

## Breeding system and spatial variation in the pollination biology of the heterocarpic *Thymelaea velutina* (Thymelaeaceae)

M. C. de la Bandera<sup>1,2</sup> and A. Traveset<sup>1</sup>

<sup>1</sup>Institut Mediterrani d' Estudis Avançats (CSIC-UIB), Esporles, Mallorca, Balearic Islands, Spain

<sup>2</sup>Fundació Jardí Botànic de Sóller, Ctra Palma–Port de Sóller, Sóller, Mallorca, Balearic Islands, Spain

Received September 10, 2004; accepted September 8, 2005

Published online: December 14, 2005

© Springer-Verlag 2005

**Abstract.** *Thymelaea velutina* (Thymelaeaceae) is a unique dioecious and heterocarpic shrub, each female producing both dry and fleshy fruits. It is endemic to the Balearic Islands (western Mediterranean) and has suffered an important regression in recent decades, especially due to habitat loss. It lives in coastal areas, mainly in dunes, but a few populations occur up to 1300 m in the mountains of Mallorca. In the present study, we examined its breeding system and pollen vectors, determined whether seed production is pollen-limited, and quantified the relative importance of different insect visitors at coastal and mountain sites. Selective exclusions showed no differences between populations in the relative importance of insects and wind as pollen vectors. Pollen was limiting at both populations, though not consistently in time. Flowers of *T. velutina* were visited mostly by generalist insects, and species composition and abundance of flower visitors varied both in space and time. The flowering period is about a month later in the mountain than in the dune, and this results in a higher frequency of insect visits to plants in the mountain due to the higher insect abundance; insects were also more diverse in the mountain, although they visited a proportionally lower number of flowers than in the dune. Despite this spatio-temporal variation in the frequency of insect visits and in the importance of a particular insect group, the ambophilous system in this

species (previously described as anemophilous) ensures a relatively high fruit set at the two different sites.

**Key words:** Ambophily, Balearic Islands, breeding system, Mediterranean vegetation, insect and wind effectiveness, generalized pollination, *Thymelaea velutina*.

Although anemophily and entomophily have traditionally been considered separately (Faegri and van der Pijl 1979), the phenomenon of ambophily – a combination of the two – has been documented rather frequently (Meeuse 1978, Stelleman 1978, Sacchi and Price 1988, Meeuse et al. 1990, Vroege and Stelleman 1990, Karrenberg et al. 2002). Insects may play an important role in the reproduction of species with typically anemophilous flowers, i.e., unisexual, small in size, rather inconspicuous, and with a low reward for pollinators (e.g. Gullías et al. 2004). Likewise, typically entomophilous flowers have proven to be also pollinated by wind (Dafni and Dukas 1986, Scariot et al. 1991, Bullock 1994). Ambophily has indeed shown to be more common than previously thought, although it is still controversial whether it is

a stable system or is a transient stage towards absolute anemophily or entomophily (Culley et al. 2002). For the genus *Salix*, for instance, Karrenberg et al. (2002) have recently argued that ambophily is the ancestral stage. The importance of either pollinator vector may vary spatially, and even temporally, and thus both systems may be maintained through time without any strong selection against either one (Vroege and Stelleman 1990, Gómez et al. 1996, Tamura and Kudo 2000, Totland and Sottocornola 2001). For a large number of species, wind pollination may well compensate a low frequency of insect visits, due for instance to an early flowering period in a particular population or to a low insect abundance in a given year (Lázaro and Traveset, in press).

We wanted to examine this in *Thymelaea velutina* (Pourr. ex Cambess.) Endl., a species that has been considered until now to be exclusively anemophilous (Tébar and Llorens 1992). Its inconspicuous flowers and the fact that it lives in habitats in which wind is important, has probably suggested this characterization, although some preliminary insect observations on the flowers of the two sexes and small amounts of nectar at the base of the flowers indicated us that entomophily could also aid in its reproduction. This species has the particularity of occurring only in two types of habitats, at sea level in dune systems and in open shrublands at high altitudes in mountain areas, providing an ideal system to examine the spatial variation in the importance of both types of pollination. Another particularity of this species is its special heterocarpy, the same female producing fleshy and dry fruits simultaneously. To our knowledge, this is the only species in the world presenting this phenomenon. The factors that contribute to the maintenance of heterocarpy in *T. velutina* are dealt with in another paper (De la Bandera and Traveset, in press). The specific goals of the present study were: (1) to determine the reproductive system of *T. velutina*, quantifying the relative importance of insects and wind as pollen vectors, (2) to assess whether seed production is pollen-limited, and

(3) to evaluate the relative importance of different insect pollinator taxa. We compared two populations, one in one of the two dune systems where the plant still survives (in a preserved area at northern Mallorca, Balearic Islands) and the other at a mountain site, in the highest peak (1450 m a.s.l.) of the mountain chain that extends from northwestern to southwestern Mallorca. These localities represent the two extremes of the altitudinal gradient where *T. velutina* lives.

## Material and methods

**Study species.** *Thymelaea velutina* is a perennial shrub endemic to the Gymnesic Islands (Balearic Islands), specifically Mallorca and Menorca (Alomar et al. 1997). It is mostly dioecious, although a few individuals may bear the two sexes simultaneously (pers. obs.). It can reach up to 1.2 m height, mostly in dunes. Inflorescences are axillary capitula bearing three to five flowers. Male flowers are yellow, bear eight orange stamens in two series, present a rudimentary ovary, and are similar in length in the two populations ( $2.8 \pm 0.03$  mm,  $N=45$  in the dune and  $2.9 \pm 0.04$  mm,  $N=50$  in the mountain; Mann Whitney U-test:  $U=885.0$ ,  $P>0.05$ ); however, they are somewhat wider in the dune ( $2.0 \pm 0.06$  mm,  $N=45$  vs  $1.8 \pm 0.04$  mm,  $N=50$ ;  $U=1459.0$ ,  $P=0.01$ ). Female flowers are greenish, have only one ovule, and they have a very similar size in both localities ( $2.0 \pm 0.03$  mm long and  $1.4 \pm 0.04$  mm wide,  $N=50$ , in the dune, and  $2.0 \pm 0.03$  mm long  $1.5 \pm 0.03$  mm wide,  $N=50$ , in the mountain;  $U=1212.5$  and  $U=1172.5$ , respectively,  $P>0.05$ ) (Fig. 1). Both flower sexes produce small amounts of nectar (pers. obs.). Flowering synchrony between males and females is almost absolute, although in some years the former may begin flowering several days earlier. The lifetime of a flower is three to four days. A detailed description of the species can be found in Pedrol (1997). Most of its populations in coastal habitats have disappeared due to human disturbance, mainly urbanizations (Alomar et al. 1997).

**Study sites.** The dune population extends throughout the northern coast of Mallorca; we chose a locality near Ca'n Picafort (UTM: 31S EE 01), within S'Albufera Natural Park. *Thymelaea velutina* is rather abundant here (several hundred



**Fig. 1.** Details of a female and a male flower of *Thymelaea velutina*. L and W are the parameters that were measured and that correspond to flower length and width, respectively

individuals) and found towards the interior, on already fixed dunes, along with species from Ericaceae, Cistaceae, Lamiaceae and Poaceae. Mean annual precipitation is 628 mm, and mean annual temperature is 17°C (data for 20 years; Guijarro 1986). Specifically, in 2000 and 2001, annual precipitation at this site was 419.3 and 530.0 mm, respectively, while mean annual temperature was 16.95°C and 17°C, respectively (data from the National Meteorological Institute). The flowering period extends from late February to early May, with a peak in late March. Sex ratio is 1 ♀ : 1.5 ♂ (N=429 plants).

The mountain population is located at Sa Coma de N'Arbona (UTM: 31S DE 08), at 1250 m, in the Puig Major mountain. This site is within a military area and, fortunately, it is also preserved from human disturbances. The main vegetation here consists of *Ampelodesmos mauritanica* (Poiret) T. Durand and Schinz, *Hypericum balearicum* L. *Teucrium marum* L. and *Rosmarinus officinalis* L., and the population of *T. velutina* consists of less than 150 individuals. Mean annual precipitation here is 1121 mm, and mean annual temperature is 9°C (data for 20 years; Guijarro 1986). During 2000 and 2001, annual precipitation was 851.4 and 1808.3 mm, respectively, while mean annual temperature was 12.65°C for the two years. The flowering period at this site is shorter than in the dune, and does not overlap with that one, the peak being in late May-early June. Sex ratio in this

population is 1 ♀ : 1.6 ♂ (N=120 plants). At this site we found one female bearing male flowers.

**Pollination experiments.** In each population we marked ten randomly chosen female individuals from which we selected nine branches with similar phenology and number of flowering buds. Branches were randomly assigned to each of three treatments: (1) hand-pollination, (2) wind-pollination (anemogamy), and (3) open pollination (control). We thus used three branches per treatment per female. An average of c. 50 flowers was used per individual and treatment. For the hand-pollination treatment, recently opened flowers were pollinated once - by means of forceps, due to the small flower size - with a mix of pollen gathered from a minimum of five males, located at least 10 m away from the target female. These flowers were bagged with museline to avoid subsequent insect visitation and possible pollen removal. The second group was bagged with cloth bags, with a mesh of 1 mm, which allowed the passage of pollen but not insects through them. Such bags, placed before anthesis, possibly lead to underestimate wind pollination, as air currents may restrain the amount of pollen deposited on the stigmas; however, our objective with this treatment was to merely assess whether wind accounts for pollination in the absence of insects. The control group of flowers were marked and left until the time of fruit collection. Flowers of all treatments were monitored every three to four days, recording the number of buds, open, and

withered flowers in each branch. The pollination experiments were performed between March 4 and April 30, 2000, and between February 6 and April 22, 2001 at the dune locality, whereas at the mountain site they were carried out between May 6 and June 19, 2000 and between May 8 and June 13, 2001.

In order to avoid losing fruits that can be removed by ants or dropped by the plant, once the flowering period was over, we bagged all marked branches with a gauze that allowed the passage of light and air but not fruits. During the whole fruiting period, we monitored plants weekly, recording in each visit the number of fruits of each type, fleshy or dry, on each branch.

The female plants used in the pollination experiments were the same both years of the study, except for three individuals at the dune site that had to be replaced in 2001 because they died during the summer of 2000. For each individual, we measured its size (height, maximum crown diameter and trunk diameter), its standing flower crop (estimated as the maximum number of simultaneously open flowers), and the distance to the nearest reproductive male, in order to assess a possible association between fruit set and any of these parameters. In 2000, we could only include five individuals from the dune in the fruit set analysis, as storms and vandalism caused the loss of some treatments in the rest of individuals.

**Pollinator censuses.** To determine whether pollination composition, frequency of insect visits to plants and flower visitation rates differed between populations, we performed censuses of pollinators to individual plants during the flowering peak in the springs of 2002 and 2003. Some preliminary data were gathered in 2001. During four to six days at each population and each year, we carried out censuses of 10 min on randomly chosen plants, alternating sexes. At the dune, censuses were performed on 20 ♀ and 25 ♂ in 2002 and 21 ♀ and 22 ♂ in 2003. In the mountain site, we used 9 ♀ and 10 ♂ in 2002 and 7 ♀ and 9 ♂ in 2003. Such censuses were conducted at the time of highest insect activity: between 10:00 h and 17:00 h. In 2003, pollinator censuses were expanded until 20:00 in order to detect any crepuscular flower visitor. In each census we recorded (a) insect identity (insect order, except for ants, for which we recorded the family Formicidae), (b) the duration of each visit

to a plant, and (3) the total number of flowers visited per plant. A visit was considered to be an approach to a plant by an insect contacting at least one flower, and thus for each plant we obtained the total number of visits per unit time ( $n^\circ$  visits / min; FIV hereafter). On the other hand, we estimated flower visitation rate (flower / min; FVR hereafter) as the total number of flowers contacted by all insect individuals per unit time on a single plant. Both years, all observations were done on sunny, not windy days during March and April at the dune, and during June at the mountain. Pooling both years, a total of 1930 min of observations at the dune and 3240 min at the mountain were carried out. A lower number of censuses were performed at the dune locality in 2002 due to the frequent rains and strong winds during the flowering period. We wanted to test the possible association of flower visitation rates by insects with plant traits such as size and flower crop. For this, we measured dimensions and standing flower crop in ten males and ten females from each population. A number of specimens from each insect species were captured for identification.

We further determined the capacity of different pollinators to transport pollen of *T. velutina*. For this purpose, we captured several individuals of each most frequent flower visitor on male plants and counted the pollen load on their bodies by means of a particle counter (model Coulter Z2). Each specimen was introduced into a glass vial that contained 2 ml of an isotonic solution (ISOTON). Pollen grains were counted from a 25 ml isotonic solution, and five measurements were made for each individual insect.

**Statistical analysis.** We used a General Linear model (GLM) to test for differences between populations and treatments in fruit set for each year. Data from 2000 and 2001 were tested separately because not all individuals from one population were the same the two years of the study. A single value of fruit set was obtained for each treatment and individual by adding the number of flowers and fruits in the three marked branches. This variable was normalized by means of the angular transformation. The model included population as a fixed factor, treatment as a within-subject (i.e. repeated measures) fixed factor and plant individual as a random factor (subject). The Tukey's test was employed as the *a*

*posteriori* test to compare differences among treatments.

To determine inter-annual differences in fruit set within populations, we carried out two more GLM analyses, one for each population. Year and treatment were included as fixed factors and individual was a random factor in both cases. For the mountain population, individual was a repeated subject, as the same ten females were studied both years. By contrast, most individuals in the dune could not be compared the two years (they were either lost due to vandalism or they naturally died), and in that case individual was not included as a repeated subject in the model.

Following Tamura and Kudo (2000), we defined an index of efficiency of wind pollination as  $N/P$ , where  $N$  and  $P$  are the fruit (seed) set of the anemogamy and hand-pollination treatment, respectively, and another index of efficiency of insect pollination as  $(C - N) / P$ , where  $C$  is the fruit (seed) set of the control flowers. Differences in such indexes between populations and years were tested with Mann-Whitney's  $U$  tests.

Differences in plant dimensions (plant height, crown and trunk diameter) and in flower crop between populations and sexes were tested by means of two-way ANOVAs. Trunk diameter and flower crop were transformed with logarithms and squared-roots, respectively. A Pearson correlation was calculated to determine if plant size and flower crop were associated with fruit set (normalized with the angular transformation).

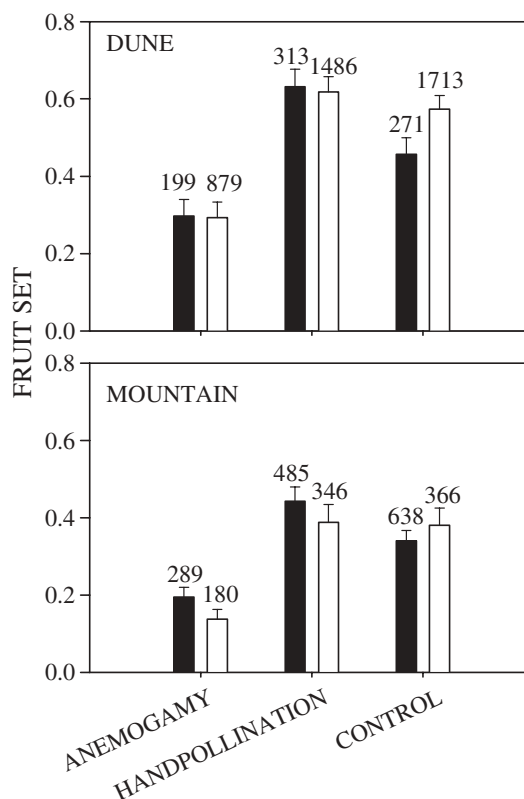
The frequency of insect visits (FIV) and flower visitation rate (FVR) were compared between populations and sexes with an analysis of covariance, using the standing flower crop of each individual as a covariate for each year separately. This analysis was performed after testing for homogeneity of slopes. The dependent variables were transformed with the logarithmic transformation to improve normality. The duration of insect visits, also normalized with the logarithmic transformation, was analyzed by means of ANOVAs followed by Tukey's tests.

Differences in the frequency of each group of insects between populations and sexes were tested using a Chi-square's test. Duration of an insect visit to a plant was compared among insect groups with a one-way ANOVA, whereas both the number of flowers visited by each group and pollen loads were compared with the Kruskal Wallis test, as data could not be normalized with any transformation. A

Pearson correlation was calculated to determine if pollen load was associated with frequency of visits. Analyses were performed with STATISTICA (v. 6.0).

## Results

**Relative importance of insects and wind as pollen vectors of *T. velutina*.** Both years of study, the proportion of flowers that set fruit was significantly higher in the dune than in the mountain ( $F_{1,13}=9.02$ ,  $P=0.01$ , and  $F_{1,18}=9.51$   $P < 0.01$ , for 2000 and 2001, respectively; Fig. 2). Likewise, differences in fruit set were found among treatments ( $F_{2,26}=37.15$  and  $F_{2,36}=28.07$ ,  $P < 0.001$ , for 2000 and 2001, respectively). Wind-pollination consistently produced a lower fruit set



**Fig. 2.** Proportion of flowers that set fruit in each treatment in the two study populations of *Thymelaea velutina*. Data are from 2000 (black bars) and 2001 (white bars). Bars of one standard error are shown. Numbers above bars represent sample sizes (no. of flowers)

**Table 1.** Mean and standard error of plant size and fecundity variables for male and female individuals of *Thymelaea velutina* in the two populations. Numbers in parentheses indicate sample sizes. \*\*\*  $P < 0.001$ . For the first three variables, data of the two sexes have been pooled as they were not significantly different (see text for details). Data on flower crop is from 2001

Variable	Sex	Dune	Mountain	F-ratio
Height (cm)	♂	57.9 ± 3.6 (12)	25.5 ± 1.4 (28)	$F_{1,85} = 213.9$ ***
	♀	59.2 ± 2.6 (27)	23.8 ± 1.8 (20)	
Crown diameter (cm)	♂	110.8 ± 11.9 (12)	67.6 ± 4.5 (28)	$F_{1,85} = 19.0$ ***
	♀	91.3 ± 6.1 (27)	63.9 ± 5.5 (20)	
Trunk diameter (cm)	♂	1.8 ± 0.2 (12)	1.4 ± 0.1 (28)	$F_{1,85} = 16.0$ ***
	♀	1.8 ± 0.1 (27)	1.3 ± 0.2 (20)	
Flower crop	♂	6947.7 ± 1040.8 (18)	4787.9 ± 1040.1 (14)	$F_{1,30} = 1.8$ ns
	♀	11651.4 ± 2873.6 (16)	3733.0 ± 558.1 (14)	$F_{1,28} = 8.0$ ***

than either open- or hand-pollination. In 2000, pollen was limiting, as fruit set increased significantly when it was added to stigmas (Fig. 2). Such increase in fruit set may drain the resources of the plant and result in reduced flowering or fruiting in subsequent years; however, we found neither such reduction in fecundity ( $r=0.483$  and  $r=-0.524$  for the dune and the mountain population, respectively,  $P>0.05$ ) nor pollen limitation in 2001 (Tukey's test; Fig. 2). The effect of treatment was similar in both populations, as there was no interaction between the two factors ( $F_{2,26}=1.86$  and  $F_{2,36}=0.53$ ,  $P>0.05$ , for 2000 and 2001, respectively).

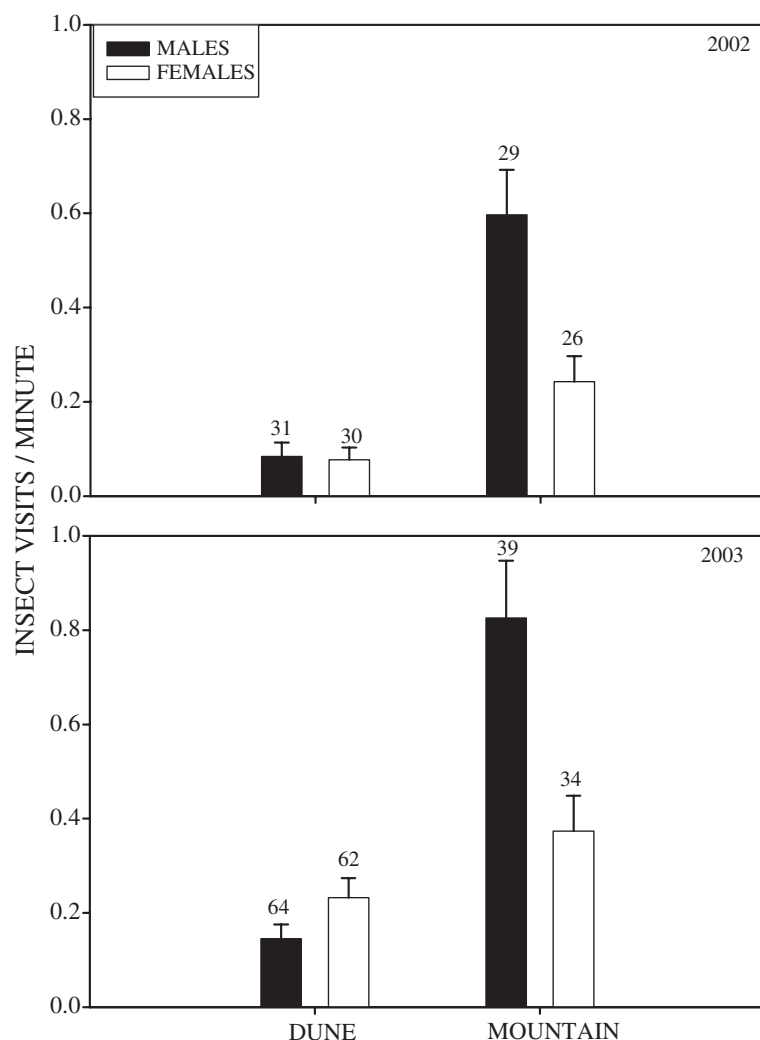
Despite fruit set was consistently greater at the dune than at the mountain, the indices of efficiency of wind and insect pollination were similar at either population, and this was consistent both study years. The values of such indices were  $0.412 \pm 0.059$  and  $0.391 \pm 0.116$ , respectively, for the dune population and  $0.416 \pm 0.044$  and  $0.447 \pm 0.090$ , respectively, for the mountain population (data of both years pooled) (all  $P>0.05$ ).

When performing separate analysis for each population, fruit set was similar between years, both in the dune and the mountain population ( $F_{1,10}=2.44$  and  $F_{1,9}=1.79$ ,  $P>0.05$ , respectively). The effect of treatment was again highly significant in both populations ( $F_{2,20} = 16.01$  and  $F_{2,18} = 41.83$ ,  $P < 0.001$ , respectively), and

there was no interaction between year and treatment ( $F_{2,20}=0.28$  and  $F_{2,18}=0.20$ ,  $P>0.05$ , respectively), although fruit set from hand-pollinations was more similar to fruit set from open pollination in 2001 than in 2000 (Fig. 2).

Shrubs in the dune were significantly larger and females produced a greater number of flowers than females in the mountain (Table 1). However, fruit set was not associated to any of the variables describing either plant size or flower crop in either population. We also found no evidence that females closer to a reproductive male had a greater fruit set (all  $P>0.05$ ; the two populations pooled). No significant differences between sexes were observed in plant height, crown diameter, trunk diameter or flower crops within each locality (all  $P>0.05$ ).

**Spatial variation in the pollinator assemblage.** The analysis of covariance, using flower crop as covariate, showed that FIV differed significantly between populations, being greater at the mountain site than at the dune ( $F_{1,292} = 123.38$ ,  $P < 0.001$ ; Fig. 3). Differences between sexes were not statistically significant ( $F_{1,292} = 0.68$ ,  $P=0.41$ ), although they were greater at the mountain than at the dune ( $F_{1,292} = 21.57$ ,  $P < 0.001$ ). Neither the effect of year ( $F_{1,292} = 0.05$ ,  $P=0.82$ ) nor the interactions between this factor and the other two were significant (all  $P>0.05$ ). Preliminary data gathered during the spring of 2001 also showed



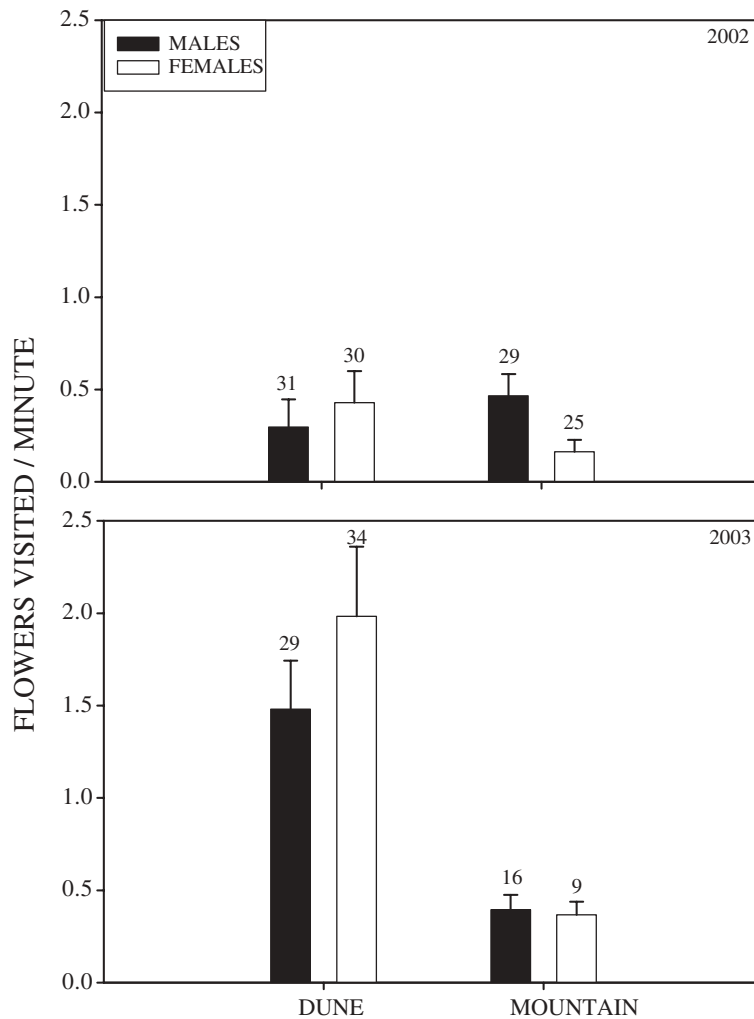
**Fig. 3.** Insect visitation rate (FIV) to male (black bars) and female (white bars) individuals of *Thymelaea velutina* for each population and study year. Bars of one standard error are shown. Numbers above bars represent sample sizes (insect censuses)

a greater FIV in the mountain ( $5 \pm 1.5$  (SE),  $N = 12$ ) than in the dune ( $0.3 \pm 0.2$ ,  $N = 30$ ) and a higher FIV to males than to females, especially in the mountain population ( $7 \pm 2.5$ ,  $N = 6$  in males vs.  $2 \pm 1.3$ ,  $N = 6$  in females).

Contrary to what was expected given the differences in FIV between populations, FVR did not differ significantly either between populations or sexes after controlling for standing flower crop (all  $P > 0.05$ ; Fig. 4).

Among the assemblage of pollinators of *T. velutina*, we found insects belonging to the

orders Hymenoptera, Diptera, Coleoptera and Lepidoptera (Table 2). The first three groups feed mostly upon pollen whereas butterflies search for nectar. Although not all insect pollinators could be identified to the species level, a minimum of nine taxa in the dune population and a minimum of 13 taxa in the mountain site were detected. The relative frequency of visits of the various groups differed significantly between populations both study years (2002:  $\chi_4^2 = 32.17$ ,  $P < 0.001$ ; 2003:  $\chi_4^2 = 107.44$ ,  $P < 0.001$ ; Fig. 5). In 2002, hymenopterans were the



**Fig. 4.** Flower visitation rate (FVR) to male (black bars) and female (white bars) individuals of *Thymelaea velutina* for each population and study year. Bars of one standard error are shown. Numbers above bars represent sample sizes (censuses in which flower visitation was monitored)

most frequent group in the dune, whereas dipterans were the most abundant in the mountain. In contrast, hymenopterans were the most frequent visitors at both sites in 2003 (Fig. 5). When comparing the frequency of visits by insect order between sexes we also observed significant differences, both in 2002 ( $\chi_4^2 = 11.24$ ,  $P < 0.05$ ) and in 2003 ( $\chi_4^2 = 37.20$ ,  $P < 0.001$ ): except for hymenopterans, males were more frequently visited than females by all insect groups (Fig. 5), what we attribute to the larger floral display of the former.

The number of flowers visited in each visit to a plant differed significantly among insect groups (2002:  $\chi_3^2 = 12.80$ ,  $P < 0.01$ ; 2003:  $\chi_4^2 = 3.82$ ,  $P = 0.43$ ), being highest for hymenopterans ( $7 \pm 0.8$  (SE),  $N = 125$ ), and followed by dipterans ( $5 \pm 0.5$ ,  $N = 124$ ), coleopterans ( $3 \pm 0.6$ ,  $N = 14$ ) and lepidopterans ( $2 \pm 0.8$ ,  $N = 8$ ). Ants usually stay on an individual for long periods of time, always longer than a ten minute census, and visit an indeterminate number of flowers. The duration of flower visits varied significantly among the other groups (2002:  $F_{3, 105} = 8.10$ ,  $P < 0.001$ );



**Table 2.** Insect species recorded visiting the flowers of *Thymelaea velutina* in 2002 and 2003 in the two populations

Order	Insect species	Dune	Mountain
Coleoptera	<i>Oedemera</i> sp. (Oedemeridae)	–	+
	<i>Oxythyrea funesta</i> Poda (Cetonidae)	–	+
Diptera	<i>Calliphora vicina</i> Robineau-Desvoidy (Calliphoridae)	+	+
	<i>Eristalinus aeneus</i> Scopoli (Syrphidae)	+	–
	<i>Eristalis tenax</i> L. (Syrphidae)	–	+
	<i>Sarcophaga</i> sp. (Sarcophagidae)	+	–
	<i>Sphaerophoria scripta</i> L. (Syrphidae)	–	+
Hymenoptera	<i>Apis mellifera</i> L. (Apidae)	+	+
	<i>Bombus terrestris</i> L. (Apidae)	–	+
	<i>Crematogaster scutellaris</i> Olivier (Formicidae)	+	–
	<i>Dasyscolia ciliata</i> Fabricius (Scoliidae)	+	–
	<i>Eucera</i> sp. (Apidae)	–	+
	<i>Halictus</i> sp. (Halictidae)	–	+
	<i>Panurgus</i> sp. (Andrenidae)	+	–
	<i>Plagiolepis pigmaea</i> Latreille (Formicidae)	+	–
	<i>Podalonia hirsuta</i> Scopoli (Sphecidae)	–	+
	<i>Polistes omisus</i> L. (Vespidae)	+	–
Lepidoptera	<i>Cynthia cardui</i> L. (Nymphalidae)	–	+
	<i>Lycaena phlaeas</i> L. (Lycaenidae)	–	+
	<i>Maniola jurtina</i> L. (Nymphalidae)	–	+
	<i>Polyommatus icarus</i> Rottentburg (Lycaenidae)	–	+

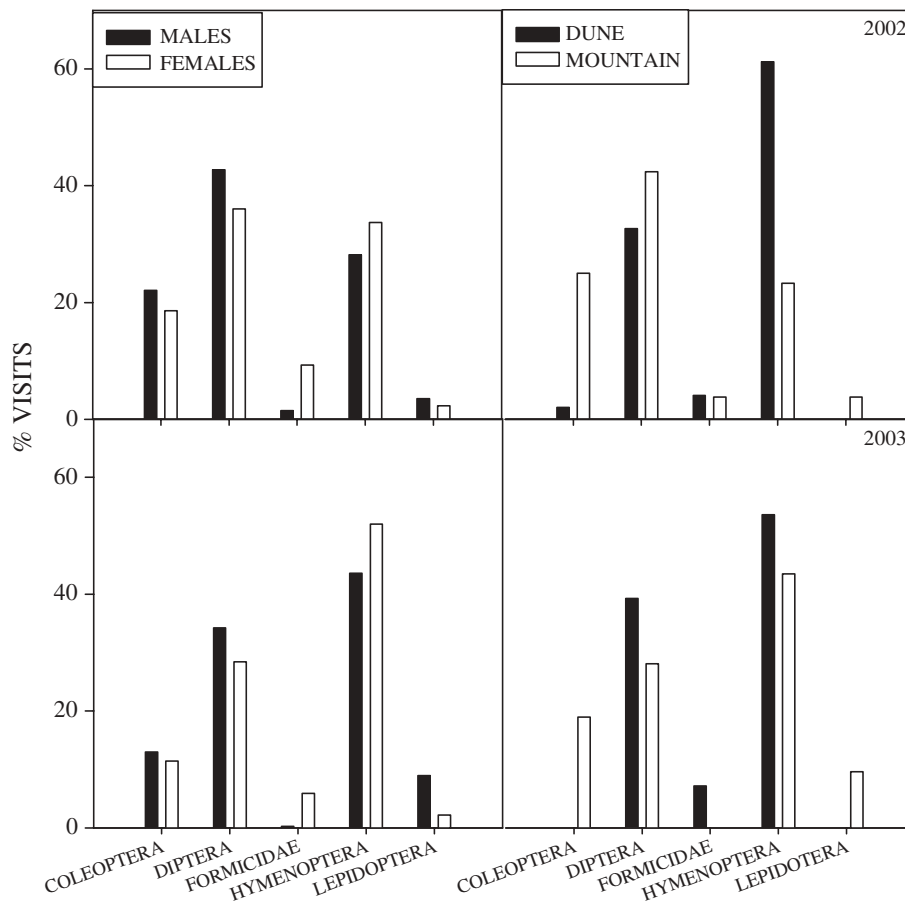
2003:  $F_{3,234} = 3.74$ ,  $P < 0.05$ ), being longest for coleopterans ( $189 \pm 26$  s (SE),  $N = 20$ ), followed by dipterans ( $100 \pm 9$  s,  $N = 141$ ), hymenopterans ( $95 \pm 9$  s,  $N = 600$ ) and lepidopterans ( $68 \pm 14$  s,  $N = 18$ ).

Dipterans were the insect group transporting the greatest pollen load in both populations, followed by flying hymenopterans and ants in the dune, and by lepidopterans, hymenopterans and coleopterans in the mountain. Such differences among insect groups were significant in the dune ( $\chi_2^2 = 22.46$ ,  $P < 0.001$ ) but not in the mountain;  $\chi_3^2 = 3.90$ ,  $P > 0.05$ ). Significant differences in pollen load were also found among species at both sites ( $\chi_8^2 = 71.79$ , and  $\chi_9^2 = 60.82$ ,  $P < 0.001$ , respectively). *Calliphora vicina* and *Eristalis tenax* were the species transporting the greatest quantities of pollen grains at a given time in the dune and mountain sites, respectively (Table 3). Curiously, these two species were the only shared in both habitats but had contrasting pollen loads depending on the habitat. However, we did not find a corre-

lation between frequency of visits of each species and its pollen load. Combining the frequency of visits, the number of flowers contacted in each visit and the number of pollen grains transported, the quantitatively most important pollinators for *T. velutina* were *Sarcophaga* sp. followed by *Polistes omisus*, *Eristalinus aeneus*, *Apis mellifera*, *Calliphora vicina*, *Crematogaster scutellaris*, *Dasyscolia ciliata*, and *Eristalis tenax* in the dune and *Eristalis tenax*, *Halictus* sp., *Oedemera* sp., *Cynthia cardui*, *Podalonia hirsuta*, *Oxythyrea funesta*, *Lycaena phlaeas*, and *Sphaerophoria scripta* in the mountain.

## Discussion

**Ambophily and fruit set.** In recent years, a number of studies has shown that many plant-animal mutualistic interactions are generalist and dynamic (Jordano 1987; Thompson 1994; Herrera 1995, 1996; Romeo et al. 1996; Waser et al. 1996; Johnson and Steiner 2000; Karrenberg et al. 2002), and some authors



**Fig. 5.** Percentage of visits by each insect group to the two plant sexes of *Thymelaea velutina* and in the two populations during 2002 and 2003. Although the family Formicidae belongs to the order Hymenoptera, we consider it separately here

have argued that these generalist systems are not only common but the rule (Polis and Strong 1996, Bascompte et al. 2003). In the case of *T. velutina*, although floral morphology fits perfectly with the description of a typically anemophilous flower, i.e. unisexual, small in size, rather inconspicuous, and with a low reward for pollinators, the results of our study demonstrate that a wide variety of insect species are playing an important role in the reproductive system of this plant.

Wind is intense in both study populations during the flowering period, although its relative importance as pollen vector was similar to that of insects at either locality. Despite this, pollen limitation was detected at

least one of the years, indicating that the number of fruits produced could potentially be higher. In the dune, the frequency of insect visits was rather low, probably due to the early flowering period at this site. In the mountain, by contrast, such frequency of insect visits was higher, despite the shorter (about three weeks) flowering period which may sometimes decrease the probability of a plant-pollinator interaction, as found in the late winter flowering species, *Daphne laureola* (Alonso 2004).

However, resource limitation is also likely to cause a low fruit set, especially at high elevations. In our case, the low winter temperatures, the strong winds that promote stomata closure, and the low depth and stoniness of

**Table 3.** Mean and standard error of number of pollen grains of *Thymelaea velutina* transported by pollinators in 2003 in the two populations. Numbers in parentheses indicate sample sizes

Insect Species	Pollen load	Population
<i>Apis mellifera</i>	7506 ± 374.556 (2)	Dune
<i>Calliphora vicina</i>	10683.333 ± 717.248 (2)	Dune
<i>Crematogaster scutellaris</i>	1427.143 ± 154.996 (3)	Dune
<i>Dasycolia ciliata</i>	4581.692 ± 474.945 (3)	Dune
<i>Eristalinus aeneus</i>	8172 ± 502.648 (4)	Dune
<i>Eristalix tenax</i>	3909.600 ± 172.124 (2)	Dune
<i>Plagiolepis pigmaea</i>	3060 ± 535.239 (2)	Dune
<i>Polistes omisus</i>	1818.667 ± 380.420 (3)	Dune
<i>Sarcophaga</i> sp.	6230 ± 516.324 (3)	Dune
<i>Calliphora vicina</i>	931.500 ± 55.662 (2)	Mountain
<i>Cynthia cardui</i>	1278.400 ± 298.874 (3)	Mountain
<i>Eristalix tenax</i>	17064 ± 2395.005 (2)	Mountain
<i>Halictus</i> sp.	2988.588 ± 200.565 (4)	Mountain
<i>Lycaena phlaeas</i>	4977 ± 678.972 (2)	Mountain
<i>Manilola jurtina</i>	918 ± 216 (2)	Mountain
<i>Oedemera</i> sp.	1666.800 ± 194.119 (3)	Mountain
<i>Oxythyrea funesta</i>	4329 ± 512.368 (2)	Mountain
<i>Podalonia hirsuta</i>	1911.600 ± 88.071 (2)	Mountain
<i>Sphaerophoria scripta</i>	1879.200 ± 468.713 (5)	Mountain

the soil at the mountain site may cause a lower photosynthetic rate and, ultimately, a lower fruit set compared to plants at sea level, where winter (period in which most plants in Mediterranean climate accumulate reserves that will be used later during flowering and fruiting) is milder. A tendency to decrease fruit set at a higher altitude has also been reported in *Saxifraga oppositifolia* (Gugerli 1998) and in *D. laureola* (Alonso 2004). The question of whether pollen or resource availability limits fruit set in nature has stimulated a number of field studies, the results of which have been variable (Pyke 1982, Pyke and Paton 1983, Bawa and Webb 1984, Whelan and Goldingay 1986, Horvitz and Schemske 1988). Moreover, different studies on the same plant species have sometimes yielded opposite results (Pyke 1982, Whelan and Goldingay 1989). In the case of *T. velutina*, the fact that females in the dune (1) produce about three times more flowers than in the mountain and (2) have a greater fruit set (even though flower visitation rate by insects is similar in the two populations), suggests that resource limitation is more important in the

mountain than in the dune. Moreover, the higher frequency of insect visitation to flowers in the mountain did not translate into a greater fruit set, further supporting such resource limitation hypothesis.

**Quantitative of insect pollinators.** The different frequency of visits (FIV) to *T. velutina* by the different groups of pollinators might be related to the relative abundances of each insect group in a given habitat. The plant establishes interactions with the most frequent group of insects in each locality. Olesen (2000) reasoned that plants interact with the most abundant pollinator in the area, and he estimated that around two thirds of all plant species have a random pollinator fauna compared to the local species pool. In our case, hymenopterans were the most frequent insect group in the dune in 2002 whereas dipterans predominated at the mountain. This result is concordant with that found in a study that compares the pollination networks between mountain and coastal communities in Mallorca Island (Lázaro and Traveset, unpubl. data). A greater abundance of hymenopterans in lowlands and of dipterans

in highlands has also been documented in other studies (Arroyo et al. 1990). However, such pattern may not be consistent through time, as hymenopterans were the most frequent group in both sites in 2003. A number of factors, such as differences in population cycles, feeding behaviour, and delay in plant phenology, may explain the variation in pollinator assemblages (Herrera 1988).

The greatest diversity and frequency of visits was found at the mountain locality. Even though some authors support the hypothesis that the greatest insect species richness is found at low altitudes (Lawton et al. 1987), others have proposed that the activity peak is found at mid elevations (Janzen 1973, Janzen et al. 1976). In a review of 20 studies, McCoy (1990) tried to reconcile these two positions, arguing that the results obtained by both groups of authors could be due to the effect of latitude and sampling method. According to McCoy's results, our mountain population would be nearer to the altitude at which the peak activity would be found; over 1200 m, the richness and activity of insects would descend. Another factor that certainly influences the richness and abundance of pollinators in both populations is the time of flowering, which at the mountain site occurs well within spring time when insect activity is greater.

Insect visitation rate to flowers (FVR) was similar in males and females of *T. velutina*, in contrast to what has been found in other plant species in which the former receive proportionally more visits (Baker 1976, Bawa et al. 1982, Armstrong and Drummond 1986, Barfod et al. 1987). Such result could suggest a case of deceit pollination, as male and female flowers are very similar. In *T. velutina*, female flowers apparently offer a lower reward to pollinators as they do not possess pollen and they produce similar amounts of nectar. Such lower reward has been found to be compensated by a high and simultaneous production of flowers in some species (Gómez et al. 1996), although this does not seem to be the case here, as flower crops did not differ significantly between sexes within each locality. Other

studies have shown that the degree of discrimination can differ among floral visitors (Le Corff et al. 1998, and references therein). In *T. velutina*, all insect groups except hymenopterans visited males more frequently than females. Females were mostly visited by ants and flying hymenopterans, which in turn contact the highest number of flowers in each visit; the former require less energy than flying (Gómez and Zamora 1992), whilst the latter might be able to compensate the lower reward in female flowers by visiting more flowers in a single bout. Unfortunately, we could not obtain data on nectar sugar concentration.

Distinguishing between visitors and pollen vectors is essential to any investigation of a pollination system (Schemske and Horvitz 1984, Fishbein and Venable 1996). Usually, effectiveness of pollinators is assessed by direct observations of pollinator behaviour and by identification of pollen loads (Johnson and Steiner 2000). However, flower visitors with the highest pollen load on their bodies are not necessarily those that transfer the highest pollen loads to stigmas (Gómez and Zamora 1992). In our case, dipterans were the group carrying the greatest pollen load in both populations, and *Sarcophaga* sp. and *Eristalix tenax* would be the most quantitatively effective pollinators of *T. velutina* at the dune and mountain, respectively, only if they would transfer also the highest pollen load to stigmas.

In short, *T. velutina* showed to have an ambophilous reproductive system, ensuring thus a relatively high fruit set in either of the two types of habitats where the species is found. The relative importance of wind and insects as pollen vectors of this species was similar between the two different localities. Even though the diversity and frequency of insect visits on the flowers were greater in the mountain than in the dune, fruit set was lower in the former, what we attribute to the harsh environmental conditions, especially in winter. Pollen may limit fruit production in some years, especially if insects are scarce, and/or rains are frequent during the flowering period. Hymenopterans and dipterans appear to be frequent

pollinators of the plant, while several species of flies are those carrying most pollen grains in their bodies.

The authors thank the “Escuadrón de Vigilancia Aérea”, especially commander Moratinos, and the staff of S’Albufera Natural Park for facilitating our work at Puig Major and Ca’n Picafort, respectively, Antonio Moreno for their valuable help during fieldwork, Javier Gulías and an anonymous reviewer for useful comments on an earlier version of the manuscript, and Luis Santamaría and Javier Rodríguez for statistical advice. Gori Salvareche kindly provided the photo of the male flower for Fig. 1. This work was partially supported by projects PB97-1174 and BOS2001-0610 financed to A.T.

## References

- Alomar G., Mus M., Roselló J. A. (1997) Flora endémica de les Balears. Consell Insular de Mallorca, FODESMA. Palma.
- Alonso C. (2004) Early blooming’s challenges: extended flowering season, diverse pollinator assemblage and the reproductive success of gynodioecious *Daphne laureola*. *Ann. Bot.* 93: 61–66.
- Armstrong J. E., Drummond B. A. (1986) Floral biology of *Myristica insipida* (Myristicaceae). *Biotropica* 18: 32–38.
- Arroyo M. T. K., Rozzi R., Squeo F., Belmonte E. (1990) Pollination in tropical and temperate high elevation ecosystems: hypotheses and the Asteraceae as a test case. In: Winiger M., Wiesmann U., Rheker J. R. (eds.) Mount Kenya area: Differentiation and dynamics on a tropical mountain ecosystem. UNESCO-IUBS, Berne, pp. 21–31.
- Baker H. G. (1976) “Mistake” pollination as a reproductive system with special reference to the Caricaceae. In: Burley J., Styles B. T. (eds.) Tropical trees: variation, breeding system and conservation. Academic Press, London, pp. 161–169.
- Barfod A., Henderson A., Balslev H. (1987) A note on the pollination of *Phytelephas microcarpa* (Palmae). *Biotropica* 19: 191–192.
- Bascompte J., Jordano P., Meliá C. J., Olesen J. M. (2003) The nested assembly of plant-animal mutualistic networks. *Proc. Nat. Acad. Sci.* 100: 9383–9387.
- Bawa K. S., Webb C. J., Tuttle A. F. (1982) The adaptative significance of monoecism in *Cnidocolus urens* (L.) Arthur (Euphorbiaceae). *Bot. J. Linn. Soc.* 85: 213–223.
- Bawa K. S., Webb C. J. (1984) Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. *Amer. J. Bot.* 71: 736–751.
- Bullock S. H. (1994) Wind pollination of neotropical dioecious trees. *Biotropica* 26: 172–179.
- Culley T. M., Weller S. G., Sakai A. K. (2002) The evolution of wind pollination in angiosperms. *Trends Ecol. Evol.* 17: 361–369.
- Dafni A., Dukas R. (1986) Insect and wind pollination in *Urginea maritima* (Liliaceae). *Pl. Syst. Evol.* 154: 1–10.
- Faegri K., van der Pijl L. (1979) The principles of pollination ecology. Pergamon Press, Oxford.
- Fishbein M., Venable D. L. (1996) Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* 77: 1061–1073.
- Gómez J. M., Zamora R. (1992) Pollination by ants: consequences of the quantitative effects on a mutualistic system. *Oecologia* 91: 410–418.
- Gómez J. M., Zamora R., Hódaz J. A., García D. (1996) Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. *Oecologia* 105: 236–242.
- Gugerli F. (1998) Effect of elevation on sexual reproduction in alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). *Oecologia* 114: 60–66.
- Guijarro J. A. (1986) Contribución a la bioclimatología de Baleares. PhD thesis, Universitat de les Illes Balears. Palma.
- Gulías J., Traveset A., Mus M., Riera N. (2004) Critical stages in the recruitment process of *Rhamnus alaternus* L. *Ann. Bot.* 93: 723–731.
- Herrera C. M. (1988) Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. *Biol. J. Linn. Soc.* 35: 95–125.
- Herrera C. M. (1995) Plant-vertebrate seed dispersal systems in the Mediterranean: ecological, evolutionary and historical determinants. *Annual Rev. Ecol. Syst.* 26: 705–727.
- Herrera C. M. (1996) Floral traits and plant adaptation to insect pollinators: a devil’s advocate approach. In: Lloyd D. G., Barret S. C. H.

- (eds.) Floral biology. Chapman and Hall, New York, pp. 65–87.
- Horvitz C. C., Schemske D. W. (1988) A test of the pollinator limitation hypothesis for a neotropical herb. *Ecology* 69: 200–206.
- Janzen D. H. (1973) Sweep samples of tropical foliage insects: effects of seasons, vegetation types, elevation, time of day, and insularity. *Ecology* 54: 687–708.
- Janzen D. H., Ataroff M., Farinas M., Reyes S., Rincón N., Soler A., Soriano P., Vera M. (1976) Changes in the arthropod community along an elevational transect in the Venezuelan Andes. *Biotropica* 8: 193–203.
- Johnson S. D., Steiner K. E. (2000) Generalization versus specialization in plant pollination systems. *Trends Ecol. Evol.* 15: 140–143.
- Jordano P. (1987) Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence, asymmetries, and coevolution. *Amer. Naturalist*. 143: 677–697.
- Karremberg S., Kollman J., Edwards P. J. (2002) Pollen vectors and inflorescence morphology in four species of *Salix*. *Pl. Syst. Evol.* 235: 181–188.
- Lawton J. H., McGarvin M., Heads P. A. (1987) Effects of altitude on the abundance and species richness of insect herbivores on bracken. *J. Anim. Ecol.* 56: 147–160.
- Lázaro, A., Traveset, A. (2005) Spatio-temporal variation in the pollination mode of *Buxus balearica* (Buxaceae), an ambophilous and selfing species: mainland-island comparison. *Ecography* (in press).
- Le Corff J., Ågren J., Schemske D. W. (1998) Floral display, pollinator discrimination, and female reproductive success in two monoecious *Begonia* species. *Ecology* 79: 1610–1619.
- McCoy E. D. (1990) The distribution of insects along elevational gradients. *Oikos* 58: 313–322.
- Meeuse A. D. J. (1978) Entomophily in *Salix*: theoretical considerations. In: Richards A. J. (ed.) *The pollination of flowers by insects*. Academic Press, London, pp. 47–50.
- Meeuse A. D., De Meijer, A. H., Mohr O. W. P., Wellinga S. M. (1990) Entomophily in the dioecious gymnosperm *Ephedra aphylla* Fursk (= *E. alte* C.A. Mey), with some notes on *Ephedra campylopoda* C.A. Mey. III. Further anthecological studies and relative importance of entomophily. *Israel. J. Bot.* 39: 113–129.
- Olesen J. M. (2000) Exactly how generalised are pollination interactions?. *Mat.- Naturv. Klasse Skrifter Ny Serie No. 39* (Scandinavian Association for Pollination Ecology honours Knut Faegri): 161–178.
- Pedrol J. (1997) *Thymelaea* Mill. (nom. cons) In: Castroviejo S., Aedo C., Benedí C. M., Muñoz Garmendia F., Nieto Feliner G., Paiva J. (eds.) *Flora Ibérica: plantas vasculares de la Península Ibérica e Islas Baleares*, vol. 8. Real Jardín Botánico, CSIC, Madrid, pp. 50–51.
- Polis G. A., Strong D. R. (1996) Food web complexity and community dynamics. *Amer. Naturalist* 147: 813–846.
- Pyke G. H. (1982) Evolution of inflorescence size and height in the waratah (*Telopea speciosissima*): the difficulty of interpreting correlations between plant traits and fruit set. In: Armstrong A., Powell J. M., Richards A. J. (eds.) *Pollination and evolution*. Royal Botanic Gardens, Sydney, pp. 91–94.
- Pyke G. H., Paton D. C. (1983) Why are waratah inflorescences so high and so large?. In: Williams E. G., Knox R. B., Gilbert J. H., Bernhardt P. (eds.) *Pollination*. University of Melbourne, Melbourne, pp. 57–68.
- Romeo J. T., Saunders J. A., Barbosa P. (1996) Phytochemical diversity and redundancy in ecological interactions. Plenum Press, New York.
- Sacchi C. F., Price P. W. (1988) Pollination of the arroyo willow, *Salix lasiolepis*: role of insects and wind. *Amer. J. Bot.* 75: 1387–1393.
- Scariot A. O., Lleras A., Hay J. D. (1991) Reproductive biology of the palm *Acrocomia aculeata* in Central Brazil. *Biotropica* 23: 12–22.
- Schemske D. W., Horvitz C. C. (1984) Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* 255: 519–521.
- Stelleman P. (1978) The possible role of insects visits in the pollination of reputedly anemophilous plants, exemplified by *Plantago lanceolata*, and syrphid flies. In: Richard A. J. (ed.) *The pollination of flowers by insects*. Academic Press, London, pp. 41–46.
- Tamura S., Kudo G. (2000) Wind pollination and insect pollination of two temperate willow species, *Salix miyabeana* and *Salix sachalinensis*. *Plant Ecol.* 147: 185–192.

- Tébar F. J., Llorens L. (1992) Variations dans la phénologie de trois espèces des communautés xérochaméphytiques de l'île de Majorque. Bull. Soc. Bot. France, Lett. Bot. 139: 161–174.
- Thompson J. N. (1994) The coevolutionary process. University of Chicago Press, Chicago.
- Totland Ø., Sottocornola M. (2001) Pollen limitation of reproductive success in two sympatric alpine willows (Salicaceae) with contrasting pollination strategies. Amer. J. Bot. 88: 1011–1015.
- Vroege P. W., Stelleman P. (1990) Insect and wind pollination in *Salix repens* L. and *Salix caprea* L. Israel. J. Bot. 39: 125–132.
- Waser N. M., Chitka L., Price M. V., Williams N. M., Ollerton J. (1996) Generalization in pollination systems, and why it matters. Ecology 77: 1043–1060.
- Whelan R. J., Goldingay R. L. (1986) Do pollinators influence seed set in *Banksia paludosa* Sm. and *B. spinulosa* R.Br.? Austral. J. Ecol. 11: 181–186.
- Whelan R. J., Goldingay R. L. (1989) Factors affecting fruit set in *Telopea speciosissima* (Proteaceae): The importance of pollen limitation. J. Ecol. 77: 1123–1134.

Addresses of the authors: M. Carmen de la Bandera\* and Anna Traveset (e-mail: atraveset@uib.es), Instituto Mediterráneo de Estudios Avanzados (CSIC-UIB), C/ Miquel Marqués 21, 07190 Esporles, Mallorca, Balearic Islands, Spain. \*Current address: Fundació Jardí Botànic de Sóller, Ctra Palma–Port de Sóller, Km 30.5, 07100 Sóller, Mallorca, Balearic Islands, Spain.