

Mating patterns and spatial distribution of conspecific neighbours in the Mediterranean shrub *Myrtus communis* (Myrtaceae)

Juan P. González-Varo · Rafael G. Albaladejo · Abelardo Aparicio

Received: 26 March 2008 / Accepted: 17 October 2008 / Published online: 9 November 2008
© Springer Science+Business Media B.V. 2008

Abstract Mate abundance is one of the most important sources of variation in plant mating systems. We examined within-population heterogeneity in the pollen pool at two spatial scales (sites and plants), and investigated the mating pattern variation in *Myrtus communis* under diverse situations of conspecific neighbourhood, using allozyme electrophoresis of naturally pollinated progeny arrays. For mating analyses, plants sampled were classified into four neighbourhood groups (from high to low) based on the local density around them and the distance to their nearest neighbour. The pollen pool was much more genetically heterogeneous among mother plants ($\sim 21\%$) than among sites ($\sim 2\%$), probably because of the high levels of selfing found (average $s = 0.65$). Outcrossing rates differed significantly among neighbourhood groups and showed a marked trend towards higher values from the lowest ($t_m = 0.26$) to the highest ($t_m = 0.45$) degree of conspecific aggregation. However, the lowest levels of biparental inbreeding and correlated paternity were found in the most isolated group of plants, indicating that these plants crossed with more and less genetically related fathers. Our study provides a clear demonstration of positive correlation between conspecific aggregation and the outcrossing rates. We discuss the ecological

implications of these results in the context of Mediterranean ecosystems.

Keywords Mating system · Conspecific neighbourhood · Outcrossing rates · Biparental inbreeding · Insect pollination · *Myrtus communis* · Mixed mating

Introduction

The plant mating system (i.e. the pattern of pairing between gametes and their genetic relatedness) is a determining factor for progeny performance, the genetic structuring and long-term viability of populations (Barrett and Harder 1996; Hamrick and Godt 1996; Husband and Schenske 1996; Duminil et al. 2007). Therefore, estimating outcrossing rates at different spatial scales is a central issue to understand the reproductive biology and landscape genetics of a species (Sork et al. 1999; García et al. 2005). Although mating systems are primarily controlled by the plant reproductive characteristics that permit or prevent self-fertilization (i.e. breeding system; see Neal and Anderson 2005), they are influenced by diverse ecological factors such as landscape architecture, vegetation structure and abundance of conspecific plants (Van Treuren et al. 1993; Franceschinelli and Bawa 2000; García et al. 2005). Further, in animal-pollinated species mating systems are directly affected by the abundance and behaviour

J. P. González-Varo (✉) · R. G. Albaladejo · A. Aparicio
Departamento de Biología Vegetal y Ecología,
Universidad de Sevilla, C/Profesor García González n 2,
41012 Seville, Spain
e-mail: juanpe@us.es

of pollinators (Franceschinelli and Bawa 2000; Kalisz et al. 2004).

Among these factors, conspecific density has been found to be one of the most important parameters determining the mating system of a species, being in some cases a more important determinant than plant population size (see Van Treuren et al. 1993). In fact, a number of studies have reported positive relationships between plant density and outcrossing rates at the patch and population levels (e.g. Murawski and Hamrick 1992; Van Treuren et al. 1993; Franceschinelli and Bawa 2000). Mate density is usually measured as the distance among conspecific plants (e.g. Hodgins and Barrett 2006), because the distance to the nearest neighbours and conspecific density around plants are expected to be negatively correlated due to the pattern of spatial aggregation of individuals, from sparse to clumped (García et al. 2005). Thus, the distance to the nearest neighbour has been reported as a good predictor of mating patterns in some wind-pollinated (Robledo-Arnuncio and Gil 2005) and animal-pollinated species (Oddou-Muratorio et al. 2006).

Such spatial distribution of individual plants within a population can influence not only the quantity, but also the quality of matings among them. For example, Jordano and Godoy (2002) detected a higher genetic similarity among *Prunus mahaleb* trees located up to 20 m apart, which they attributed to the seed rain pattern. As these authors showed, the distance among neighbours (spatial distribution) is associated with their level of genetic relatedness. Consequently, biparental inbreeding (i.e. mating among genetically related individuals) may increase in dense patches of conspecific plants (Stacy et al. 1996; Hodgins and Barrett 2006).

In this study, we investigated within-population mating pattern variation in *Myrtus communis* L. (myrtle) under diverse situations of conspecific neighbourhood, using naturally pollinated progeny arrays. Myrtle is a self-compatible, hermaphroditic and insect-pollinated Mediterranean shrub. During its flowering peak (late spring to early summer), myrtle dominates the entomophilous flora of the study sites, providing an excellent model that avoids interference from other local flowering species (see García et al. 2005). Conspecific neighbourhoods of mother plants sampled were characterized combining local density and the distance to the nearest neighbour; thus,

incorporating the potential interactive effects of both parameters on mating patterns. Specifically, (1) we examined within-population heterogeneity in the pollen pool at two spatial scales (sites and plants), and (2) we sought to determine whether the variation among plants in their conspecific neighbourhood composition translated into differences in outcrossing rates and biparental inbreeding. Given the scarcity of data regarding the mating system in Mediterranean woody species, we then examined the ecological implications of these results in a Mediterranean context.

Materials and methods

Study species

The myrtle is a common shrub in the Mediterranean region. It grows up to 4 m in height, occurs in fertile soils in low and warm habitats, and is the only representative of the Myrtaceae in the flora of the Mediterranean Basin. In southern Spain, it is a main component of late successional forests of helm oak (*Quercus ilex* subsp. *ballota*), cork oak (*Q. suber*), and pine (mainly *Pinus pinea* and *P. halepensis*). The flowers are white, have one style and multiple stamens (>50), open in June–July, and are mainly pollinated by bees and flies (González-Varo et al. unpublished). The myrtle fruit is a multi-seeded berry that is dark-blue when fully matures in November. In the study area, berries contain an average (\pm SD) of 5.6 (\pm 3.2) seeds ($n = 620$ fruits from 31 plants). Frugivorous passerine birds and some mammals (belonging to Order Carnivora) are the most important seed dispersal vectors (Traveset et al. 2001). Although self-compatibility in myrtle has been inferred from hand-pollination experiments (Mulas and Fadda 2004; González-Varo et al. unpublished), no study has assessed the myrtle mating system in wild populations.

Study area

The study area is located close to the Doñana National Park in the Guadalquivir River Valley (south-western Spain) and is 20–30 m above sea level. The study focused on two forest stands less than 1.5 km apart (Fig. 1), with myrtle abundances higher than many

other locations surveyed in the region (González-Varo, unpublished data). On the basis of the proximity and physiognomic similarity among stands, we considered the myrtles in these stands to be a single large population growing at two different sites (see below).

Both stands are mixed forests of stone pine (*P. pinea*), cork oak (*Q. suber*) and helm oak (*Q. ilex* subsp. *ballota*), and cover areas of 244 (VMQ1) and 87 (VMQ2) hectares. The shrub cover is 23% in VMQ1 and 25% VMQ2, and the most common species in the shrub layer are *Cistus salvifolius*, *M. communis*, *Pistacia lentiscus*, *Rosmarinus officinalis* and *Genista hirsuta*.

The number of reproductive adult myrtles within each stand, estimated by extrapolating myrtle densities, was approximately 8,900 individuals in VMQ1 and 2,100 in VMQ2. The myrtle density in each stand (36.7 and 24.2 individuals/ha, respectively) was obtained by counting all adult plants within a 10-m sampling band on either side of each of 10 linear transects (500 m) randomly located within each forest stand (the total area covered in each stand was 10 ha). We used a GPS to measure the 500 m transect length, and a laser distance meter (Leica DISTO™ A3) to determine whether individual myrtle plants were within the 20 m sampling band.

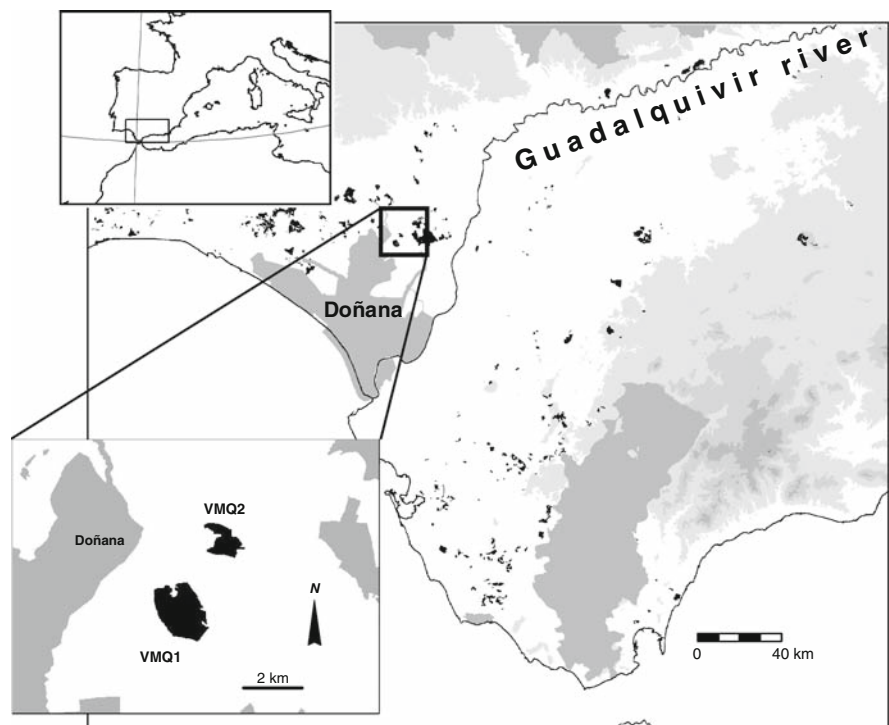
Neighbourhood characterization

Within each forest, stand myrtle shrubs can occur with different levels of local aggregation tending to clump more when growing in moist soils or in less managed areas. In autumn 2006, we marked 28 individual plants (16 in VMQ1 and 12 in VMQ2) that differed in the apparent level of aggregation than other conspecifics. Whereas the shortest distance between both sites is 1,302 m, distance between marked individuals range between 35 and 887 m within each one. For each marked individual, we measured two neighbourhood variables: the distance to the nearest neighbour (NEAREST) and the number of myrtles within a 15 m radius (DENSITY). We chose a 15 m radius because it was the longest distance that allowed an unbiased measurement of DENSITY in the densest patches of shrub vegetation. In order to integrate both measurements in a single metric, we developed an individual conspecific neighbourhood index (C_n), expressed as

$$C_n = (1 + \text{DENSITY})/\text{NEAREST}.$$

C_n values increase as the local density increases and the distance to the nearest neighbour decreases (see an analogous index in Ivey and Wyatt 1999). Note

Fig. 1 Geographic location of the study area in the south-west Iberian Peninsula. The white area represents the Guadalquivir River Valley, where the remaining woodland habitats are shown in black; mountain and protected areas are shaded grey. The inset illustration shows the two forest stands of the study in black and other woodland habitats in grey



that this index depends on the radius used in measuring the local density, and only C_n values calculated using the same radius could be compared (15 m in this study). From the C_n values obtained, we classified the plants sampled into four neighbourhood groups: high (H), mid-high (MH), mid-low (ML) and low (L) (see Table 1), based on the threshold values provided by the inter-quartile range of the distribution of C_n values. Each group consisted of seven mother plants.

Sampling and genotyping of progeny

We collected 20 fruits from throughout the entire canopy of each of the 28 marked plants. We performed allozyme analysis of the progeny arrays using horizontal starch gel electrophoresis; only one seed per fruit was used, to avoid the effects of correlated mating in single pollination events. Twenty myrtle seeds per mother plant were germinated on distilled water-saturated filter paper in Petri dishes. Germination of myrtle seeds is rapid in Petri dishes, and germination rates are very high (Traveset et al. 2001). We obtained germination rates well over 90%, if seeds were not fungus infected. Emergent seedlings were crushed in four drops of extraction buffer (Werth 1975) and the resulting homogenates were absorbed on chromatography paper wicks (Whatman, 3 mm) and stored at -80°C until electrophoresed. Electrophoresis was carried out in 9% starch gels, following the general protocols of Wendel and Weeden (1989) and the staining recipes of Soltis et al. (1983). We initially screened for 14 enzyme systems that have been previously assessed in adult myrtle leaves in our laboratory. However, only four produced reproducible and consistent bands that could be scored following electrophoresis of

seedling extracts. A histidine-citrate gel with an electrode buffer at pH 6.5 was used to resolve isocitrate dehydrogenase (*Idh-2*, EC 1.1.1.41), phosphoglucumutase (*Pgm-1*, EC 5.4.2.2), uridine diphosphoglucose pyrophosphorilase (*Ugpp-2*, EC 2.7.7.9) and phosphoglucosomerase (*Pgi-2*, EC 5.3.1.9). We found a total of 11 alleles: two in each of the *Idh-2* and *Pgm-1* loci, three in the *Ugpp-2* locus, and four in the *Pgi-2* locus. Overall, multilocus allozyme profiles were generated for 426 seedlings (9–20 per family; mean \pm SD = 15.2 ± 2.6), 229 from VMQ1 and 197 from VMQ2.

Scales of pollen pool variation

In order to examine heterogeneity in allele frequency of pollen pools between the two sampled forest stands (two sites), and among mother plants nested within them, we performed a two-level analysis (Φ_{ST} and Φ_{FS} , respectively) of the molecular variance (AMOVA). To perform an AMOVA with respect to the male gametes (i.e. pollen that had sired a mother), the female contribution derived from the mother genotypes was subtracted from the diploid multilocus genotypes of the seedlings. The AMOVA was performed with GenAlEx 6.0 (Peakall and Smouse 2006).

Analysis of mating patterns

Mating patterns in each neighbourhood group were characterized by three mating variables: multilocus outcrossing rates (t_m), biparental inbreeding ($t_m - t_s$; t_s being the average of single locus outcrossing rates) and correlated paternity (r_p), defined as the proportion of progeny sired by the same father. Methods for obtaining mating estimates at the individual

Table 1 Mean values (\pm SD) of NEAREST (distance to the nearest adult myrtle) and DENSITY (number of myrtles within a 15 m radius) for each C_n neighbourhood group

Neighbourhood group	Quartile range (C_n)	C_n values	NEAREST	DENSITY	n (n_1/n_2)
H (high)	Q ₇₅ –Q ₁₀₀	>4.3	1.6 \pm 0.8	12.0 \pm 5.2	7 (5/2)
MH (mid-high)	Q ₅₀ –Q ₂₅	0.93–4.3	4.0 \pm 2.3	4.6 \pm 1.3	7 (5/2)
ML (mid-low)	Q ₂₅ –Q ₅₀	0.22–0.93	8.8 \pm 3.5	3.0 \pm 1.7	7 (3/4)
L (low)	Q ₀ –Q ₂₅	<0.22	26.1 \pm 12.9	0.14 \pm 0.4	7 (3/4)

n = sample size for each group; values in parentheses indicate the number of mother plants sampled in VMQ1 and VMQ2 forests (n_1 and n_2 , respectively)

plant-level are usually inaccurate (Ivey and Wyatt 1999). We sought individual estimates of outcrossing rates using the method of moments, available in the software MLTR v 3.0 (Ritland 2002), but the results included many negative estimates and values of $t_m > 1$, and high error values. A larger sample both of seeds per progeny and loci would be needed to obtain reliable individual estimates (see also García et al. 2005). For this reason, mating variables (t_m , $t_m - t_s$ and r_p) were estimated at the neighbourhood group level using MLTR v 3.0 (Ritland 2002), pooling the four groups in the same analysis; thus, providing equal allele frequencies to every group. We also calculated the number of effective pollen donors, as $N_{ep} = 1/r_p$, for each group. We used the Newton–Raphson algorithm since missing data in our data set were infrequent. As in the AMOVA (see above), the female contribution derived from the mother genotypes was subtracted from the diploid multilocus genotypes of the seedlings. The errors for estimates were obtained by bootstrap methods (setting 1,000 replicates), with families as the unit of re-sampling. As the errors did not fit a normal distribution, the occurrence of significant differences among neighbourhood groups in the mating estimates was tested with a Kruskal–Wallis ANOVA. Significant differences between pairs of neighbourhood groups were tested with the Nemenyi multiple comparisons test, a non-parametric Tukey-type test (Zar 1999).

Results

Spatial distribution of conspecific neighbours

The average distance to the nearest conspecific plant (NEAREST) for the 28 marked plants was 10.1 m (range 0.3–50 m), while the average density of conspecifics (DENSITY) within the 15 m radius was 4.9 individuals (range 0–20 individuals). The average values (\pm SD) of NEAREST and DENSITY for each neighbourhood group (based on the C_n index) are shown in Table 1. NEAREST and DENSITY were negatively correlated in a logarithmic fashion (see Fig. 2), with the Pearson's correlation coefficient between log-NEAREST and log-DENSITY being highly significant ($r = -0.64$; $n = 28$, $P < 0.001$).

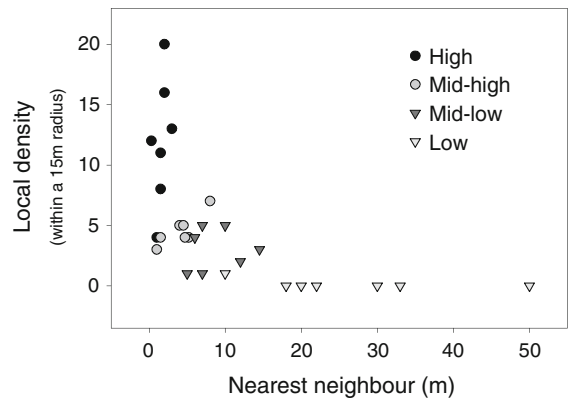


Fig. 2 Relationship between DENSITY (within a 15 m radius) and NEAREST in the 28 mother plants studied. The four types of symbols show the neighbourhood groups

Scales of pollen pool variation

Although significant, pollen pool genetic differentiation between sites (forest stands) was close to zero, accounting for just $\sim 2\%$ ($\Phi_{ST} = 0.022$; $P < 0.002$) of the molecular variance and indicating that the pollen cloud received by mother plants in both forest stands had nearly the same genetic composition for the loci analysed. However, a considerable amount of genetic difference ($\sim 21\%$) was detected among pollen pools received by each maternal plant ($\Phi_{FS} = 0.211$; $P < 0.001$), although most of the genetic variation in the pollen clouds was attributable to the within-mother plant level (77%).

Mating patterns

The estimate of average outcrossing rate ($t_m = 0.35$) for myrtle was significantly different from a $t_m = 1$ (95% CI = 0.21, 0.50; $n = 4$ groups). Among neighbourhood groups, outcrossing rates (t_m) ranged from 0.26 to 0.45, indicating the high prevalence of selfing in myrtle. Both outcrossing rates (K–W test; $\chi^2_3 = 867$, $P < 0.001$) and biparental inbreeding (K–W test; $\chi^2_3 = 572$, $P < 0.001$) were significantly different among neighbourhood groups. There was a marked trend to higher levels of outcrossing from the lowest (group L) to the highest (group H) degree of conspecific aggregation (Table 2). Thus, selfing was higher for sparse and isolated plants lacking close neighbours in the landscape. Biparental inbreeding was low ($\leq 3.2\%$), but significantly different from

zero for all groups except group L ($t_m - t_s = -0.001$; 95% CI = $-0.003, 0.001$). Correlated paternity (r_p) also differed significantly among groups (K–W test; $\chi^2_3 = 256, P < 0.001$) and was higher under situations of high conspecific aggregation (groups MH and H), indicating that a higher proportion of progeny shared the same father in these groups (Table 2).

Discussion

Mating patterns and conspecific neighbourhood

The pollen pool influencing the studied plants was strongly structured at the individual plant level, finding nearly no differences ($\sim 2\%$) between the study sites. For example, the among-mother component of variance found in myrtle ($\sim 21\%$) contrasts with the almost 0% variance ($\Phi_{FS} = -0.015$) reported by O’Connell et al. (2006) for the outcrossing and wind-pollinated conifer, *Picea glauca*. This large difference can be explained by the high level of selfing detected in myrtle (average $s = 0.65$; $s = 1 - t_m$). When most of the progeny is produced by self-fertilization, the difference in the pollen pool received by each mother plant is expected to be high because of the particular genetic differences among mothers. The opposite situation is expected when outcrossing rates are high (close to 1) and correlated paternity is low (i.e. the progeny is sired by many fathers), diluting these particular differences among mothers (e.g. O’Connell et al. 2006). Furthermore, in myrtle, the among-mothers variation in the pollen pool received was related to the conspecific neighbourhood (Cn index), which was found to be a critical factor in the outcrossing rate estimates, decreasing

nearly 50% (from 0.45 to 0.26) from more aggregated to less aggregated plants. This means that isolated plants received a smaller amount of xenogamous pollen than aggregated ones. In self-compatible and animal-pollinated species, the levels of cross pollination, and consequently the outcrossing rates, depend on the frequency of pollinator visitation and foraging behaviour (Levin and Kerster 1969; Kunin 1997; Franceschinelli and Bawa 2000). During its maximum flowering peak, myrtle is the most abundant and almost the only flowering species in the study sites. A single myrtle shrub can simultaneously have several thousand open flowers; so in a situation of high conspecific aggregation, strongly clumped patches can act as large floral displays that are attractive to pollinators (Kunin 1997). However, for isolated myrtle shrubs, the large quantity of flowers produced by a single plant may favour pollinators spending more time moving among flowers within the same individual, favouring geitonogamy. In fact, honeybees *Apis mellifera* and other hymenopterans, such as *Amegilla quadrifasciata*, *Colletes fodiens* or *Megachile pilidens*, have been frequently observed foraging on different flowers on the same plant during several minutes (González-Varo et al. unpublished). Based on optimal foraging behaviour, it is expected that self-pollination occurs more frequently in more isolated plants, where pollinators stay longer visiting flowers within the same individual because of the higher energetic cost of travelling to another plant (Charnov 1976).

Levels of genetic relatedness are associated with conspecific aggregation (Vekemans and Hardy 2004). Nevertheless, pollen dispersal distance is expected to be greater for isolated plants because pollinator foraging distances are generally negatively correlated with plant density (Levin and Kerster 1969). In a

Table 2 Estimates (\pm SD) for t_m (multilocus outcrossing rates), $t_m - t_s$ (biparental inbreeding) and r_p (correlated paternity) of each neighbourhood group

Neighbourhood group	n	t_m	$t_m - t_s$	r_p	N_{ep}
H (high)	106	0.454 (0.171) ^a	0.026 (0.028) ^b	0.281 (0.486) ^a	3.6
MH (mid-high)	106	0.404 (0.121) ^b	0.029 (0.036) ^b	0.379 (0.488) ^a	2.6
ML (mid-low)	104	0.293 (0.132) ^c	0.032 (0.025) ^a	0.175 (0.443) ^b	5.7
L (low)	110	0.263 (0.086) ^d	-0.001 (0.026) ^c	0.097 (0.477) ^c	10.3

The number of effective pollen donors ($N_{ep} = 1/r_p$) is also showed. n = number of seedlings sampled

Different letters indicate significant differences between neighbourhood groups ($P < 0.05$, Nemenyi test)

study of pollen dispersal in three insect-pollinated tropical tree species, Stacy et al. (1996) found that for sparse and isolated flowering trees pollen came from at least several hundred metres away, far beyond nearest neighbours. Conversely, where trees were clumped they found most mating occurred among near neighbours. Oddou-Muratorio et al. (2006) also found that the progeny of isolated trees of the obligate outcrossing species *Sorbus torminalis* derived from higher rates of long-distance pollen dispersal, were genetically more diverse, and were sired by a higher number of fathers also displaying low genetic relatedness. Interestingly, in myrtle, the lowest levels of biparental inbreeding and correlated paternity were found in the group of most isolated plants (group L), indicating that despite these plants had low outcrossing rates, they crossed with more and less genetically related fathers. Although the limitations of Ritland's method for accurate estimation of r_p have been questioned (see Fernández-Manjarrés et al. 2006), the pattern of correlated paternity found in myrtle was consistent with the patterns of conspecific aggregation. Therefore, outcrossing in isolated individual plants may represent an important source of genotypic variation in the population (Oddou-Muratorio et al. 2006): a mother plant not only bears fruits to disperse by frugivores, but also genotypes (Jordano and Godoy 2002), reinforcing that the patterns of pollen and seed dispersal originate patterns of spatial genetic structure (Vekemans and Hardy 2004).

A mixed mating system in *Myrtus communis*

Our estimates of outcrossing indicated that the myrtle has a mixed mating system ($0.2 < t_m < 0.8$; see Vogler and Kalisz 2001) with a large proportion of selfing events. The average outcrossing rate was 0.35, indicating that 65% of the progeny were produced by self-fertilization, which is also common in other animal-pollinated tropical woody Myrtaceae (e.g. Yates et al. 2006; Schmidt-Adam et al. 2000).

Most research about mating systems in animal-pollinated species has been conducted in tropical tree species, which even at low densities, show a predominance of outcrossing ($t_m > 0.80$; Ward et al. 2005). However, in temperate zones most studies have focused in wind-pollinated trees (mainly conifers and oaks; e.g. Robledo-Arnuncio and Gil 2005;

Fernández-Manjarrés et al. 2006). *M. communis* is an animal-pollinated species belonging to a pre-Mediterranean lineage (Herrera 1992), and in this study, we can report a low frequency of outcrossing even under the highest conspecific abundance. The Mediterranean basin, as a whole, and the Guadalquivir River Valley, in particular, can be considered an example of historical climate change and intensive human management (Grove and Rackham 2001; Aparicio 2008). Here, myrtle persists in small populations within forest patches embedded in a highly agricultural matrix (Albaladejo et al. 2008). So, a mixed mating system, strongly determined by the availability of mates (density-dependent), may be advantageous (see Goodwillie et al. 2005), reducing pollen limitation and providing reproductive assurance (Kalisz et al. 2004). In fact, seed production in myrtle can be more pollen limited in quantity than in quality (i.e. self vs. cross) (González-Varo et al. unpublished).

Conclusions

Habitat heterogeneity (including topography, micro-habitat and perturbations) generates non-uniform spatial distributions of plants, which ultimately affects individual mating patterns. Our study provides a clear demonstration that habitat heterogeneity influences progeny diversity and relatedness through a positive relation between conspecific aggregation and outcrossing rates. These results are consistent with the findings of previous studies demonstrating a positive relationship between plant density and outcrossing rates and variation in mating patterns among individuals, and contribute to the growing body of empirical data that will enable generalizations to be made. Furthermore, our study also contributes to filling existing gaps in the knowledge of mating systems within Mediterranean ecosystems.

Acknowledgements We thank Silvia Otero and Laura F. Carrillo for their help with the fieldwork and electrophoresis, respectively. Sebastien Lavergne provided useful discussion of ideas. Cristina García and Juan Arroyo critically reviewed an earlier version of this manuscript. The helpful comments by Andrew Lowe and an anonymous reviewer significantly improved the manuscript. This work has been financially supported by the Fundación Banco Bilbao Vizcaya Argentaria (FBBVA), the Spanish Ministerio de Educación y Ciencia (CGL2004-00022BOS) and the Consejería de Innovación,

Ciencia y Empresa (Junta de Andalucía) (Proyecto de Excelencia P06-RNM-01499).

References

- Albaladejo RG, Fernández J, Aparicio A, Fernández L, González-Varo JP (2008) Population genetics of myrtle *Myrtus communis* in a chronic fragmented landscape: can gene flow counteract anthropic disturbance? *Plant Biol* (in press)
- Aparicio A (2008) Descriptive analysis of the 'relictual' Mediterranean landscape in the Guadalquivir River valley (southern Spain): a baseline for scientific research and the development of conservation action plans. *Biodivers Conserv* 17:2219–2232
- Barrett SCH, Harder LD (1996) Ecology and evolution of plant mating. *Trends Ecol Evol* 11:73–79
- Charnov EL (1976) Optimal foraging, the marginal value theorem. *Theor Popul Biol* 9:129–136
- Duminil J, Fineschi S, Hampe A, Jordano P, Salvini D, Vendramin GG et al (2007) Can population genetic structure be predicted from life-history traits? *Am Nat* 169:662–672
- Fernández-Manjarrés JF, Idol J, Sork VL (2006) Mating patterns of black oak *Quercus velutina* (Fagaceae) in a Missouri oak-hickory forest. *J Hered* 97:451–455
- Franceschinelli EV, Bawa KS (2000) The effect of ecological factors on the mating system of a South American shrub species (*Helicteres brevispira*). *Heredity* 84:116–123
- García C, Arroyo JM, Godoy JA, Jordano P (2005) Mating patterns, pollen dispersal and the ecological maternal neighbourhood in a *Prunus mahaleb* (L.) population. *Mol Ecol* 14:1821–1830
- Goodwillie C, Kalisz S, Eckert CG (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Ann Rev Ecol Syst* 36:47–79
- Grove AT, Rackham O (2001) *The nature of Mediterranean Europe: an ecological history*. Yale University Press, New Haven, Connecticut
- Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. *Philos Trans R Soc Lond B* 351:1291–1298
- Herrera CM (1992) Historical effects and sorting processes as explanations for contemporary ecological patterns: character and syndromes in Mediterranean woody plants. *Am Nat* 140:421–446
- Hodgins KA, Barrett SCH (2006) Mating patterns and demography in the tristylous daffodil *Narcissus triandrus*. *Heredity* 96:262–270
- Husband BC, Schemske DW (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70
- Ivey CT, Wyatt R (1999) Family outcrossing rates and neighbourhood floral density in natural populations of swamp milkweed (*Asclepias incarnata*): potential statistical artefacts. *Theor Appl Genet* 98:1063–1071
- Jordano P, Godoy JA (2002) Frugivore-generated seed shadows: a landscape view of demographic and genetics effects. In: Levey DJ, Silva WR, Galetti M (eds) *Seed dispersal and frugivory: ecology, evolution and conservation*. CABI Publishing, Wallingford, UK, pp 305–320
- Kalisz S, Vogler DW, Hanley KM (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* 430:884–887
- Kunin WE (1997) Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *J Ecol* 85:225–234
- Levin DA, Kerster HW (1969) Dependence of bee-mediated pollen and gene dispersal upon plant density. *Evolution* 23:560–571
- Mulas M, Fadda A (2004) First observations on biology and organ morphology of myrtle (*Myrtus communis* L.) flower. *Agric Mediterr* 134:223–235
- Murawski DA, Hamrick JL (1992) The mating system of *Calliandra platyfolia* under extremes of flowering-tree density: a test of predictions. *Biotropica* 24:99–101
- Neal PR, Anderson GJ (2005) Are 'mating systems' 'breeding systems' of inconsistent and confusing terminology in plant reproductive biology? Or is it the other way around? *Plant Syst Evol* 250:173–185
- O'Connell LM, Mosseler A, Rajora OP (2006) Impacts of forest fragmentation on the mating system and genetic diversity of white spruce (*Picea glauca*) at the landscape level. *Heredity* 97:418–426
- Oddou-Muratorio S, Klein EK, Demesure-Musch B, Austerlitz F (2006) Real-time patterns of pollen flow in the wild-service tree, *Sorbus torminalis* (Rosaceae). III. Mating patterns and the ecological maternal neighbourhood. *Am J Bot* 93:1650–1659
- Peakall R, Smouse PE (2006) Genalex 6: genetic analysis in excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295
- Ritland K (2002) Extensions of models for the estimation of mating systems using n independent loci. *Heredity* 88:221–228
- Robledo-Arnuncio JJ, Gil L (2005) Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. *Heredity* 94:12–22
- Schmidt-Adam G, Young AG, Murray BG (2000) Low outcrossing rates and shift in pollinators in New Zealand pohutukawa (*Metrosideros excelsa*; Myrtaceae). *Am J Bot* 87:1265–1271
- Soltis DE, Hauffler CH, Darrow DC, Gastony GE (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and standing schedules. *Am Fern J* 73:9–27
- Sork VL, Nason J, Campbell DR, Fernández JF (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends Ecol Evol* 14:219–224
- Stacy EA, Hamrick JL, Nason JD, Hubbell SP, Foster RB, Condit R (1996) Pollen dispersal in low-density populations of three neotropical tree species. *Am Nat* 148:275–298
- Traveset A, Riera N, Mas R (2001) Ecology of fruit-colour polymorphism in *Myrtus communis* and differential effects of birds and mammals on seed germination and seedling growth. *J Ecol* 89:749–760
- Van Treuren R, Bulsma R, Ouborg NJ, van Delden (1993) The effects of population size and plant density on outcrossing

- rates in locally endangered *Salvia pratensis*. *Evolution* 47:1094–1104
- Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol Ecol* 13:921–935
- Vogler DW, Kalisz S (2001) Sex among the flowers: the distribution of plant mating systems. *Evolution* 55:202–204
- Ward M, Dick CW, Gribel R, Lowe AJ (2005) To self, or not to self. A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* 95:246–254
- Wendel JF, Weeden NF (1989) Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS (eds) *Isozymes in plant biology*. Dioscorides Press, Portland, Oregon, USA, pp 5–45
- Werth CR (1975) Implementing an isozyme laboratory at a field station. *Virg J Sci* 36:53–76
- Yates CJ, Coates DJ, Elliott C, Byrne M (2006) Composition of the pollinator community, pollination and the mating system for a shrub in fragments of species rich kwongan in south-west Western Australia. *Biodivers Conserv* 16:1379–1395
- Zar JH (1999) *Biostatistical analysis*. Prentice Hall International, New Jersey, USA