



Effects of fragmentation on pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (*Myrtus communis*)

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ABSTRACT

Pollinator assemblages may shift as a consequence of the destruction and fragmentation of natural habitats. The scarcity of mates and pollinators can lead plant populations to suffer from pollen limitation and a decrease in reproductive performance within fragmented areas. We studied the shift in pollinator assemblages along with pollen limitation and seed production patterns in the Mediterranean shrub *Myrtus communis*. Our study included six populations contrasting in patch and population size (Large vs. Small) within a fragmented landscape characterized by ~1% of potential forest coverage. The breeding system in *Myrtus communis* was self-compatible, but compared with natural pollination, fruit set increased with pollen addition (quantity limited), and seed set (brood size) increased with outcross pollen addition (quality limited). While the pollinator assemblage in Large patches was taxonomically diverse, it was almost monopolized by honeybees in Small patches, where visitation rates were highest and wild bee species were almost absent. In general, Small populations were less pollen limited for fruit set than Large populations, particularly those that received the highest rates of honeybee visits. However, despite differences in fragmentation and pollinators between Large and Small populations, seed production patterns (brood size and seed mass) were rather similar among them, in agreement with similar pollen limitation levels found for brood size. A higher susceptibility of native pollinators to the presence of honeybee hives was found in Small patches, suggesting that the pollinator assemblage may be severely altered when fragmentation occurs in combination with beekeeping. We discuss its implications and effects on plant reproduction in fragmented areas.

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1. Introduction

Habitat fragmentation is one of the main results of current global change and thus is considered a major threat for biodiversity of terrestrial ecosystems, compromising the long-term persistence of species in small and isolated patches (Fahrig, 2003; Lindenmayer and Fischer, 2006). Plants, as sessile organisms, are prone to suffer these threats because of the spatial arrangement of habitat patches, which affects the ecological processes occurring within them, such as genetic bottlenecks, alteration of biotic interactions and biological invasions (Hobbs and Yates, 2003).

One of the main threats to plant reproduction within fragmented habitats is pollination failure (Wilcock and Neiland, 2002). Fragmentation usually leads to a correlated decrease in plant population size, and plant species occurring at low population size or density are more sensitive to pollen limitation, defined as a lower fruit and seed production caused by a scarce pollen receipt (Burd, 1994). Generally, pollen limitation is a consequence of

reduction in quantity and/or quality, e.g., self- or cross-pollination, of pollen deposited on stigmas, which provokes lower ovule fertilization and seed production or less vigorous offspring (Knight et al., 2005; Aizen and Harder, 2007). Thus, pollen-limited plant populations have been documented to experience disruptions in seed production (e.g., Aizen and Feinsinger, 1994a), which, in an extreme case, may compromise their long-term viability (Lamont et al., 1993; Groom, 1998).

Besides the scarcity of mates for cross-pollination, in animal-pollinated plants, pollen limitation is also caused by low pollinator visitation rates (Cunningham, 2000). Pollinators are also vulnerable to habitat fragmentation, and the pollinator assemblage may be shifted because different pollinator species may respond differently to landscape changes (Steffan-Dewenter et al., 2002; Aizen and Feinsinger, 2003; Ashworth et al., 2004). In consequence, fragmentation may reduce the abundance of pollinators (e.g., Steffan-Dewenter and Tschardtke, 1999), shift the pollinator guilds (e.g., Donaldson et al., 2002) and facilitate non-native species to access floral resources (e.g., Aizen and Feinsinger, 1994b).

However, despite numerous studies over the last two decades, results do not show clear patterns on the effects of fragmentation on pollination failure (reviewed in Hobbs and Yates, 2003; Aguilar

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et al., 2006; for pollinators see Steffan-Dewenter and Westphal, 2008). In general, self-incompatible plants are more likely to show lower fecundity than self-compatible ones (Aguilar et al., 2006), although most studies were biased to self-incompatible herbs and tree species in tropical or temperate ecosystems. Therefore, results of particular studies are a consequence of the several possible combinations of plant traits, e.g., breeding systems, degree of specialization in pollinators, rarity, and ecosystem properties (evolutionary setting of the biota, history of anthropogenic disturbance, sensitivity of pollinator fauna).

Despite the long-standing process of habitat fragmentation in the Mediterranean (Grove and Rackham, 2001), its effects on ecological processes, in general, and plant reproduction, in particular, have received little attention (but see Santos et al., 1999 for studies on seed dispersal by vertebrates). Additionally, the Mediterranean region has been recognized as likely to have more intense climate change in the near future (IPCC, 2007), which may foster reduction of suitable habitats and increasing fragmentation. Thus, there is a need for more empirical studies to detect the effects of habitat fragmentation on critical ecological processes such as the reproductive performance of plant species, particularly if these are important components of the natural vegetation. These studies will provide a sound basis for establishing conservation guidelines in regions prone to deforestation and fragmentation, such as those in the Mediterranean.

We investigated these issues in Mediterranean myrtle (*Myrtus communis* L., Myrtaceae), an insect-pollinated shrub, which has the typical traits of sclerophyllous Mediterranean plants and is a main component in many oak woodland understoreys and late successional shrublands across the Mediterranean Basin. The study was carried out in the Guadalquivir River Valley (SW Spain), a large and fertile lowland area devoted to intensive agriculture, where forest vegetation was virtually eliminated by humans several centuries ago. Nowadays, the landscape in this area can be considered of 'relictual' type (*sensu* McIntyre and Hobbs, 1999), where overall patch area is very low and spatial connectivity among patches is mostly lacking (Aparicio, 2008). Among them, small, isolated patches supporting small myrtle populations (<50 inds.) are frequent.

In this study, we addressed pollinator assemblage, pollen limitation and seed production in myrtle populations with contrasting levels of habitat and population size. Because fruit and seed set may depend on abiotic factors and resources, we performed a pollen supplementation experiment to ascertain the degree to which differences in fruit and seed set are caused by pollen limitation (Knight et al., 2005). Specifically, we aimed to answer the following questions: (1) are the pollinator assemblages and the visitation rates different among Large and Small patches?; (2) are Small myrtle populations more pollen limited than Large populations?; (3) is there any relationship between local pollinator assemblages and pollen limitation?; and (4) are seed production patterns influenced by population size (Large or Small) or pollinator visitation rate?

For a correct understanding and interpretation of the effects of variation in pollinator assemblages on myrtle fecundity, we determined the breeding system of the species, which had not been previously reported.

2. Methods

2.1. Study species

The myrtle is a common sclerophyllous shrub and the sole representative of the Myrtaceae in the flora of the Mediterranean Basin. It grows up to 4 m in height in fertile soils in low and warm habitats. In southern Spain, it is a main component of late succes-

sional woodland understoreys, blooming massively during late June–early July. The flowers are hermaphrodite, with a white open dish corolla up to 3 cm in diameter with a life span of 1–3 days (Fig. 1), have one style and many stamens (>50) and contain a mean (\pm SD) of 72 ± 12 ovules ($n = 150$ flowers from 15 plants). Fruits are ellipsoidal berries, dark blue when ripe from November, with a mean diameter (\pm SD) of 8.8 ± 1.1 mm, containing 5.6 ± 3.2 seeds (González-Varo et al., 2009) that account for an average natural seed set of $7 \pm 2\%$ ($n = 15$). The number of seeds per fruit (hereafter, brood size) is not significantly correlated with the number of ovules per flower ($r = 0.28$, $P = 0.32$, $n = 15$ plants), and given the large number of ovules per flower and their low level of variation among plants (CV = 16%), brood size may be considered a useful surrogate for seed set as shown by the highly positive correlation among both variables ($r = 0.84$, $P < 0.001$, $n = 15$ plants).

Self-compatibility in myrtle has been inferred by means of bagged pollinations in cultivar clones (Mulas and Fadda, 2004). A mating system analysis using isozyme markers has revealed a mixed-mating system in large and dense populations (González-Varo et al., 2009). Endozoochorous seed dispersal is carried out by frugivorous passerine birds and carnivorous mammals (Traveset et al., 2001). However, many aspects of the pre-dispersal reproductive biology of the species, such as its breeding system and pollinators (but see Herrera, 1988), remain unknown to date.

2.2. Study area and populations

The Guadalquivir River Valley is a large lowland area of ca. 21,000 km² located in south-western Spain and long devoted to intensive agriculture, where 535 natural or semi-natural (holm-oak, cork-oak and stone-pine) forest patches covering about 1% of the valley area can be found (see Aparicio, 2008, for a detailed description). Myrtle is known to occur in 162 of these patches, and during its late flowering peak (early summer) is virtually the only dominant species in full bloom (see González-Varo et al., 2009). We selected six patches contrasting in both area and population size: three large patches (>90 ha) supporting large myrtle populations (>2000 inds.) and three small patches (<30 ha) supporting small populations (<70 inds.) (hereafter Large and Small patches/populations, respectively; see Table 1). None of selected patches had animal-pollinated co-flowering crops in their surroundings (spring flowering olive and orange orchards, and cereal and cotton fields). The number of reproductive adult myrtles in each patch was estimated by extrapolating myrtle density to the patch area in Large populations and by direct counting in Small

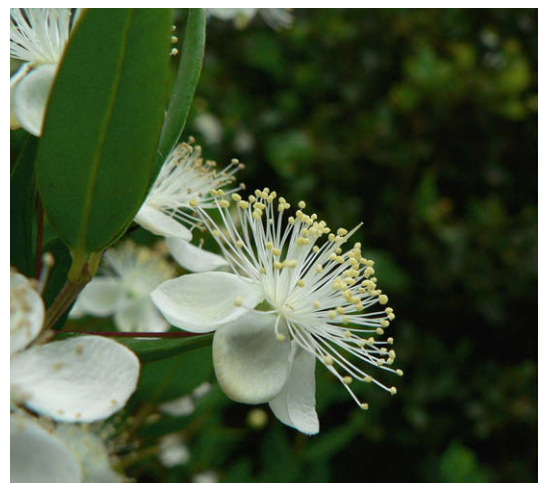


Fig. 1. Flower of Mediterranean myrtle (*Myrtus communis*).

Table 1
 Characteristics of the six myrtle (*Myrtus communis*) populations in this study. NND denotes the average nearest neighbour distance, and Conspecific density the number of adult myrtles within a 15 m radius, both measured over 16–34 individuals per population.

Population	Coordinates (lat. N–long. W)	Type	Patch size (ha)	Approx. pop. size	Nearest large pop. (km)	NND (m)	Conspecific density
DBL	37°13'20"–6°18'17"	Large	240	8900	–	5.1	8.8
CHP	37°14'35"–6°17'01"	Large	90	2100	–	7.4	7.2
DHY	36°33'15"–6°08'14"	Large	90	3200	–	4.6	5.1
GBL	37°23'23"–6°51'56"	Small	30*	50	>20	11.7	2.1
CRB	37°43'39"–4°55'20"	Small	10	70	>20	13.9	2.1
PTR	36°34'12"–6°07'30"	Small	2	30	~1	7.9	5.6

* Area exceeded that of the other small patches, but most of this patch (approx. 2/3) is covered by oak Mediterranean savanna lacking of understorey, and the myrtle population was restricted to a <10 ha area.

populations (Table 1). Myrtle density in Large populations was obtained by counting all adult plants within 10 linear transects (500 × 20 m = 1 ha) randomly located within each patch.

2.3. Breeding system

We performed controlled pollinations in order to determine the breeding system of the species. Pollinations were conducted in June 2007 on five flowering individuals in one of the Large populations (CHP, Table 1). On each plant, we applied six pollination treatments: (1) "Agamospermy": flowers were emasculated and bagged; (2) "Spontaneous selfing": flowers were bagged before anthesis without further manipulation; (3) "Self-pollination": flowers were bagged and hand pollinated with pollen from other flowers of the same plant (i.e. geitonogamous self-pollination); (4) "Control": flowers were left to natural pollination by insects; (5) "Xenogamy": open pollinated flowers were hand pollinated with outcross pollen; and (6) "Forced xenogamy": emasculated flowers were bagged and hand pollinated with pollen from other plants. Each treatment was replicated on eight flowers per plant, over 2–4 branches. Small branches containing about 6–10 flowers were covered with gauze bags (<0.1 mm mesh light) for the bagging treatments. Floral buds were emasculated one day before opening. Flowers were pollinated by gently touching the stigma with anthers from two distant myrtles (collected more than 20 m away) in the population or from other flowers of the same plant, depending on the treatment. Bags were removed one week after hand pollinations. Overall, 259 flowers were marked (40–50 flowers per treatment) with plastic rings in the flower pedicel and regularly monitored until fruit production. The effects of pollination treatments on fruit set and brood size were tested with Generalized Linear Mixed Models (GLIMMIX procedures of SAS 9.1; SAS, 2000), with pollination treatment as the fixed factor and individual plant as a random-effect factor. Binomial distribution and logit-link function were used for modelling fruit set and Poisson distribution and log-link function for brood size. Post-hoc tests (differences of least squares means) were used to identify treatments that differed.

2.4. Pollinator observations

We surveyed floral visitors in the 2007 flowering season. We only considered insects touching flower sex organs and thus being potential pollinators. We performed censuses in 5-min observation periods over individual shrubs at their flowering peak. We identified insect visitors as morphospecies in the field, which included insects of similar morphology and behaviour in the flowers. These insects were later identified by specialists. Censuses were performed ~1 m apart from the shrub canopy, and the number of individuals of each morphospecies observed during the census time visiting flowers on each focal plant was recorded. Censuses were carried out during 3–4 days from 1000 to 1400 h, the maximum pollinator activity period, as determined by previous observations, on calm, sunny or slightly cloudy days. In Large patches, focal

shrubs for censuses were randomly chosen along a ~500 m walking transect. Total observation time was 80–140 min per forest patch. In each patch, 50% of morphospecies were recorded after 5–30 min, 75% after 30–45 min of census, 90% after 45–100 min, whereas the 100% was reached after 45–115 min. Thus, reported insect visitors and their frequency should confidently reflect the actual pollinator fauna of the patches.

The number of visits recorded per census (mean ≈ 4 insects 5-min⁻¹) and the high number of flowers contacted by some visitors (up to 25 flowers min⁻¹) made it very difficult to take data at the single flower level; therefore, visitation rates were recorded at the plant level. We calculated the visitation rate to individual plants (V/H: visits/hour) and the relative frequency of visits by each insect morphospecies (%: percentage of visits). Differences among Large and small patches in V/H were tested with Generalized Linear Mixed Models (GLIMMIX procedures of SAS 9.1; SAS, 2000), with patch size as the fixed factor and population as a random-effect factor. Data were square-root transformed to improve normality. A χ^2 contingency test was performed to assess differences in frequency of visits by different insect taxa in Large and small patches.

2.5. Pollen limitation

We marked 6–8 myrtle shrubs in June 2007 at their flowering peak in each population ($n = 42$ plants). On each plant, we applied two pollination treatments: natural pollination (Control) and addition of xenogamous pollen (Xenogamy) in the same manner as described above (see section *Breeding system*). The flowers used for the Xenogamy treatment were recently opened (identified by the light colour of the anthers) and hand pollinated in the morning. Flowers used as Control were floral buds chosen the day before opening, aiming to avoid flower alteration, e.g., fallen petals, when marking them with plastic rings. Each pollination treatment (Xenogamy and Control) was applied on 18–22 flowers per individual plant distributed on 2–4 branches. A total of 1732 flowers were marked and monitored for fruit set and brood size, 876 and 856 as Control and Xenogamy, respectively. Branches within a plant were pooled for each treatment for data analyses.

In early September 2007, all developed fruits were collected, counted and dissected. We collected unripe, fully swollen fruit to avoid consumption by frugivores. For each individual plant, fruit set and brood size obtained in each treatment were used to calculate a pollen limitation index expressed as: $PL = 1 - C/X$, where C and X represent the fruit set or brood size of Control and Xenogamy treatments, respectively (following Tamura and Kudo, 2000). This standard index has a straightforward biological interpretation (e.g., Tamura and Kudo, 2000; Lázaro and Traveset, 2006), ranging from zero when both treatments produce the same fruit set and/or brood size, i.e., no pollen limitation, to one when natural pollination (Control) does not produce seeds (full pollen limitation). To estimate the total pollen limitation throughout both stages (fruit set and brood size), we also calculated the cumulative

pollen limitation index in each individual as $PL_{cumulative} = 1 - (C_{fruit\ set} C_{brood\ size}) / (X_{fruit\ set} X_{brood\ size})$. For each group of Large and small populations, the pollen limitation index was compared with zero by means of *t*-tests. Differences among Large and small populations were tested with Generalized Linear Mixed Models (GLIMMIX procedures of SAS 9.1; SAS, 2000), with patch size as the fixed factor and population as a random-effect factor. Three individual plants (from DBL, GBL and CRB) that did not produce any fruits were excluded from data analyses.

2.6. Seed production

Two variables were considered at the plant level: brood size and seed mass. We sampled fruits naturally produced from 12–14 plants in each of the six study patches during the fruiting seasons of 2006 and 2007, except for population DHY, sampled only in 2007. We randomly collected 20 ripe fruits from around the canopy of each individual. Fruits were dissected, and the brood size was counted. We randomly sampled one seed per dissected fruit and weighed it to the nearest 0.1 mg to obtain the average seed mass of each individual plant ($n = 20$ seeds per individual plant). In total, 2900 fruits were dissected, and the same number of seeds was weighed, 1320 and 1580 in the 2006 and 2007 seasons, respectively. Differences among Large and small patches in seed production (brood size and seed mass) were tested with Generalized Linear Mixed Models (GLIMMIX procedures of SAS 9.1; SAS, 2000), with patch size and year as the fixed factors and population as a random-effect factor. Year was included as a fixed factor in order to test possible between-year differences in seed production among types of populations (i.e. interaction 'Patch size \times Year').

3. Results

3.1. Breeding system

Pollination treatments produced significant effects on fruit set ($\chi^2_4 = 24.4$, $P < 0.001$) and brood size ($\chi^2_4 = 35.6$, $P < 0.001$; Fig. 2). Agamospermy was excluded from analyses because this treatment did not produce any fruit, and consequently any seed. Compared with natural pollination (Control = 57%), fruit set was higher for the hand-pollination treatments Xenogamy and Self-pollination (87% and 77%, respectively) and lower for Spontaneous selfing and Forced xenogamy treatments (49% and 45%, respectively; Fig. 2a). Naturally pollinated flowers (Control) produced an average brood size of 3.5 seeds. Compared with this, brood sizes were higher for treatments with xenogamous pollen addition (brood size = 4.3 and 4.9 seeds per fruit in Xenogamy and Forced xenogamy, respectively) and lower for both selfing treatments (brood size = 2.7 and 3.1 in Spontaneous selfing and Self-pollination, respectively; see Fig. 2b).

We acknowledge that our experiments may have underestimated the fruit set in the Forced xenogamy treatment (FX in Fig. 2a) because of some damage to flowers when emasculated. However, brood size showed that flowers properly hand outcrossed clearly set more seeds (Fig. 2b). Similarly, the Spontaneous selfing treatment (SS in Fig. 2a) may have overestimated fruit set without insect visits because of the dense pollen cloud caught within the bags. In a trial performed using in a wider mesh light (1 mm) in SS treatment, fruit set dropped to 7.5%, much lower than the 45.7% of natural pollination).

3.2. Pollinator assemblage and visitation rates

A total of 520 insects belonging to at least 26 taxa, including unidentified bees and flies, were observed visiting myrtle flowers

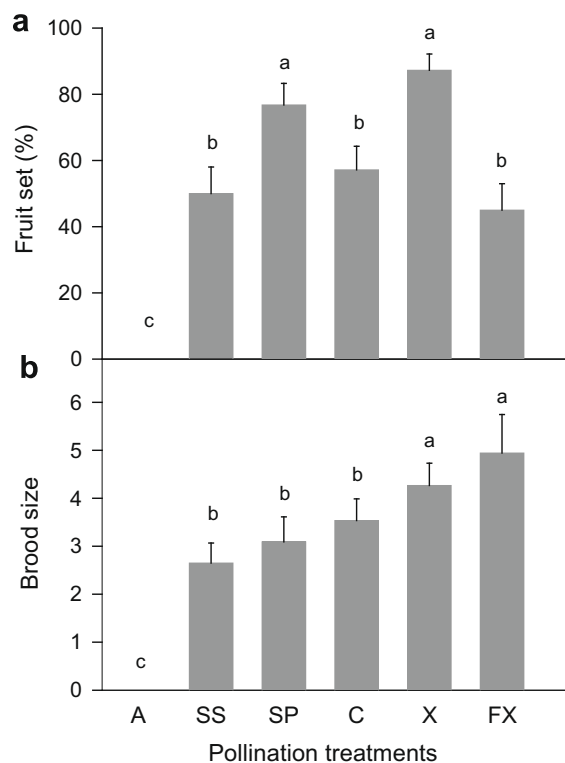


Fig. 2. Effect of controlled pollinations on the mean of (a) fruit set and (b) brood size for assessing the breeding system of *Myrtus communis*. Vertical bars denote standard errors. Pollination treatments: A, Agamospermy; SS, Spontaneous selfing; SP, Self-pollination; C, Control; X, Xenogamy; FX, Forced xenogamy (see text for details). Bars with the same letter do not differ significantly ($P > 0.05$).

(Table 2). *Cetonia aurata* beetles (Coleoptera) were rarely observed preying on myrtle flowers outside the census time; therefore, they were not considered as pollinators. Bees (Hymenoptera: Apoidea) accounted for 74–93% of visits in each patch, and the remaining were flies (Diptera). A greater visitor diversity was observed in Large patches (10, 14 and 14 taxa at DBL, CHP and DHY, respectively) compared with the small patches (9, 5 and 9 taxa at GBL, CRB and PTR, respectively; see Table 2). Marked differences in relative frequency of taxa occurred for bees (Fig. 3a). Honeybees (*Apis mellifera*) were consistently more frequent in small patches (>3-fold higher), to the detriment of other bee species (>3.5-fold lower, Fig. 3a). Wild bees and flies were more frequent in Large patches and honeybees were as frequent as other bee species (Fig. 3a, Table 2). A χ^2 contingency test revealed that the frequency of visits in Large and small patches by the honeybees, other hymenopterans and dipterans differed significantly ($\chi^2 = 24.0$, $P < 0.001$; Fig. 3a).

Insect visitation rates (V/H) were ~2-fold higher in small patches compared to the Large patches (Fig. 3b), although the difference was not significant ($F_{1,126} = 1.22$, $P = 0.27$). This lack of significance was caused by a high variability in visitation rates among patches, particularly among the small patches (see Fig. 3b), since a one-way ANOVA revealed highly significant differences among them ($F_{5,126} = 6.95$, $P < 0.001$); the small patches CRB and GBL having significantly the highest rates (Tukey's test: $P < 0.05$).

We searched for honeybee hives and found them within or in the immediate vicinity (<100 m) of the Large patches DBL and CHP and the small patches GBL and CRB.

3.3. Pollen limitation

The addition of outcross pollen significantly increased the fruit set in the experimental plants (all populations pooled) from 57% to

Table 2
Insects visiting myrtle (*Myrtus communis*) flowers in the six studied populations during June–July 2007. V/H is visits/hour. % = Percentage of visits (relative to the total number of visits) attributable to a given taxa for each population. * = Observed outside the census time. The number of taxa is also shown for each population with taxa observed visiting myrtle flowers out of census time in parentheses. *n* = number of 5-min observation periods.

Floral visitors	Large patches						Small patches					
	DBL <i>n</i> = 24		CHP <i>n</i> = 24		DHY <i>n</i> = 20		GBL <i>n</i> = 16		CRB <i>n</i> = 28		PTR <i>n</i> = 20	
	V/H	%	V/H	%	V/H	%	V/H	%	V/H	%	V/H	%
Hymenoptera	22.5	73.8	34.5	84.1	23.4	84.8	59.3	92.9	74.6	86.1	24.0	90.0
<i>Apis mellifera</i> (Apidae)	10.5	34.4	7.5	18.3	4.8	17.4	57.0	89.4	73.7	85.1	12.0	45.5
<i>Anthidium</i> sp. (Megachilidae)							*	*			1.2	4.5
<i>Amegilla quadrifasciata</i> (Anthophoridae)	2.0	6.6	3.5	8.5	1.2	4.3	*	*			1.8	6.8
<i>Bombus terrestris</i> (Apidae)			*	*	*	*					3.0	11.4
<i>Ceratina cucurbitina</i> (Anthophoridae)	4.5	14.8	7.5	18.3	3.6	13.0	1.5	2.4				
<i>Colletes fodiens</i> (Colletidae)			0.5	1.2	1.8	6.5						
<i>Halictus</i> sp. (Halictidae)												
<i>Lasioglossum</i> sp. (Halictidae)	3.0	9.8	1.5	3.7								
<i>Megachile ligniseca</i> (Megachilidae)					6.0	21.7					2.4	9.1
<i>Megachile pilidens</i> (Megachilidae)	2.0	6.6	7.5	18.3	4.8	17.4						
<i>Nomioides</i> sp. (Halictidae)	0.5	1.6			1.2	4.3						
<i>Pseudapis bispinosa</i> (Halictidae)			1.5	3.7			0.8	1.2			3.6	13.6
<i>Xylocopa violacea</i> (Anthophoridae)			*	*	*	*						
Hymenoptera indet.			1.0	2.4					0.9	1.0		
Diptera	8.0	26.2	6.5	15.9	4.2	15.2	4.5	7.1	12.0	13.9	2.4	10.0
<i>Anthrax</i> spp. (Bombyliidae)	3.0	9.8	3.0	7.3	*	*	1.5	2.4				
<i>Eristalinus taeniops</i> (Syrphidae)			0.5	1.2	*	*	1.5	2.4				
<i>Eristalis tenax</i> (Syrphidae)							0.8	1.2	*	*		
<i>Paragus</i> sp. (Syrphidae)											0.6	2.3
<i>Sphaerophoria scripta</i> (Syrphidae)	1.0	3.3	0.5	1.2			0.8	1.2				
<i>Stomorhina lunata</i> (Calliphoridae)									7.7	8.9		
<i>Syritta pipiens</i> (Syrphidae)	0.5	1.6										
<i>Villa</i> spp. (Bombyliidae)	3.5	11.5	2.5	6.1	1.2	4.3			4.3	5.0	1.2	4.5
<i>Xanthogramma marginale</i> (Syrphidae)					2.4	8.7					0.6	2.3
Diptera indet.					0.6	2.2						
Total	30.5		41.0		27.6		63.5		86.6		26.4	
Number of hymenopterans species	6		8 (2)		7 (2)		3 (2)		2		6	
Number of dipterans species	4		4		3 (2)		4		2 (1)		3	
Total number of species	10		12 (2)		10 (4)		7 (2)		4 (1)		9	

72% and the brood size from 3.0 to 4.2 seeds per fruit (Wilcoxon paired tests: $Z \geq 4.08$, $P < 0.001$, $n = 39$). In Large patches, mean fruit set (\pm SD) resulting from natural pollination (Control treatment) was $53 \pm 19\%$ ($n = 21$), and $58 \pm 19\%$ ($n = 18$) in the small patches. PL index values were higher in the Large patches for fruit set, and consequently for cumulative seed production, however, PL values for brood size were almost equal in both types of patches (Fig. 4). Although the differences between Large and small patches were not significant ($F_{1,33} < 2.18$, $P > 0.1$), Large patches were significantly pollen limited at all levels, whereas small patches were not for fruit set (see Fig. 4).

Insect visitation rates were negatively associated with pollen limitation for fruit set ($r = -0.805$, $P = 0.05$; Fig. 5a) and cumulative seed production ($r = -0.792$, $P = 0.06$; Fig. 5b); the small patches GBL and CRB, which showed the highest insect visitation rates showed the lowest PL index values. Pollen limitation for brood size, however, was not associated with insect visitation rates ($r = -0.468$, $P = 0.35$).

3.4. Seed production

Neither patch size, year nor their interaction (Patch size \times Year) showed any significant effect in brood size ($F_{1,137} < 1.78$, $P > 0.18$; Fig. 6a). Seed mass did not differ among Large and small patches ($F_{1,137} = 0.00$, $P = 0.98$), although it differed between years ($F_{1,137} = 11.60$, $P < 0.001$); seeds being heavier in 2006 compared with 2007 (Fig. 6b). However, the interaction Patch size \times Year was not significant ($F_{1,137} = 0.08$, $P = 0.78$), indicating that between-year differences for seed mass occurred in both Large and small populations (Fig. 6b). Such similarity in seed production

vastly contrasts with the high variability found in brood size and seed mass among individual plants within populations in both years (variance partitioning: 51–76% explained).

4. Discussion

4.1. Breeding system, pollen limitation and pollinator assemblages

The breeding system has been documented as the most important trait in determining plant fecundity susceptibility to habitat fragmentation (Aguilar et al., 2006). Our results consistently indicated that myrtle has a self-compatible breeding system that needs (1) pollinator service to increase the fruit set and (2) cross-pollination to increase the brood size. In other words, fruit set in myrtle is favoured by the quantity of pollen arrival, whereas brood size is favoured by the quality of such pollen (self- or cross-pollination; see Aizen and Harder, 2007). Considering these two facts in the myrtle breeding system is essential for understanding the effects of fragmentation and pollinator assemblage on pollen limitation and seed production.

Pollination limitation may occur as a consequence of the disruption in the pollination environment (Wilcock and Neiland, 2002; Knight et al., 2005). This pollination environment can be dissected in (1) the plant population side, including population size and density and kinship relationships (e.g., Heschel and Paige, 1995; Cunningham, 2000; Duncan et al., 2004), and in (2) the pollinator environment, including abundance, efficiency and diversity of pollinators (e.g., Aizen and Feinsinger, 1994b; Steffan-Dewenter and Tschardt, 1999; Donaldson et al., 2002). In the studied

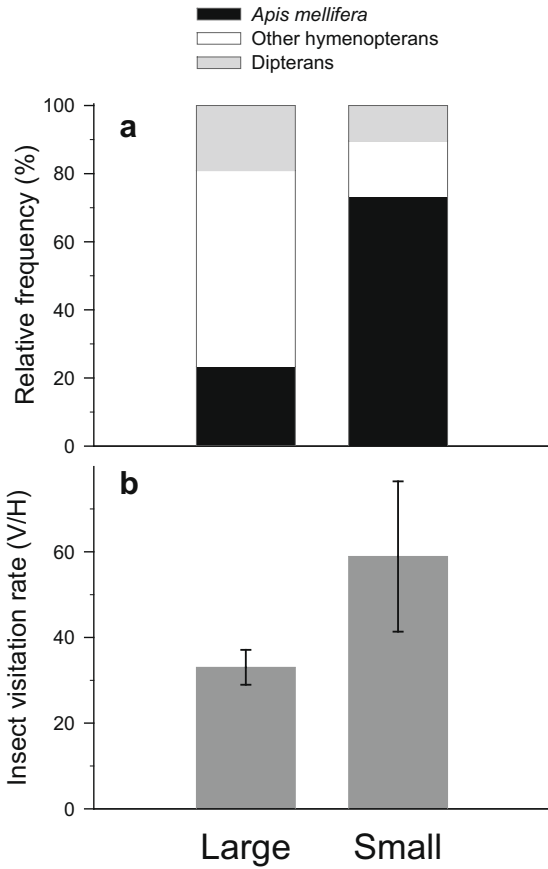


Fig. 3. Differences between Large and small patches in (a) relative frequency of visits to myrtle (*Myrtus communis*) flowers of honeybees (*Apis mellifera*), other bees species (other hymenopterans) and flies (dipterans) and (b) mean (\pm SE) insect visitation rates (visits/hour) to myrtle shrubs.

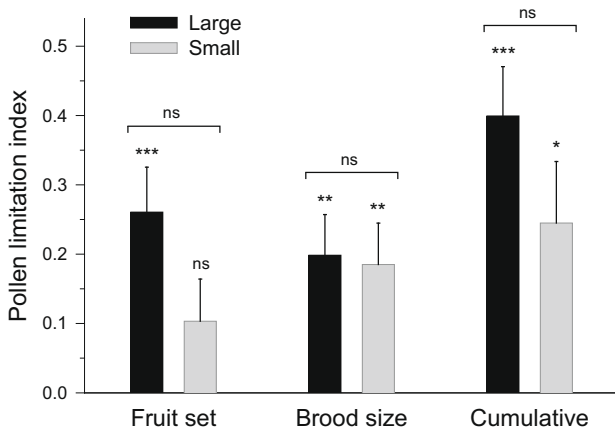


Fig. 4. Pollen limitation index values (\pm SE) in Large and Small myrtle (*Myrtus communis*) populations at the fruit set and brood size level, and cumulative pollen limitation throughout both stages. Significant differences from zero (*t*-tests) are shown above the bars. Differences between Large and small patches are shown above horizontal square brackets. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns, no significant differences.

myrtle populations, pollen limitation seems to depend greatly upon the local pollinator environment and less on fragmentation. Contrary to predictions, Large populations were in general more pollen limited than small populations. Such differences in fecundity were correlated with pollinator visitation rates in each popu-

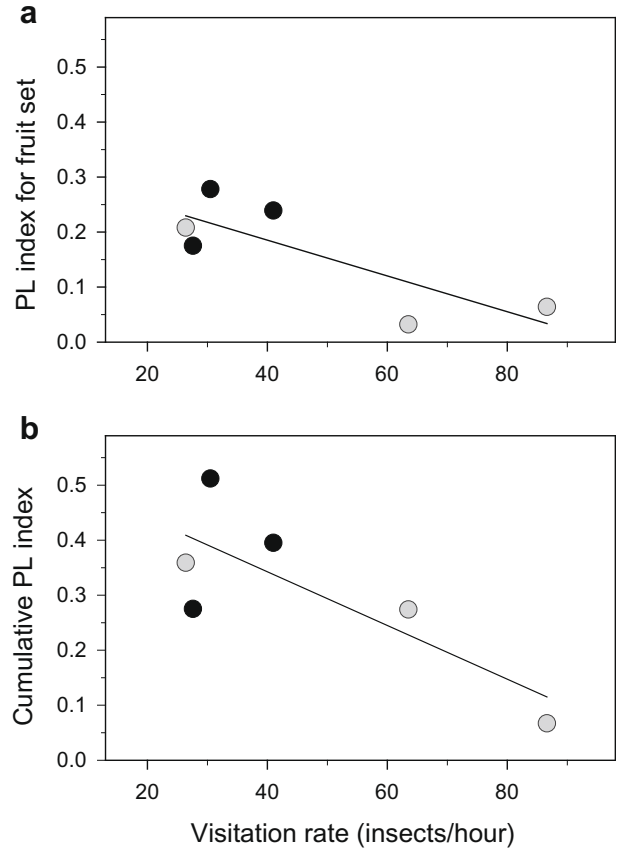


Fig. 5. Relationships between the pollen limitation index for (a) fruit set and (b) cumulative seed production, and the insect visitation rates to myrtle (*Myrtus communis*) shrubs. Black circles represent Large patches; grey circles small patches. For descriptive purposes, least squares regression lines are shown.

lation: the higher the rate, the lower the pollen limitation (Fig. 5). However, the higher visitation rates received by two out of three small populations were not associated with higher levels of cross-pollination, as indicated by the similar pollen limitation (PL) index values for brood size among types of populations (Fig. 4). Thus, small populations received a larger quantity of pollen than Large populations, but pollen quality seemed to be similar in both types, at least in terms of seed production.

The major differences in visitation rates were caused by honeybees. In small populations, where myrtle flowers were much visited by honeybees, the species richness in the pollinator fauna decreased, as previously reported in other regions (Aizen and Feinsinger, 1994b). This reduction was especially severe for wild bees, probably because of stronger competition for floral resources between insects of similar behaviour and requirements (Goulson, 2003), and the different tolerances of wild and honeybees to anthropogenic disturbances (see Steffan-Dewenter et al., 2002; Winfree et al., in press). The honeybee is not an exotic species in the Mediterranean region, but it occurs at very elevated densities because of the presence of domestic hives (Goulson, 2003). Although honeybee hives were present in two Large and two small patches, their impact on the pollinator assemblage was only evident in the latter. Honeybees might intensively have used all the floral resources (mostly myrtle) in small patches, whereas the higher flower availability in Large patches probably diluted their effect. This was supported either by the high similarity in pollinator assemblage found between the three Large patches or by the lower honeybee abundance found in the Small patch PTR, which lacked hives in its surroundings (Table 2). This suggests that, in our region, the pollinator assemblage may be severely altered

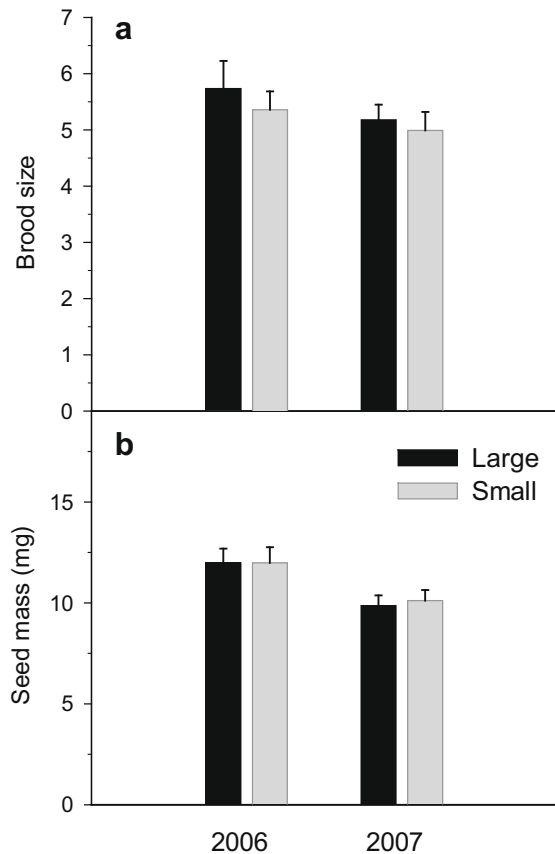


Fig. 6. Estimates of mean (a) brood size and (b) seed mass in myrtle (*Myrtus communis*) populations occurring in Large and small patches in the 2006 and 2007 fruiting seasons. Vertical bars denote standard errors.

when habitat fragmentation occurs in combination with beekeeping. The study area is an important source of honey, pollen and beeswax production within the region (Andalusia) and the country (Spain), which ranks first among European producers. For example, almost half a million hives were legally settled in Andalusia in 2005, and about half of them were located in the Guadalquivir River Valley (Anon., 2005).

Pollination of native plants by honeybees has been generally reported to be effective in terms of female plant fecundity across different regions (reviewed by Goulson, 2003). Honeybees have even reported to 'rescue' pollination and raise plant fecundity in fragmented systems (Dick, 2001), but they have also proved to be poor pollinators even when they were locally more abundant than native pollinator fauna (Aizen and Feinsinger, 1994a, 1994b). In the studied patches, the higher visitation rates by domestic honeybees reduced pollen limitation in myrtle; thus, they 'rescue' seed production in the species as effective pollinators. However, our results also show that honeybees can threaten the presence and abundance of wild bee species in small patches; thus, the pollination of more specialized plant species that rely on wild bees for pollination might be affected (see Ashworth et al., 2004). The fact of most wild bees observed usually visit many flower species (i.e. non-oligolectic; F.J. Ortiz, personal communication) does not necessarily imply that pollination of these flowers also rely in many bee species (Ashworth et al., 2004).

4.2. Seed production patterns

Despite the contrasting levels of fragmentation and population size, and the differences found in pollinator visitation rates, seed

production variables considered (brood size and seed mass) were similar in Large and small patches in both study years. These results are supported by the PL index values found for brood size, which were also almost equal between Large and small patches. However, fruit set was lower in Large patches, thus, total seed production per individual (reproductive output) may be lower in the larger fragments.

As shown, brood size in myrtle is limited by cross-pollination. Yates et al. (2007) suggested that the smaller brood size produced by the shrub *Calothammus quadrifidus* (Myrtaceae) in the smallest populations was a consequence of inbreeding depression, since pollinator visits were similar across patches. Also, Heschel and Paige (1995) found a lower seed mass in the smallest populations of the perennial herb *Ipomopsis aggregata* (Polemoniaceae) as a consequence of inbreeding depression. In myrtle, Albaladejo et al. (2009) found a positive correlation between genetic diversity and population size in 14 populations in the same study area. It could be argued that individuals in Small populations are more susceptible to selfing and outcrossing with relatives, and therefore suffer a lower seed production because of inbreeding depression, either in seed number or mass. However, brood size and seed mass were similar in Large and small populations. Probably, the mixed-mating system of myrtle, which even within Large patches shows a low frequency of outcrossing (mean $t_m = 0.35$; González-Varo et al., 2009), makes difficult detecting effects of inbreeding depression in seed production (Husband and Schemske, 1996). These effects could be expressed in comparatively less vigorous progenies in small than in Large patches, but this possibility remains unexplored.

4.3. Conclusions and implications for management

Our study shows that (1) Large patches supported a greater flower visitor diversity, but small patches tended to have higher insect visitation rates, which were performed almost exclusively by honeybees to the detriment of wild bee species; (2) pollen limitation was lower in small patches for fruit set and cumulative seed production; (3) pollen limitation was lower at the highest visitation rates; and (4), brood size and seed mass were rather similar between Large and small populations. Taken together, our findings indicate that self-compatible breeding system and generalist pollination are key traits in Mediterranean myrtle, making this species less vulnerable to pollination failure under the circumstances of severe fragmentation.

Besides the effects of habitat fragmentation due to size and isolation of fragments, the direct impact of management of forest patches and the surrounding matrix may play a stronger role. In our study, this was reflected by the effects of beekeeping on the pollinator assemblage and pollen limitation of our study species. The implication for management is that the diversity of the pollinator assemblage in small habitat patches can be reduced and monopolized by domestic bees. Although honeybees improved sexual reproduction on our studied species, fecundity was also sustained where hives were not found. This may be different for other plant species in the community, thus, it would be convenient to implement similar studies for plant species relying on more specialized pollination and/or with self-incompatibility, just to ascertain the direct role of beekeeping and decreasing natural pollinators. Moreover, there may be some more subtle effects of honeybee pollination on the genetic structure of progenies and hence on the vigour of future generations. Ongoing research will determine these effects. Finally, changes in pollinator array composition open new challenges that go beyond the effects on particular species, as complex interaction networks may be affected.

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