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RESEARCH PAPER

Microspatial population genetic structure of the Mediterranean shrub *Fumana thymifolia*

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ABSTRACT

Fumana thymifolia **(Cistaceae) is an insect-pollinated, gravity-dispersed evergreen shrub, which is a common component of fire-prone Mediterranean shrubland ecosystems. Despite the availability of basic knowledge on its ecology, little is known of its breeding system and no information is available on its population genetic structure. We explored the within-population genetic structure of this species using amplified fragment length polymorphism (AFLP) molecular markers and related this to predictions based on its breeding system, pollen and seed dispersal. Existing information on the reproductive ecology of *F. thymifolia* was supplemented by artificial pollination experiments. We determined that self-fertilisation can occur in *F. thymifolia* but results in reduced fruit set. Significant genetic structuring was detected within the population, a likely consequence of localised seed dispersal in combination with a mixed mating system. In a study site covering approximately 0.5 ha, AMOVA revealed that approximately 9% of genetic variability was distributed among population subsamples. Significant spatial genetic structure was detected, with kinship coefficients being significantly elevated above the null expectation in the first six distance classes (maximum 5 m), and a value of *Sp* of up to 0.0342, comparable with species having similar ecological characteristics. Weak isolation by distance at the plot scale was detected, suggesting that insect-mediated pollen flow is non-random, despite being more extensive than seed dispersal. *Fumana thymifolia* provides a promising model for the investigation of both short- and long-term population dynamics in relation to fire frequency within this plant community.**

INTRODUCTION

Within natural plant populations, genetic diversity is rarely distributed homogeneously; individuals often become less genetically similar as the distance between them increases. Such fine-scale genetic structure may result from the interaction of a number of factors, such as adult density, population history, selection and limited seed and pollen dispersal (Loveless & Hamrick 1984; Merzeau *et al.* 1994; Linhart & Grant 1996; Streiff *et al.* 1998). The relative importance of each factor is not easy to determine. Vekemans & Hardy (2004) suggested that, on a fine spatial scale, the most prevalent cause of spatial genetic structure (SGS) is likely to be the formation of local pedigree structures due to limited gene flow. As the degree of intra-population gene flow (whether *via* pollen, propagules or both) is a key factor in the development of SGS, the breeding system, pollination syndrome and dispersal mechanism of a species are important determinants of the level of SGS that develops within its populations

(Loveless & Hamrick 1984; Vekemans & Hardy 2004). The magnitude of SGS is expected to be greater in selfing as opposed to predominantly outcrossing species. However, significant SGS has been detected even in outcrossing, wind-pollinated trees, despite the expectation that wind-pollinated species should show very high levels of gene flow (and hence low levels of genetic structure) even among populations (Merzeau *et al.* 1994; Leonardi & Menozzi 1996; Cottrell *et al.* 2003; Jump & Peñuelas 2007). This apparent contradiction may be explained by the typically more limited dispersal of seed when compared with that of pollen, such that the resulting restricted maternal gene flow may result in pronounced spatial genetic structure within populations, even if pollen flow is extensive (Jump & Peñuelas 2007).

Determination of the degree of spatial genetic structure within populations is a key component of understanding the ecology of a species. High levels of SGS are indicative of the development of patch structures within otherwise continuous populations. Adequate knowledge of SGS is therefore important for the conservation and management of natural populations in order not to misrepresent species or population diversity (Epperson 1989; Shapcott 1995). Accurate estimates of SGS can inform decisions on the targeting of conservation resources at particular populations and the sampling area necessary to represent the genetic diversity within them (Escudero *et al.* 2003). If SGS is ignored, particular genotypes may be over- or under-represented within samples, and potentially important genetic variation may therefore be missed (Epperson 1989).

To quantify SGS, spatial autocorrelation techniques have been used to identify correlations among the genotypes of geographically referenced individuals occurring within a population. Such methods have the advantage that they do not require the delimitation of sub-populations and may therefore identify the scale of genetic structure within a population without prior knowledge of that scale (Dewey & Heywood 1988; Heywood 1991). In the methods presented by Loiselle *et al.* (1995) and Hardy (2003) and discussed by Vekemans & Hardy (2004), the probability that genes in different individuals within the sample are identical by descent is estimated from alleles identical in state, using the calculation of a coefficient of kinship or co-ancestry. The expectation being, that under an isolation-by-distance (IBD) model, the kinship coefficient between two individuals should decline as the distance between them increases (Loiselle *et al.* 1995; Hardy 2003). The resulting relationship may be viewed as a correlogram, whether for individual genes or a multilocus average. As there is no need to predetermine the scale, and because the analysis can be based on individual genotypes and distances between individual plants, such analysis of SGS is valuable for investigating the genetic consequences of dispersal over fine spatial scales within populations (Dewey & Heywood 1988; Loiselle *et al.* 1995).

Fumana thymifolia (L.) Spach ex Webb (Cistaceae) is a common component of early successional Mediterranean shrubland communities that typically establish after agricultural abandonment. This species grows up to 25-cm high and occurs in open shrublands around the Mediterranean basin on calcareous soils. The species is insect-pollinated (Petanidou & Vokou 1990), flowering occurs in spring and summer, and abundant seedling establishment occurs after autumn or winter rains. Flowers are diclinous, actinomorphic, with five yellow 6–9-mm petals. The inflorescence is typically a cyme of three to nine flowers. Seeds are small (*ca*. 1 mg) and lack obvious adaptations for dispersal, although herbivorous ungulates, such as sheep, may disperse the seeds when consumed during grazing (Ramos *et al.* 2006).

Fumana thymifolia was chosen as the subject of this study as it shares many traits with typical 'Mediterranean' taxa that evolved during the Quaternary under the current Mediterranean climatic conditions of the region (Herrera 1992a). The Cistaceae family is highly diversified and widely distributed in the Mediterranean basin; it is comprised of mostly woody species (short-lived shrubs) with large, animal-pollinated flowers preceding the production of dry fruits that are not widely dispersed (Herrera 1992b; Aronne & Wilcock 1994). Shrubby Cistaceae are major and typical constituents of sclerophyllous shrublands that cover large areas of the Iberian Peninsula and other Mediterranean countries, and are characteristic of the vegetation that develops after disturbance (typically by fire) of evergreen sclerophyllous forests (Herrera 1992b).

In many Mediterranean areas, such as the Eastern Iberian Peninsula, early successional shrublands have become dominant in the last few decades as a result of secondary succession after the abandonment of croplands and pastures (Baeza *et al.* 2002) and of a regressive impact on forest ecosystems as a consequence of the increase in wildfires resulting from changes in climate and land use (Lloret *et al.* 2002). *Fumana thymifolia* therefore provides a convenient model to study the spatial patterns of genetic diversity and population development of Mediterranean woody species.

Given the gene dispersal mechanisms outlined above, and based on data summarised for species with similar ecological characteristics by Vekemans & Hardy (2004), we expected *F. thymifolia* to be characterised by a strong intra-population genetic structure. However, despite basic information being available on the ecology of this species and its pollination syndrome, we were unable to find reports of its breeding system, or any estimation of population structure. We therefore sought to characterise the spatial pattern of the population genetic structure in *F. thymifolia* and to determine whether self-fertilisation is possible in this species. This information will enable comparison of SGS in *F. thymifolia* with available estimates for similar plant species and is fundamental to ongoing research into the structure and function of these dynamic Mediterranean shrubland ecosystems.

MATERIALS AND METHODS

Study site

The sampled population was located within the Garraf Natural Park, Barcelona, Spain, (41°8′ N, 1°54′ E, at 300 m a.s.l). The climate is Mediterranean, with cool, moderately wet winters and hot, dry summers. Stony and basic soils have developed over Cretaceous limestone. The vegetation is a short (less than 1-m high) shrubland growing in old agricultural terraces (vineyards), which were abandoned about 100 years ago. This shrubland is typical of Mediterranean coastal shrublands that occupy extensive areas of the Mediterranean region. The vegetation is subject to periodic fires, the last of which occurred in 1994. The vegetation therefore represents an early successional community, dominated by woody species such as the nanophanerophytes *Erica multiflora* L. and *Globularia alypum* L, and the chamaephytes *Fumana thymifolia*, *Fumana ericoides* (Cav.) Gandg. (syn *Fumana ericifolia* Wallr. in Linnaea) and *Coris monspeliensis* L. Although *F. thymifolia* co-occurs on the study site with *F. ericoides*, no hybrids have been reported between these species (Castroviejo *et al.* 1993). This study was conducted in a network of nine vegetation plots, each of 20 m^2, located within an area of the Garraf shrubland of around 0.5 ha. The distance between plots varied from 6.3 m to 75.2 m, with a mean of 39.7 m (Fig. 1).

AFLP analysis

One hundred and fourteen young (1–7 years old) *Fumana thymifolia* individuals were sampled from within the nine plots (3–16 individuals per plot, Table 1). Sampled leaf tissue was frozen in liquid nitrogen and stored at −80 °C.

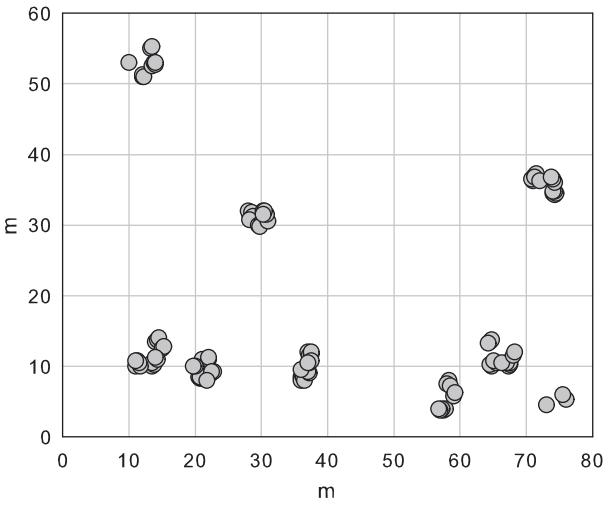

Fig. 1. Location of *Fumana thymifolia* samples. Gridlines represent 10-m divisions of the study site.

Table 1. Summary of *Fumana thymifolia* genetic diversity per plot.

plot	n	heterozygosity	% polymorphic loci
1	15	0.31 (±0.014)	85
2	15	0.30 (±0.013)	88
3	15	0.27 (±0.014)	85
4	15	0.28 (±0.014)	80
5	10	0.27 (±0.014)	83
6	15	0.28 (±0.015)	79
7	16	0.29 (±0.015)	73
8	3	0.15 (±0.015)	38
9	10	0.18 (±0.014)	57

n = no. of individuals per plot; Genetic diversity values are based on 164 AFLP loci with a maximum allele frequency of 0.95 over all 114 individuals studied. Mean heterozygosity over loci is presented ±1 SE.

Approximately 0.5 cm^2 of leaf tissue was ground in liquid nitrogen for 30 s at 30 × *g* using a mixer mill (Tissue Lyser, Qiagen) and two glass beads. Genomic DNA was extracted from the ground tissue using the DNeasy Plant Mini kit (Qiagen) and quantified using a NanoDrop ND-1000 spectrophotometer running software v3.0.1 (NanoDrop Technologies) following the manufacturers' instructions.

Amplified fragment length polymorphism (AFLP) molecular markers were used for this study because of their high utility for investigating spatial genetic structure (Jump & Peñuelas 2007) and the paucity of genetic resources available for the study species. The AFLP protocol followed is detailed in Jump *et al.* (2008). Briefly, after digestion of genomic DNA with *Eco*RI and *Mse*I, adaptors were ligated on both ends of the DNA fragments. Two sequential PCR amplifications were performed. The first PCR used *Eco*-A and *Mse*-C primers and the second PCR used one of five *Eco*-ACG/*Mse*-CNN primer combinations (where N represents any nucleotide). *Eco*-ACG primers carried a VIC fluorochrome (Applied Biosystems, ABI). *Mse*-CNN primer extensions were: CAG, CAT, CTG, CTC and CTA. Oligonucleotide sequences for PCR adapters and primers are as published by Vos *et al.* (1995).

Electrophoresis of fluorescent labelled PCR products was performed on an ABI3130xl genetic analyser according to the manufacturer's instructions. Fragment sizes were determined with reference to a LIZ-500 size standard (ABI) using GeneMapper v 4.0 (ABI). Fragment presence/absence was scored by hand and subsequently checked by a second investigator without reference to sample ID. A binary matrix of band presence/absence was then created. Each set of 96 reactions included two positive (known genotype) and two negative (H_2O or PCR mix without DNA) controls that were carried from restriction digest through to selective AFLP-PCR.

Pollination experiments

Self-fertilisation in *F. thymifolia* was determined by experimental manipulation of flowers. In June 2005, we

selected 56 plants growing in the study site, but outside the plots from which DNA samples were collected. The following treatments were randomly applied to one previously emasculated flower of each of 14 plants: (i) deposition of pollen on the stigma of the same flower (autogamous self-pollination), followed by bagging of flowers with cellophane bags; (ii) deposition of pollen from another flower of the same individual and bagging (geitonogamous self-pollination); (iii) deposition of pollen from another individual and bagging (allogamy or out-crossing); and (iv) open-pollination (unmanipulated control). Hand-pollinations were done in (i), (ii) and (iii) by rubbing several dehisced anthers, held with forceps, all over the stigma. The treatments were applied immediately after sunrise of the same day to fresh flowers that were just opening. The cellophane bags were removed the day after the treatment because flowers only remain fertile for 1 day. Around 3 weeks later, remaining mature fruits were collected (four fruits were lost in the geitonogamy treatment, six fruits were lost in the open-pollination treatment). Since dehiscence had occurred in some fruits, we only considered the proportion of swollen fruits with filled seeds as an estimation of reproductive success.

Data analysis

Genetic analysis

Loci were screened using a genome scan (Beaumont & Nichols 1996) to identify any loci that might violate assumptions of selective neutrality. Five potentially non-neutral loci were identified; these loci were subsequently excluded from all of the genetic analyses presented here [full procedure reported in Jump *et al.* (2008)]. All analyses were performed using those loci that met a polymorphism criterion of a maximum allowable allele frequency of 0.95. Allele frequencies, percentage polymorphic loci per plot and heterozygosity was calculated using GenAlEx v. 6 (Peakall & Smouse 2006).

Population structure

To investigate the distribution of genetic diversity within the study site, we used AMOVA (Excoffier *et al.* 1992) to partition total genetic diversity within and among plots, based on calculation of genetic differentiation, Φ_{pt}, between individuals. Statistical significance of Φ_{pt} was based on 10,000 random permutations of individuals among plots. To determine whether isolation-by-distance (IBD) was detectable at the plot level, we analysed the relationship between plot-based genetic distance and the corresponding geographic distance. The relationship between linearised Φ_{pt}, calculated as $\Phi_{pt}/(1 - \Phi_{pt})$, between plots and the corresponding natural logarithm of geographic distance between all possible pair-wise plot comparisons (Rousset 1997) was assessed using a Mantel test and 10,000 permutations of plots among locations. Both analyses were conducted using GenAlEx.

Spatial genetic structure

Analysis of spatial genetic structure (SGS) included all sampled individuals. SGS analysis was conducted based on calculation of the kinship coefficient between individuals (F_{ij}), which summarises the genetic co-ancestry between individuals i and j and can be defined as $F_{ij} = (Q_{ij} - Q_m)/(1 - Q_m)$ (where Q_{ij} is the probability of identity in a state for random genes from i and j, and Q_m is the average probability of identity in the state for genes coming from random individuals from the sample). Kinship coefficients were calculated according to the dominant marker estimator described by Hardy (2003). We have no estimate of the inbreeding coefficient (F_{is}) in *F. thymifolia*, therefore, we repeated the SGS analysis with F_{is} set to 0.1 and 0.25, as the pollination experiments we report here showed that self-fertilisation is possible in this species. However, estimation of kinship using this method is relatively robust to errors in F_{is} estimation (Hardy 2003). To determine whether our data were sufficient to accurately estimate SGS in this species, we reduced the size of our dataset by the random exclusion of loci and individuals and used the Pearson product-moment correlation coefficient (r) to compare the mean estimate of F_{ij} over pairs of individuals within a given distance interval ($F_{(d)}$), calculated from reduced numbers of individuals and loci with $F_{(d)}$ based on the full dataset (see Cavers *et al.* 2005; Jump & Peñuelas 2007 for examples of this method).

$F_{(d)}$ was computed based on 14 intervals from 0 m to 70 m and plotted against distance to visualise spatial autocorrelation patterns. Given that *F. thymifolia* lacks obvious adaptations for seed dispersal, interval size was selected to allow investigation of SGS across the full range of distances inherent within our data whilst providing additional resolution at short distances. The maximum distance for each interval was 0.5, 1, 2, 3, 4, 5, 7.5, 10, 20, 30, 40, 50, 60 and 70 m. Ninety-five per cent confidence intervals for $F_{(d)}$ were calculated based on 10,000 random permutations of individuals among geographical locations. To test the hypothesis that there was significant spatial structure, the observed regression slope (b) of F_{ij} on $\ln(r_{ij})$ [where $\ln(r_{ij})$ represents the natural logarithm of the physical distance between individuals i and j] was compared with those obtained after 10,000 random permutations of individuals among locations, and the statistical significance determined using a Mantel test. This procedure has the advantage that all of the information is contained in a single test statistic and the results are not dependent on arbitrarily set distance intervals (Vekemans & Hardy 2004). Spatial genetic structure was also quantified by the *Sp* statistic, which represents the rate of decrease of pair-wise kinship with distance (Vekemans & Hardy 2004). *Sp* is calculated as $-b/(1 - F_{(1)})$ (where $F_{(1)}$ is the mean F_{ij} between individuals in the first distance class). All analyses of SGS were conducted using the program SPAGeDi V1.2d (Hardy & Vekemans 2002).

Pollination experiments
The effect of the pollination treatments on the number of filled fruits (discrete variable) was tested using contingency tests with the G statistic (Zar 1996). We performed a test considering the four treatments and further tests comparing each pair of treatments.

RESULTS

Genetic analyses

A total of 337 polymorphic AFLP markers were recorded over 114 individuals using five primer combinations. The number of polymorphic markers (allele frequency < 1) per individual primer combination was as follows: *Eco*-ACG plus *Mse*-CAG, 83 markers; *Mse*-CAT, 63; *Mse*-CTG, 89; *Mse*-CTC, 44; and *Mse*-CTA, 58. We set a polymorphism criterion of a maximum allowable allele frequency of 0.95 for all analyses, resulting in 164 loci being used over all 114 individuals for assessment of genetic structure within the investigated population. Heterozygosity ranged from 0.15 to 0.31 per plot, with an average over all plots of 0.26 (Table 1).

Population structure
Analysis of molecular variance showed that the greatest proportion of genetic variability within the study site (91%) was found within the treatment plots studied. However, a significant differentiation between plots was observed (P < 0.001), with 9% of variation being distributed at the between-plot scale (Table 2). A weak but significant pattern of IBD was evident at the plot level within the study site (r^2 = 0.087, P = 0.041, Fig. 2).

Spatial genetic structure
Significant SGS was detected over all individuals sampled within the study site (P < 0.001, Fig. 3). AFLP markers revealed mean kinship coefficients per distance class ($F_{(d)}$) that were significantly elevated above zero for distance classes of less than a maximum of 5 m. $F_{(d)}$ declines rapidly, from a value of 0.144 for the distance class 0–0.5 m to 0.051 at 4.0–5.0 m, with a further significant deviation from zero in the 10.0–20.0-m distance class. The value of the *Sp* statistic was 0.0342 when calculated over the full distance inherent within our dataset. The above values of $F_{(d)}$ and *Sp* are calculated with F_{is} = 0.25. If F_{is} is reduced to 0.1, *Sp* = 0.0294 and $F_{(d)}$ for the first distance class falls to 0.126; however, the general pattern of decline of $F_{(d)}$ with increasing distance class and the distance classes within which $F_{(d)}$ is significantly elevated above zero remain unaffected. Mean sample size per distance class varied from 60 to 1604, with a mean of 457. Following Cavers *et al.* (2005) and Jump & Peñuelas (2007), reduction of sample sizes to 100 loci or 80 individuals by the random exclusion of loci or individuals, indicated that our data are adequate to accurately estimate SGS in this species, since correlations of $F_{(d)}$ calculated from reduced datasets with $F_{(d)}$ based on the full dataset were 0.98 and 0.94, respectively.

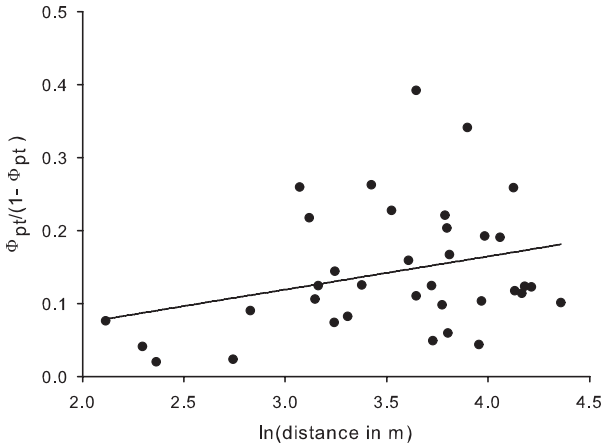

Fig. 2. Genetic differentiation plotted against the logarithm of geographical distance (in m) for all pairwise combinations of *Fumana thymifolia* plots. The significant positive relationship is indicated by the regression line: $y = 0.0454x - 0.0173$, $r^2 = 0.087$, P = 0.041. A significant positive relationship indicates that samples are spatially genetically structured at the plot level, with isolation by distance playing an important role.

Pollination experiments

In the autogamy treatment, five out of 14 fruits contained filled seed, in comparison with six out of ten fruits in the geitonogamy treatment, 11 out of 14 in the allogamy treatment, and seven out of eight in the open-pollination treatment (unmanipulated control) (Fig. 4). The G contingency table test indicated a significant effect of pollination treatments (G = 8.32, P = 0.040). Pair-wise comparisons between treatments showed that the autogamy treatment produced a significantly lower proportion of fruits with filled seed than allogamy and open-pollination treatments (G = 5.44, P = 0.022, and G = 6.04, P = 0.014, respectively).

Table 2. Hierarchical analysis of molecular variance (AMOVA) in *Fumana thymifolia* based on 164 AFLP loci.

	DF	SS	MS	VC	% var	Φ_{pt}	P
variation among plots	8	507.89	63.49	2.84	9	0.0922	<0.001
variation within plots	105	2935.95	27.96	27.96	91		

df = degrees of freedom; SS = sum of squares; MS = mean squares; VC = variance component; % var = % variation; Φ_{pt} represent differentiation between plots; (P < 0.05).

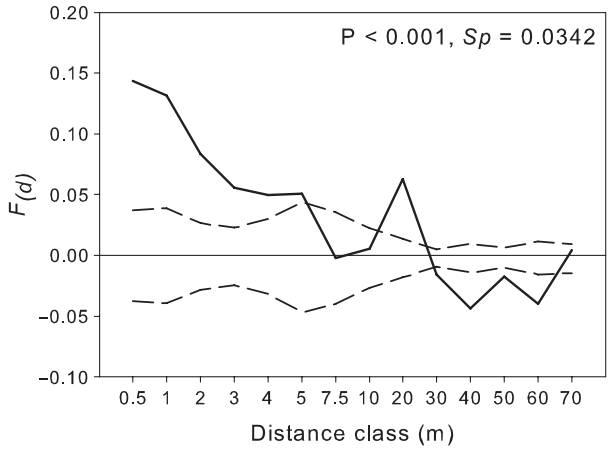

Fig. 3. Analysis of spatial genetic structure in *Fumana thymifolia* from AFLP markers with the inbreeding coefficient, F_{is} = 0.25. Solid lines indicate the mean kinship coefficient per distance class ($F_{(d)}$) and dashed lines indicate the limits of its 95% confidence interval. Individuals are significantly more similar than would be expected by chance when $F_{(d)}$ lies above its 95% confidence limit. The P-value is given for the regression slope of individual pair-wise kinship coefficients on the corresponding logarithmic geographic distance between individuals (see text). *Sp* represents the rate of decrease of pair-wise kinship with distance.

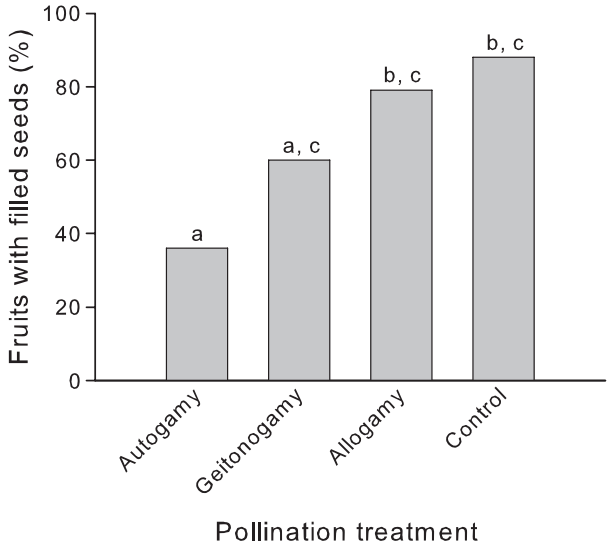

Fig. 4. Percentage of *Fumana thymifolia* fruits having filled seed resulting from experimental pollination treatments and an unmanipulated control. Bars not sharing the same letter differ significantly when compared using the log likelihood G statistic (P < 0.05).

DISCUSSION

Fruit losses in the pollination trials decreased our power to test for differences between treatments. However, given the possibility of successful self-fertilisation in *F. thymifolia*, it is likely that this species possesses a mixed mating system, although we cannot determine the absolute contribution that self-pollination makes to seed production or recruitment within the study population.

The spatial dispersal of pollen is important ecologically as it determines the relative genetic isolation of individual plants (Ellstrand 1992). In this insect-pollinated species, pollinator behaviour will affect the spatial extent of SGS by determining the spatial extent of pollen flow within the population. In situations where intra-population gene flow is extensive, local patch structuring within populations may be absent (Dewey & Heywood 1988). Likewise, self-fertilisation is expected to result in a greater magnitude of spatial genetic structure, in contrast with outcrossing species in which SGS is likely to be more reduced (Vekemans & Hardy 2004). The impact of a mixed mating system on the magnitude of SGS in *F. thymifolia* will depend on the relative contribution of successful outcrossed and self-fertilised pollination. Despite the reduced probability that seed production will result from self-fertilisation as opposed to outbreeding (Fig. 4), the proportion of seed resulting from outcrossed rather than selfed pollination will also be dependent on pollinator behaviour, *i.e.* foraging time spent within plants *versus* foraging time spent between plants.

Although we have no data on pollinator foraging habits within the study population, *F. thymifolia* is most commonly pollinated by bees (Petanidou & Vokou 1990), which exhibit non-random searching behaviour, typically following a systematic pattern both at the plant and patch scale (Goulson 2003). We expected, therefore, that the pollination ecology of this species would result in a pronounced genetic structure within the population, both in terms of magnitude and of spatial scale. The visitation of several flowers on the same plant by individual pollinators is likely to elevate levels of geitonogamous self-fertilisation, whereas systematic searching by bees within patches will elevate the probability that pollen flow will be local, rather than random. Short-distance seed dispersal will also be a highly important factor in the development of genetic structure within populations of this species (Loveless & Hamrick 1984; Hamrick *et al.* 1993; Loiselle *et al.* 1995; Mahy *et al.* 1999), and *F. thymifolia* lacks both specialised mechanisms for long-distance seed dispersal or secondary seed dispersal by animals.

Results of the AMOVA suggest significant differentiation at the level of individual plots, indicating that genetic diversity is not distributed randomly across the site. Approximately 9% of diversity is distributed at the between-plot level, in support of the prediction of restricted gene flow occurring between *F. thymifolia* plants within the study site. Furthermore, genetic structure at the plot level corresponds to the isolation-by-distance model (r^2 = 0.087, P = 0.041, Fig. 2), indicating decreasing genetic similarity of plots with increasing spatial separation. When the pattern of spatial autocorrelation is evaluated between individual genotypes, we find significant levels of SGS. Estimates of co-ancestry differed

significantly from zero in the first six distance classes included in our study (maximum 5 m), with a further significant deviation from zero in the 10.0–20.0 m distance class. The highest value of $F_{(d)}$, 0.144, was detected in the shortest distance class, $F_{(0-0.5)}$, $F_{(d)}$ fell steeply thereafter, in agreement with the expectation of rapidly declining gene dispersal with increasing distance from source (Cresswell 2006 and references therein).

The detection of genetic structure at the level of both sampling plot and individual genotype, as outlined above, demonstrates that gene flow is non-random across the study site, although the signature of restricted gene flow differs depending on the spatial scale of the analysis. When compared with strong SGS at short distances, weak IBD at the plot level may be explained by the distances between individual plots (6.3 m to 75.2 m, mean 39.7 m), being generally larger than the maximum extent of SGS reported here between individuals (5 m), or by low numbers of individuals within plots leading to a large variance of pair-wise Φ_{pt} between plots. However, the contrast between weak IBD across the site and the pronounced SGS at much smaller scales may also provide information on the differential contributions of seed and pollen dispersal within the population.

The magnitude of SGS detected within the shortest distance class in our study (0.126–0.144, 0.5 m) is approximately equivalent to the relatedness expected for half sibs (0.125 assuming random mating) (Loiselle *et al.* 1995), and strongly suggests very limited seed dispersal within the population (Kalisz *et al.* 2001). Lack of SGS at larger distances and weak IBD at the plot scale suggest that gene flow by pollen is more extensive. However, the detection of IBD between plots even within the relatively small area covered by our study site supports the expectation of non-random pollinator movement (Goulson 2003), and indicates that gene flow is still restricted at spatial scales beyond the seed shadow of individual plants. Consequently, although highly localised seed dispersal is likely to play the major role in genetic differentiation of individual plots, limited dispersal of both seed and pollen have contributed to the development of the observed genetic structure within the population.

Using the *Sp* statistic to compare our results with those of other published studies calculated by Vekemans & Hardy (2004), it is clear that SGS in *F. thymifolia* is comparable to that recorded for species with similar growth form and reproductive ecology. *Sp* in *F. thymifolia* in this study was 0.0294–0.0342 (depending on the value of F_{is} used for calculation), similar to values of *Sp* reported for two other animal-pollinated shrubs and small trees with a mixed mating system: 0.0327 for *Ancistrocladus korupensis* (Foster & Sork 1997) and 0.0529 for *Helicteres brevispira* (Franceschinelli & Kesseli 1999). Similarly, *Sp* in *F. thymifolia* is comparable with mean values of *Sp* given for all small trees and shrubs ($Sp = 0.0259$), mixed mating ($Sp = 0.0372$), or gravity dispersed ($Sp = 0.0281$) plant species that Vekemans & Hardy (2004) reviewed, although the mean *Sp* for animal-pollinated plants ($Sp = 0.0171$) is somewhat lower.

The magnitude and scale of spatial structure within populations is strongly influenced by adult density (Hamrick *et al.* 1993; Franceschinelli & Kesseli 1999; Vekemans & Hardy 2004). Given that periodic fires occur in the area within which the study population occurs, it will be interesting to identify how SGS within the population changes over time, both gradually as the population ages, and *via* a 'before and after' comparison to investigate the immediate impact of fire. Variation in SGS between age classes in established populations occurs, but is not readily predictable since it may both increase (Tonsor *et al.* 1993; Ueno *et al.* 2000; Jacquemyn *et al.* 2006; Yamagishi *et al.* 2007) and decrease (Hamrick *et al.* 1993; Chung *et al.* 2003; Yamagishi *et al.* 2007) with increasing plant age. Thus, investigation of how spatial genetic structure in *F. thymifolia* varies over time as a consequence of plant maturation and community development will be highly rewarding in this dynamic, fire-prone ecosystem.

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