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Experimental study of pollination by ants in Mediterranean high mountain and arid habitats

Abstract In this paper, we report the results of an experimental study on ant pollination of three plant species inhabiting the Mediterranean high mountains (*Alyssum purpureum*, *Arenaria tetraquetra* and *Sedum anglicum*) and four species inhabiting the aridlands (*Lepidium subulatum*, *Gypsophyla struthium*, *Frankenia thymifolia* and *Retama sphaerocarpa*) of South-eastern Spain. We determined several plant and ant traits, as well as the composition and abundance of the pollinator assemblage. Insects belonging to 29 families and five orders visited the flowers of the plant species studied. In all but two, *L. subulatum* and *G. struthium*, the ants comprised 70–100% of the flower visitors. The results clearly show that five out of seven of these plant species were pollinated by ants. The role of the ants as pollinators seems to depend heavily on the relative abundance of the ants with respect to the other species of the pollinator assemblage, ant pollination becoming evident when ants outnumber other floral visitors. The ant-pollination systems analysed in this study may be the result of prevailing ecological conditions more than an evolutionary result of a specialized interaction.

Key words Ant pollination · High-mountain ecology · Mediterranean habitats · Mutualism

Introduction

The role of ants as pollinators is the subject of a great controversy, fueled by growing empirical evidence which is mainly observational and often contradictory (Hickman 1974; Petersen 1977a, b; Wyatt 1980, 1981; Fritz and Morse 1981; Wyatt and Stoneburner 1981; Pant et al. 1982; Galen 1983; Peakall et al. 1987, 1990,

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1991; Peakall and Beattie 1989; Gómez and Zamora 1992). In spite of this, and with respect to plant species whose flowers are visited by ants, most authors assume that ants are poor pollinators, if not simply nectar robbers. Consequently, there is a paucity of recorded pollinator systems involving ants (Beattie et al. 1984, 1985).

Several hypotheses have been used to explain why ants are ineffective pollinators, but the most accepted one is the "antibiotic hypothesis", according to which ants can carry pollen between different plants but secrete fungicide liquids, mainly from metapleural glands, leaving pollen grains unviable (Beattie et al. 1984, 1985; Hull and Beattie 1988; Peakall 1989; Peakall et al. 1990, 1991; Peakall and Beattie 1991). However, in detailed studies of pollen inhibition by liquid fungicides, a certain percentage of pollen grains invariably remain viable (Beattie et al. 1984; Sanderson and Wright 1989), and can even exceed the percentage of pollen grains transported by winged insects considered to be true pollinators (Harder and Thomson 1989; Thomson and Thomson 1989; Holsinger and Thomson 1994). Addressing this issue, Peakall and Beattie (1991) stated that to appreciate the true role of the ants in pollination it is necessary, at the very least, to observe ants carrying pollen grains and depositing them on a conspecific stigma, and, most importantly, to test experimentally the effect of ant presence on plant-seed production. Nevertheless, only a few studies have experimentally studied the effect of ants on seed production (Fritz and Morse 1981; Galen 1983; Peakall et al. 1987; Peakall and Beattie 1989; Gómez and Zamora 1992).

There is a high number of plant species whose flowers are habitually visited by ants in many Mediterranean habitats of Spain, such as coastal scrublands and mixed pine forests (Herrera et al. 1984), grasslands (Retana et al. 1987), high mountains (Gómez and Zamora 1992; Fernández Escudero 1994; Gómez 1994) and arid shrubsteppes (Sánchez-Piñero 1994). Furthermore, Gómez and Zamora (1992) have

experimentally demonstrated that ants act as true pollinators of *Hormathophylla spinosa*, a plant living in the Mediterranean high mountain regions. In this study, we tested whether the results of our previous study were indicative of only one case reflecting extremely local ecological conditions or, on the contrary, exemplified a kind of insect-flower interaction that may be widespread among several Mediterranean habitats. We experimentally studied ant pollination in seven additional plant species inhabiting the Mediterranean high mountain regions and the aridlands of South-eastern Spain, observing ant activity in flowers. The main aims of this work were: (1) to quantify the pollinator assemblages of each plant species studied and numerically evaluate the relative importance of ants with respect to the remaining floral visitors; (2) to analyse the plant and ant traits possibly related to ant pollination; (3) to ascertain, by means of exclusion experiments, whether ants contribute to seed production in these plant species.

Material and methods

Sites and species studied

For this study, we selected two sites situated in two contrasting open habitats of southern Spain, where we had previously observed ants visiting flowers of many plant species. The first site was located above the timberline in the Sierra Nevada (Parque Natural de Sierra Nevada, Granada Province, Spain), in an undisturbed sector of the Rio San Juan, at 2550 m elevation. In a typical year, this site is under snow from November or December to May. During summer, temperatures range from 3 to 27.2 °C (mean = 16.6 °C), rising to 60 °C in the soil. Wind velocity reaches 35 m/s, and the annual rainfall averages 750 mm, falling mainly in autumn and winter. The average relative humidity is about 36%. In this sector, the most abundant plant species are *Hormathophylla spinosa* (L.) Küpfer (Cruciferae), *Arenaria tetraquetra* L. (Caryophyllaceae), *Genista versicolor* Boiss. (Fabaceae), *Sedum anglicum* Hudson (Crassulaceae) and *Juniperus communis* L. (Cupressaceae).

The second site was situated in a seasonal watercourse 750 m a.s.l. in the Baza basin (Granada province), named Barranco del Espartal. The substrate is composed of silt with gypsum sediment, forming a typical badlands landscape. This arid zone is also highly seasonal, with hot summers, temperatures usually ranging from 10 to 42 °C (mean = 25 °C) and rising to 60 °C in the soil, and cold winters (down to −8.6 °C). The arid climate has sporadic rainfall (300 mm annually), mainly during the cold months. In this area, the most abundant plant species are *Artemisia* spp. (Compositae), *Salsola vermiculata* L. (Quenopodiaceae), *Ononis tridentata* L. (Fabaceae), *Gypsophyla struthium* L. (Caryophyllaceae), *Retama sphaerocarpa* (L.) Boiss. (Fabaceae), and *Stipa tenacissima* L. (Gramineae).

We chose seven plant species which were abundant in our study sites and whose flowers are habitually visited by several species of ants. Thus, we studied the pollination biology of *Alyssum purpureum* Lag. and Rodr. (Cruciferae), *Arenaria tetraquetra* and *Sedum anglicum* in the alpine sector of the Sierra Nevada, and *Lepidium subulatum* L. (Cruciferae), *Gypsophyla struthium*, *Frankenia thymifolia* Desf. (Frankeniaceae) and *Retama sphaerocarpa* in the aridlands of the Baza basin.

For each plant species, we determined the height, life habit, flowering time and inflorescence type, as well as flower colour, shape (whether actinomorphic or zygomorphic) and size (using a digital caliper; see Table 1).

Composition and abundance of flower visitor assemblage

The composition and abundance of the pollinator assemblage was determined for 20 flowering *Arenaria tetraquetra*, *Alyssum purpureum*, *S. anglicum*, *F. thymifolia* and *L. subulatum* specimens. In the case of *R. sphaerocarpa* and *G. struthium*, the pollinator assemblage was determined in eight and ten flowering plants, respectively. The sampling procedure was to count all flower visitors seen in 1-min periods on each labelled plant, considering as one pollinator census the overall series of 1-min periods for all labelled plants every time. We conducted 40 (*Arenaria tetraquetra*), 36 (*G. struthium*), 24 (*L. subulatum*), 20 (*Alyssum purpureum*, *S. anglicum*), and 12 (*F. thymifolia*, *R. sphaerocarpa*) censuses, for a total of 800, 720, 480, 400, 400, 240 and 96 min of observation, respectively, distributed evenly from sunrise to sunset, and spanning the entire flowering period of each plant species. Any insect capable of making contact with the anthers and/or stigma was considered to be a flower visitor. With a magnifying glass, we could observe whether ants made contact with the anther or the stigma and whether ants carried pollen grains. We collected samples of all ant species appearing in the censuses, and, in the lab, measured these specimens under a binocular microscope with a micrometer (× 10), also determining the presence or absence of metapleural glands.

Experimental determination of the role of ants as pollinators

We experimentally determined whether the ants visiting each plant species acted as true pollinators or only as nectar robbers by selectively excluding ants from the flowers. We used a factorial design for assessing the proportional contribution of both worker ants and winged insects to the reproductive success of every plant species. The following four treatments were carried out on each plant species: (1) "ants-only treatment", in which flowers were visited only by ants. For this, we covered the flowers with nylon-mesh bags (0.25 mm mesh) that excluded all winged insects but allowed ants to crawl up the stem. (2) "Winged-only treatment", in which flowers were visited only by winged insects. We excluded ants by the application of glue (Tanglefoot) on the stem of the inflorescence (*L. subulatum*, *G. struthium*, *F. thymifolia*), on the twig (*R. sphaerocarpa*) or surrounding the whole plant by means of a ring of Plasticine put on the ground (*Alyssum purpureum*, *Arenaria tetraquetra*, *S. anglicum*). (3) "No-pollinator treatment", in which flowers were visited by no insect. We excluded all insects by combining the two previously described procedures. (4) "All-pollinators or control treatment", in which flowers were left to open pollination (see Gómez and Zamora 1992 for a detailed description). Experiments were periodically checked (every 2 or 3 days) to ensure that the exclusion of winged insects did not affect normal ant foraging on flowers.

For these experiments, plants were arbitrarily selected from among the reproductive individuals of the population, with a similar size and appearance, and at the same phenological stage (beginning of the flowering period). We tried to apply all four treatments to the same plant. However, four species (*F. thymifolia*, *Alyssum purpureum*, *Arenaria tetraquetra* and *S. anglicum*) were too small to permit the four treatments in each individual. In these species, several reproductive individuals were selected and arbitrarily divided into four groups, one group per treatment. Each treatment was replicated 8 times in *R. sphaerocarpa*, 10 in *L. subulatum*, *F. thymifolia* and *G. struthium*, and 15 in *Alyssum purpureum*, *Arenaria tetraquetra* and *S. anglicum*. However, in four individuals of *L. subulatum* the no-pollinator and ants-only treatments were disrupted by domestic sheep, and in one individual the all-pollinators and winged-only treatments were also lost to domestic sheep. Furthermore, we lost ants-only and no-pollinator treatments in one labelled plant, and the winged-only treatment in two plants of *G. struthium* due to strong autumn storms. Finally, the plants belonging to the no-pollinator treatment in *S. anglicum* were killed

by mammal trampling, and therefore we could only use three treatments.

At the beginning of the treatment, we counted the number of flowers by experimental units (inflorescence, twig or whole plant), and at the end of the ripening period we counted the number of fruits, to calculate the fruiting success corresponding to each treatment. One sample of fruits per treatment was collected at the end of the ripening period in each plant species before seed dispersal, for a laboratory count of the number of mature seeds, aborted seeds and unfertilized ovules within each of those fruits.

We used female fertility – the percentage of ovules becoming viable seeds in relation to the total number of ovules produced by each plant (Charlesworth 1989) – as an estimate of the reproductive success. To obtain the initial number of ovules per plant, we multiplied the initial number of flowering buds by the number of ovules in each ovary, and then divided the total number of viable seeds in each plant by the initial number of ovules calculated (see Gómez and Zamora 1992 for a detailed description). However, since it was not possible to count the initial number of ovules per mature fruit in *F. thymifolia*, we estimated the reproductive success of this plant species by the fruit set. In addition, *G. struthium* lacks a fixed number of ovules per flower, and since the flowers wither after fruit maturation, it is not possible to individualize them; consequently, we could not determine how many flowers set fruit per inflorescence or how many ovules were present in each flower. For this reason, we expressed the reproductive success of these plants as the number of seeds divided by the number of flowers on each stem (seed per inflorescence, hereafter). We believe that this way of estimating reproductive success had no affect on the conclusions of the experiments, given that the analysis was comparative between treatments, all of these expressed using the same method.

To test differences among treatments, we used a one-way ANOVA with a posteriori pair-wise comparision (Bonferroni-Dunn test). To avoid pseudoreplication and to ensure the independence of the data, we used individual plants as the sample unit for our analyses. The variables were arcsin transformed prior to statistical analysis (Zar 1984). We performed all statistical analyses using a type III sum of squares.

Results

Plant traits

The features analysed in the experimental group varied between plant species. The life habit was diverse, including herbaceous plants, shrubs and one treelet (Table 1). All species flowered in early summer, except *G. struthium*, which flowered during autumn. All but one species had actinomorphic flowers, ranging in colour from white to purple (Table 1). The group was dominated by mass-flowering species such as *F. thymifolia*, *Arenaria tetraquetra* and *R. sphaerocarpa* (Table 1). In all species, the flowers were less than 1 cm in diameter (Table 1) and produced nectar.

Ant traits

We found two species of anthophilous ants in the high mountain of the Sierra Nevada, although only *Proformica longiseta* Collingwood was abundant (Table 3). This ant species visited flowers only to

Table 1 Certain morphological traits displayed by the experimental plant species (% ants percentage of ants in flowers of each plant species with respect to the total floral visitor assemblage, Fl. size floral diameter ± 1 SE, sample size in parentheses)

Species	Flowers/ plant	Flowering time	% ants	Flower shape	Ovule number	Fl. size (mm)	Height (cm)	Flower colour	Life habit
Alyssum purpureum	15–225	June	97.1	Actinomorphic	4	5.90 ± 0.09 (57)	2–5	Purple	Dwarf shrub
Arenaria tetraquetra	20–1000	July	82.1	Actinomorphic	4–29	7.38 ± 0.13 (358)	4–10	White	Cushion plant
Sedum anglicum	1–10	July	100	Actinomorphic	15	3.99 ± 0.05	7–10	White	Perennial herb
Gypsophyla struthium	>1000	Sept–Oct	12.3	Actinomorphic	?	5.01 ± 0.07 (104)	150	White	Shrub
Lepidium subulatum	>1000	May–June	14.4	Actinomorphic	2	4.12 ± 0.03 (70)	40–60	White	Shrub
Frankenia thymifolia	500–700	May–June	90.2	Actinomorphic	?	4.93 ± 0.04 (31)	10–15	Pink	Dwarf shrub
Retama sphaerocarpa	>>1000	May–June	74.2	Zygomorphic	1–2	4.90 ± 0.04 (21)	100–300	Yellow	Treelet

Table 2 Anthophilous ants traits (n number of ants examined, Size length as mean ± 1 SE)

Species	n	Size (mm)	Metapleural gland	Pollen presence
Camponotus foreli	30	5.14 ± 0.18	No	Yes
Camponotus sp.	16	4.80 ± 0.13	No	Yes
Crematogaster auberti	18	3.96 ± 0.11	Yes	No
Leptothorax fuentei	3	3.17 ± 0.09	No	Yes
Plagiolepis schmitzii	16	1.50 ± 0.09	Yes	No
Proformica longiseta	32	2.95 ± 0.08	Yes	Yes
Tapinoma nigerrimum	8	2.80 ± 0.10	Yes	No

collect nectar, whereas *Tapinoma nigerrimum* (Nylander) visited flowers primarily to prey upon tiny herbivorous insects. In the aridlands of Baza there were five ant species visiting flowers. In *L. subulatum* and *G. struthium* the main anthophilous ants were *Crematogaster auberti* Emery, *Plagiolepis schmitzii* Forel and *Camponotus foreli* Emery, whereas on the remaining two plant species, *Camponotus foreli* was more abundant (Table 3). The other two ant species in the aridlands were *Leptothorax fuentei* Santschi and *Camponotus* prope *foreli* (A. Tinaut, personal communication; *Camponotus* sp. hereafter). These five ant species collected nectar during the flowering period, although *Crematogaster auberti*, whilst abundant on *G. struthium* and *R. sphaerocarpa*, was mainly tending aphids, only visiting a few flowers of the former plant species. The size of anthophilous ants ranged between *Camponotus foreli* or *Camponotus* sp. to *P. schmitzii* (Table 2). Of all the ant species studied, only *Camponotus foreli*, *Camponotus* sp. and *L. fuentei* lacked metapleural glands (Table 2). We observed pollen grains on the body of *P. longiseta*, *Camponotus foreli*, *Camponotus* sp. and *L. fuentei*, but not on *T. nigerrimum*, *Crematogaster auberti* or *P. schmitzii* (Table 2).

Flower visitor assemblage

Insects belonging to 29 families and five orders visited the flowers of the plant species studied. At the family taxonomic level, the most diverse assemblages were those of *R. sphaerocarpa* in the aridlands, with insects belonging to 12 families, and *Arenaria tetraquetra* in the high mountain, with insects belonging to 10 families. The least diverse flower visitor assemblage was that of *S. anglicum*, a plant species visited only by *P. longiseta* during censuses. At the species level, *R. sphaerocarpa* was also notable in having 18 insect species visiting its flowers and *Arenaria tetraquetra* with 15 insect species. On the contrary, *Alyssum purpureum* was visited only by six insect species and *S. anglicum* only by ants (Table 3).

The results show that ants accounted for more than 70% of the flower visits in five of the seven plant species (*Alyssum purpureum*, *Arenaria tetraquetra*, *S. anglicum*, *F. thymifolia* and *R. sphaerocarpa*; Table 3). On the contrary, ants accounted for less than 15% of the visits to the flowers of *G. struthium* and *L. subulatum*, the most abundant insects being beetles belonging to the family Mordellidae (*Mordellistena* sp. and Mordellidae gen. sp.; Table 3). We did not observe thrips in the flowers of these plant species during censuses.

Exclusion experiments

The exclusion experiments indicate that all the plant species studied in the high mountain were pollinated by *P. longiseta* (Table 4). Thus, the flowers of *Alyssum purpureum* and *Arenaria tetraquetra* visited only by ants produced significantly more seeds than flowers not visited by pollinators (Table 4). Even in *Arenaria tetraquetra* the flowers without ants (winged-only treatment) produced significantly fewer seeds than flowers visited by all pollinators (control treatment). In *S. anglicum*, flowers excluded from ants produced significantly fewer seeds than flowers accesible to ants (Table 4).

In the aridlands, *R. sphaerocarpa* and *F. thymifolia* were clearly pollinated by ants, mainly *Camponotus foreli* (Table 4). In fact, flowers of *R. sphaerocarpa* visited only by ants produced significantly more seeds than flowers excluded from all pollinators (Table 4). In addition, flowers of *F. thymifolia* visited by ants produced significantly more fruit than did flowers excluded from ants, and those with no pollinators and even with winged insects only (Table 4). On the contrary, *G. struthium* was not pollinated by ants (Table 4), given that flowers under the ants-only treatment did not produce seeds, and flowers of the winged-only treatment produced more seeds than did the control. In this plant species the effect of the ants on seed production seems to have been negative. Similarly, in *L. subulatum* the ants-only treatment resulted in the same quantity of seeds as the no-pollinator treatment; however, flowers excluded from ants produced significantly fewer seeds than did controls (Table 4).

Finally, the exclusion experiments also show that flying insects acted as pollinators for every plant species studied, although in the species pollinated by ants, the pollination efficiency of winged insects was similar to the efficiency of ants (Table 4). In addition, the quantity of seeds produced without any pollinator was much lower than the quantity of seed produced in any of the other three treatments in the five plant species pollinated by ants, although *Arenaria tetraquetra* and *Alyssum purpureum* bore a few seeds in the no-pollinator treatment (Table 4).

Discussion

The experimental results show that at least five of the seven plant species studied were pollinated by ants. With regard different habitats, all the plant species studied in the high mountain of the Sierra Nevada (*Alyssum purpureum*, *Arenaria tetraquetra*, *S. anglicum*) were pollinated by ants. In the aridland, *R. sphaerocarpa* and *F. thymifolia* were clearly pollinated by ants. However, our study has shown that in the aridlands *G. struthium* was not pollinated by ants, whereas the results with *L. subulatum* are inconclusive, since seed production decreased when we excluded ants from this crucifer, but the seed production in flowers visited only by ants was similar to seed production in flowers visited by no pollinators (Table 4). No studied plant species, except maybe *S. anglicum*, was exclusively pollinated by ants,

Table 3 Composition and abundance (average number of insects per 10 min and per plant) of the flower visitor assemblage for every plant species (means ± 1 SE)

Species	Alyssum purpureum	Arenaria tetraquetra	Sedum anglicum	Lepidium subulatum	Gypsophila struthium	Retama sphaerocarpa	Frankenia thymifolia
HYMENOPTERA							
Formicidae							
Camponotus foreli				1.58 ± 1.2	4.10 ± 0.5	46.46 ± 1.7	36.50 ± 3.4
Camponotus sp.							1.17 ± 0.5
Crematogaster auberti				2.83 ± 1.2	0.07 ± 0.1		
Leptothorax fuentei				0.58 ± 0.3	+		0.25 ± 0.2
Plagiolepsis schmitzii				2.08 ± 0.7	8.00 ± 1.3		
Proformica longiseta	15.15 ± 1.2	16.50 ± 1.8	2.20 ± 0.4				
Tapinoma nigerrimum		0.10 ± 0.1					
Synphytae					0.07 ± 0.1		
Scolidae						0.10 ± 0.1	
Icneumonidae		0.05 ± 0.1					
Andrenidae					0.14 ± 0.1	2.29 ± 1.0	
Halictidae						0.62 ± 0.3	3.00 ± 0.8
Anthophoridae						0.52 ± 0.2	
Apidae		0.20 ± 0.1					
DIPTERA							
Syrphidae		0.20 ± 0.1					
Bombyliidae					17.43 ± 1.7	0.21 ± 0.3	0.08 ± 0.8
Calliphoridae		0.01 ± 0.1			0.42 ± 0.2	0.73 ± 0.3	
Muscidae	0.10 ± 0.1	0.80 ± 0.3				0.31 ± 0.2	
Cloropidae		1.95 ± 0.6					
Mycetophylidae		0.12 ± 0.1					
Nematocera n.i.				0.08 ± 0.1			0.17 ± 0.2
Diptera n.i.							0.25 ± 0.1
LEPIDOPTERA							
Lycenidae					0.14 ± 0.1	0.10 ± 0.1	
COLEOPTERA							
Nitidulidae						0.10 ± 0.1	
Cleridae						0.21 ± 0.1	
Tenebrionidae				0.25 ± 0.1		10.21 ± 1.7	
Mordellidae				38.33 ± 13	70.90 ± 5.6		0.17 ± 0.1
Dasytidae	0.20 ± 0.1	0.10 ± 0.1		0.50 ± 0.2			
Malachidae		0.17 ± 0.1		0.08 ± 0.1			
Meloidae				0.08 ± 0.1			
Curculionidae				0.17 ± 0.1			
Chrysomelidae				0.25 ± 0.2			
Bruchidae	0.05 ± 0.1						
Phalacridae	0.05 ± 0.1	0.02 ± 0.1					
HEMIPTERA							
Hemiptera n.i.	0.10 ± 0.1						

since flying insects were as effective pollinators as ants.

The main hypothesis to explain the inefficacy of ants as pollinators is the antibiotic hypothesis (see Introduction). However, *P. longiseta*, an ant visiting the flowers of many plant species growing in the high mountain of the Sierra Nevada (Gómez and Zamora 1992; Fernández Escudero 1994; Gómez 1994), pollinates *H. spinosa* (Gómez and Zamora 1992), *Alyssum purpureum*, *Arenaria tetraquetra* and *S. anglicum* (Table 4), despite the fact that it has metapleural glands producing secretions that reduce pollen viability (Gómez and Zamora 1992). On the other hand, *Camponotus foreli*, an ant species living almost exclusively on floral nectar of a high number of plant species in some Mediterranean savanna-like grasslands (Retana et al. 1987), pollinates several plant species in the aridlands of Baza, such as *R. sphaerocarpa* and *F. thymifolia*. This ant species has no metapleural glands, and this absence of metapleural glands seems to be characteristic of almost all the species in the genus *Camponotus* (Hölldobler and Engel-Siegel 1984); nevertheless, this ant genus reduces pollen viability in some plant species (Beattie et al. 1985; Hull and Beattie 1988). We have not observed any morphological trait either in ants or in the flowers which safeguards the pollen grains against the ant secretions, these grains making direct contact with the ant integument. In view of all the above, our findings suggest that the production of metapleural secretions does not determine the ant's effectiveness as a pollinator, contradicting the antibiotic hypothesis for these plant species. Another

Table 4 Results of the exclusion experiments for each plant species. Numbers represent female fertility (means ± 1 SE), expressed as the percentage of initial ovules setting mature seed in each treatment and plant species, except in *Gypsophyla struthium*, where numbers represent "seeds per inflorescence" and in *Frankenia thymifolia*, where numbers represent fruit set (see Methods). Sample size (number of plants) are given in *parentheses*; the number of labelled flowers and fruits are the first and the second figures within *brackets*, respectively. *F-test* refers to the *F*-ratio in an one-way ANOVA. Means followed by different *superscript letter* in each row are significantly different at $P < 0.05$, according to Bonferroni-Dunn's *t*-test

Species	Control	Winged-only	Ants-only	No-pollinator	*F*-test
Alyssum purpureum	17.1 ± 1.6[a] (15) [1373, 337]	15.2 ± 2.0[a] (15) [649, 257]	12.0 ± 1.8[a] (15) [1511, 207]	4.2 ± 1.7[b] (15) [952, 82]	10.09****
Arenaria tetraquetra	29.4 ± 1.7[a] (15) [7669, 319]	16.8 ± 2.6[b] (15) [3820, 296]	12.5 ± 3.2[b] (15) [5305, 286]	7.6 ± 2.0[c] (15) [3372, 130]	24.28****
Sedum anglicum	71.5 ± 2.3[a] (15) [70, 70]	40.2 ± 3.2[b] (15) [68, 68]	50.8 ± 5.3[b] (15) [50, 50]	–	13.21****
Gypsophyla struthium	06.6 ± .21[a] (10) [2302, –]	09.9 ± 3.6[a] (8) [1634, –]	00.0 ± 0.0[b] (9) [2137, –]	0.3 ± 0.3[b] (9) [1884, –]	10.97****
Lepidium subulatum	62.2 ± 3.6[a] (9) [3801, 836]	41.8 ± 5.5[b] (9) [3187, 772]	6.2 ± 4.5[c] (6) [1954, 486]	8.8 ± 3.3[c] (6) [1806, 546]	32.07****
Frankenia thymifolia	49.0 ± 8.5[a] (11) [680, 277]	36.3 ± 7.5[a] (10) [908, 408]	40.1 ± 6.8[a] (10) [568, 195]	0.1 ± 0.1[b] (10) [941, 10]	18.24****
Retama sphaerocarpa	02.1 ± 1.7[a] (8) [2426, 50]	00.6 ± 0.8[b] (8) [2266, 15]	00.6 ± 1.3[b] (8) [2293, 12]	0.0 ± 0.0[b] (8) [2495, 0]	06.02***

***$P < 0.001$; ****$P < 0.0001$

hypothesis explains the rarity of ant pollination by their small size and aptery, preventing them from carrying pollen between different plants (Rico Gray 1989; Peakall and Beattie 1991). Although we have not specifically studied the foraging behaviour and flight distance of both ants and flying insects, we believe, on the basis of prior study, that both types of pollinator move similarly among flowers as demonstrated in *Hormathophylla spinosa* (Gómez and Zamora 1992), a plant with a habit and floration similar to most of the seven plants of the present study. This can be inferred from the fact that the quantity of seeds in flowers visited only by ants was similar to that in flowers pollinated by flying insects.

An outstanding result from our field experiments is that the relative abundance of the ants in the pollinator assemblage determined their effect on seed production, ant pollination becoming evident only when ants outnumber other floral visitors. In fact, ants represented from 70 to 100% of the total flower visitors in all plants pollinated by these insects (Table 1; see also Gómez and Zamora 1992 for *H. spinosa*). Thus, ants affected plant reproductive success to a similar degree as all the remaining pollinator species. By contrast, ants represented only 12.3% of the flowers visits in *G. struthium* and 14.4% of the visits in *L. subulatum*, the two plant species which were not pollinated by ants (Table 4).

All plants highly visited by ants flowered in early summer (Table 1), when the high temperatures and low humidity characteristic of the Mediterranean climate can limit many winged pollinators (Unwin and Corbet 1991). This stressful effect is intensified in the high mountain by strong winds (Hickman 1974; Petersen 1977a, b). On the other hand, the abundance and activity of ants is higher during summer in our study sites,

both in the high mountains (Zamora 1987; Fernández Escudero 1994) and the aridlands (Hódar 1993; Sánchez-Piñero 1994), favouring the presence of these insects in flowers. For this reason *G. struthium* flowering during autumn, when nectarivorous ant abundance is low (personal observation), is visited primarily by insects other than ants. Not only flowering phenology, but also abundant flowers per plant surface, and accessible nectaries (determined partially by flower size and form), increase the probability of ant visits. In general, ants visit plants having abundant flowers close to the ground and high flowering synchrony, as in the case of *Arenaria tetraquetra* and *Alyssum purpureum* in the high mountain and *F. thymifolia* in the aridlands. However, *R. sphaerocarpa* reaches 3 m in height, but is also visited by ants, probably due to its mass flowering strategy; on the contrary, ants also visit plants with few flowers, such as *S. anglicum*, but with a clustering growth pattern and low growth habit. Given that ants may prefer flowers which are numerous, with accessible nectaries and, ideally, close to the ground, plants with these traits would have more probability of being pollinated by ants.

Finally, our experimental results support the suggestion of Hickman (1974) and Beattie (1991) that ant pollination is probable in aridlands, high mountains and other open and dry habitats, where many plant species have growth and reproductive traits (e.g. stunted habit, mass flowering) strongly determined by abiotic factors, and where ants outnumber winged insects during the flowering period. From an evolutionary point of view, all of these plant traits related to stressful abiotic environments can actually favour the maintenance of a low specialized pollination system, in which ants can play a notable role. In this ecological context, ants visiting flowers should be judged potential

pollinators until proven otherwise, as in the case of winged floral visitors. The relationship between *P. longiseta* and high-mountain plants exemplifies the importance of this kind of mutualistic interaction, since only one ant species is the primary pollinator of many alpine plants in the Sierra Nevada. In brief, the ant-pollination systems analysed in this study may be the result of prevailing ecological conditions more than an evolutionary result of a specialized interaction.

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