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Part 1 - General application information

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Research Title
The effect of fire on spatial and temporal changes in the genetic structure of perennial plants: Pinus halepensis in Israel as a case study
Keywords
Fire, spatial genetic structure, dispersal kernels, mechanistic model, pinus halepensis, seed dispersal by wind, pollen dispersal by wind, plant recruitment, spatial dynamics

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Signature & Stamp	Signature & Stamp

Part 2 scientific abstract

The research project proposed here seeks to study the effect of fire on the temporal and spatial dynamics of population genetic structure in an obligate post-fire seeding tree species. More specifically, our aims are: (1) to compare the Fine-scale Spatial Genetic Structure (FSGS) and dispersal kernels of post-fire regenerating stands for the seed, seedling, sapling and adult stages with those of pre-fire or near unburned stands; (2) to analyze the temporal changes in FSGS and dispersal kernels as a function of post-fire age; (3) to study the spatial patterns of seed dispersal from mature large trees in recently burned sites immediately after fire; and (4) to study pollen dispersal, pollen receipt and fertilization patterns in post-fire versus fire-free stands.

Fire is a major, intensively-studied, disturbance in Mediterranean-type ecosystems, where plants exhibit adaptations to survive and reproduce after fire. Fire has drastic ecological and evolutionary consequences as a driver of massive post-fire regeneration and as a selective force for the evolution of several life history traits. Although population genetics is the scientific discipline bridging between ecology and evolution, and the study of FSGS (the clustering of similar genotypes at small spatial scales) is currently in the fore front of research, fire-driven population genetics in plants has been largely under-investigated and some key questions remain unresolved. In particular, we know very little about how fire affects the FSGS of plants: only a few quantifications of FSGSs after fire are available, and investigations of the underlying mechanisms are even less frequent. We propose to address this challenge by applying a mechanistic approach to study how fire affects the genetic structure of plant populations, focusing on *Pinus halepensis* in Israel as a study case. We selected *P. halepensis* as a model tree species because of the large amount of knowledge about its reproductive biology, fire ecology, seed dispersal, post-dispersal establishment, population expansion and fire-free population genetics; practically, quantification of FSGS for this species in facilitated by known DNA microsatellite markers, and its seed dispersal by wind has successfully been simulated by advanced mechanistic wind dispersal models.

Our proposed mechanistic approach (section A2) emphasizes the role of early recruitment processes in determining spatial patterns in plant populations. Seed and pollen dispersal, the principle mechanisms of gene flow in plant populations, have intensively been investigated in many ecosystems but much less so in post-fire environments. Here we propose to evaluate the dispersal kernels (the probability of dispersal distance) of pollen/seeds/seedlings/saplings/young trees, in order to assess the role of key early recruitment processes in determining FSGS. To comprehensively represent the spatial and temporal dimensions of fire-driven FSGS dynamics, we propose to combine the traditional 'space-for-time' (chronosequence) approach in *P. halepensis* stands with known fire history, with the practically challenging hence very uncommon 'time-for-space' approach (section A3), in the Nir Ezyon site (Mt. Carmel), where we have been studying *P. halepensis* dispersal and establishment both before (1992-1998) and after (1998-2001) a major fire event.

We envisage our findings to significantly contribute to basic and applied science related to population dynamics following disturbances, filling a major gap in our understanding of how disturbances affect population genetics. We further expect the development of a capacity to predict the genetic consequences of post-fire regeneration to be highly relevant for such salient applied questions as the population dynamics in fragmented landscapes and the conservation of the genetically unique population of *P. halepensis* in Israel.

Research Program
A Scientific background
A1. How does fire affect the genetic structure of plant populations?

Disturbances play a major role in shaping plant traits, species composition, ecosystem stability and landscape structure (e.g. Lavorel and Cramer 1999; Wilcox et al. 2006). Fire, either natural or anthropogenic, is a major disturbance in many ecosystems worldwide, including Mediterranean-type ecosystems (e.g. Naveh 1975; Trabaud 1987; Bond and Keeley 2005). Plants in these ecosystems exhibit a variety of adaptations to survive and/or reproduce in fire-prone ecosystems; e.g., resilience to fire, resprouting, or mass recruitment promoted by fire-related cues at seed dispersal or germination stages (e.g. Trabaud 1987; Whelan 1995; Bond and Van Wilgen 1996; Pausas and Verdu 2005; Pausas et al. 2006). Although fire effects on ecosystems – including soil characteristics (e.g. Neary 1999), community and population composition (e.g. Whelan 1995; Bond and Van Wilgen 1996; Menges and Quintana-Ascencio 2003) and spatial aspects of post-fire regeneration (Ne'eman and Izhaki 1998; Eshel et al. 2000; Bahulikar et al. 2004) – have been studied in detail, several key topics remained understudied. Here we propose to investigate an unexplored key question in population genetics, lying in the interface between ecology and evolutionary biology – how does fire affect the spatial genetic structure of plants?

With the advent of efficient molecular techniques and highly polymorphic molecular markers, studies of plant population genetics have recently shown dramatic progress in quantifying spatial dynamics of gene flow and genetic structure, and elucidating their major determinants (Gamache et al. 2003; Meirmans et al. 2003; Burczyk et al. 2004). Fine-scale Spatial Genetic Structure (hereafter **FSGS**), the non-random distribution, or clustering, of like genotypes at relatively small spatial scales, has recently been a subject of intensive research (e.g., Vekemans and Hardy 2004; Hardesty et al. 2005; Sato et al. 2006). Knowledge of FSGS is important for basic research because FSGS can influence population dynamics and persistence by affecting levels of adaptation to micro-environmental variation (Epperson 1992), mating patterns (Young and Merriam 1994), intensity of density-dependent processes (Hamrick et al. 1993), effective population size and inbreeding and consequently progeny fitness (Hamrick and Nason 1996; Schnabel et al. 1998). Knowledge of FSGS is also important for applied research, e.g., for management and planning of forests and natural reserves (Epperson 1992; Chung et al. 1998).

Recent emphasis in quantifying FSGS in colonizing plants (Litrico et al. 2005; Williams et al. 2005; Jones et al. 2006; Krauss et al. 2006; Troupin et al. 2006; Ward 2006) sets the stage for evaluating FSGS in disturbed environments. Although post-fire regeneration is a critical stage for many plants in fire-prone ecosystems, during which selection for fire-related traits like serotiny are expected to take place (e.g. in pines Perry and Lotan 1979; Ne'eman et al. 2004), few studies have explored the consequences of fire or other disturbances to the genetic structure of plant populations (e.g. Truesdale and McClenaghan 1998; Parker et al. 2001; Céspedes et al. 2003; Namroud et al. 2005; Premoli and Kitzberger 2005).

Comparisons between neighboring burned and unburned populations revealed significant FSGS only in **burned** stands of *Pinus clausa* (Parker et al. 2001) and only in **unburned** stands of *Nothofagus dombeyi* (Premoli and Kitzberger 2005). Allelic diversity and time after fire were **positively** correlated for *Cupressus*

forbesii (Truesdale and McClenaghan 1998) and **negatively** correlated for *Swietenia macrophylla* (Céspedes et al. 2003). Such contrasting patterns are commonly interpreted as signatures for an individualistic site- and/or species-specific response and lack of generality is often evoked. We argue, however, that although the research approach taken in these studies is useful to characterize and compare the resulting patterns, **it has very limited ability to provide general insights into the mechanisms underlying these patterns**, because different processes can generate the same pattern, and the same process can generate conflicting patterns (Jeltsch et al. 1999, Nathan and Casagrandi 2004).

A2. A mechanistic approach to study fire effects on genetic structure of plant populations

Our proposed mechanistic approach emphasizes the importance of early recruitment processes on spatial patterns of individual plants and genotypes (Harper 1977; Schupp and Fuentes 1995; Nathan and Muller-Landau 2000). This general principle is probably valid for most plant species in nearly all ecosystems, and presumably for both pre- and post-fire conditions. Indeed, the basic classification of fire survival strategies follows this principle, being based on post-fire resprouting or seedling recruitment abilities (Bond and van Wilgen 1996; Pausas et al. 2004). These strategies apparently have differential impacts on the genetic characteristics of the post-fire generation, as compared to the pre-fire population, and on the FSGS of the post-fire plant populations. Adult individuals of post-fire resprouting species have a high probability to survive fires and consequently fire has probably little impact on their genetic composition or FSGS. In contrast, most adult plants of post-fire obligate seeding species die during canopy fires and a new seedling generation is recruited after fire (Keeley 1991). Such populations are subjected to immediate selection by fire and thus fire could affect their genetic composition and FSGS. To the best of our knowledge, the temporal change in the genetic structure of tree populations during post-fire regeneration has not been studied yet.

Seed and pollen dispersal are the principle mechanisms of gene flow in plant populations, and are therefore the key determinants of genetic patterns (Wells and Young 2002; Heuertz et al. 2003; Bialozyt et al. 2006). The patterns and processes of seed dispersal have been intensively investigated (e.g. reviews in Harper 1977; Nathan and Muller-Landau 2000; Bullock et al. 2002; Levey et al. 2002; Nathan et al. 2003; Levin et al. 2003; Levine and Murrell 2003), but studies on post-fire seed dispersal are much less common (Enright and Lamont 1989; Saracino et al. 1997; Hammill et al. 1998). Comparisons of pre- versus post-fire seed dispersal patterns for the same tree species are very rare (but see Saracino et al. 1997; Whelan et al. 1998). As far as we know, the impact of the seed dispersal on the FSGS has never been investigated in post-fire conditions. The allocation of research efforts is even more asymmetric for studies of pollen dispersal: a plethora of investigations in fire-free conditions (e.g., Latta et al. 1998; Dick et al. 2003; Robledo-Arnuncio and Gil 2005), but no single study, to our knowledge, on pollen dispersal and fertilization of post-fire populations and its effect on the genetic composition and FSGS of their progenies. Overall, the proposed research approach does not only offer a more comprehensive way to investigate fire effects on plant population genetics, but also helps to fill up a gap in our knowledge of post-fire regeneration.

We propose to incorporate the effects of plant regeneration processes on FSGS in our mechanistic approach by evaluating the **dispersal kernels** of successive stages. Dispersal kernels portray the probability

density function of the distances between the source and the deposition sites of dispersed individuals evaluated for different reproduction or recruitment stages. Dispersal kernels can be quantified for pollen (distances between pollen donor and pollen receipt) and seeds (distances between seed source and seed deposition site). They can also be quantified for seedling/sapling/young adults (distances between the parent plant and the sites of established seedlings/saplings/young adults), giving rise to *effective* (dispersal and establishment) dispersal kernels (sensu Nathan et al. 2003). The different dispersal kernels reflect the operation of the principle distance- and density-dependent processes shaping the development of spatial structure during early recruitment stages, thus effectively summarize the key processes affecting FSGS. In addition, FSGS can be affected by factors such as inbreeding level and relative reproductive success (Troupin et al. 2006). To assess the potential role of these factors in shaping FSGS, measures such as expected and observed heterozygosity and inbreeding coefficient should be evaluated.

A3. Merging the space-for-time and the time-for-space approaches

Post-fire succession typically expends over several decades, making it difficult to follow the dynamics of successional populations by the time-for-space approach of sequential observations in fixed plots. Applying this approach for a sequence covering fire-free dynamics, a large fire event, and post-fire dynamics in the same site is even more challenging, and to our knowledge has never been accomplished (but see sect. C3). Therefore, the prevailing approach to study successional dynamics is the space-for-time (or "chronosequence") approach of parallel observations in sites differing in their successional age, the time elapsed from the last fire (e.g., Foster and Tilman 2000; Bjornstad 2001). This approach has been taken also in the few studies examining fire effects on population genetics (sect. A1). However, the basic assumption of the space-for-time approach, that the spatial variation (among sites) in post-fire dynamics is equivalent to the temporal dynamics (between times) has been questioned (Bjornstad 2001); similar arguments can also be raised against attempts to generalize from time-for-space studies alone. We therefore propose to combine the two approaches, taking advantage of a unique opportunity, made available as a result of our long-term study, to implement the rarely feasible time-for-space approach (see sect. C3).

A4. The proposed case study: *Pinus halepensis* populations in Israel

The Mediterranean region is one of the worlds most fire prone areas, where fire shaped plant species and landscapes (Bond and van Wilgen 1996). Post fire regeneration was extensively studied in many Mediterranean countries including Israel and Mt. Carmel in particular (Ne'eman and Trabaud 200). Therefore, we propose to study effect of fire on changes in the genetic structure of *P. halepensis* in Israel. We selected *Pinus halepensis*, a common wind-pollinated and -dispersed Mediterranean tree, as a model species for studying the effects of fire on population genetics of an obligate seeder, for the following main reasons: **(1)** The species' reproductive biology, including the effects of age, fire and other environmental conditions, has been intensively investigated in Israel (Ne'eman et al. 1992; Ne'eman 1997, 2000; Ne'eman and Izhaki 1998; Goubitz et al. 2004; Nathan and Ne'eman 2004) and elsewhere (Agee 1998; Barbero et al. 1998; Arianoutsou and Ne'eman 2000; Trabaud 2000). **(2)** The key recruitment processes of seed dispersal and post-dispersal establishment have been intensively studied in both fire-free (Nathan et al. 1999, 2000;

Nathan and Ne'eman 2000, 2004) and post-fire (Ne'eman et al. 1992, 2004; Ne'eman and Izhaki 1998, 1999; Arianoutsou and Ne'eman 2000; Trabaud 2000; Ne'eman 2000) conditions. **(3)** Several mechanistic wind dispersal models were successfully applied to simulate dispersal in this species (Nathan et al. 2001, Nathan and Ne'eman 2004), including theoretical investigation of the role of dispersal versus disturbance by a comprehensive genetic-demographic simulation model (Bohrer et al. 2005). **(4)** FSGS has successfully been quantified in an Israeli population (Troupin et al. 2006), using specific DNA microsatellite markers and well-established protocols (Vendramin et al. 1996; Keys et al. 2000). **(5)** The species is considered an excellent colonizer within its native range (e.g. Acherar et al. 1984; Nathan et al. 2000; Lavi et al. 2005) and an invasive species elsewhere (Richardson 2000). Finally, **(6)** Studies of large-scale genetic variation around the Mediterranean Basin showed that the Israeli population is genetically distinct (Schiller 2000). The last two topics emphasize the importance of studying the species' population genetics for practical aspects associated with disturbances, habitat fragmentation, invasions and conservation.

B. Research objectives & expected significance

The research project proposed here seeks to study the effect of fire on the temporal and spatial dynamics of gene flow and population genetic structure in an obligate post-fire seeding tree species, using *P. halepensis* as a model species (sect. A4). To this end, we aim to analyze how fire affects the fine-scale spatial genetic structure (FSGS) and dispersal kernels (sect. C2.4 & C2.7.4) in *P. halepensis* populations in Israel.

Our specific objectives are: **(A)** to compare the FSGS and dispersal kernels of post-fire regenerating stands for different (seed, seedling, sapling and adult) stages with those of pre-fire or unburned stands (sect. C2.4); **(B)** to analyze the temporal changes in FSGS and dispersal kernels as a function of post-fire age (sect. C2.4); **(C)** to quantify the spatial patterns of seed dispersal from mature large trees in recently burned site immediately after fire, and compare them to seed dispersal pattern from unburned trees in fire-free conditions, by combining field measurements and mechanistic wind dispersal models (sect. C2.5); **(D)** to study pollen dispersal, pollen receipt and fertilization patterns in post-fire versus fire-free stands (sect. C2.6).

For our methodology we propose to combine the following two approaches: **(1)** the space-for-time approach, by focusing on several post-fire regenerating *P. halepensis* populations with different fire histories on Mt Carmel, in comparison to nearby unburned stands (sect. C2.3) and **(2)** the time-for-space approach, by focusing on the population at Nir-Ezyon site, burned in 1998, where we intensively studied seed dispersal and recruitment in both fire-free and post-fire conditions (sects. C2.3 and C3).

The proposed research combines innovative approaches to address important yet difficult to study hence poorly-explored questions. We expect the findings of our study to contribute significantly both to basic and applied research relevant to plant population dynamics in disturbed environments. On the basic side, studying the effects of fire on the spatiotemporal dynamics of population genetic structure will fill a major gap in our understanding of how major disturbances affect population genetics and, consequently, life history evolution and plant community composition. One of the main contributions of the proposed study is development of a general research approach integrating the phenomenological (describing patterns) and mechanistic (modeling processes) approaches. Similarly, we propose to develop and evaluate one of the first

combinations of the space-for-time and the time-for-space approaches for studying population genetics following succession.

On the applied side, fire has many important implications for management and conservation. Developing a capacity to predict the genetic consequences of plant regeneration after fire is highly relevant to applied questions of population dynamics in fragmented landscapes and invasion by alien species (e.g. Higgins and Richardson 1998; Clark et al. 1999; With 2002; Cannas et al. 2003). This is especially important under current climate changes, expected to increase fire hazard in the Mediterranean Basin (Davis and Michaelson 1995). Also of great practical importance is the danger of genetic contamination of endangered natural forest populations, as is *P. halepensis* in Israel, from nearby planted stands (e.g. Ellstrand et al. 1999; Thompson et al. 2003). Understanding how fire affects gene flow is critical for addressing these concerns.

C. Detailed description of the proposed research

C1. Hypotheses and predictions

H1: The post-fire spatiotemporal dynamics of **dispersal kernels** during early recruitment stages differ from the corresponding dynamics in the absence of fire. Stage-specific predictions, summarized in Table 1, are:

Pollen dispersal: **P1A:** The mean pollen dispersal (donor-to-recipient) distance in post-fire conditions is higher than the corresponding distance in the absence of fire, because of stronger winds in canopy-burned stands and the low density of pollen donors in a burned stand.

Seed Release: **P1B:** The post-fire initial density of dispersed seeds **_near_** adult trees is higher than the corresponding seed density in the absence of fire, because fire induces massive seed release.

Seed dispersal: **P1C:** The post-fire initial density of dispersed seeds **_far from_** adult trees is higher than the corresponding seed density in the absence of fire, because of stronger winds in canopy-burned stands.

Seed survival: **P1D**: The post-fire survival of seeds, **_both near and far from_** adult trees, is higher than that in the absence of fire, because of higher seed number and reduced number of seed predators.

Seedling emergence: **P1E**: The post-fire seedling density, **_both near and far from_** adult trees, is higher than the corresponding seedling density in the absence of fire, because of the combination of P1B, P1C and P1D.

Seedling/sapling survival: **P1F**: The post-fire seedling/sapling survival **_near_** adult trees is higher than the corresponding seedling/sapling survival in the absence of fire, because of the favorable ash patches.

Seedling/sapling survival: **P1G:** The post-fire seedling/sapling survival **_far from_** adult trees is lower than the corresponding seedling/sapling survival in the absence of fire, because of strong intraspecific competition.

H2: The post-fire spatiotemporal dynamics of **FSGS** during early recruitment processes differ from the corresponding dynamics in the absence of fire. In Table 2, we summarize our specific predictions, following the mechanisms described in P1A to P1G above. Overall, we predict the following general trends:

P2A: FSGS will strongly increase during post-fire dynamics.

P2B: FSGS might decrease, remain or increase during fire-free dynamics.

P2C: The increase in FSGS during fire-free dynamics, if occurs, will be much less pronounced in comparison to the corresponding increase during post-fire dynamics.

C2. Experimental design & methods

C2.1. Overall research strategy

The proposed research integrates field observations/measurements, surveys, laboratory analyses and experiments, genetic analyses based on molecular markers, and modeling. We have gained extensive expertise with these methods during our previous and ongoing ISF-supported projects. To cover the longest possible post-fire time span we will establish 12 study sites with known fire history (sect. C2.3). **The requested funding for 4 years is mandatory to accommodate the high inter-annual variation in seed dispersal and post-dispersal recruitment processes characterizing the study system** (Nathan et al. 2000).

C2.2. Study species

Pinus halepensis is a tree with dual life strategy expanding its populations into disturbed areas by long distance seed dispersal (Nathan et al. 2000, 2001, 2002) but is also a typical post-fire obligate seeding species (Ne'eman and Trabaud 2000). The adult trees die after severe canopy fire, but many seedlings are recruited from seeds dispersed from serotinous and regular cones. Most of the seeds land in the vicinity of the mature trees, where the thick post-fire ash layer inhibits their germination. Yet, inter- and intra-specific competition is reduced at these sites, leading to relatively high survival and growth rates compared to seedlings that germinate far from the adult burned trees (Ne'eman 2000). Because these seeds are likely to be siblings, the enhanced survival at ash layers is likely to largely determine dispersal kernels and FSGS. The fast-growing saplings reach reproductive age after 5 years, producing first young female cones that will develop into serotinous cones, which help avoiding immaturity risk of being burned at young age before the establishment of a reasonable size of canopy stored seed bank (Goubitz et al. 2004). In large burned areas all these cones are probably pollinated by pollen dispersing long distances from few survived pines, which should be reflected in the dispersal kernels and FSGS.

C2.3. Study sites

To cover sufficiently long post-fire temporal sequences, we plan to establish 12 study plots in 7 burned and 5 unburned sites (Figure1, Table 3). The 12 plots include: (1) Three sites burned in 1978, 1989 and 1998; (2) Two recently (2006) burned plots; (3) Three plots with long no-fire history, located close to the plots burned in 1978, 1989 and 1998 (Figure 1). All the study plots were approved by the Nature and Parks Authority. The remaining 4 plots – 2 recently burned plots and 2 control unburned plots nearby – must be selected during the first or second year of our study, to follow recruitment dynamics immediately after fire. We do not expect to encounter difficulties in establishing these additional plots in the first year of our study, based on the documented fire frequency of 2-3 large fires every year in the study region.

We shall quantify FSGS and dispersal kernels in all plots, for comparisons by the space-for-time approach (sect. A3). In one plot (Nir-Ezyon) we shall also use the time-for-space approach. Our intensive studies of the isolated pine population in this site, started in 1992, elucidated the temporal dynamics and spatial patterns of seed dispersal and establishment (Nathan et al. 1999; Nathan et al. 2000), and the mechanisms of seed dispersal by wind (Nathan et al. 2001, 2002). In October 1998, the site was burned by a large fire that destroyed almost all adult trees. We sampled seed dispersal after fire by counting seeds in seed

traps placed in their pre-fire locations (Figure 2). Seedling establishment was sampled in one m² plots near each seed trap (see sect. C3 for preliminary results).

Each study plot will consist of about 3000 m² and contain 50-80 adult pines, and determine post-fire tree ages using tree ring dating following Troupin et al. (2006). Adult trees and recruitment sampling plots will be mapped using a sub-meter DGPS, an elevated antenna and a laser rangefinder. Lessons from our experience in mapping ~2000 pine trees in a 60ha plot – as part of our recently completed ISF-supported project (ISF 474/02 to RN) – will be implemented to facilitate this demanding task.

C2.4. Fieldwork

In general, the proposed fieldwork is designed to enable comparing FSGS and dispersal kernels between burned) and adjacent unburned (4, 7, 8, 11, 12) plots (Table 3, Figure 1). The fieldwork is designed to provide data for estimating FSGS and dispersal kernels by molecular techniques (sect. C2.5), and dispersal kernels by models (sect. C2.6). For the molecular work, the specific activities described below will provide DNA samples from up to 100 individuals (seeds, seedlings, saplings or adults) in sampling session in each plot, summing up to 5200 samples (Tables 4 and 5).

Pollen dispersal (testing P1A). We shall examine pollen dispersal, pollen receipt and fertilization patterns in the post-fire developing stand in Nir Ezyon burned (plot 3) and an adjacent unburned isolated plot (plot 4). A total of 550 samples for DNA extraction will be taken from each plot (Table 5). This includes samples from ~150 living adult trees in the plot and its vicinity which are the potential pollen donors for the seeds in the early post-fire cone cohorts. We shall germinate a random sample of 25 seeds from each of 4 randomly selected cones from the 10 largest post-fire young trees, and extract DNA from young leaves of the 5 largest emerging seedlings for each cone. The same procedure will be repeated for the last cone cohort. We will perform parallel sampling in saplings of equivalent age in an adjacent unburned plot.

Seed release and dispersal (testing P1B and P1C). Post-dispersal seed density will be sampled by counting seeds on the ground (in the first day after fire) and by seed traps, to be established immediately after fire in the 2 newly-burned plots (9, 10) and in the control unburned plots (11, 12). Seed traps will be arranged in a regular grid (~33 traps per plot, 10m apart), covering both locations at immediate proximity to adult trees and in the gaps between trees. Seed traps will be monitored in weekly to monthly intervals during 3 successive years, and collected seeds will be kept in dry conditions. In all other plots (1-8) we shall sample seeds from the soil see bank. Seeds will be carefully sampled at randomly-stratified plots, covering locations both under mature living and burned pine trees and canopy gaps amongst them. We shall sample the upper soil layer (0-5 cm) in 20x20cm squares (Ne'eman and Izhaki 1999) in autumn before germination, and sieve out the seeds. This procedure will be repeated in 3 successive years. We shall germinate a set of randomly selected seeds as a fixed proportion of the total seed crop collected in each sampling station, summing up to 100 germinated seeds per plot, to be used for DNA extraction (Table 4).

Seed survival, seedling emergence, seedling and sapling survival (testing P1D to P1G). We shall establish an array of predation protected plots (against ants, rodents and birds) next to the seed trap stations (see above), using methods established and thoroughly tested in our previous studies (Nathan and Ne'eman

2004). Seedling emergence and survival, as well as sapling survival will be monitored for 3 successive years in 5 1m^2 plots adjacent to each seed trap station, using the design we applied in the Nir Ezyon plot after the 1998 fire (sect. C2.3). Seedlings and saplings will be sampled for DNA extraction as described above.

Adult trees (testing P2A to P2C). We shall randomly sample up to 100 mature (cone-producing) pines in each plot (Table 4).

C2.5. Molecular Work

This section describes the main procedures for processing and analyzing molecular data for estimating FSGS and dispersal kernels. In our recently-completed ISF-supported project (ISF 474/02 to RN), we have successfully applied these techniques for *P. halepensis* at Mt. Pithulim (Troupin 2005, Troupin et al. 2006).

DNA extraction, amplification and sequencing*.* Genomic and chloroplast DNA from seeds, needles and if possible also dead xylem following Liepelt et al (2006) will be extracted using the DNeasy Plant Mini Kit (Qiagen) according to manufacturer's protocol. Extracted DNA samples will be quantified by Nanodrop ND-1000 Spectrophotometer (requested permanent equipment, sect. D). DNA samples will be scanned for maternal and paternal microsatellites using primers described by Vendramin et al. (1996) and Keys et al. (2000). PCR reactions will be performed as described by Troupin et al. (2006). Microsatellite analysis will be preformed at the Center for Genomic Technologies of the Life Science Institute at the Hebrew University of Jerusalem (http://www.bio.huji.ac.il/bio-genome.html).

Heterozygosity. For assessing effects of several key factors not accounted by dispersal kernels (sect. A2), we shall follow Troupin et al. (2006) in using the 'Genalex 6.0' software (Peakall & Smouse 2005) to calculate expected heterozygosity, observed heterozygosity, and the effective number of alleles. The inbreeding coefficient within individuals relative to the population (F_{IS}) (Weir & Cockerham 1984) and its significance will be calculated using the 'Genepop 3.4' software (Raymond & Rousset 1995). All measures will be calculated only for nuclear markers.

Parentage analyses*.* Parentage analysis assigns putative parents to given offspring. We shall use the software 'FAMOZ' (Gerber et al. 2003) which is based on maximum likelihood methods (Meagher 1986) and has the possibility of incorporating cytoplasmic markers in addition to nuclear ones. The program calculates the 'Logarithm of the odds ratio' (LOD) scores for each possible parentage relationship (single parent and parent pair). The higher the LOD score for a given parent-offspring pair, it is more likely that the given relationship is true. For each analysis the corresponding F_{IS} value will be entered (sect. C2.3.2.), as a measure of departure from the Hardy-Weinberg equilibrium. Various values of LOD score calculation errors (mistyping error) and simulation errors (randomly replacing an allele from the genotyped parents) will be tested. Exclusion probabilities for co-dominant markers (Jamieson and Taylor 1997) will be calculated, as the difference between the highest LOD score and second highest LOD score (Δ statistic, Marshal et al. 1998). Since the chloroplast genome is paternally inherited in conifers (Wagner 1992), we can determine whether a successfully assigned parent is the seed source or the pollen donor. In our previous ISF-supported project we successfully applied these techniques for *P. halepensis* trees at Mt. Pithulim (Troupin 2005). In the proposed research we also plan to apply a technique for assigning maternal parentage to single dispersed

seeds (from seed traps, sect. C2.4.) from the maternally-originated wing tissue. This technique has recently been applied for a few angiosperm species (Godoy and Jordano 2001, Ziegenhagen et al. 2003, Jones et al. 2005, Pairon et al. 2006). Ziegenhagen et al. (2003) also applied this technique for a coniferous species (*Abies alba*), using both the wing and the endosperm to assign the maternal source. Dispersal distance will be calculated as the Euclidean distance between the trap and the assigned maternal tree.

FSGS analyses. Levels of FSGS will be determined for all adult (cone-producing) individuals sampled in our plots. Following our experience with the focal species (Troupin et al. 2006), we shall use standard spatial autocorrelation methods (Sokal and Oden 1978) as well as the '*Sp*' statistic (Vekemans and Hardy 2004) that is less sensitive to the sampling design used, and allows the comparison of FSGS magnitude among different populations and developmental stages (seeds, seedlings, saplings and adults) (Vekemans and Hardy 2004). For spatial autocorrelations, we shall apply the multivariate autocorrelation procedure, which strengthens the spatial signal and reduces the allele to allele stochasticity and locus to locus noise (Smouse and Peakall 1999), using the 'Genalex 6.0' software (for detailed description see: Smouse & Peakall 1999; Peakall et al. 2003; Peakall and Smouse 2005). The '*Sp*' analyses will be calculated using the software 'Spagedi' (Hardy & Vekemans 2002).

C2.6. Modeling work

In addition to the direct method of estimating dispersal kernels from parentage analysis based on molecular markers (sect. C2.5), we shall apply and compare two indirect methods, inverse modeling and mechanistic wind dispersal models. Although all three methods are increasingly being used in dispersal research (Nathan et al. 2003, Nathan 2006), only a few studies have applied more than one approach. In a recent collaboration, we provided one of the first comparisons between dispersal kernels estimated by parentage analysis and inverse modeling techniques, showing remarkable similarity for *Pinus pinaster* sapling dispersal kernels (Gonzáles-Martínez et al., in press). Although these results are encouraging, such convergence is no guarantee (e.g., Pairon et al. 2006) and rigorous comparisons are critically needed.

Inverse modeling techniques require knowledge of the spatial arrangement of offspring (seeds, seedlings etc.) in sampling plots and of all the potential seed sources (adult plants) and their relative fecundity (the number of seeds dispersed). Although the spatial location of offspring and potential seed sources in sampling plots are known (sect. C2.4), the actual dispersal distances of observed offspring remain unknown. Thus, inverse modeling techniques estimate dispersal distances only indirectly, using maximum likelihood techniques to fit a certain sampling distribution to the observed offspring number in the sampling plots. Previous studies assumed either Poisson (e.g. Clark et al. 1999) or negative binomial (e.g. Clark et al. 1998) sampling distributions. Fecundity is commonly assumed to be a linear function of the tree basal area (Clark et al. 1998, 1999); we shall test this assumption by regressing the expected number of seeds dispersed (for methods, see Nathan et al. 1999, Nathan and Ne'eman 2000) on measurement of basal area in 100 randomly selected trees in our plots. Candidate functions for the dispersal kernels include the exponential-family function (Clark et al. 1998), the 2Dt distribution (Clark et al. 1999), the lognormal and the Weibull distributions (Greene et al. 2004), and the inverse Gaussian distribution (Katul et al. 2005). We have

successfully applied this technique to estimate dispersal kernels of *Pinus pinaster* (Gonzáles-Martínez et al., In Press), and *P. halepensis* (Schurr, Nathan and Steinitz, unpublished).

Mechanistic wind dispersal models use data on the biological and physical factors affecting the distance of dispersal of pollen or seeds by wind. We have extensive experience with various kind of wind dispersal model, including simple ballistic models that work very well for local scale (Nathan et al. 2001, 2002), Lagrangian simulations that account for the effects of turbulence (Nathan et al. 2002a) and canopy structure (Nathan and Katul 2005), complicated Large-Eddy Simulations that can capture the effects of landscape heterogeneity (Nathan et al. 2005, Bohrer et al. in preparation), as well as analytical models which are much faster than the simulation models listed above, and still provide faithful description of both short and long-distance dispersal (Katul et al. 2005). These studies include successful applications of several mechanistic models to our focal species (Nathan et al. 2001, 2002, Nathan and Ne'eman 2004). In the study proposed here, we plan to apply new models of wind dispersal in heterogeneous landscape and complex topography, currently being developed in collaboration with Prof. Gabriel Katul (Duke University) as part of our ongoing ISF-FIRST project (1316/05 to RN). We emphasize that our model has been developed and tested mostly for seed dispersal, but also has been successfully applied for pollen dispersal (Katul et al. 2006). We are therefore in the favorable position of having an array of models well-suited for simulating the key dispersal process, which have been evaluated for our focal species.

C3. Preliminary Results

Intensive seed dispersal was observed at Nir-Ezyon site (sect. C2.3) in the first day after fire, reaching a pick of local density of 308 seeds m^{-2} day^{-1}, compared to a maximum of 20 seeds m^{-2} day^{-1} recorded during the pre-fire sampling period (Nathan et al. 1999). Post-fire seed dispersal continued for three months. Seedlings densities reached a local maximum of 14 seedlings m^{-2}. The total number of seedlings of the first post-fire cohort declined from 394 (in the total 390 m^2 sampling area) in the first post-fire year to 113 in the third year. The number of seedlings of the second post-fire cohort was 44 in second post-fire year. In the third year, 14 newly-established seedlings were observed. These seedlings might have originated from seeds dispersed from the few adults that survived the fire; alternatively, they could reflect long-distance dispersal from individuals >500 m from the site, cryptic dispersal from cones on standing-dead individuals, or the existence of persistence post-fire seed bank. In September 2006 we observed tens of young trees with cone cohorts of up to 4 years, implying that these trees became reproductive only 4 years after fire. The combination of genetic analyses for all the trees (including the burned ones, sect. C2.5) and detailed monitoring of post-fire seed dynamics in other recently burned sites will help decipher the alternatives.

C4. Conditions available for research performance

All equipment needed for the field work in available in G.N. lab in Oranim campus. R.N. lab at The Hebrew University of Jerusalem has 15 workstations and GIS software and a laser rangefinder and other accessories required for mapping and spatial analyses. Nathan's lab also includes a functional molecular laboratory, including 2 PCR machines, centrifuge, various pipettes and pH meters, 2 gel systems, UV table, 500L -85°C freezer, 390L -20°C and 390L Refrigerator.

D. Tables and Figures

Table 1. Predicted dynamics in dispersal kernels in post-fire versus fire-free conditions. The < and > symbols refer to the mean dispersal distance.

Conditions / Stage	Post-fire		Fire-free
Parents	Unknown		Unknown
Seeds	Dispersal kernels strongly leptokurtic: most seeds are dispersed under mother's canopy, yet a relatively large proportion disperse long distances by stronger winds in canopy-burned stands	>	Dispersal kernels less leptokurtic: seeds dispersed relatively short distances because winds are weaker in full-canopy stands
Seedlings	Dispersal kernels became thin-tailed, because most seed deposited far from adult trees do not germinate	<	Dispersal kernels become more fat-tailed, because seeds dispersed far from adult trees have higher probability to escape predation and emerging seedlings have lower competition
Saplings	Dispersal kernels became more thin-tailed, because most seedlings germinated far from adult trees do not survive	<<	Dispersal kernels become more fat-tailed, because seedlings far from adult trees have higher survival probability
Adults	Dispersal kernels became more thin-tailed, because most saplings germinated far from adult trees do not survive	<<	Dispersal kernels become more fat-tailed, because saplings far from adult trees have higher survival probability

Table 2. Predicted **FSGS** (Fine-scale Spatial Genetic Structure) dynamics in post-fire versus fire-free conditions.

Conditions / Stage	Post-fire		Fire-free
Parents	Unknown		Unknown
Seeds	High FSGS, but lower than in fire-free conditions due to longer dispersal distance	<	High FSGS, due to limited seed dispersal
Seedlings	FSGS higher than the previous stage, because germination is mostly under (burned) adult trees	>	FSGS lower than the previous stage, because of positive density-dependent mortality of seeds
Saplings	FSGS similar or higher than the previous stage, because seedlings survive mostly under (burned) adult trees	>	FSGS lower than the previous stage, because of positive density-dependent mortality of seedlings
Adults	FSGS considerably higher compared to pre-fire generation	>	FSGS considerably lower, and perhaps even lacking, compared to the previous generation

Table 3. Location, fire history, name and number of our proposed study sites and plots.

Sites	Plot # and name	Location (ITM)	Last burned	Years since fire
Lubim	1. Lubim 1,	1994 7393	2006	1
Lubim	2. Lubim 2	1994 7393	2006	1
Nir Ezyon	3. Nir Ezyon 1	1984 7333	1998	9
Nir Ezyon	4. Nir Ezyon 2	Near site 3	No fire	>50
Hai Bar	5. Hai Bar	2028 7394	1989	18
Mitla	6. Mitla	1996 7382	1978	29
Antenna	7. Antenna	2033 7393	No fire	>50
Beit Oren	8. Beit Oren	2002 7377	No fire	>50
X	9. X1	Unknown	2007	
Y	10. Y1	Unknown	2007	
X	11. X2	Near site 9	No fire	>50
Y	12. Y2	Near site 10	No fire	>50

Table 4. DNA sampling design of mature burned or living trees, seeds, seedlings and saplings in the research plots. Burned or living adults, seeds, seedlings and saplings.

| Plots #	# of plots	Items	# of samples per plot	1st y	2nd y	3rd y	4th yr	Total
1, 2, 9, 10	4	Burned trees	100	200	100	100		400
1, 2	2	Seeds (soil)	100	100	100			200
1, 2, 9, 10	4	Seedlings	100		200	100	100	400
9, 10	2	Seeds (traps)	100		100	100		200
3, 5, 6	3	Burned trees	100	150	150			300
3, 4, 5, 6	4	Seedlings	100	200	200			400
3, 4, 5, 6	4	Saplings	100	200	200			400
4, 7, 8, 11, 12	5	Adults	100	100	100	200	100	500
4, 7, 8, 11, 12	5	Seeds	100	100	100	200	100	500
7, 8, 11, 12	4	Seedlings	100	100	100	100	100	400
7, 8, 11, 12	4	Saplings	100	100	100	100	100	400
3,4	2	pollen receipt	550			550	550	1100
Total				**1250**	**1450**	**1450**	**1050**	**5200**

Table 5. DNA sampling design of mature unburned trees and seeds from the earliest and last produced cones of the post-fire saplings for the pollen dispersal, pollen receipt and fertilization study in the burned Nir Ezyon 1 plot and an unburned adjacent Nir Ezyon 2 plot (data included in Table 4).

Sampling unit	Number of units	Samples per unit	Total samples
Surviving adults	150	1	150
1st cone cohort	40	5	200
Last cohort	40	5	200
Total per plot			550
Total for 2 plots			**1100**

Figures

Figure 1. Map of the proposed burned and unburned study plots, for more details see table 3.
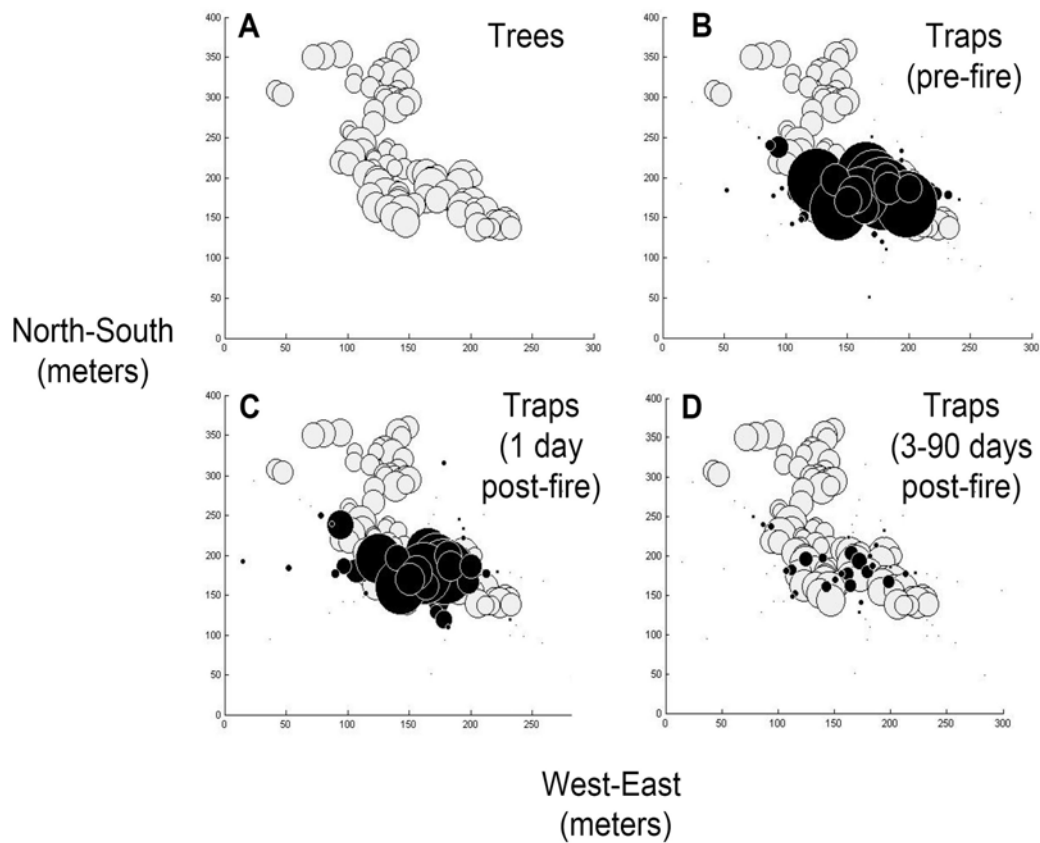
Figure 2. The location of trees (grey) and seed trap yield (black) before (**A** and **B** respectively), in the first day (**C**) and during a period of 3-90 days (**D**) after the 1998 fire after in Nir Ezyon site. Circle size is relative to the canopy projection of the trees and to the seed yield in traps.

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Time schedule and work-plan

Objective	Beginning	End
Establishment of plots (1,2) and sampling soil seed bank (preliminary work)	09/2006	10/2006
Establishment of plots (3-8) and sampling of plots (1-8)	10/2007	07/2008
Mapping and sampling of plots (1-8)	10/2007	07/2008
DNA extraction and analysis	10/2007	07/2008
Annual report	08/2008	08/2008
Establishment of plots (9-12) and sampling	09/2008	07/2009
Monitoring of seedling and saplings in all plots	09/2008	07/2009
DNA extraction and analysis	09/2008	07/2009
Seed trapping and predation experiments	09/2008	07/2009
Modeling	09/2008	07/2009
Annual report	08/2009	08/2009
Supplementary sampling	09/2009	07/2010
Monitoring of seedling and saplings in all plots	09/2009	07/2010
DNA extraction and analysis	09/2009	07/2010
Seed trapping and predation experiments	09/2009	07/2010
Modeling	09/2009	07/2010
Annual report	08/2010	08/2010
Supplementary sampling	09/2010	08/2011
Monitoring of seedling and saplings in all plots	09/2010	08/2011
DNA extraction and analysis	09/2010	08/2011
Final data analyses	09/2010	08/2011
Modeling	09/2010	08/2011
Preparation of MS: The effect of fire on spatial and temporal changes in dispersal kernels and FSGS in tree populations	09/2010	08/2011
Preparation of MS: Mechanistic models for post-fire tree seed dispersal by wind	09/2010	08/2011
Preparation of MS: Effective pollination distance and FSGS in a post-fire pine forest	09/2010	08/2011
Preparation of MS: Post-fire Seed predation in an obligate seeding tree	09/2010	08/2011
Final report	09/2011	09/2011

Explanatory Notes:

We request support for four years to accommodate roughly one year of preparations in establishing the 12 study plots (this is a rather demanding task), followed by at least three years of sampling and monitoring the patterns of recruitment in these plots, required because of the strong inter-annual variation typical of the underlying processes. We emphasized that 4 plots can only be established after the approval of this proposal, to enable quantification of the post-fire dynamics starting from the first day after first, and lasting for at least 3 years, thus covering the most sensitive period during which the spatial dynamics are most pronounced.