations, management should also include *ex-situ* conservation strategies. While further research should investigate potential interactions between migration load and genetic rescue in Aleppo pine populations, any effective *ex-situ* or *in-situ* conservation effort for the species should take into consideration that naturally regenerated young trees within native Aleppo pine stands cannot be considered as autochthonous, emphasizing the importance of assisted regeneration using controlled-pollination progenies, as well as the need for further development of vegetative propagation methods from adult-tree tissues (Madmony *et al.* 2007).

Genetic introgression into wild populations and genetic homogenization, two processes demonstrated in this study, are not exclusive problems of forest trees. The consequences of these processes are important also for other kinds of man-induced biota translocations, such as agriculture and revegetation (Byrne *et al.* 2011). Deployment of extensive agricultural crops in uncultivated areas may pose serious threats to the genetic variation in nearby conspecific wild populations. These potential genetic effects of agriculture, in addition to the escape of transgenic elements from genetically modified organisms (Smouse *et al.* 2007), bring to stage a neglected aspect of the conflict between agriculture and nature that calls for specific research and management. This challenge requires precise knowledge on gene flow and the distribution of genetic variation among cultivated and wild populations.

Acknowledgements

We thank Rafi Kent, Hemdat Banai, Orr Spiegel, Omri Finkel, Tal Avgar, Royi Zidon, David Troupin, Mariela Leiderman, Oz Nathan, Mati Caspi, Maayan Harel, Ofir Altstein and Shalev Bodenheimer for help with field work, Natan Elbaz and Dotan Rotem from the Israel Nature and Parks Authority for help with collection of DNA samples, Mira Korner and the team of The Center for Genomic Technologies at the Hebrew University of Jerusalem for help with genotyping, Adena Brickman and Avihai Zolti for help with laboratory work, Israel Tauber from Keren Kayemeth LeIsrael for providing forest maps, Adi Ben-Nun and Nir Horvitz for technical assistance, Galya Shalit for assistance with graphic design and Nechama Ben-Eliahu for valuable comments of earlier version of the manuscript. Support for this study was available through grants from the Israel Nature and Parks Authority, Israel Science Foundation (ISF-474/02, ISF-150/07 and ISF-FIRST-1316/05), the Ring Foundation, the Adelina and Massimo Della Pergola Chair of Life Sciences and the Friedrich Wilhelm Bessel Research Award of the Humboldt Foundation to R. Nathan, and the Rieger Foundation, Minerva foundation, Wolf foundation and European Molecular Biology Organization (EMBO) to O. Steinitz. J.J. Robledo-Arnuncio was partly supported by CGL2009-09428 project from the Spanish Ministry of Science and Innovation.

References

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution*, **16**, 613–622.

Ashkenazi S (2004) Management of the Arboreal Flora of the Carmel with emphasis on the management of *Pinus halepensis* Mill, Internal Report, Hakeren Hakayemet LeIsrael; Israel Nature and Parks Authority.

Atzmon N, Moshe Y, Schiller G (2004) Ecophysiological response to severe drought in *Pinus halepensis* Mill. trees of two provenances. *Plant Ecology*, **171**, 15–22.

Ayllon F, Martinez JL, Garcia-Vazquez E (2006) Loss of regional population structure in Atlantic salmon, *Salmo salar* L., following stocking. *ICES Journal of Marine Science*, **63**, 1269–1273.

Barbéro M, Loisel R, Quézel P *et al.* (1998) Pines of the Mediterranean basin. In: *Ecology and Biogeography of Pinus* (ed Richardson DM), pp. 153–170. Cambridge University Press, Cambridge.

Bittencourt JVM, Sebbenn AM (2007) Patterns of pollen and seed dispersal in a small, fragmented population of the wind-pollinated tree *Araucaria angustifolia* in southern Brazil. *Heredity*, **99**, 580–591.

Bonneh O (2000) Management of planted pine forests in Israel: past, present and future. In: *Ecology, Biogeography and Management of* Pinus halepensis *and* P. brutia *Forest Ecosystems in the Mediterranean Basin* (eds Ne'eman G, Trabaud L), pp. 377–390. Backhuys, Leiden, The Netherlands.

Bucci G, Anzidei M, Madaghiele A, Vendramin GG (1998) Detection of haplotypic variation and natural hybridization in *halepensis*-complex pine species using chloroplast simple sequence repeat (SSR) markers. *Molecular Ecology*, **7**, 1633–1643.

Byrne M, Stone L, Millar MA (2011) Assessing genetic risk in revegetation. *Journal of Applied Ecology*, **48**, 1365–1373.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L) Skeels] endemic to Morocco. *Theoretical and Applied Genetics*, **92**, 832–839.

Fenster CB, Galloway LF (2010) Inbreeding and outbreeding depression in natural populations of *Chamaecrista fasciculata* (Fabaceae). *Conservation Biology*, **14**, 1406–1412.

González-Martínez SC, Robledo-Arnuncio JJ, Collada C *et al.* (2004) Cross-amplification and sequence variation of microsatellite loci in Eurasian hard pines. *Theoretical and Applied Genetics*, **109**, 103–111.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. Available from http://www2.unil.ch/popgen/softwares/fstat.htm

Grivet D, Sebastiani F, González-Martínez SC, Vendramin GG (2009) Patterns of polymorphism resulting from long-range colonization in the Mediterranean conifer Aleppo pine. *New Phytologist*, **184**, 1016–1028.

Grunwald C, Schiller G, Conkle MT (1986) Isozyme variation among native stands and plantations of Aleppo pine in Israel. *Israel Journal of Botany*, **35**, 161–174.

Guevara MA, Chagné D, Almeida MH *et al.* (2005) Isolation and characterization of nuclear microsatellite loci in *Pinus pinaster* Ait. *Molecular Ecology Notes*, **5**, 57–59.

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, **8**, 461–467.

Keys RN, Autino A, Edwards KJ *et al.* (2000) Characterization of nuclear microsatellites in *Pinus halepensis* Mill. and their inheritance in *P-halepensis* and *Pinus brutia* Ten. *Molecular Ecology*, **9**, 2157–2159.

Korol L, Shklar G, Schiller G (2002) Genetic variation within *Pinus halepensis* Mill. provenances growing in different microenvironments in Israel. *Israel Journal of Plant Sciences*, **50**, 135–143.

Kremer A, Ronce O, Robledo-Arnuncio JJ *et al.* (in press) Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters*, in press.

Laikre L, Palme A, Josefsson M, Utter F, Ryman N (2006) Release of alien populations in Sweden. *Ambio*, **35**, 255–261.

Larsen JB (1995) Ecological stability of forests and sustainable silviculture. *Forest Ecology and Management*, **73**, 85–96.

Lavi A, Perevolotsky A, Kigel J, Noy-Meir I (2005) Invasion of *Pinus halepensis* from plantations into adjacent natural habitats. *Applied Vegetation Science*, **8**, 85–92.

Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology and Evolution*, **17**, 183–189.

Liphschitz N, Biger G (2001) Past distribution of Aleppo pine (*Pinus halepensis*) in the mountains of Israel (Palestine). *Holocene*, **11**, 427–436.

Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, **45**, 622–629.

Madmony A, Riov J, Zehavi A *et al.* (2007) Genetic conservation of native *Pinus halepensis* in Israel: 1. Ha-Masreq nature reserve. *''Forest'': Journal of Forestry, Woodlands & Environment''*, **9**, 15–19. (In Hebrew with English abstract).

Melzack RN, Grunwald C, Schiller G (1981) Morphological variation in Aleppo pine (*Pinus halepensis* Mill.) in Israel. *Israel Journal of Botany*, **30**, 199–205.

Mendel Z (2000) The phytophagous insect fauna of *Pinus halepensis* and *P. brutia* forests in the Mediterranean. In: *Ecology, Biogeography and Management of* Pinus halepensis *and* P. brutia *Forest Ecosystems in the Mediterranean Basin* (eds Ne'eman G, Trabaud L), pp. 217–236. Backhuys, Leiden, The Netherlands.

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5446–5451.

Nathan R, Ne'eman G (2000) Serotiny, seed dispersal and seed predation in *Pinus halepensis*. In: *Ecology, Biogeography and Management of* Pinus halepensis *and* P. brutia *Forest Ecosystems in the Mediterranean Basin* (eds Ne'eman G, Trabaud L), pp. 105–118. Backhuys, Leiden, The Netherlands.

Nathan R, Ne'eman G (2004) Spatiotemporal dynamics of recruitment in Aleppo pine (*Pinus halepensis* Miller). *Plant Ecology*, **171**, 123–137.

Nathan R, Safriel UN, Noy-Meir I, Schiller G, Steinberger Y (1996) Samara's aerodynamic properties in *Pinus halepensis* Mill., a colonizing tree species, remain constant despite considerable variation in morphology. In: *Preservation of Our World in the Wake of Change* (ed Steinberger Y), pp. 553–556. Israel Society for Ecology and Environmental Quality Sciences, Jerusalem.

Nathan R, Safriel UN, Noy-Meir I, Schiller G (1999) Seed release without fire in *Pinus halepensis*, a Mediterranean serotinous wind-dispersed tree. *Journal of Ecology*, **87**, 659–669.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Noy-Meir A (2003) *Invasion of* Pinus halepensis *from Planted Forests into Natural Habitats*. The Hebrew University of Jerusalem, Jerusalem. (in Hebrew with English summary).

Olden JD (2006) Biotic homogenization: a new research agenda for conservation biogeography. *Journal of Biogeography*, **33**, 2027–2039.

Olden JD, Poff NL, Douglas MR, Douglas ME, Fausch KD (2004) Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology and Evolution*, **19**, 18–24.

Osem Y, Ginsberg P, Tauber I, Atzmon N, Perevolotsky A (2008) Sustainable management of Mediterranean planted coniferous forests: an Israeli definition. *Journal of Forestry*, **106**, 38–46.

Osem Y, Zangy E, Bney-Moshe E *et al.* (2009) The potential of transforming simple structured pine plantations into mixed Mediterranean forests through natural regeneration along a rainfall gradient. *Forest Ecology and Management*, **259**, 14–23.

Panetsos CP (1975) Natural hybridization between *Pinus halepensis* and *Pinus brutia* in Greece. *Silvae Genetica*, **24**, 163–168.

Paquette A, Messier C (2010) The role of plantations in managing the world's forests in the Anthropocene. *Frontiers in Ecology and the Environment*, **8**, 27–34.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Perevolotsky A, Sheffer E (2009) Forest management in Israel – the ecological alternative. *Israel Journal of Plant Sciences*, **57**, 35–48.

Quézel P (2000) Taxonomy and biogeography of Mediterranean pines. In: *Ecology, Biogeography and Management of* Pinus halepensis *and* P. brutia *Forest Ecosystems in the Mediterranean Basin* (eds Ne'eman G, Trabaud L), pp. 1–12. Backhuys, Leiden, The Netherlands.

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83–109.

Richardson DM, Rundel PW, Jackson ST *et al.* (2007) Human impacts in pine forests: past, present, and future. *Annual Review of Ecology Evolution and Systematics*, **38**, 275–297.

Robledo-Arnuncio JJ, Gil L (2005) Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. *Heredity*, **94**, 13–22.

Robledo-Arnuncio JJ, Navascues M, González-Martínez SC, Gil L (2009) Estimating gametic introgression rates in a risk assessment context: a case study with Scots pine relicts. *Heredity*, **103**, 385–393.

Robledo-Arnuncio JJ, González-Martínez SC, Smouse PE (2010) Theoretical and practical considerations of gene flow. In: *Forests and Genetically Modified Trees* (ed. El-Kassaby Y), pp. 147–162. IUFRO-FAO, Rome.

Sathyan P, Newton RJ, Loopstra CA (2005) Genes induced by WDS are differentially expressed in two populations of aleppo pine (*Pinus halepensis*). *Tree Genetics and Genomes*, **1**, 166–173.

Savolainen O, Pyhajarvi T, Knurr T (2007) Gene flow and local adaptation in trees. *Annual Review of Ecology Evolution and Systematics*, **38**, 595–619.

Schiller G (1977) Interrelations between site factors and performance of Aleppo pine in the Sha'ar HaGay forest. *La-Yaaran*, **27**, 13–23.

Schiller G, Atzmon N (2009) Performance of Aleppo pine (*Pinus halepensis*) provenances grown at the edge of the Negev desert: a review. *Journal of Arid Environments*, **73**, 1051–1057.

Schiller G, Waisel Y (1989) Among-provenance variation in *Pinus halepensis* in Israel. *Forest Ecology and Management*, **28**, 141–151.

Schiller G, Ne'eman G, Korol L (1997) Post-fire vegetation dynamics in a native *Pinus halepensis* Mill. forest on Mt. Carmel, Israel. *Israel Journal of Plant Sciences*, **45**, 297–308.

Schuster WSF, Mitton JB (2000) Paternity and gene dispersal in limber pine (*Pinus flexilis* James). *Heredity*, **84**, 348–361.

Smouse P, Robledo-Arnuncio J, González-Martínez S (2007) Implications of natural propagule flow for containment of genetically modified forest trees. *Tree Genetics & Genomes*, **3**, 141–152.

Steinitz O (2010) Gene flow between and within Aleppo pine (*Pinus halepensis*) populations. PhD thesis, The Hebrew University of Jerusalem, Jerusalem.

Steinitz O, Troupin D, Vendramin GG, Nathan R (2011) Genetic evidence for a Janzen–Connell recruitment pattern in reproductive offspring of *Pinus halepensis* trees. *Molecular Ecology*, **20**, 4152–4164.

Thanos CA, Daskalakou EN (2000) Reproduction in *Pinus halepensis* and *P. brutia*. In: *Ecology, Biogeography and Management of* Pinus halepensis *and* P. brutia *Forest Ecosystems in the Mediterranean Basin* (eds Ne'eman G, Trabaud L), pp. 79–90. Backhuys, Leiden, The Netherlands.

Troupin D, Nathan R, Vendramin GG (2006) Analysis of spatial genetic structure in an expanding *Pinus halepensis* population reveals development of fine-scale genetic clustering over time. *Molecular Ecology*, **15**, 3617–3630.

Valbuena-Carabaña M, Gonzàlez-Martìnez SC, Sork VL *et al.* (2005) Gene flow and hybridisation in a mixed oak forest (*Quercus pyrenaica* Willd. and *Quercus petraea* (Matts.) Liebl.) in central Spain. *Heredity*, **95**, 457–465.

Voltas J, Chambel M, Prada M, Ferrio J (2008) Climate-related variability in carbon and oxygen stable isotopes among populations of Aleppo pine grown in common-garden tests. *Trees – Structure and Function*, **22**, 759–769.

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the analysis of population-structure. *Evolution*, **38**, 1358–1370.

O.S. is an ecologist interested in dispersal, biodiversity and conservation genetics. J.J.R.-A. is interested in developing methods for gene flow estimation in plants and applying them to empirical investigation of the interaction between gene flow and local adaptation. R.N. is interested in various aspects of movement ecology, especially the interactions of aerial organisms (seeds, birds and bats) with their real-life complex environments during dispersal, foraging and migration.

Data accessibility

DNA sequences: GenBank accessions AY304043, AY304036, CR354476, CR377943, AJ012087, AF195535, AF195543, JF803635, JF803641; Sample locations and microsatellite data: DRYAD entry doi:10.5061/dryad.db8tv34s.

Supporting information

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

Fig. S1 Change in nuclear microsatellite allele frequencies between adult and young trees in the native populations of Mt. Pithulim and of Mt. Shfanim.

Table S1 Comparison of the average (for nine nuclear microsatellite markers) allelic richness, observed heterozygosity and gene diversity (standard error in parentheses) between the adults and the young in Mt. Pithulim, Mt. Sumak and

Mt. Shfanim native populations and surrounding planted populations.

Table S2 Average (standard error in parentheses) inbreeding coefficient (F_{is}) for adult and young trees in Mt. Pithulim, Mt. Sumak and Mt. Shfanim, based on nine nuclear microsatellite markers.