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The reproductive ecology of *Medicago citrina* (Font Quer) Greuter (Leguminosae): a bee-pollinated plant in Mediterranean islands where bees are absent

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Abstract. The reproductive ecology of *Medicago citrina* was studied in the Columbretes archipelago (Mediterranean Sea), where bees are absent. The flowers were self-compatible, homogamous, nectar producers, depended on large floral visitors for tripping and pollination. Several characteristics of the reproductive biology of *M. citrina* indicate an adaptation to an isolated environment characterised by scarcity of appropriate pollinators. As a result of this scarcity, fruit and seed set under natural conditions were significantly lower compared to hand-pollination treatments. The main flower visitors were mostly flies and blowflies, with *Eristalis tenax* (Syrphidae) and *Calliphora vicina* (Calliphoridae) being the most efficient pollinators. We argue that because *M. citrina* faces pollen limitation in the small islets of Columbretes, it presents an interesting case study of pollination related to island endemism and diversity.

Key words: Blowflies, endemism, hoverflies, isolation, Mediterranean islands, migratory pollinators, nectar secretion, reproductive ecology, tripping.

Introduction

The Mediterranean Region probably has more isolated plant populations than most other regions. Heterogeneous geographical barriers (e.g. high mountains, many small islands and archipelagos), extreme climates and intensive land use by man over millennia, have sculptured a diverse patchwork of ecosystems. On small Mediterranean islands, pioneer populations may originate from a few founder individuals and develop under conditions of complete geographical and genetic isolation (Blondel and Aronson 1999). Such islands provide unique natural laboratories for studying the process of evolution (Carlquist 1974). However, many islands are difficult to access and, as a consequence, the reproductive biologies of their plants and animals are poorly studied (Anderson et al. 2001).

Geographical isolation of plants on islands is often associated with pollen limitation of seed and fruit set due to small pollinating faunas or lack of adequate pollinators (McCullen 1990, Howarth and Mull 1992, Anderson et al. 2001), as well as with Allee-effects that can occur in small plant populations (Petanidou et al. 1991, 1995a, b; Oostermeijer et al. 1994, 2000). Pollinator scarcity and reduced pollinator services are often associated with high selfing-rates (Ramsey and Vaughton 1996, Fausto et al. 200l, Anderson et al. 2001, references cited therein), or a high frequency of anemophily (Berry and Calvo 1989, Anderson et al. 2001). On the other hand, small and isolated populations of predominantly outbreeding, self-compatible species may lose genetic variability by genetic drift and inbreeding (Barrett and Kohn 1991, Raijmann et al. 1994, Fischer and Matthies 1998, Menges and Dolan 1998, Young et al. 1999). This "genetic erosion", when not counteracted by gene flow may result in reduced fitness, which may lead to local extinction even if the ecological conditions are relatively suitable for the species (Ouborg et al. 1991; Treuren et al. 1991; Ouborg 1993; Oostermeijer et al. 1992, 1994, 1998; Groom 2001).

We examine the pollination ecology of *Medicago citrina*, an endangered species that is usually dependent upon bees for pollination, but is also found in areas where bees are lacking (Lesins and Lesins 1979). One such area is the volcanic archipelago of Columbretes, located *c.* 55 km east of the coast of Castellón (eastern Spain) where no permanent bee population exists (Español 1958, García Marí et al. 1991). Our aim is to describe the pollination ecology of *M. citrina* within this insular community, and identify possible adaptations to the absence of bees. Specifically, we (1) examine the reproductive ecology of *M. citrina* under natural conditions in Columbretes; (2) measure the pollination effectiveness of non-bee pollinators; and, (3) compare nectar rewards and reproductive characteristics (pollen viability, floral fecundity, selfing rate, etc.) of these island populations with their mainland con-specifics. We discuss our findings in the context of *in situ* species management and maintenance of biodiversity in isolated environments. The results presented here are part of a major study on the reproduction ecology of plants in insular ecosystems, with the aim to explore possible viability constraints associated with their isolation and to pinpoint the vulnerability and risks related to their conservation (Pérez-Bañón 2000, Juan 2002).

Material and methods

The study species and its distribution

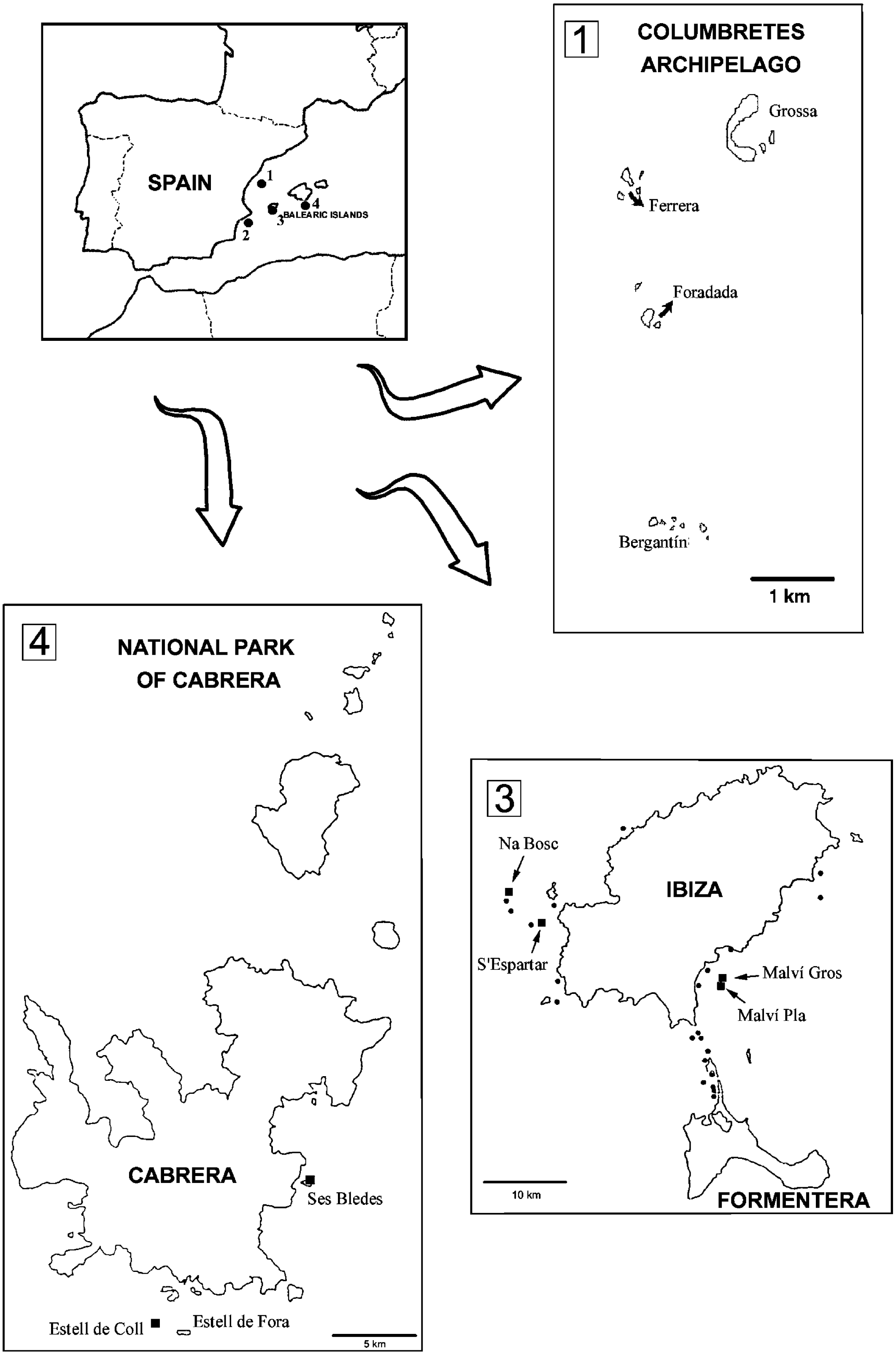
Medicago citrina (Font Quer) Greuter [≡*M. arborea* var. *citrina* Font Quer; ≡*M. arborea* subsp. *citrina* (Font Quer) O. Bolòs & Vigo], is a perennial woody leguminous shrub which grows up to 2 m in height.

Flowers have a typical papilionoid leguminous structure and are born in axillary racemes of 8–17 flowers, with hundreds of racemes on each branch. Nectaries are located at the bottom of the corolla tube. *M. citrina*, like many papilionoid leguminous flowers is dependent on a process known as "tripping" for its pollination (Free 1993). At anthesis, the style and the staminal column remain enclosed in the keel until pressed down by the weight of a specialised visiting insect (normally a medium- to large-sized bee) so that the style and staminal column quickly move out of the keel, and the flower is tripped.

The species is known only from a few Mediterranean islands of the Spanish coast, the Columbretes archipelago, some islets around Ibiza (Juan et al. 1999), around Cabrera (Bibiloni et al. 1993) within the Balearic Islands, and the small islet Escull del Cap near Alicante (Serra et al. 2001) (Fig. 1).

In the Columbretes archipelago, *M. citrina* only grows on three small islets: Grossa, Ferrera and Foradada. The establishment dates from after the volcanic events that gave rise to the archipelago,

Fig. 1. Location of the four study populations (dots) of *M. citrina*. 1. Marine Reserve of Columbretes archipelago. 2. Escull del Cap (Alicante). 3. Ibiza. 4. National Park of Cabrera. Subpopulations are marked with either solid squares or arrow heads


SPAIN
1
2
3
4
BALEARIC ISLANDS
1
COLUMBRETES ARCHIPELAGO
Grossa
Ferrera
Foradada
Bergantín
1 km
4
NATIONAL PARK OF CABRERA
CABRERA
Ses Bledes
Estell de Coll
Estell de Fora
5 km
3
Na Bosc
S'Espartar
IBIZA
Malví Gros
Malví Pla
10 km
FORMENTERA

between 1 and 0.3 mya (Aparicio et al. 1991). Most likely, these populations originated from the Balearic Islands, following bird dispersal (Juan, unpublished data). Its limited distribution (few hundreds of plants on a few tiny islands) and vulnerability of habitats have lead to *M. citrina* being listed as an endangered plant in Spain (Spanish Royal Decree 439/1990) and assigned as endangered by IUCN (Aizpuru et al. 2000).

Nomenclature of plant taxa follows Greuter et al. (1989) and Mateo and Crespo (2001).

Study site

The fieldwork was carried out from February to June in 1997 and 1998, in the Marine Reserve of Columbretes archipelago which has prevailing Mediterranean climate (mean annual temperature 18.6 °C, mean annual rainfall 315 mm). All experiments and observations were carried out during the flowering peak of the species (late February–April).

Medicago citrina is the dominant woody species in Foradada and Ferrera (Fig. 1), located mainly on the northern slopes. On Grossa, there are approximately 100 individuals introduced by man from the nearby Ferrera and Foradada, after the native population went extinct following overgrazing (rabbits) and fires. The pollination treatments, experiments and observations were conducted mainly on the island Grossa. Systematic fieldwork on the large native populations of Foradada and Ferrera was prevented by the strong winds prevailing in the archipelago during most days of the flowering period.

The dominant vegetation of Grossa island is composed of *Suaeda vera* Forssk., *Lavatera* sp., and *Lobularia maritima* (L.) Desv. subsp. *columbretensis* R. Fern. (endemic to the archipelago), together with herb communities dominated by *Euphorbia terracina* L. and *Medicago littoralis* Rohde.

Flowering characteristics

Flowering phenology of *M. citrina* was recorded in the Grossa population from February to June 1997. We counted the number of inflorescences bearing open flowers on randomly selected 4 permanent branches of 15 plants throughout the season. Counting was carried out every 3–5 days from the end of February to April. At other times counts were carried out at least once every fifteen days, when weather and sea conditions allowed visits to the archipelago.

Flower life span (= duration of anthesis) was studied on flowers selected at random and located at different positions on two plants ($N = 31$; $N = 32$, respectively). The flowers were marked at the bud stage and observed daily until they withered. Commencement of flower anthesis was defined by the opening of floral petals, when insect access to the flower was made possible.

Nectar secretion

Flowers were selected at random at different positions of the plant/branch. They were marked at the bud stage during the blooming peak and each branch was covered with a metal cage (70 cm length, 15 cm diameter) with fine gauze to prevent insect access. Nectar secretion rates were measured by emptying the whole quantity of nectar accumulated at the bottom of the flower tube with 2-μl to 10-μl calibrated "Drummond" microcapillary tubes. Nectar concentration (expressed in % w/w sucrose) was measured immediately after nectar retrieval for each flower with a "Bellingham and Stanley" pocket refractometer. Sugar content (in mg of equivalent sucrose) was calculated as: volume × (concentration/100) × nectar density (Dafni 1992).

Within day nectar secretion. Daily nectar secretion rate was studied on 1st-day flowers ($N = 47$) on several caged branches. Freshly opened flowers were emptied of their nectar at 10:00 h and sampled thereafter at *c.* 4-h intervals (14:00 and 17:00–18:00 h, hereafter called "noon" and "evening" samples, respectively). Emptying the flowers earlier than 10:00 h was not possible because dew covered the flowers every morning and diluted the nectar.

Nectar secretion in relation to flower age. To assess the nectar secretion pattern during flower anthesis, we sampled the nectar from the first day of opening until withering (N = 121, 64, 44, 35, 14, 15, 20 flowers for 1st to 7th day -the last possible day of flower anthesis-, respectively). Sampling was carried out on the caged flowers once per day, between 10:00 and 11:00 h.

Pollen viability

The effect of flower age on pollen viability was examined using flowers enclosed at the bud stage in metal cages covered with fine gauze ($N = 42$). Over the following seven days, all opening flowers from three plants were marked each day with a different colour of waterproof paint. On the seventh day three flowers of each age were collected on separate Petri dishes. Flowers were artificially tripped by exerting a slight pressure on the keel, and the exposed pollen was checked for viability. Forty-two samples were tested (7 days × 3 flowers × 2 samples per flower).

To assess the effect of pollen exposure on its viability, 1^{st}-day flowers from three plants were collected in a Petri dish ($N = 21$), and then artificially tripped. Pollen was sampled from anthers from three flowers daily for the next seven days (at *c*. 10:00 h) and tested for viability.

Pollen viability was assessed using a germinability test. Preliminary tests with different sucrose solutions, showed that a 30% solution was the most appropriate for the germination of *M. citrina* pollen; this solution was used for the preparation of Brewbaker-Kwack medium. Under a dissecting microscope, each pollen sample (*c*. 500 grains) was mixed well with a droplet of Brewbaker-Kwack medium (Kearns and Inouye 1993). The droplets (= samples) were left hanging from the lid of a Petri dish at room temperature and after 48 h, germination was stopped by freezing (Dafni 1992). Each sample was transferred onto a slide, permanently mounted and examined under a microscope. Only the pollen grains that germinated successfully and produced tubes were considered viable. Pollen viability was expressed as the percentage of germinating pollen grains.

Reproductive success after different pollination treatments

We conducted the following pollination treatments: spontaneous self-pollination, manual self- and cross-pollination, and simple tripping without further manual pollination. Insects were excluded from flowers with metal cages (70 cm length, 15 cm diameter) covered with fine gauze, which enclosed whole branches. The cages remained closed after the pollination treatments were applied until the beginning of fruit set. Two sets of treatments were carried out, one at the beginning (late February) and the other at the end (mid April) of the 1998 flowering peak.

The results from only 5 plants were used, out of the 15 originally enclosed, because the cages on the remaining 10 were destroyed by bad weather. All flowers used were selected at random at different positions of the plants and branches.

Spontaneous self-pollination: The flowers and inflorescences at the bud stage were colour-marked and enclosed in metal cages to be left undisturbed until fruit harvest ($N = 234$).

Hand self-pollination: Flower buds were caged and marked, and then tripped and self-pollinated by hand on the first day they opened ($N = 100$). Self-pollination was carried out by removing anthers of other 1^{st}-day flowers of the same plant with a pair of fine forceps and rubbing them gently over the stigma of the 1^{st}-day flowers to be treated.

Hand cross-pollination: A total of 107 flower buds were covered and marked as above. The 1^{st}-day flowers were tripped and cross-pollinated manually by rubbing newly opened floral anthers of 1^{st}-day flowers of neighbouring plants, over the stigmas of the study plant.

Hand-pollination of emasculated flowers: These treatments allowed us to reduce the risk of unwanted self pollen transfer and self-pollination during the hand-pollinations described above, and hence check for existing differences between hand self- and cross-pollinations. The treated flowers were carefully emasculated at the bud stage, when corolla length was equal to calyx length. All the branches were covered as described above. Emasculated flowers were either self-pollinated with fresh pollen from flowers of the same plant ($N = 64$), or cross-pollinated with fresh pollen from neighbouring plants ($N = 49$).

Pollination after tripping: A total of 173 flower buds were bagged, marked and tripped during their first day of anthesis. The tripped flowers were left untouched until harvest inside metal cages covering the whole branches.

To determine whether tripping was essential for pollination (and subsequent fruit set), all the flowers used to measure flower life span ($N = 63$) were followed until fruit set. We assessed the percentage of fruit set for flowers that had been tripped naturally.

Tripping rate under natural conditions was estimated using 351 flowers marked at the bud stage and left untouched. The number of flowers tripped naturally by any means, e.g. flower visitors, wind or high temperatures (Free 1993), was counted at the stage of withering.

Natural pollination: Natural pollination success was measured using flowers ($N = 2735$) marked at the bud stage from 4 plants and left uncaged to be freely visited by insects.

Reproductive success and flower age

A second set of experimental treatments to investigate fecundity in relation to flower age was carried out at the beginning and the end of the flowering season, using 152 and 237 flowers, respectively. The flowers were caged at the bud stage, and were marked as soon as they opened, which resulted in a continuum of flowers that had been open for different lengths at time (1–7 days). On the seventh day all marked flowers were artificially tripped and self-pollinated by hand.

In all the above treatments, fruits were harvested when fully ripe approximately 2–3 months after pollination treatments. After harvesting, seeds were counted and were of two types: badly formed (shrivelled or empty) or full, normal seeds. The first category was considered unfertilised and/or aborted, and the second as viable. The reproductive success of these flowers was estimated as fruit set (percentage of fruits over the total number of flowers marked), and seed set (number of seeds/fruit).

Flower visitors

Observations of flower visitors were carried out during both years of the study. Systematic surveys (insect visitor counts) were performed in 1997 by two observers recording the number of insects visiting the flowers of three mature plants on a 2 × 2 m permanent plot for 15-min periods. The counts were conducted twice per day, during the morning (between 9:00 and 11:00 h) and the early afternoon (14:30–16:00 h) for 22 days during the flowering peak (27 February–22 March). The pollination efficiency of flower visitors was assessed by measuring both pollen transfer by different insect species and fruit and seed set resulting from their visits.

Pollen loads on insects: Pollen (con- and heterospecific) adhering to the insect body of the main flower visiting species was collected and examined. The loads were sampled from insects captured after they had just visited a flower of *M. citrina* in the vicinity of the study plot during peak flowering. Immediately after capture, the insects were killed and the pollen from all body parts was removed with a sticky piece of fuchsin gel (Beattie 1972). The pollen was counted under a light microscope and identified by using reference material from the field. Microphotographs of non examined insects, taken with a scanning electron microscope (SEM; JEOL 840, at 20 kV), illustrated where *M. citrina* pollen adhered on the insect.

To check for the dependence of insects on *M. citrina* pollen, a series of gut content analyses was carried out. After pollen removal from the external body, the insects were dissected under a microscope and pollen from different parts of the gut was collected (Gilbert 1993). The samples were mounted and stained with a sticky piece of fuchsin gel (Beattie 1972) and the pollen grains identified and counted as above. The number of individuals examined varied among species ($N = 15$–20). We examined the hoverflies *Eristalis tenax* (Linnaeus 1758) and *Eupeodes corollae* (Fabricius 1794), and the blowflies *Calliphora vicina* Robineau-Desvoidy, 1830 and *Lucilia sericata* (Meigen 1826). The total number of pollen grains counted per sample varied between 200–9000.

Fruit and seed set resulting from floral visits by different insects: Assuming that caged pollinators may not show the same levels of efficiency as those in natural conditions and may act atypically as either making few visits because they are disturbed or visiting flowers by necessity, we carried out the following experiment in order to assess the pollination efficiency of the flower visitors. Insects were enclosed in metal cages, like the ones described previously. When in anthesis, 1–2 insects of *Eristalis tenax*, *Eupeodes corollae*, *Calliphora vicina* or *Lucilia sericata* were kept in each cage until all flowers withered (number of flowers caged in 1997: $N_{E.\ tenax} = 125$, $N_{E.\ corollae} = 52$, $N_{C.\ vicina} = 219$, $N_{L.\ sericata} = 132$; in 1998: $N_{E.\ tenax} = 165$, $N_{E.\ corollae = 97,\ NC.\ vicina} = 119$, $N_{L.\ sericata} = 103$). Considering that the variability in flower numbers in the cages may bias the pollination efficiency measurements, we decided to consider not only the fruit/seed set, but also the tripping rates caused by the respective insects. These results would be interesting only in the case that fruit/seed set in all cages were at the same time non proportional to flower numbers and tripping rates. As, indeed, this assumption was met (they were not proportional: see Results section and Fig. 10), the efficiency results were considered. When necessary, dead insects were replaced with living insects. Pollination effectiveness was measured as percentage of fruit set in 1997 and 1998 and as seed set in 1998.

The transient character of some flower-visiting insects in the Columbretes archipelago was indicated by their irregular presence relative to other more constant floral visitors. This was assessed by regular counts of *Eristalis tenax* and *Calliphora vicina* visiting the flowers of the dominant plants *M. citrina* and *Daucus gingidium* L. on Grossa island (late February–mid April and May–mid June 1997, respectively). The counts were made by two observers on a daily basis from 10:00 to 11:00 h by observing insects visiting the flowers of either species in a randomised transect.

Data analysis

Prior to statistical analysis, the data were tested for normality using Kolmogorov-Smirnov one-sample tests. In several cases the data were not normally distributed, and therefore we applied non-parametric tests (Sokal and Rohlf 1981). Variation in reproductive success with pollination treatments was tested by Kruskal–Wallis ANOVA (K–S) followed by separate Mann–Whitney U-tests (M–W) after application of the Bonferroni correction. Percentages obtained by pollen germination treatments for tripped and non-tripped flowers were evaluated using *G*-tests. Throughout the paper, the means and standard errors are given.

Results

Flowering phenology and anthesis characteristics

The flowering period of *M. citrina* was long, lasting from the end of January until the end of May, with a peak from mid February to mid April. The flowers are homogamous and the anthers ripened about 2 days before anthesis, when the corolla was about twice as long as the calyx. Flower life span of non-tripped flowers was significantly longer than that of tripped ones (8.0 ± 0.2 days, $N = 27$ and 5.7 ± 0.4 days, $N = 25$; M–W $U_{[27,25]} = 114.5$ $P < 0.000$). Post-tripped flowers remained attractive with a brilliant lemon yellow colour and turgent corolla for 1.9 ± 0.2 days ($N = 15$), after which they showed a loss of floral colour and turgor.

Nectar secretion

Daily pattern. Nectar of 1st-day flowers was mainly secreted in the morning (Fig. 2). Volume followed the same pattern as sugar content. Morning volume and sugar content were significantly higher than in the afternoon (volume: $0.0 < U < 16.0$, $P < 0.0002$; sugar content: $0.0 < U < 37.0$, $P < 0.0051$).

Nectar secretion in relation to flower age. Nectar was secreted throughout anthesis (Fig. 3). The highest nectar secretion was recorded during the first three days, with a peak on the second day, although it did not differ significantly from the nectar secreted on the first and the third day (M–W *U* tests with Bonferroni corrections: $P > 0.05$). A moderate amount was secreted during the fourth day, thereafter followed by a dramatic decrease. These values were significantly lower than those of the first three days (M–W *U* tests, $P < 0.05$).

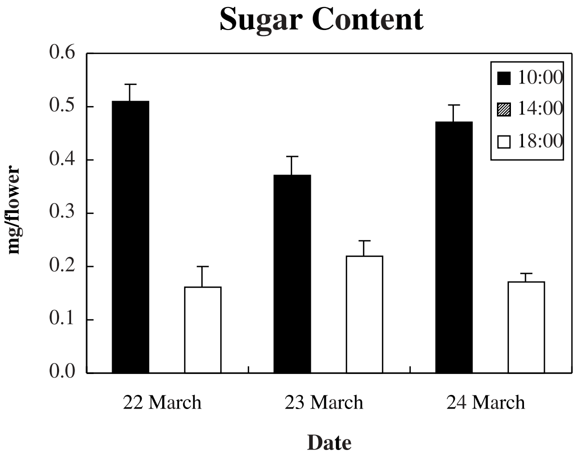


Fig. 2. Daily nectar secretion pattern of 1st-day flowers of *M. citrina*. Samples were taken three times per day over three consecutive days ($N_{22\ \text{March}} = 14$; $N_{23\ \text{March}} = 14$; $N_{24\ \text{March}} = 19$)

Pollen viability

The overall pollen viability differences between tripped and non-tripped flowers were not significant (G = 9.627, $P > 0.05$; Fig. 4). However, viability of tripped flowers dropped dramatically after the second day of anthesis, whereas that of the non-tripped flowers remained high until the fifth day.

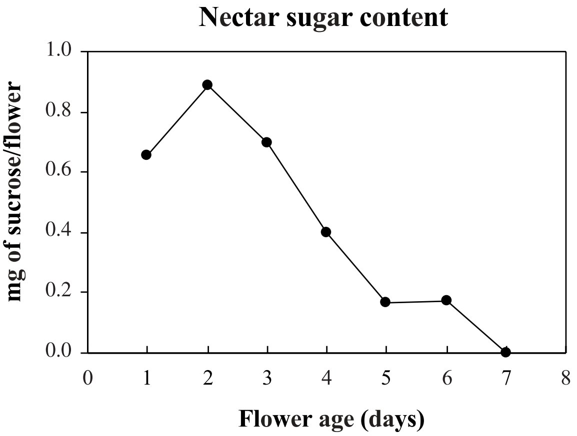


Fig. 3. Nectar secretion pattern of virgin, non-tripped flowers of *M. citrina*. We present the sugar content secreted daily throughout the whole flower anthesis (N = 121, 64, 44, 35, 14, 15, 20 flowers for 1st to 7th day, respectively)

Reproductive success of M. citrina

From February to April the flowers remained fecund from the first to the sixth day of anthesis, whereas no seeds were produced when flowers were pollinated from the seventh day onwards (Fig. 5). In both periods fecundity, calculated as seed set, steadily decreased from the second day onwards, showing a strong dependence on flower-age (K–W $H_{[5,\ 152]} = 20.3815$, $P < 0.0011$; K–W $H_{[6,\ 237]} = 29.3643$, $P < 0.0001$, for February and April, respectively). In both cases, flowers that had been tripped and pollinated during the first day of anthesis yielded the highest seed set, compared to those tripped and pollinated later. However, after the Bonferroni correction was applied most of these differences were found to be non-significant. There was no difference in floral fecundity between the two periods studied ($P > 0.05$).

There were no significant differences between seed sets resulting after the treatments of February and April (M–W *U* tests; $P > 0.05$). Seed set after different pollination treatments carried out in April varied greatly (Fig. 6; K–W $H_{[4,\ 1414]} = 488.0$, $P < 0.000$ for the total number of seeds; K–W $H_{[4,\ 1414]} = 492.4$, $P < 0.000$ for viable number of seeds). Manual self- and cross-pollination yielded a greater

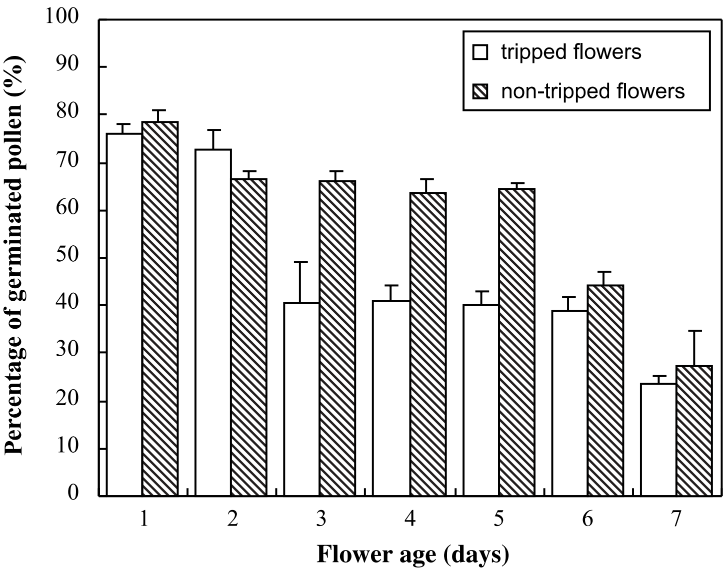


Fig. 4. Pollen viability expressed as percentage of germinated pollen grains throughout the whole life span of non-tripped and tripped flowers of *M. citrina* (3 flowers and 6 samples for each day and case). Standard errors are given. Pollen in tripped flowers was continuously exposed, whereas it was protected in non-tripped flowers

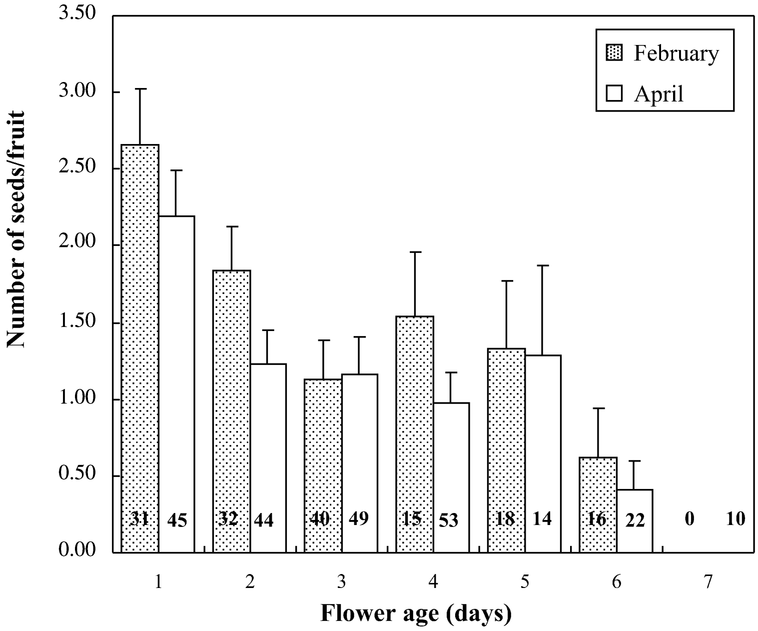


Fig. 5. Reproductive success of *M. citrina* after synchronous tripping and self-pollination of flowers of different ages in February and April. We give the mean number of seeds per fruit (columns), the sample number and the standard error (bars)

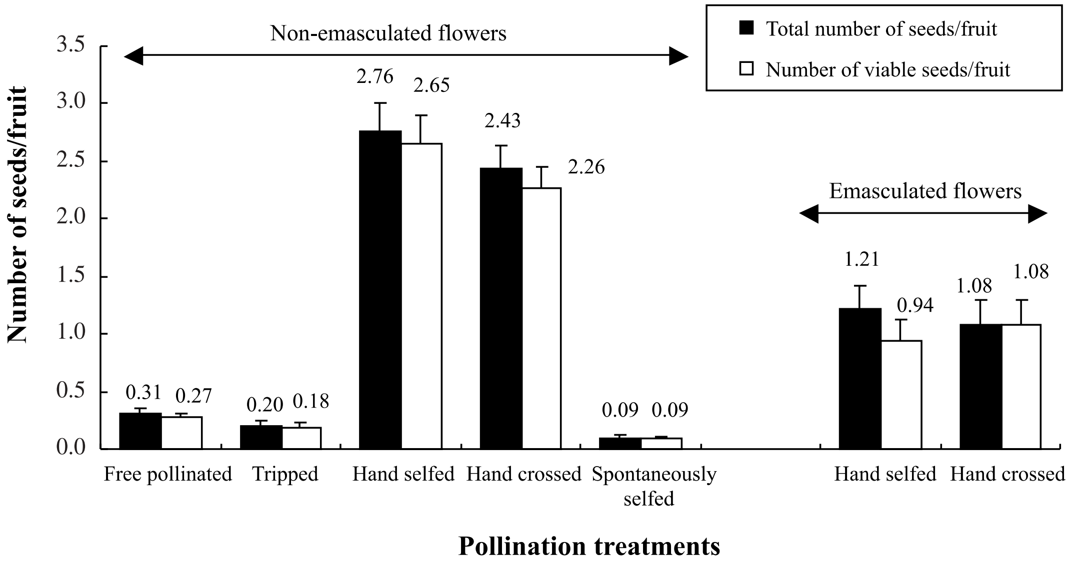


Fig. 6. Reproductive success of *M. citrina* for all pollination treatments. Columns represent the mean number of seeds per fruit (total = viable + aborted, and only viable). Percent seed set is given at the top of each column. Bars are standard error

number of seeds (total or viable) than any of the other treatments (M–W *U* tests with Bonferroni corrections, $P < 0.000$). No significant difference in the number of seeds was found between free-pollinated and simply tripped flowers, i.e. without intentional pollen transfer (M–W *U* test, $P > 0.05$). Spontaneous self-pollination resulted in a much lower success than free-pollination or simple tripping ($P < 0.05$). Percent fruit set showed the same pattern: 13.6% for naturally-pollinated, 10.4% for simply tripped, 72.5% for hand-selfed, 70.1% for hand-crossed and 4.2% for spontaneously-selfed flowers. It is noteworthy that fruit set after natural-pollination was only 13.6%, equivalent to a small fraction of the flowers that were freely tripped under natural conditions (45.3%). No fruit was produced among the untripped flowers ($N = 63$).

There were no differences in seed set (total or viable) between hand self- and cross-pollinated flowers (Fig. 6; M–W *U* tests, $P > 0.05$). Similar results were obtained for flowers that had been originally emasculated in order to control for pollen source ($P > 0.05$).

Flower visitors and their efficiency

Our investigation of the existing pollinator pool in the archipelago confirmed the absence of permanent bee-colonies from Columbretes. *Medicago citrina* flowers were mainly visited by flies, mostly Calliphoridae (89%) and Syrphidae (11%). *Calliphora vicina* was the most dominant visitor among blowflies (86.5%), whereas the frequency of *Lucilia sericata* was much lower (2.5%). Six hoverfly species visited *M. citrina*, but visited flowers less frequently than *C. vicina*. Most frequent were *Eupeodes corollae* (5.6%) and *Eristalis tenax* (4.5%), whereas *Episyrphus balteatus*, *Meliscaeva auricollis* and *Scaeva pyrastri* were observed only occasionally on the flowers (0.9%). In fact, our long-term observations carried out on a daily basis demonstrated that some of the existing hoverfly populations in the archipelago are very irregular flower visitors. Such insects are *E. tenax* and other hoverflies that may not appear to the flowers for a couple of consecutive days (Fig. 7). Considering their anthophilous character, this irregularity may imply that the populations of these insects are so variable in the archipelago that it can be explained only by their transient habit. All the observed species visited flowers mainly during the morning (9:00–11:00 h) when dew covering the flowers started to evaporate, and the volume and sugar content of nectar was high (Figs. 2, 8). The rate of visits decreased dramatically early in the afternoon, when the flies shifted to flowers of other species, mainly *Lobularia maritima* and *Euphorbia terracina*.

Among the other occasional visitors, the migratory butterfly *Vanessa cardui* (Linnaeus 1758) and the migratory hawkmoth *Macroglossum stellatarum* (Linnaeus 1758) visited the flowers for nectar, but very rarely tripped them.

Analysis of gut contents showed that calliphorids contained almost no pollen grains in their guts. Syrphids, on the other hand, were not great pollen consumers of *M. citrina*, and the two main syrphid visitors, *Eristalis tenax* and *Eupeodes corollae*, had only 2.6% and 1.0%, respectively, of *M. citrina* pollen in their guts. The syrphids were probably visiting *M. citrina* flowers mainly for nectar and *L. maritima* flowers for pollen as indicated by

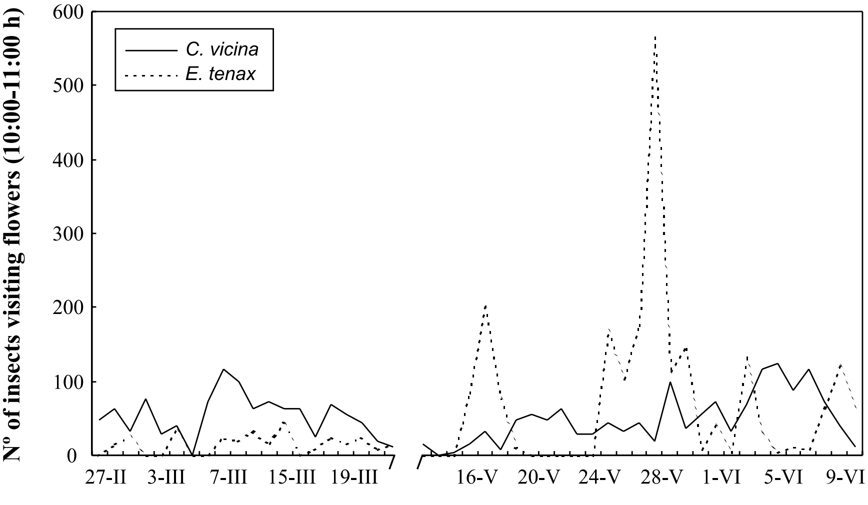


Fig. 7. Daily counts of *Calliphora vicina* and *Eristalis tenax* visiting the flowers of *M. citrina* and *Daucus gingidium* on the island Grossa in the Columbretes archipelago throughout the flowering season of 1997. Values are the numbers of insects visiting flowers in a transect between 10:00–11:00h

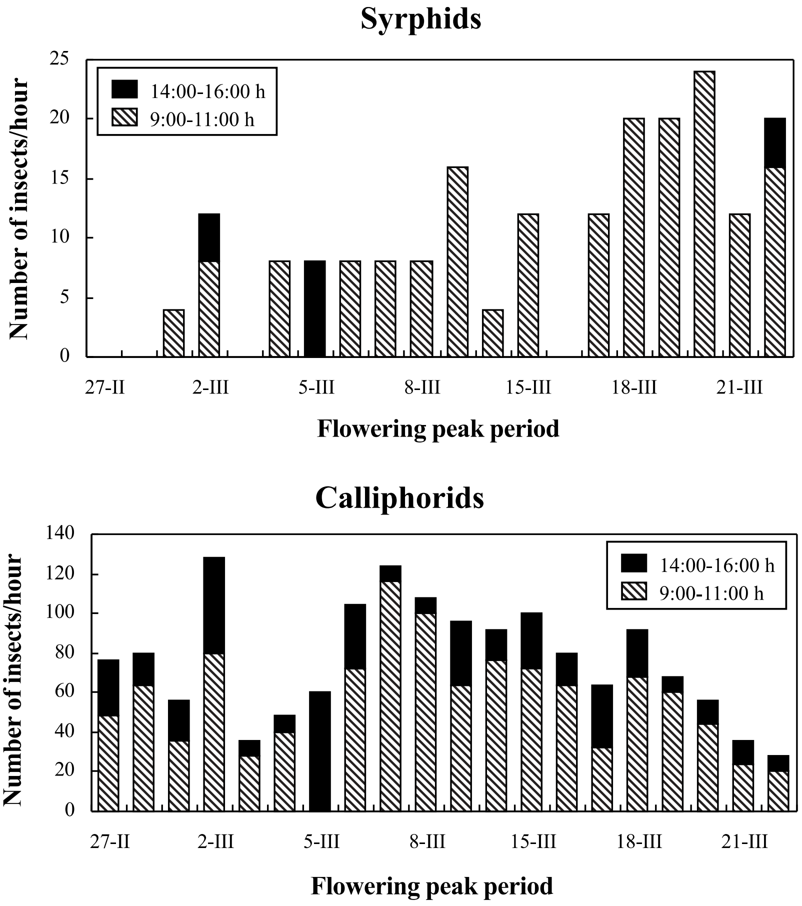


Fig. 8. Visitation rates of the two main insect groups recorded in the permanent plot during the flowering peak (27 February–22 March 1997) in the morning (9:00–11:00) and early in the afternoon (14.00–16.00)

the gut content analyses (*E. tenax*: 97.4%; *E. corollae*: 99.0%).

Examination of the pollen that adhered to the bodies of the insects visiting *Medicago* flowers, showed that *E. tenax* bore the highest absolute number and percentage, followed by calliphorids (Table 1). The microphotographs show that the largest quantities of *M. citrina* pollen were located on the ventral part of the thorax of *E. tenax* (Fig. 9). This implies that pollen transfer and deposition occurred sternotribically, as is the case for *M. sativa* (Small et al. 1987).

Enclosure experiments used to assess the pollination efficiency of flower visitors showed that the highest seed sets were produced by *E. tenax* and *C. vicina*. *E. tenax* was more efficient for setting seed than *C. vicina* (M–W *U* tests: $P < 0.05$). In all cases, seed set and seed production were significantly higher than spontaneous pollination (Fig. 10; M-W *U* tests based on the viable number of seeds: $P < 0.001$). Similar results were obtained from fruit sets from enclosure experiments carried out in 1997 and 1998 (Fig. 11): fruit set was highest in the case of *E. tenax*, followed by *C. vicina*, *E. corollae*, and *L. sericata*. The tripping rates affected by the insects inside the enclosures were as follows: *E. tenax* (66.4% and 55.8% for 1997 and 1998, respectively), *C. vicina* (50.2%; 54.6%), *L. sericata* (17.2%; 13.6%), *E. corollae* (14.6%; 11.3%). Spontaneous (automatic) tripping rate was 19.8% in 1997 and 16.5% in 1998.

Discussion

Breeding system of M. citrina

Medicago citrina is a fully self-compatible species bearing homogamous but non-autoga-

Table 1. Quantitative and qualitative analysis of the pollen load adhered on the bodies of the main flower visitors of *M. citrina* during the flowering peak. The insects were captured in flight, after having visited a *M. citrina* flower. The other plant species are *Lobularia maritima* subsp. *columbretensis*, *Suaeda vera*, *Sonchus tenerrimus*, and *Phagnalon saxatile*

	Total number of pollen grains				Plant species (% of the total)	
	Mean	SE	Range	n	*M. citrina*	Main other species
Calliphoridae						
Calliphora vicina	178.0	31.5	21–418	20	12.7	82.3
Lucilia sericata	328.0	136.0	24–1264	20	2.8	78.6
Syrphidae						
Eristalis tenax	715.0	291.0	96–1500	15	18.2	75.6
Eupeodes corollae	85.1	52.9	10–606	18	2.1	88.7

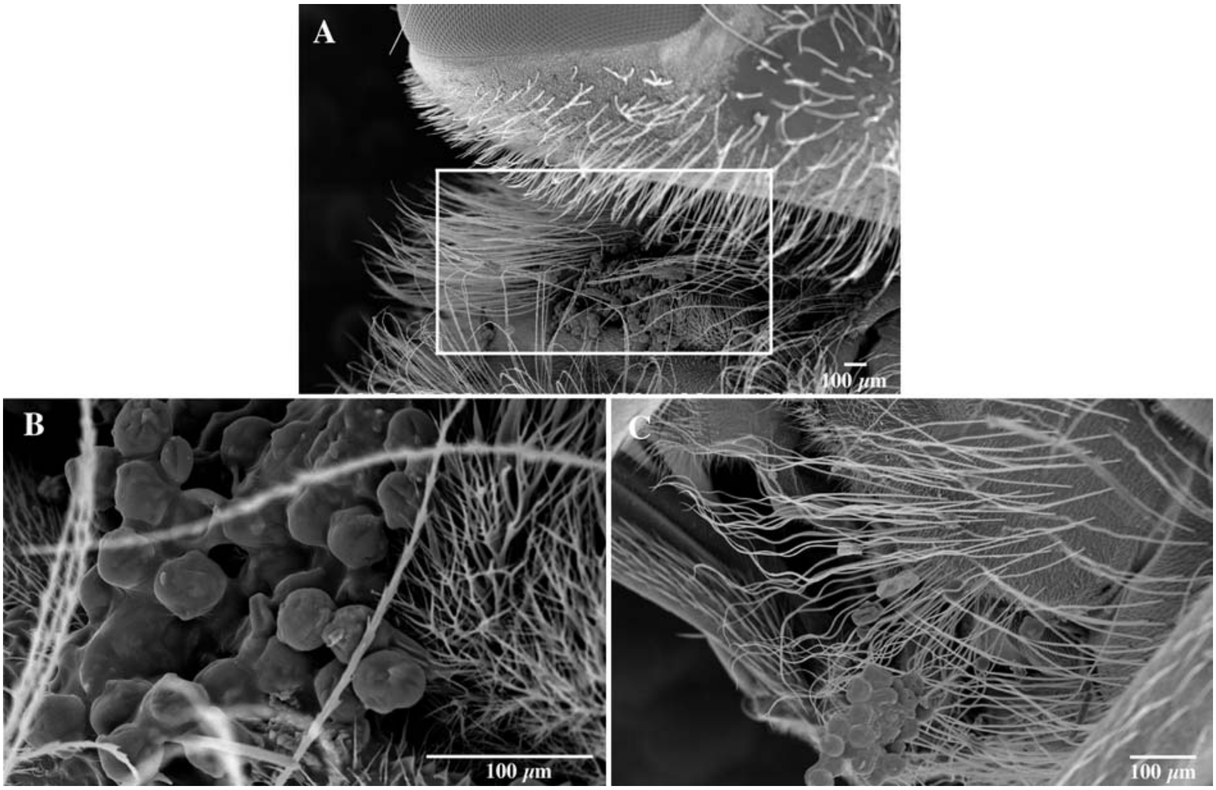


Fig. 9. Thorax images of *Eristalis tenax*. **A** Ventral part of the thorax anterior half, where staminal column of *M. citrina* strikes during tripping; **B** and **C** Pollen load of *M. citrina* adhered to thorax tegument

mous flowers. Based on experimental evidence presented here, we conclude that even though the Columbretes archipelago is isolated, the pollination of *M. citrina* depends entirely on external pollination agents. Pollinators are necessary to set off the flower's tripping mechanism to allow the transfer of self or cross pollen for seed set. Indeed, our results show that both hand-pollination treatments (self and cross-pollination) led to higher seed

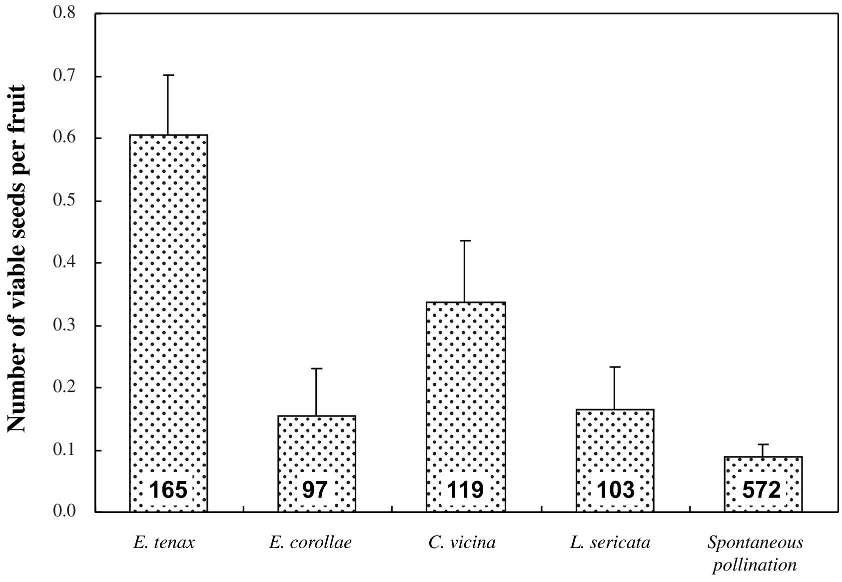


Fig. 10. Reproductive success of *M. citrina* in experimental enclosures with different insects in 1998. Each column gives the mean number of viable seeds per fruit as a result of the pollinating activity of 1–2 individual insects in the enclosure. Sample sizes and standard errors are given

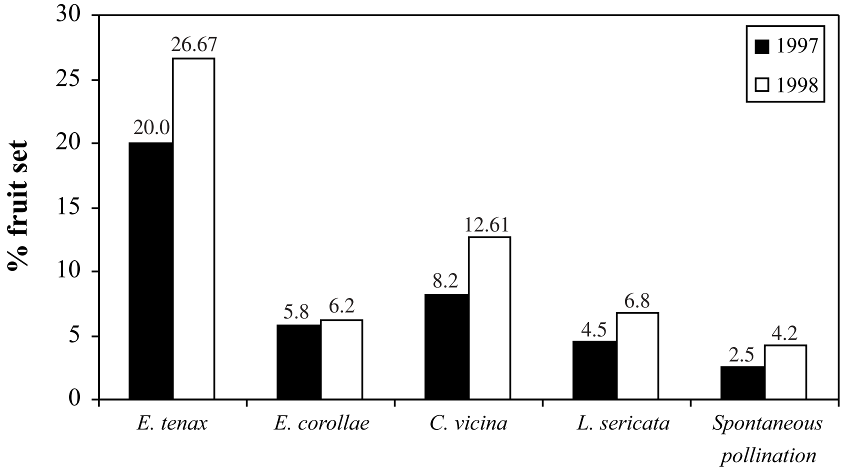


Fig. 11. Percentage of fruit set of *M. citrina* due to the different insects used in the enclosure experiments during the years 1997 and 1998

set than either simple tripping or spontaneous selfing. This difference can be explained by the very low rates of auto-tripping (<20% observed) and self-deposition of pollen on the stigma when tripping is not the result of insect visits (Fig. 6). Tripping is a predominant mechanism for the reproduction of *M. citrina*, as it is probably for all perennial *Medicago* species (Lesins and Lesins 1979, Free 1993). This phenomenon in *M. citrina* is almost entirely the result of insect visits and, very rarely, caused by other factors, such as temperature and humidity (Juan et al. unpubl. data). Interestingly, our results indicate that tripping may be caused by agents other than insects (as previously mentioned by wind or high temperatures), that might enhance natural pollination and seed set.

To ensure reproductive success, both tripping and active pollen transfer are required. This is illustrated by the extremely low seed set after natural pollination (45.3% tripping rate) compared with tripping by hand (100% tripping rate) and hand-pollination (Fig. 6). These results imply that the species either suffers from pollen limitation due to low insect visitation rate (Syrphidae and Calliphoridae) or inefficient pollen transfer, or from interference from heterospecific pollen adhering to the bodies of visiting insects (Table 1). Such interference may come from pollen from the abundantly co-flowering *Lobularia maritima* subsp. *columbretensis*, whose pollen was often found on the pollinator's body and therefore effectively competes *M. citrina* pollen on the floral stigma (Waser 1978, Petanidou et al. 1995a) or may simply clog the floral stigma.

Medicago citrina starts flowering early and extends throughout most of the season, which is unusual under Mediterranean conditions (Petanidou et al. 1995c). The extremely long flowering period is combined with a long flower life span, even in flowers that have been tripped since their first day of anthesis (8.0 days vs 5.7 in tripped flowers). This span is much higher than that recorded in similar species in other Mediterranean communities, which usually range between 2.3 and 3.2 days (Petanidou 1991). The long flower life span may indicate a lack of appropriate pollinators in the community. A further response to the absence of its usual pollinators (bees) is the pattern of nectar secretion. Nectar is secreted mainly early in the morning, a period when large-sized thermoregulating bees can forage, and less in the evening and minimally during the noon period (Heinrich 1993, Stone 1994). The flowers are more attractive during the first three days of anthesis, the period of the maximal nectar secretion, coinciding with the period of the highest potential floral fecundity. Indeed, our results show that floral fecundity is maximal during the first day of anthesis, whereas it decreases steadily from the second day onwards. The same holds true for pollen viability that is highest during the first and second day of anthesis. However, in non-tripped flowers pollen maintains relatively high viability for 5 days, whereas in tripped ones (i.e. exposed since the first day of anthesis) the viability drops dramatically after the second day. All the above results indicate a likely pollination partnership in which large efficient bees may play a major role, as have been also observed in all *M. citrina* populations of the Balearic Isles (Juan 2002). This is also indicated by the morphological characteristics of *M. citrina* which exhibits classic characteristics of many bee-flowers (i.e. vivid yellow, zygomorphic-papilionoid) which require obligatory tripping for their pollination (Fig. 6).

Flower visitors and efficient pollinators

Columbretes have been reported as islands without bee permanent populations (Español 1958, García Marí et al. 1991), which was consistent with our surveys during the flowering seasons of 1996–1998. The occurrence of bees on the islands is very unusual and corresponds to no more than a few wind-drifted individuals per year. The absence of bees may have an enormous impact on the pollination ecology and reproductive success of the native *M. citrina*, which appears to be a predominantly bee-pollinated species.

The flower visitors of *M. citrina* in the Columbretes archipelago were almost exclusively flies. Blowflies visited the flowers only for nectar, whereas hoverflies also visited the flowers, but for pollen. In both groups, insect visitation coincided with the high rates of nectar secretion in the morning, whereas fewer individuals visited the flowers during the second phase of nectar secretion (18:00 h) which had a lower rate.

Based on results for the *M. citrina* pollen transfer on insect bodies (Table 1), the effectuated tripping rates compared to spontaneous tripping rates (50–66% vs. 16–20%), and the high seed set in the enclosure experiments (Fig. 10), it is concluded that the most efficient pollinators of *M. citrina* flowers in Columbretes were *Eristalis tenax* and *Calliphora vicina*. *Eupeodes corollae* and *L. sericata* were less frequent trippers and showed extremely low pollinator efficiencies when compared to spontaneous pollination. Based on field data (surveys and enclosure experiments), we suggest that *E. tenax* and *C. vicina* are the usual tripping agents because of their size and proboscis length (5–7 and 4–5 mm, respectively, compared to 2–3 mm of *L. sericata* and *E. corollae*). This morphological character allows them to reach the nectar accumulated at the bottom of the corolla tube and agitate the auricles of the wing petals, and therefore initiate the tripping process.

An evaluation of flower visitors, based on an analysis of pollen transfer by insects, percentage of effectuated tripping and reproductive success in enclosures, indicates that the pollen vector of the highest relative importance was *E. tenax*. This efficiency as a pollinator may be enhanced by the presence of many palynophilic hairs on the insect body. According to Holloway (1976), direct pollen feeding in *E. tenax* has been supplemented and probably replaced by a pollen-collecting system operating as the fly collects nectar. According to the same author, the effectiveness of *E. tenax* is increased by the fact that, during the tripping process, the pollen grains are mainly widespread on the anterior half of the ventral surface of the thorax, where they can remain for several days. However, the efficiency of *E. tenax* in absolute terms is still quite low to be truly considered an efficient pollinator of *M. citrina* flowers.

Absence of well adapted pollinators and consequent low pollinator efficiency is not the only problem *M. citrina* faces with respect to its reproduction in Columbretes. In addition, the major pollinator *E. tenax*, is a migratory species and an accidental visitor to the archipelago (Fig. 7; Aubert and Goeldlin de Tiefenau 1976, 1981; Aubert and Jaccard 1981). Our 2-year data show some fluctuations of *E. tenax* populations during the flowering period implying that the populations are not permanent in Columbretes, and probably originating from the adjacent coastal populations. The presence of the necrophagous blowfly *Calliphora vicina*, which is of marginal importance for the reproduction of *M. citrina*, depends mainly on the availability of bird carcasses, as well as the human activities on the islands.

Our results show that the reproduction of *M. citrina* in Columbretes is mainly based on autogamy. This holds true even in cases when floral tripping is carried out by casual pollinators (*E. tenax* and *C. vicina*), although it is difficult to ascertain the extent of self- or cross-pollination. According to our experience and the resulting data (Fig. 6), during the tripping process some self pollen is deposited on the stigma either autonomously or by insects. The high degree of inbreeding is also supported by pollen loads on the flies, which consist of very low percentages of *M. citrina* pollen.

Conservation biology and management

On the Grossa islet, *M. citrina* has been facing serious problems of survival due to non-sustainable management in the past, but nowadays those problems have disappeared. On Foradada and Ferrera, the plants suffer from "outbreaks of herbivores". For example, during the period 1995–98 a severe attack by *Icerya purchasi* Maskell, 1878 (Hemiptera, Margarodidae) destroyed *c.* 44% of the flowering individuals of Foradada (Sánchez Codoñer, pers. comm.).

Columbretes constitute an environment where bee pollinators are virtually absent, and, as a result, the plants are pollen limited. Although the lack of bees can be perceived as a deficit of the island community, when all of the populations within the greater archipelago are considered, it appears that the populations are still viable. The natural absence of bees may be a common intrinsic phenomenon to the island evolutionary system, which may constitute a trigger factor for local adaptations and high endemic rates (Brown and Lomolino 1998, Anderson et al. 2001).

Regarding the observed plant-pollinator partnership under the existing isolation constraints, the migratory hoverfly *E. tenax*, and the blowfly *C. vicina* are very important in providing pollination services, hence supporting the persistence of *M. citrina* in the archipelago. Such casual relationships of "casual" pollinator agents with small isolated populations in tiny Mediterranean islands are worthy of systematic study. For instance, it would be interesting to know to what extent plant characteristics like endemism, rarity or population size are related to these fragile, informal plant-pollinator partnerships like that of *M. citrina*. Perhaps in the future, biogeographers should draw more attention to these informal cases of island pollination, focusing on aspects illustrating the subtle pollinating territories (e.g. migratory paths and strips), which have evolved and maintained to date the splendid Mediterranean nature garden.

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