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ORIGINAL PAPER

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Paternity analysis using microsatellite markers to identify pollen donors in an olive grove

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Abstract Olive (*Olea europaea* L.) is a wind-pollinated, allogamous species that is generally not considered to be self-compatible. In addition, cross-incompatibilities exist between cultivars that can result in low fruit set if compatible pollinisers are not planted nearby. In this study, microsatellite markers were used to identify 17 genotypes that were potential pollen donors in a commercial olive orchard. DNA typing with the same primers was also applied to 800 olive embryos collected from five cultivars in the grove over 2 years of study. Pollen donors for the cultivars Barnea, Corregiola, Kalamata, Koroneiki, and Mission were estimated by paternity analysis, based on the parental contribution of alleles in the genotypes of the embryos. The exclusion probability for the marker set was 0.998 and paternity was assigned on the basis of the 'most likely method'. Different pollen donors were identified for each of the maternal cultivars indicating that cross-compatibilities and incompatibilities varied between the genotypes studied. Cross-pollination was the principal method of fertilization, as selfing was only observed in two of the embryos studied and both of these were from the cultivar Mission. This is the first report where these techniques have been applied to survey the pollination patterns in an olive grove. The results indicate that careful planning in orchard design is required for efficient pollination between olive cultivars.

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Introduction

Olives have been in cultivation for thousands of years, yet compatibility relationships between cultivars and the mechanism of self-incompatibility have not been fully investigated. In common with many woody, forest trees, the olive is a wind-pollinated, allogamous species. Several studies on the identification of compatible cultivars have led to conflicting results, possibly due to different environmental conditions under which the studies were conducted or to confusion in cultivar identity (Mekuria et al. 1999). For example, the cultivar Moraiolo was identified as self-compatible by Singh and Kar (1980) and as self-incompatible by Bini and Lensi (1981). Manzanillo has been reported variously to be self-compatible (Androulakis and Loupassaki 1990; Sibbett et al. 1992) and mainly self-incompatible (Griggs et al. 1975; Lavee and Datt 1978; Cuevas and Polito 1997). In addition, self-compatibility and the response to pollen donors can vary between seasons (Lavee et al. 2002).

The most commonly used methods for identification of compatible cultivars are based on artificial cross-pollination using pollination bags to regulate the pollen load and subsequent observation of pollen tube growth (Wu et al. 2002) and/or fruit set (Lavee et al. 2002). However, conditions inside the pollination bag may affect fruit set (Rallo et al. 1990) and pollination bags may leak, allowing pollen from other genotypes to enter and fertilize the egg cell (de la Rosa et al. 2004). The results will also vary depending on whether the initial or final yield is considered because of the high rate of fruit drop in olive (Rallo et al. 1990).

The identification of the paternal parent using molecular techniques is a reliable method because the genetic contribution of alleles is traced from the parents to the offspring. Microsatellite markers are suitable for this purpose because of their codominant segregation and high level of polymorphism in olive (Sefc et al. 2000; Cipriani et al. 2002; de la Rosa et al. 2002).

Several reports have used microsatellite markers and paternity analysis to study pollen movement in tree populations (Chaix et al. 2003; Isagi et al. 2004; Robeldo-Arnuncio and Gil 2005). The effectiveness of microsatellite markers in identification of paternal parents of progeny obtained from an olive breeding program has been demonstrated by de la Rosa et al. (2004). The 'most likely method' of paternity analysis identifies the maternal contribution of alleles in the genotype of the embryo and then compares the remaining alleles with the genotypes of potential fathers to identify the most likely father (Thompson and Meagher 1987). This method has been successfully used by Gerber et al. (2000) and Chaix et al. (2003) and has been incorporated into the computer program, FaMoz (Gerber et al. 2003), which was used for paternity assignment in this study.

The aim of this study was to determine the pollen donors for five cultivars in a commercial olive grove, using paternity analysis. Bloom time, pollen vitality, and weather conditions during the flowering period were also recorded. The study was done over 2 years and different compatibilities between cultivars were identified.

Materials and methods

Study site and plant material

The study was conducted in a commercial, mixed olive orchard at Gumeracha (34°49′33″ S, 138°52′10″ E, 355 m above sea level) in South Australia (Fig. 1). The cultivars present were Barnea, Corregiola, Kalamata, Katsourella, King Kalamata, Koroneiki, Leccino, Manzanillo, Mission, Pendolino, Sevillano, UC13A6, and Verdale. Among the cultivars several individuals were identified with atypical phenotypes. These were named atypical Corregiola, atypical Kalamata, atypical Koroneiki, and atypical Pendolino and were also found to be genotypically different from the standard cultivars after DNA fingerprinting.

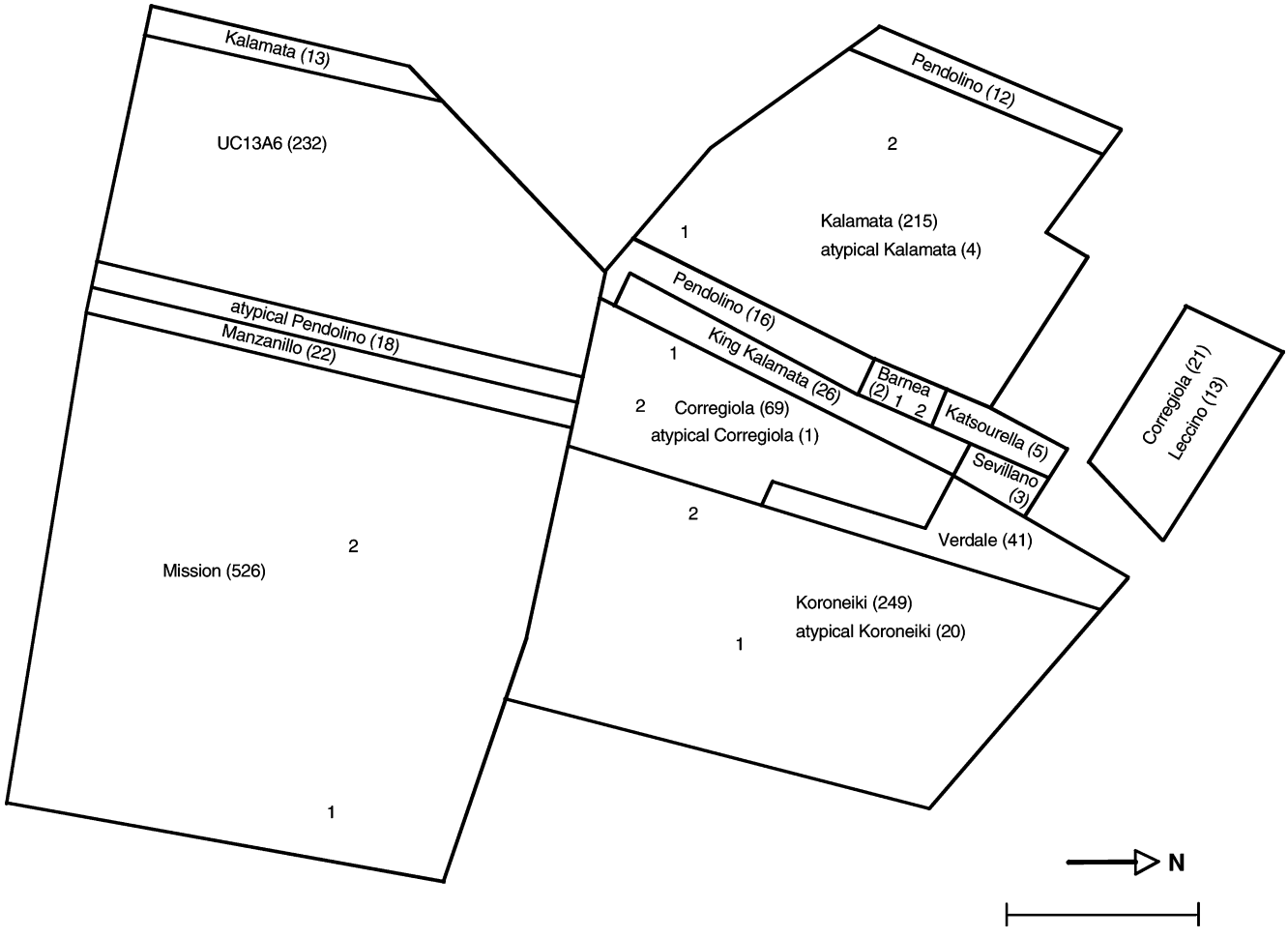

Fig. 1 Field plan of the orchard showing the position of cultivars and the selected mother trees (indicated by *1* and *2*). The number of trees for each cultivar is indicated in parentheses. Scale bar = 50 m

Bloom time and pollen vitality

The dates of the start of bloom (10% flowers open), full bloom (80% flowers open), and end of bloom (80% flowers spent) were recorded for each genotype. The stages were estimated by examining several panicles around the canopy, counting how many flowers were open on each, and averaging the result. For each tree, pollen samples were collected from flowers sampled from five panicles selected at random around the canopy. Anthers were left to dehisce in a small tube overnight in a box containing silica gel. Pollen vitality was measured by fluorescein diacetate staining (Pinney and Polito 1990).

DNA extraction and microsatellite analysis

DNA was extracted from leaf material for the comparison of parent genotypes against those of standard cultivars from the University of Adelaide olive database (Guerin et al. 2002). Freshly collected leaves were transferred on ice to the laboratory and stored at 4°C. DNA was extracted using the method described by Mekuria et al. (1999). Representative trees of the different genotypes in the grove, and all of the selected mother trees, were sampled for DNA fingerprinting. The total number of trees sampled from each genotype were as follows: one tree from Leccino, atypical Corregiola, atypical Kalamata, and atypical Pendolino; two from atypical Koroneiki, Barnea, Katsourella, King Kalamata, Manzanillo, Verdale, Koroneiki, and Corregiola; three from Pendolino, Sevillano, and UC13A6; four from Kalamata; and five from Mission.

Ten fruits were sampled from four canopy segments facing each direction (north, south, east, and west) from two selected trees of five cultivars in each year, making a total of 800 embryos examined over 2 years of the trial. DNA was extracted from the embryos using the following method. The embryos were ground and incubated for 10 min at 65°C in 500 µl of grinding buffer (100 mM Tris, pH 8.0, 20 mM EDTA, pH 8.0, 4 mg/ml diethyl dithio carbamic acid, 110 µg/ml DNase-free RNase), followed by addition of 500 µl of lysis buffer [100 mM Tris, pH 8.0, 20 mM EDTA, pH 8.0, 1 M NaCl, 2% (w/v) SDS, 1% (w/v) sodium metabisulphite], and further incubation for 30 min at 65°C. An equal volume of phenol–chloroform–isoamyl alcohol (25:24:1) was added and mixed, followed by centrifugation for 10 min at 14,000 rpm. The supernatant was removed and DNA precipitated using 500 µl of isopropanol. The DNA was washed in 1 ml of wash buffer [76% (v/v) ethanol, 10 mM ammonium acetate] and the pellet was dissolved in 50 µl TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0).

DNA amplification was performed in a volume of 20 µl containing 20–60 ng genomic DNA, 1.5 mM $MgCl_2$, 1 × PCR buffer (200 mM Tris–HCl, pH 8.4, 500 mM KCl), 0.2 mM of each dNTP, 0.4 µM of forward primer (fluorescent phosphoramidites FAM, HEX, or NED at 5′ position), 0.4 µM of reverse primer, and 1.25 U of Taq DNA polymerase (Invitrogen), using a Programmable Thermal Controller (M.J. Research Inc., USA). FAM and HEX-labelled primers were obtained from GeneWorks Pty Ltd, Adelaide, South Australia, and NED-labelled primers were obtained from Applied Biosystems, USA.

Eight microsatellite primers were tested for fingerprinting the parent trees and embryos: ssrOeUA-DCA3, ssrOeUA-DCA4, and ssrOeUA-DCA14 (Sefc et al. 2000), EMO2 (de la Rosa et al. 2002), UDO99-006, UDO99-008, UDO99-024, and UDO99-031 (Cipriani et al. 2002). Five of the primers, ssrOeUA-DCA3, EMO2, UDO99-006, UDO99-008, and UDO99-031, were used on the samples for identification as these were sufficient to distinguish between the standard genotypes used.

The steps for PCR amplification were: initial denaturation at 95°C for 5 min, 34 cycles of 45 s at 95°C, 45 s at the published annealing temperatures (Sefc et al. 2000; de la Rosa et al. 2002; Cipriani et al. 2002), 45 s extension at 72°C, and a final extension at 72°C for 45 min to reduce the incidence of stutter peaks. PCR products were diluted 1:50 and 3 µl was mixed with 5 µl of ROX–formamide (6 µl ROX Genescan 400MD + 500 µl of Hi Di Formamide, Applied Biosystems, USA) and separated using the ABI Prism 3700 DNA Analyser (Applied Biosystems). Data were analysed using Genescan version 3.5.1 (PE Biosystems, USA). All PCR reactions were done in duplicate and if the products were not identical the process was repeated until a consensus was reached.

Paternity analysis

Paternity analysis (Gerber et al. 2003), exclusion probability (Jamieson and Taylor 1997), and identity probability (Waits et al. 2001) were generated using the software program FaMoz (http://www.pierroton.inra.fr/genetics/labo/Software/Famoz/index.html). The statistical analysis used in this program is based on the most likely method described by Meagher and Thompson (1986). When only one allele was present it could not be determined whether a homozygote or heterozygote with a null allele was measured, so the second allele was scored as missing data to avoid incorrect exclusion of potential fathers. A simulation was done using 1,000 offspring to obtain the threshold for paternity in order to minimize errors due to gene flow from inside and outside the stand. The genotype with the highest log of the odds ratio (LOD) score was considered as the most likely father. The LOD score represents the likelihood of a particular genotype being the father compared to all other genotypes.

A simple linear regression between number of embryos pollinated and number of trees was performed

for each year separately using Genstsat 7th edition (VSN Int Ltd, Herts, UK). The data for the number of embryos and number of trees was transformed on the log-scale prior to analysis in order to normalise the data.

Results

Microsatellite polymorphism in parental genotypes

Eight microsatellite loci were used for identification of the genotypes of the trees planted at the study site and for paternity analysis of embryos collected from five cultivars within the orchard. All the loci were polymorphic and the number of alleles per locus ranged from 4 to 8, with an average of 6.4 (Table 1). The high cumulative exclusion probability (0.998) indicated that the set of markers was able to adequately exclude all unlikely fathers. The low cumulative identity probability (0.000) for the set of eight primers used to identify the parental genotypes indicated a very low probability of wrongly assigning a genotype as the father (Table 1). These probability values signify that paternity analysis could be estimated with a high level of accuracy (Chaix et al. 2003).

DNA fingerprints were generated for the selected trees of each genotype and the identities of the cultivars was confirmed by comparison with standard samples from the University of Adelaide olive DNA database (Guerin et al. 2002). Some individuals that showed phenotypic differences were observed among trees planted as Kalamata, Koroneiki, Corregiola, and Pendolino. These trees were analysed with the marker set and were shown to be genetically distinct from the standard samples and were referred to as atypical Kalamata, atypical Koroneiki, atypical Pendolino, and atypical Corregiola. The genotypes could not be identified from the olive database (Guerin et al. 2002). In total, 17 genotypes were identified in the orchard and were all considered as potential pollen donors in the paternity analysis.

Weather conditions for pollen dispersal

Olives are anemophilous, requiring dry conditions and adequate air movement for pollen distribution. The weather during the bloom periods were recorded from the Bureau of Meteorology, Mt Crawford weather station, which is located at a distance of 11.5 km northeast of the study site. The bloom periods were mostly dry, with rainfall occurring only on a few days. The maximum temperatures during the bloom period of both the years ranged from 15 to 33°C with an average of 21.4°C. However, it was between 20 and 30°C on most days, which is suitable for pollen tube growth (Cuevas et al. 1994). Winds were predominantly easterly and westerly in the mornings and mainly easterly in the afternoons, with winds of lower intensities blowing in other directions throughout the day. In general, conditions were suitable for effective pollen dispersal throughout the orchard during the flowering period.

Bloom time and pollen vitality

As cross-pollination is most likely to occur when compatible cultivars flower simultaneously, the bloom periods for each genotype were recorded in the 2 years of study (Fig. 2). The onset of flowering was later, overall, in 2003. However, bloom times for all pollen donors overlapped to some extent with the maternal trees during both years of study. The only exception was in 2002 when atypical Corregiola did not begin to flower until the blooms of Barnea, Corregiola, Koroneiki, and Mission were already predominantly spent. The atypical Kalamata trees were not identified until 2003 and therefore bloom times were not recorded for these trees in 2002.

Pollen vitality was measured to confirm that the trees produced viable pollen for fertilization. In 2002, percent pollen vitality ranged from 23.5% in King Kalamata to 72.3% in Koroneiki with an average of 56.1% and from 19.7% in UC13A6 to 65.5% in Leccino with an average of 42.8% in 2003. Very little pollen was collected from Verdale and atypical Koroneiki in both years, indicating

Table 1 Number of alleles, size range in base pairs, exclusion and identity probabilities for the parental genotypes calculated using eight microsatellite loci

Locus	Number of alleles	Size range of alleles (bp)	Exclusion probability	Identity probability
EMO2 (de la Rosa 2000)	4	202–215	0.337	0.178574
UDO99-006 (Cipriani et al. 2002)	6	146–179	0.588	0.046362
UDO99-008 (Cipriani et al. 2002)	6	155–170	0.474	0.089572
UDO99-024 (Cipriani et al. 2002)	6	166–194	0.613	0.032954
UDO99-031 (Cipriani et al. 2002)	7	108–151	0.618	0.037908
SsrOeUA-DCA3 (Sefc et al. 2000)	7	230–252	0.633	0.030945
ssrOeUA-DCA4 (Sefc et al. 2000)	8	131–187	0.624	0.062935
ssrOeUA-DCA14 (Sefc et al. 2000)	7	148–188	0.543	0.034811
Cumulative	51		0.998	0.000000

Published source of the primer sequences are shown in parentheses

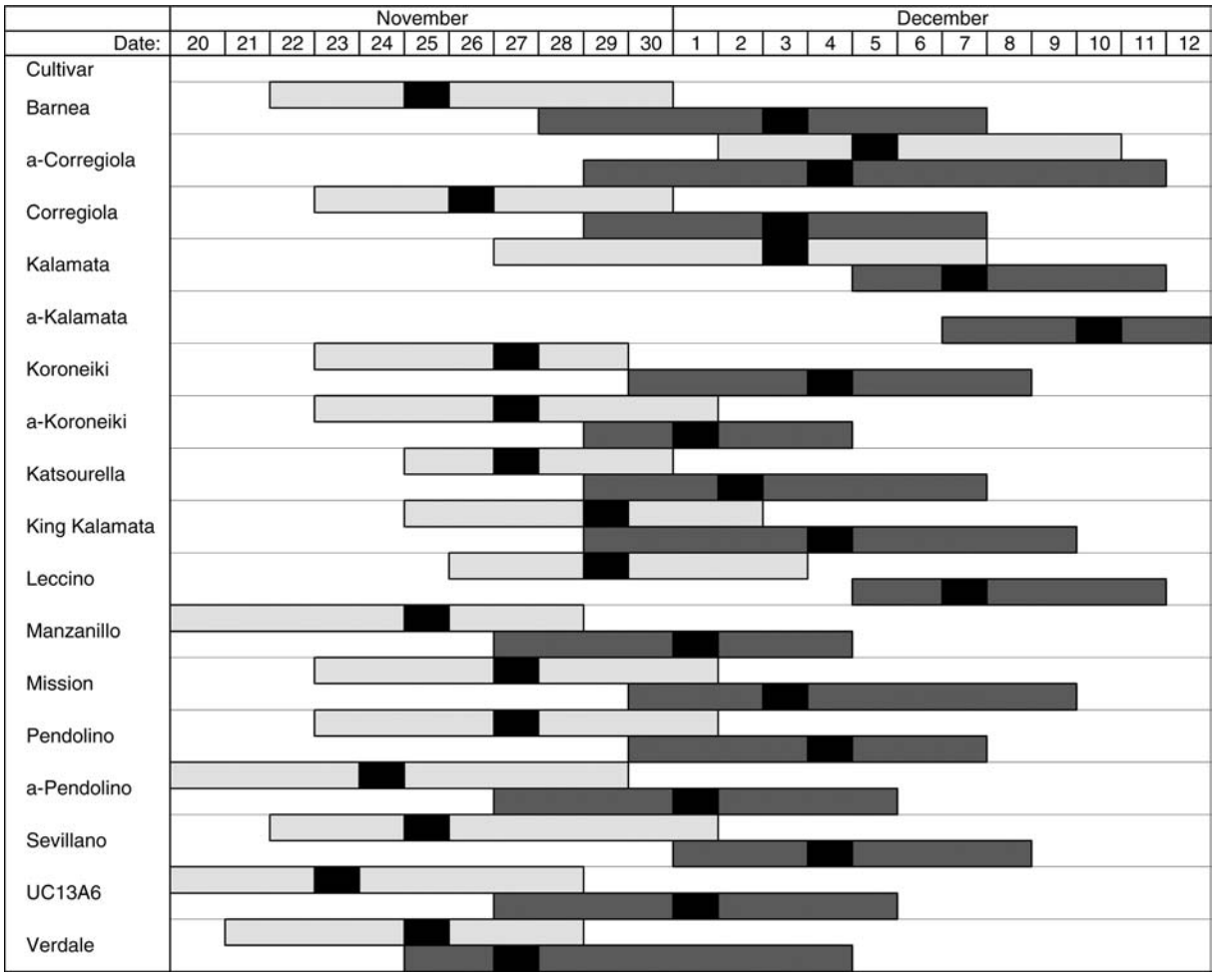

Fig. 2 Bloom time in olive cultivars during the 2 years of study, November to December 2002 (*light grey*) and 2003 (*dark grey*). *Bars* indicate the length of bloom time from beginning (10% flowers open) to the end (80% flowers spent) with full bloom shown in *black*

that, at least for the trees sampled, these two cultivars were predominantly male sterile.

Paternity analysis

The pollen donors that were assigned to the embryos sampled from the maternal trees are shown in Table 2. The LOD scores for the most likely fathers ranged from 0.10 to 10.88, with an average of 3.63, and were above the estimated threshold for paternity, which ranged from 0.01 to 0.06, with an average of 0.02. The number of embryos assigned to pollen donors ranged from 169 for Koroneiki, represented by 249 trees, to one embryo for atypical Corregiola represented by only one tree in the grove. Paternity could not be assigned to approximately

Table 2 Numbers of embryos from the mother trees assigned to each of the pollen donors over 2 years

	Pollen donor																	
Mother tree	Bar	Cor	Kor	Kal	Mis	Kat	KK	Lec	Pen	Man	Sev	UC	Ver	a-C	a-K	a-Ko	a-P	Ukn
Barnea	0	4	0	18	30	3	8	3	29	0	15	9	13	0	3	0	2	23
Corregiola	1	0	0	29	45	1	0	2	6	2	6	0	3	0	0	27	2	36
Koroneiki	3	0	0	12	68	4	1	3	8	1	2	18	2	0	2	8	2	26
Kalamata	0	26	87	0	3	0	2	2	1	0	2	3	1	1	0	8	2	22
Mission	1	6	82	0	2	1	0	0	0	0	0	0	0	0	0	0	0	68
Total	5	36	169	59	148	9	11	10	44	3	25	30	19	1	5	43	8	175

Cultivar names were abbreviated as follows: *Bar* Barnea; *Cor* Corregiola; *Kor* Koroneiki; *Kal* Kalamata; *Mis* Mission; *Kat* Katsourella; *KK* King Kalamata; *Lec* Leccino; *Pen* Pendolino; *Man* Manzanillo; *Sev* Sevillano; *UC* UC13A6; *Ver* Verdale; *a-C* atypical Corregiola; *a-K* atypical Kalamata; *a-Ko* atypical Koroneiki; *a-P* atypical Pendolino; *Ukn* unknown

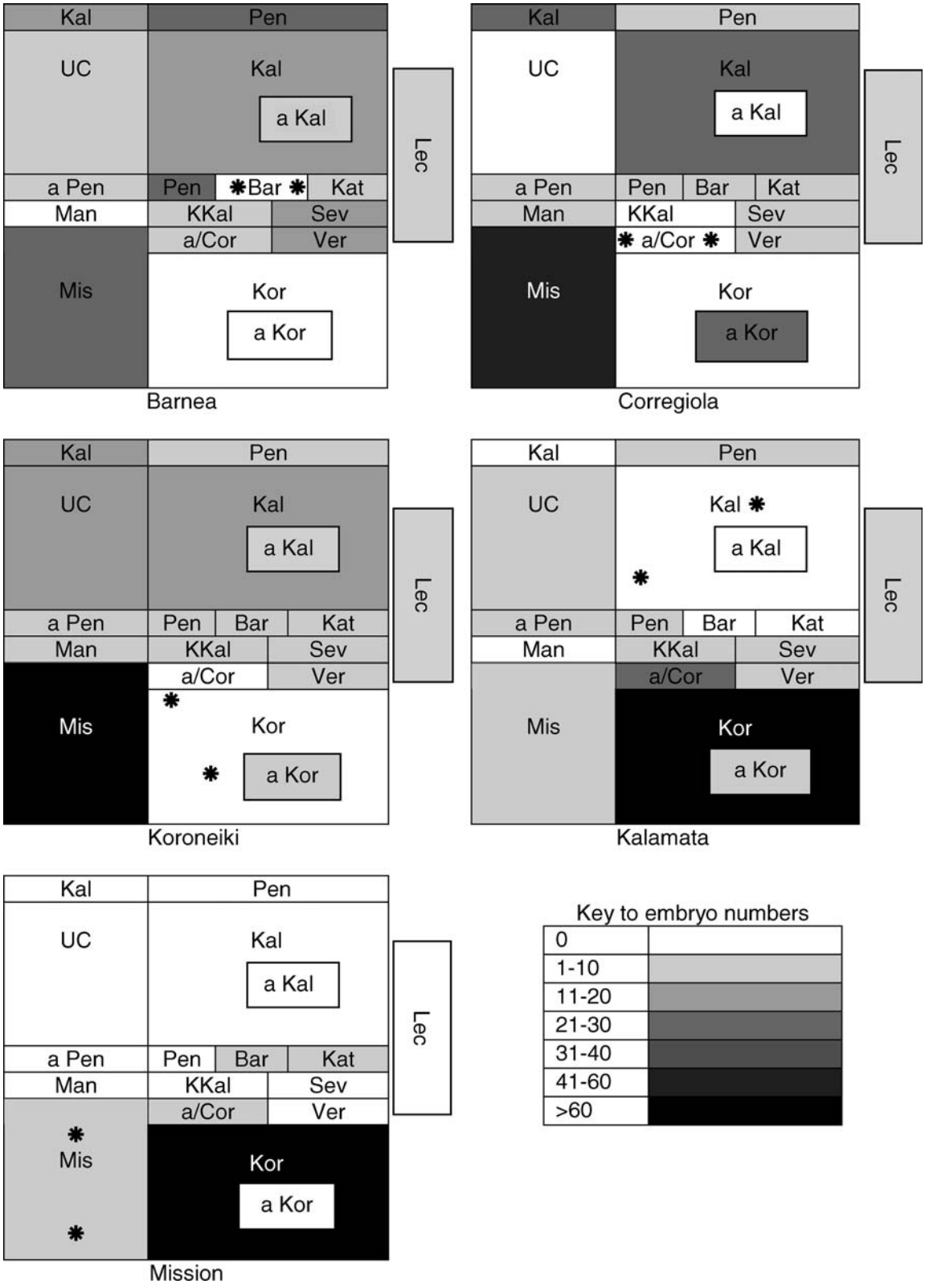

Fig. 3 Diagrammatic representation of the olive grove showing the spatial relationship between the mother trees studied (*heavy eight pointed rectilinear black stone*) and the most likely pollen donors. *Shaded areas* indicate the number of embryos pollinised by that cultivar ranging from none (*white*) to >60 (*black*) as indicated by the key. Cultivar names were abbreviated as follows: *a*/*Cor* atypical Corregiola and Corregiola; *a Kal* atypical Kalamata; *a Kor* atypical Koroneiki; *a Pen* atypical Pendolino; *Bar* Barnea; *Kal* Kalamata; *Kat* Katsourella; *KKal* King Kalamata; *Kor* Koroneiki; *Lec* Leccino; *Mar* Manzanillo; *Mis* Mission; *Pen* Pendolino; *Sev* Sevillano; *UC* UC13A6; *Ver* Verdale

22% of embryos; these are listed as having an unknown pollen donor. Unknown donors were most commonly found for Mission embryos, with only 57% having been assigned a pollen donor from the cultivars in the grove.

Simple linear regression between the number of embryos pollinated (log + 1) and the number of trees (log) of that genotype was performed for each year separately and a significant positive relationship was found for both years ($P = 0.028$ and $r^2 = 0.24$ in 2002 and $P = 0.001$ and $r^2 = 0.64$ in 2003). The major difference between the 2 years of study was due to the activity of UC13A6, which pollinised one embryo in 2002 compared to 29 in 2003. UC13A6 had the third largest number of trees after Mission and Koroneiki, yet its overall contribution to the embryos was relatively low (4%) compared to Mission (19%) and Koroneiki (21%).

Kalamata was a major pollen donor for Barnea in both the years. However, Pendolino and Sevillano were also among the top pollen donors in the first year, while Mission, Verdale, UC13A6, and King Kalamata were more significant pollinisers in the second year. The main pollen donors for Corregiola were Mission, Kalamata, and atypical Koroneiki. Kalamata was predominantly pollinised by Koroneiki and Corregiola. Mission and Koroneiki had a clearly reciprocal relationship, as each was the major pollen donor for the other. The only incidence of self-pollination detected occurred in two embryos from Mission.

The spatial relationship between the mother tree and the pollen donors and the frequencies of pollen donation are represented in Fig. 3. In some cases it appears that incompatibilities exist between the maternal trees and potential pollen donors. For example, pollen from Koroneiki fertilized many flowers from Kalamata and Mission, but did not contribute to the embryos tested for Barnea or Corregiola. Similarly, Kalamata was assigned as the pollen donor for many embryos from Koroneiki, Corregiola, and Barnea, but not for those from Mission. UC13A6 was a significant pollen donor for Koroneiki, but made a weak contribution to Kalamata and Barnea, and was not represented among the embryos from either Corregiola or Mission.

Discussion

The results presented here demonstrate that microsatellite markers and paternity analysis are effective tools to survey the frequency of pollen donors for selected maternal trees in a mixed orchard. The use of eight microsatellite primers was sufficient for paternity assignment as predicted by the high exclusion probability and low identity probability (Chaix et al. 2003). This is the first report using these techniques to study pollination patterns in an olive grove.

One of the main points of interest arising from these results is that the olive trees tested in this orchard rarely self-fertilized. Only two cases of self-pollination were observed out of the 800 embryos tested and both of these were from Mission. It has previously been reported that self-pollination in Mission resulted in very low fruit set (Griggs et al. 1975). The low incidence of self-fertilization was not unexpected as olives are predominantly allogamous, exhibiting high levels of heterozygosity and DNA polymorphism among individuals (Angiolillo et al. 1999; Rallo et al. 2000). This finding emphasizes the importance of cross-pollination for adequate fruit set in olive.

Another interesting observation was the difference in receptivity to pollen donors for each of the maternal cultivars within the orchard. The dominant pollinisers were not necessarily the closest neighbours, indicating that compatibility has a major role in pollen success. This has also been reported in other studies using isozyme markers to identify pollen donors in orchards of cherry and avocado (Brant et al. 1999; Sulaiman et al. 2004). Barnea was receptive to several pollen donor cultivars, whereas the majority of Mission embryos were pollinised by Koroneiki. Twelve of the 17 genotypes identified in the orchard did not contribute pollen to the Mission embryos, indicating that Mission may be cross-incompatible with most of the cultivars in the grove.

The two major pollen donors in the orchard were Koroneiki and Mission. These two cultivars had the largest number of trees in the grove and would therefore have a favourable bias on the overall pollen load. Koroneiki appeared to be a strong pollen donor for both Mission and Kalamata. However, the fact that none of the embryos from either Corregiola or Barnea were assigned to Koroneiki, even though the mother trees were near the Koroneiki block, indicates possible incompatibility between these cultivars. In contrast, Mission pollinated at least some embryos from all the cultivars tested. The results confirm the findings of previous reports that self-incompatibility and cross-incompatibility commonly occur in olive (Griggs et al. 1975; Moutier et al. 2001; Wu et al. 2002; Lavee et al. 2002).

Simple linear regression analysis showed that a significant positive relationship existed between the number of trees of any given genotype in the grove and the number of embryos pollinated. However, the correlation values were fairly weak indicating that other factors also influenced the number of embryos pollinated by that genotype. These factors are likely to include the number of cross-compatible trees present, pollen vitality and dispersal, stigma receptivity, and environmental conditions.

Further research is required to confirm the compatibility relationships between these and other olive cultivars. The techniques of DNA fingerprinting and paternity analysis would be useful tools for studies designed to determine compatibilities between cultivars after controlled crosses. Testing the parentage of the progeny verifies the cross as well as the compatibility between the parent cultivars. These methods have been used to confirm paternity in an olive breeding program (de la Rosa et al. 2004).

By using paternity analysis to study the distribution of pollen donors within an olive grove the actual events

of fertilization under the conditions of free, open pollination have been observed. Compatibility studies show that pollen is able to germinate on the stigma, penetrate the style, and fertilize the egg cell in the embryo sac. However, additional information is gained from this study: at the time of stigma receptivity in the maternal tree, viable pollen from the donor was present in the air, could travel to the recipient flower, and successfully compete with other pollen grains on the stigma to reach the egg cell. The successful pollen donors identified in this report are therefore not only compatible with the maternal tree but have also been demonstrated to function effectively under field conditions.

Pollen donors for a significant proportion of the embryos tested could not be identified. It is proposed that the mother trees were pollinated either by pollen from unidentified genotypes in the orchard or by wind-borne pollen from trees outside the orchard. The nearest olive trees outside the grove were planted approximately 500 m to the west. Beyond these trees the nearest grove was approximately 3 km to the southeast. The paternal alleles identified in these embryos revealed that a similar contribution was made in both years and indicated that more than one unknown genotype was involved.

The lack of pollen grains in the anthers taken from Verdale and atypical Koroneiki indicated that the trees tested were male sterile in both years. However, paternity analysis showed that these two genotypes contributed pollen to some embryos. Pollen vitality observations were taken on only a sample of flowers collected at full bloom stage. It is possible that viable pollen was present in some flowers that were not sampled, and also that viable pollen developed later in the season, after the trees had been sampled. All the other genotypes had viable pollen and therefore had the ability to cross-pollinate. The pollen donors were located in all parts of the orchard and not restricted to adjacent trees (Fig. 3). This can be attributed to adequate wind movement during the bloom period, which allowed for pollen transfer throughout the orchard.

The compatibility relationships between Corregiola, Koroneiki, Kalamata, and Mission were observed from the results. The role of Barnea as a pollen donor for the other maternal cultivars tested is difficult to determine, as Barnea would have been under-represented in the pollen load due to the low number of trees in the orchard. The cultivar pairs of Corregiola and Kalamata, Corregiola and Mission, Kalamata and Koroneiki, and Koroneiki and Mission all appeared to be reciprocally cross-compatible. Conversely, Corregiola and Koroneiki exhibited reciprocal cross-incompatibility. The relationship between Mission and Kalamata also appears to be largely incompatible as Kalamata was not identified as a pollen donor for Mission and only three of the Kalamata embryos tested were fertilized by Mission. It has been proposed that self-incompatibility in olive is gametophytic (Cuevas and Polito 1997; Ateyyeh et al. 2000; Wu et al. 2002). In general, reciprocal compatibilities/incompatibilities are observed in systems under gametophytic control (Lewis 1994; Sedgley 1994). However, other reports have shown non-reciprocal relationships between olive cultivars (Moutier et al. 2001; Lavee et al. 2002). Lavee et al. (2002) suggesting that multiple origins of the domesticated *Olea europaea* have resulted in a complex system controlling self-incompatibility. Further work is needed to understand the genetic system operating in olive and will facilitate the identification of the genes involved.

In conclusion, this study identified the most likely pollen donors for five commercially important olive cultivars growing under field conditions. Both bloom time and pollen viability varied between cultivars and seasons and may have affected the ability of the trees to contribute to the pollen load and the major pollen donors varied between the years for some cultivars. For these reasons it is recommended that olive orchards should contain more than one compatible cultivar as polliniser trees to obtain optimum yields. The results obtained are a valuable source of information for orchard design, and the techniques applied would be useful for further investigation into cross-compatibility between olive cultivars.

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