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### Is the occluded corolla of *Antirrhinum* bee-specialized?

Pablo Vargas<sup>a</sup>; Concepción Ornosa<sup>b</sup>; Francisco Javier Ortiz-Sánchez<sup>c</sup>; Juan Arroyo<sup>d</sup>

<sup>a</sup> Real Jardín Botánico de Madrid, 28014-Madrid, Spain <sup>b</sup> Departamento de Zoología y Antropología Física, Universidad Complutense de Madrid, José, Madrid, Spain <sup>c</sup> Grupo de Investigación "Transferencia de I+D en el Área de Recursos Naturales", Universidad de Almería, Almería, Spain <sup>d</sup> Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Sevilla, Spain

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## Is the occluded corolla of *Antirrhinum* bee-specialized?

Pablo Vargas<sup>a\*</sup>, Concepción Ornos<sup>b</sup>, Francisco Javier Ortiz-Sánchez<sup>c</sup> and Juan Arroyo<sup>d</sup>

<sup>a</sup>Real Jardín Botánico de Madrid, CSIC, Plaza de Murillo 2, 28014-Madrid, Spain;

<sup>b</sup>Departamento de Zoología y Antropología Física, Universidad Complutense de Madrid, Jose' Antonio Nováis 2, 28040 Madrid, Spain; <sup>c</sup>Grupo de Investigación "Transferencia de I+D en el Área de Recursos Naturales", Universidad de Almería, E-04120 La Cañada, Almería, Spain;

<sup>d</sup>Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Apartado 1095, E-41080 Sevilla, Spain

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This paper provides the first results of pollinator fauna associated to the reproductive biology of *Antirrhinum charidemi*, *Antirrhinum graniticum* and *Antirrhinum braun-blanquetii*. Censuses of over 16 hours spanning the flowering phenology of each species were drawn up. As previously hypothesized, the characteristic occluded (personate) corolla of *Antirrhinum* was exclusively pollinated by bees, but by a higher number (four) of bee families than predicted. Seven bee species (*Bombus hortorum*, *Anthidium manicatum*, *Chalicodoma lefebvrei*, *Anthidium sticticum*, *Anthophora dispar*, *Xylocopa violacea* and *Anthidium cingulatum*) account for over 90% of flower visits (2098). The flower visitation index varied between species (0.0126–0.0320), reaching relatively medium values compared to Mediterranean plants. In contrast, reproductive success was found to be high when estimated as both fruit (45.4–84.2%) and seed (75.4–98.4%) sets, suggesting that these bee guilds are effective pollinators of the three self-incompatible *Antirrhinum* species. Specialization of the personate flower of *Antirrhinum* is therefore interpreted for bees with both large bodies and high visitation indices.

**Keywords:** *Antirrhinum braun-blanquetii*; *Antirrhinum charidemi*; *Antirrhinum graniticum*; bee species; mediterranean; reproductive success

### Introduction

The *Antirrhinum* (snapdragon) genus consists of about 25 species primarily distributed throughout the western Mediterranean (Iberian Peninsula) (see Figure 1). Although variation in vegetative and flower characters is used to identify *Antirrhinum* taxa, the 25 species display a rather constant corolla shape. The five petals of *Antirrhinum* are arranged into an adaxial or upper lip (two upper petals) and a lower or abaxial lip (three lower petals) (Figure 1). The corolla lower lip typically develops a basal convexity (palate) occluding the mouth of the tube. The lower part of the corolla is formed by a long, wide tube and a saccatae spur. A similar flower is also found in other genera of the tribe Antirrhineae (Sutton 1988). In particular, flowers of the large genus *Linaria* (c. 150 species) primarily display occluded corollas, but long spurs. This special reproductive structure, with either a saccata or a spur, was early named as a different flower type (personate flower) because of its occluded corolla

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\*Corresponding author. Email: [vargas@rjb.csic.es](mailto:vargas@rjb.csic.es)

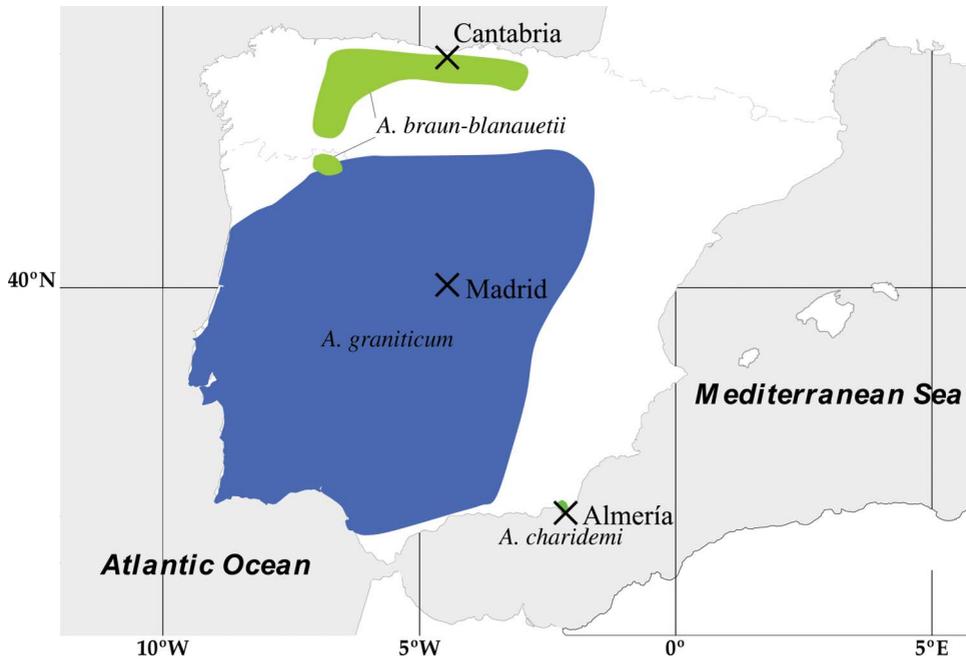


Figure 1. Distributional map and population localities (X) of the three Iberian *Antirrhinum* species used in the present study.

mouth (*persona* from Latin meaning “mask” or “covered face”) that characterizes the corolla entrance of all *Antirrhinum* species and relatives (Tournefort 1700; Sprengel 1793). The lure of this complex flower resulted in early investigation of floral development and inheritance in *Antirrhinum majus* by Mendel (1866) and Darwin (1876). A concerted study coinciding with the rediscovery of Mendel’s research established *Antirrhinum* as a model plant of flower ontogeny since the early twentieth century (Schwarz-Sommer et al. 2003).

Considerable strength is needed to enter the personate flower and collect pollen and nectar, and it has been historically hypothesized that bees are the only pollinators (Müller 1929; Sutton 1988). Indeed, bees have been observed visiting *A. majus* flowers to reach nectar and to collect pollen by touching anthers with the dorsal thorax (scutum) (Macior 1967; Gottlieb et al. 2005). Although independent results corroborate the importance of bees in personate flower pollination, detailed observations have primarily been performed in non-natural and semi-natural systems (Macior 1967; Glover and Martin, 1998; Comba et al. 2000; Gottlieb et al. 2005). A sound empirical basis is, however, necessary to explain some critical features in the evolution of certain populations (Whibley et al. 2006). For instance, previous results appear to indicate that *Antirrhinum* pollinators bring about determinant consequences in the genetic structure of endangered species (Torres et al. 2003). In fact, knowledge of the role of pollinators in propagating *Antirrhinum* species in a hybrid zone (eastern Pyrenees) has been shown to be of central importance in natural selection of flower colour (Whibley et al. 2006).

The fully occluded, personate flower of *Antirrhinum* has led authors to hypothesize that the corolla hinders the entrance of most insects other than bees because they

are not heavy or strong enough to open the corolla mouth (Sutton 1988; Endress 1994). It is, however, currently recognized that ascertaining the specificity of flower specialization by pollinator systems requires additional experimental work to assess the efficiency of plant reproductive success (Aigner 2001). To circumvent biased results deriving from lack of experimental studies, we addressed basic questions on pollinators and the personate flower by drawing up censuses of the visiting fauna in three disparate *Antirrhinum* species. In addition, estimates of reproductive success may help to evaluate the effectiveness of the most frequent *Antirrhinum* pollinators. That bees are the only *Antirrhinum* pollinators in nature appears to be a reasonable assumption, although appropriate quantitative data are required to confirm this.

The present paper addresses pollinator significance and reproductive success of three endemic species representing most of the phenotypic variation of *Antirrhinum* flowers. Based on previous results, specific questions refer to: (1) whether bees are the only pollinators; (2) the degree of adjustment made by the personate flower to particular pollinator species; and (3) whether the pollination fauna observed is effective in plant reproductive success.

## Materials and methods

### *Antirrhinum* species

Three *Antirrhinum* species (*A. charidemi*, *A. graniticum*, *A. braun-blanquetii*) were chosen to represent most of the diversity of the genus in terms of the extreme flower phenotypes of the three subsections of *Antirrhinum* (*Kickxiella*, *Streptosepalum* and *Antirrhinum*), three geographical areas (south-east, central and northern Iberia; Figure 1) and three contrasted climates (Mediterranean arid, Mediterranean continental and Eurosiberian temperate). They share, in contrast, similar rocky habitats. Climatic data were taken from the Spanish National Meteorology Institute ([www.aemet.es](http://www.aemet.es)). Two subpopulations per species were studied to include areas of the same species expected to have a similar pollinator fauna. At least two phenological periods of sampling were considered to take into account both flower and insect phenologies in the flower–pollinator relationships. During observation periods, we recorded the number of mature flowers per plant under observation, the identity of each flower visitor, the number of visits made by each of these, and whether visits were apparently legitimate, that is if the insect entered the flower touching sex organs or was involved in nectar robbing. Legitimacy of pollinators was also evaluated from flower–pollinator sizes and detailed observations of their behaviour pattern (see below).

*Antirrhinum braun-blanquetii* Rothm. is endemic to northern Iberia (Figure 1). We chose two subpopulations 15 km apart in Cantabria (Bielva, 175 m, 43°18'52" N, 4°27'54" W; Quintanilla, 260 m, 43°14'56" N, 4°28'28" W). Flowers can be found anytime in spring and summer, the blooming period of this species ranges from May to August. Our subpopulations had yellow corollas and large flowers (corolla tube about 30 mm long and 9 mm wide) (Figure 2). These flowers are some of the largest of the genus (Sutton 1988). Conditions recorded in Cantabria in 2007 (mean temperature 14°C; mean rainfall *c.* 1200 mm) were close to normal. The two localities had different numbers of individuals and flowers at each plot, ranging from 100 to 300 flowers of 18 plants (Bielva) and from 18 to 105 flowers of 11 plants (Quintanilla). Observation periods were from 07.30 to 18.15 h (Greenwich mean time; GMT).

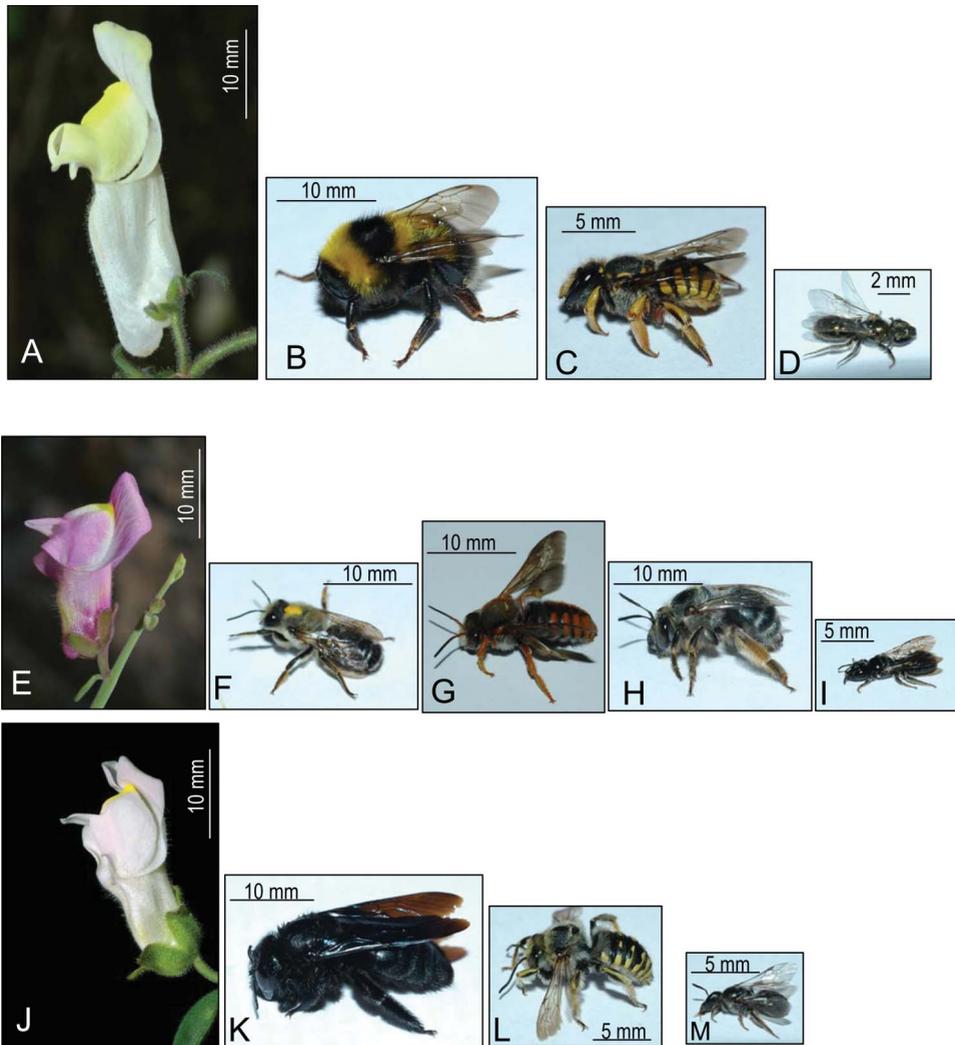


Figure 2. Photographs of flowers of the three *Antirrhinum* species and main bee pollinators (see Tables 1–3). (A) *Antirrhinum braun-blanquetii*; (B) *Bombus hortorum*; (C) *Anthidium manicatum*; (D) *Lasioglossum smeathmanellum*; (E) *Antirrhinum charidemi*; (F) *Chalicodoma lefebvrei*; (G) *Anthidium sticticum*; (H) *Anthophora dispar*; (I) *Ceratina cucurbitina*; (J) *Antirrhinum graniticum*; (K) *Xylocopa violacea*; (L) *Anthidium cingulatum*; (M) *Lasioglossum buccale*.

*Antirrhinum charidemi* Lange is endemic to an  $8 \times 4$  km strip of land in the Cabo de Gata mountains (Almería, south-eastern Spain) (Figure 1). This is one of the four endangered species of the genus recently included in the red book of Spanish flora (Bañares et al. 2003). The studied subpopulations display either purple (Vela Blanca) or purple and whitish (Barranco Dragoncillo Blanco) corollas and small flowers (corolla tubes about 19 mm long and 6 mm wide) (Figure 2). Sutton (1988) considered these flowers to be the smallest of the genus. The Cabo de Gata mountains suffer from the most arid conditions in the Mediterranean basin (Hofrichter 2001), with high temperature (mean  $18.7^{\circ}\text{C}$ ) and

low rainfall (mean *c.* 200 mm) values. We recorded pollinators at three periods in the long flowering season (March–June 2007) of the species and at two sites (Vela Blanca, 300 m, 36°43'24" N, 2°10'09" W; Barranco Dragoncillo Blanco, 240 m, 36°46'55" N, 2°07'54" W) separated by 6.5 km. A few out of season flowers are, however, found throughout the year. The study plots contained 13 plants (seven at Vela Blanca, six at Barranco Dragoncillo Blanco) with 50–300 flowers, depending on flowering stages. Observation periods were from 07.20 to 18.20 h (GTM).

*Antirrhinum graniticum* Rothm. is widely distributed in central Iberia (Figure 1). Two subpopulations 10 km apart were chosen from the Madrid area (Colmenar Viejo, 825 m, 40°29'34" N, 3°44'23" W; San Pedro hill, 1030 m, 40°43'32" N, 4°27'54" W) and visited at two stages of the flowering phenology (May–July 2007). Our subpopulations show white corollas and medium-size flowers (corolla tubes about 22 mm long and 9 mm wide) (Figure 2). This flower is of medium size in the genus (Sutton 1988). Climatic conditions in 2007 fitted the average of the continental Mediterranean area of Madrid in terms of temperature (mean 14°C) and rainfall (mean 450 mm). Three and five plants were used, producing between 60 and 500 (Colmenar Viejo) and between 50 and 300 (San Pedro hill) flowers, respectively. Observation periods were from 07.20 to 18.20 h (GTM).

### *Pollinator censuses and plant sites*

We conducted all flower visitor censuses in optimum conditions: sunny days, temperature over 20°C and no wind. However, we also visited the study sites when weather conditions were different (rain, wind, low temperature) and observed no pollinators. Observations were performed on *Antirrhinum* plots at 15-min intervals, followed by 5-min pauses, and produced around 1000 minutes (16–17 h). Time effort was similarly distributed in two or three phenological periods to record pollinator visits in each species (Tables 1–3). Only daytime censuses were performed. Judging from flower morphology and daytime observations of lepidopteran behaviour patterns, we considered it highly improbable that nocturnal visitors such as Sphingidae significantly contributed to *Antirrhinum* pollination. A visit was considered legitimate (flower contact) when the visitor used the corolla opening to access the reward and touched reproductive parts. Pollinator efficiency and visitation rates were calculated on per-flower (number) and a per-time (hour) bases. The number of flowers visited by insects was divided by the total number of open flowers found each day by time effort (number of minutes) (flower visitation index). Pollinators were identified using local faunas and taxonomic accounts (Ornosa and Ortiz-Sánchez 2004; Terzo and Ortiz-Sánchez 2004; Ornosa et al. 2006, 2007, 2008; Michener 2007). Specimens were collected, identified and deposited in the collections of the Universidad Complutense de Madrid and of one of the authors (J.J. Ortiz-Sánchez).

### *Pollinator diversity*

We applied the widely used Shannon–Wiener diversity index in ecological studies to estimate the diversity of pollinators visiting each of the three species of *Antirrhinum*, by pooling data from plots and sites for each species ( $H' = -\sum p_i \ln p_i$ , where  $p_i$  is the frequency of flower visits of a pollinator species  $i$ ). This index has been applied successfully to pollinators for comparative purposes (Mustajärvi et al. 2001; Gómez et al. 2007).

### Flower and insect sizes

Given the occluded structure of the *Antirrhinum* corolla, it is likely that insects enter flowers depending on their body size in comparison with the corolla size. Some (one to three) of the insects captured for identification were also measured (maximum length and width, excluding antennae and wings; Tables 1–3). Volume of insect bodies was calculated as an ellipsoid ( $V = 4/3\pi abc$ ). This size can be directly related to weight and strength available to open and to enter flowers. For comparison, the volume of the corolla tubes was calculated as that of a cylinder ( $V = \pi^2 h$ ).

### Fruit and seed sets

Flower duration is typically 1 week, depending on blooming stages (results not shown). Plant reproductive success was estimated by recording data from marked pedicels of flowers once visited by pollinators. We did not quantify whether fruit and seed sets were the result of one or multiple visits. A predominant self-incompatibility has been recorded for *A. graniticum* (Mateu-Andrés and Segarra-Noragues 2003) and *A. charidemi* (Carrió et al. 2009). To obtain the first data of self-compatibility in *A. braun-blanquetii*, flowers of the two subpopulations were tested for cross- and self-pollination. Considering self-incompatibility, the presence of a swollen capsule was scored to estimate at least one effective pollinator visit in these species (i.e. carrying compatible pollen), whereas one empty seed capsule was taken to represent failure in the pollination contact or fertilization. Capsules were collected as they matured typically 30–40 days after pollination (May–August 2007) depending on species phenology (see above). Fruits were directly counted, and seeds and fruit ovules were then counted from individual capsules under a microscope because of their small size.

The relationship between insect visit rate to flowers (pooling data from all censuses through the season) and reproductive success was examined by single correlation analyses between these variables. It was possible to have separate estimates of visit rate and fruit set at the plot level (except for *A. graniticum* because subpopulations were formed by tangled individuals making us unable to estimate plots separately). As a result, this analysis was performed at the level of  $n = 11$  plots. The seed-set analysis was performed at the site level ( $n = 6$ ). To determine possible differences in efficiency of insects of different size, which may have different abilities to open the corolla mouth, all these correlations were estimated separately for insects longer or shorter than 8 mm and longer or shorter than 14 mm long, and for all insects. Relationship between visit rate and insect size (volume) was also examined by simple correlation analyses.

## Results

### Predominant pollinators across species

Only bees were recorded as legitimate pollinators of the three *Antirrhinum* species (Tables 1, 2, 3). However, *Oxythyrea funesta* (Coleoptera) was observed once in one flower of *A. braun-blanquetii* and in one flower of *A. charidemi* in the vicinity of the plots. Nocturnal visits were not considered because we were only able to spot (camera-recorded) some Lepidoptera failing to enter the flowers as a result, as expected, of their low weight and flower manipulation abilities.

Table 1. Physical features of bees and number of visits to flowers of *Antirrhinum braun-blanquetii*.

Bee species (family)	Length in mm	Width in mm	Estimated volumes in mm <sup>3</sup>	2–6 July (590 min)	7–10 August (465 min)	Number of visits (%)
<i>Bombus hortorum</i> (Linnaeus, 1761) (Apidae)	17 (22)	8 (9.5)	569.4	134	290	424 (84.6)
<i>Anthidium manicatum</i> (Linnaeus, 1758) (Megachilidae)	10.5	5.5	166.2	53	0	53 (10.6)
<i>Lasioglossum smeathmanellum</i> (Kirby, 1802) (Halictidae)	6.0	1.7	9.1	12	5	17 (3.4)
<i>Ceratina cucurbitina</i> (Rossi, 1792)* (Anthophoridae)	8.0	2.5	26.2	3	0	3 (0.6)
<i>Bombus muscorum</i> (Linnaeus, 1758) (Apidae)	14.0	6.5	309.6	0	2	2 (0.4)
<i>Bombus</i> sp. (Apidae)	—	—	—	0	2	2 (0.4)
<i>Bombus pascuorum</i> (Scopoli, 1763) (Apidae)	13.5	5.75	—	+	—	—
Total	—	—	—	202	299	501

Notes: An asterisk (\*) indicates the only bee species (*Ceratina cucurbitina*) visiting the three *Antirrhinum* species, and a plus sign (+) refers to a bee species (*Bombus pascuorum*) observed in flowers in the vicinity of the study plots. Length (mm), width (mm), and volume (mm<sup>3</sup>) of adult bees captured in the field after entering corolla; queen bee measurements in parenthesis. Census periods and time effort (in parenthesis), and total number and percentage (in parenthesis) of flower visits are also indicated.

Table 2. Physical features of bees and number of visits to flowers of *Antirrhinum charidemii*.

Bee species (family)	Length in mm	Width in mm	Estimated volumes in mm <sup>3</sup>	12–16 March (375 min)	7–10 April (325 min)	12–14 May (390 min)	Number of visits (%)
<i>Chalicodoma lefebvrei</i> (Lepeletier, 1841) (Megachilidae)	14.0	4.75	165.3	0	0	156	156 (38.1)
<i>Anthidium sticticum</i> (Fabricius, 1793) (Megachilidae)	15.0	6.5	331.7	106	0	43	149 (36.4)
<i>Anthophora dispar</i> (Lepeletier, 1841) (Anthophoridae)	14.5	6.5	320.6	0	51	0	51 (12.5)
<i>Ceratina cucurbitina</i> (Rossi, 1792)* (Anthophoridae)	7.75	2.5	25.3	0	0	38	38 (9.3)
<i>Megachile deceptor</i> (Pérez, 1890) (Megachilidae)	9.0	4.0	75.4	0	0	3	3 (0.7)
<i>Apis mellifera</i> (Linnaeus, 1758) (Apidae)	13.75	4.6	152.3	3	0	0	3 (0.7)
<i>Chalicodoma sicula</i> (Rossi, 1792) (Megachilidae)	18.0	6.0	339.1	3	0	0	3 (0.7)
<i>Anthophora plumipes</i> (Pallas, 1772) (Anthophoridae)	15	6.5	331.7	2	0	0	2 (0.5)
<i>Eucera</i> sp. (Anthophoridae)	–	–	–	2	0	0	2 (0.5)
<i>Osmia submicans</i> (Morawitz, 1870) (Megachilidae)	7.75	2.75	30.7	2	0	0	2 (0.5)
Total	–	–	–	118	51	240	409

Note: An asterisk (\*) indicates the only bee species (*Ceratina cucurbitina*) visiting the three *Antirrhinum* species. Length (mm), width (mm) and volume (mm<sup>3</sup>) of adult bees captured in the field after entering corolla. Census periods and time effort (in parenthesis), and total number and percentage (in parenthesis) of flower visits are also indicated.

Table 3. Physical features of bees and number of visits to flowers of *Antirrhinum graniticum*.

Bee species (family)	Length in mm	Width in mm	Estimated volumes in mm <sup>3</sup>	1–23 June (585 min)	8–29 July (495 min)	Number of visits (%)
<i>Xylocopa violacea</i> (Linnaeus, 1758) (Anthophoridae)	24.5	9.75	1218.9	572	408	980 (82.5)
<i>Ceratina cucurbitina</i> (Rossi, 1792)* (Anthophoridae) and <i>Lassioglossum buccale</i> (Pérez, 1903) (Halictidae)	8.5	2.5	27.8	19	96	115 (9.7)
<i>Anthidium cingulatum</i> (Latreille, 1809) (Megachilidae)	11.75	5.25	169.5	0	87	87 (7)
<i>Anthophora affinis</i> (Brullé, 1832) (Anthophoridae)	18.5	7.5	544.6	2	0	2 (0.2)
<i>Anthidium</i> sp. (Megachilidae)	–	–	–	2	0	2 (0.2)
<i>Anthophora</i> sp. (Anthophoridae)	–	–	–	2	0	2 (0.2)
Total	–	–	–	597	591	1188

Notes: An asterisk (\*) indicates the only bee species (*Ceratina cucurbitina*) visiting the three *Antirrhinum* species. Data of *Ceratina cucurbitina* and *Lassioglossum buccale* are summarized together because of difficulties in distinguishing between them in the field (see text). Length (mm), width (mm) and volume (mm<sup>3</sup>) of adult bees captured in the field after entering corolla. Census periods and time effort (in parenthesis), and total number and percentage (in parenthesis) of flower visits are also indicated.

*Antirrhinum braun-blanquetii* was only visited by six bee species, of which *Bombus hortorum* accounted for most flower visits (Table 1). *Bombus hortorum* foraged nectar and transported pollen on the scutum over a wide range. Numerous flowers were visited in the plot by this bumblebee that performed long-distance flights afterwards. *Anthidium manicatum* patrolled groups of plants during the first phenological period. Interestingly, this bee species has been spotted pollinating *A. braun-blanquetii* in the Botanical Garden of Vienna (Müller 1996). *Lasioglossum smeathmanellum* hardly moved from a few flowers of the same plant (a pollinator showing flower constancy). As a whole, half of the marked flowers set fruits (mean values of 45.5% in Bielva, 53.3% in Quintanilla). The seed set also reached high mean values (Table 4). The flower visitation index was 0.0126, which was the lowest value of the three *Antirrhinum* species. Flower selfing tests revealed that *A. braun-blanquetii* was self-incompatible. We failed to obtain fruits from 29 flowers tested for selfing, whereas 14 fruits were obtained by hand pollination of 24 flowers with cross pollen.

*Antirrhinum charidemi* received two main pollinators at similar rates, but at different periods: *Chalicodoma lefebvrei* in the third sampling period and *Anthidium sticticum* in the first and third sampling periods (Table 2). In fact, the presence of *Anthidium sticticum* was noted from March to June, whereas *C. lefebvrei* appeared in the area from May onwards. Although the two bee species were seen to fly from one plant to the next, only the *Anthidium sticticum* clearly displayed a territorial behaviour pattern. *Anthidium sticticum* was also observed as a frequent pollinator on *Antirrhinum microphyllum* (Torres et al. 2003). In the second half of the blooming season, *Anthophora dispar* appeared and displayed a wide-ranging behaviour pattern, such as visiting a few flowers (3–22) and no more than three plants before flying away. *Ceratina cucurbitina* displayed marked flower constancy in one or several plants. The reproductive success of *A. charidemi* by this pollinator guild was estimated to result in approximately half of the flowers being transformed into fruits (mean values of 51% in Barranco Dragoncillo Blanco; 54% in Vela Blanca) and a considerable number of ovules into seeds (Table 4). The flower visitation index was 0.0320, which was the highest value among the three *Antirrhinum* species.

*Antirrhinum graniticum* showed similar bee specificity and reproductive success to that of *A. braun-blanquetii*. The carpenter bee (*Xylocopa violacea*) accounted for most flower visits, legitimately taking pollen on the scutum (once visiting 104 flowers in a single foraging bout) before flying away (Table 3). We did not observe robbing behaviour by this *Xylocopa* species, whereas *Xylocopa cantabrita* and *Xylocopa uclesiensis* did act exclusively as robbers on the same plants. *Xylocopa violacea* forms part of the pollination fauna of *A. majus* in a semi-natural area of the eastern Mediterranean basin with native and introduced plants (Gottlieb et al. 2005). We were not always able to distinguish *Ceratina cucurbitina* or *Lasioglossum buccale* in pollinator censuses given their small body sizes, similar coloration and their behaviour pattern of flower constancy; for this reason flower visits recorded for these species are shown together (Table 3). One more *Anthidium* species (*Anthidium cingulatum*) patrolled and visited flowers in the second half of the blooming season. The fruit set was different between subpopulations (mean values of 50.5% in Colmenar Viejo; 84.2% in San Pedro hill). High seed-set values were reached for the flowers visited by pollinators in both subpopulations (Table 4). The flower visitation index was 0.0200.

There was a large difference in taxonomic composition between the pollinator faunas on the three *Antirrhinum* species. The only pollinating bee shared by the three

Table 4. Percentage values of reproductive biology estimates (seed set, fruit set) in three *Antirrhinum* species.

	<i>A. braun-blanquetii</i> (Cantabria)		<i>A. charidemi</i> (Almería)		<i>A. graniticum</i> (Madrid)	
	Site 1 (Bielva)	Site 2 (Quintanilla)	Site 1 (Barranco D. B.)	Site 2 (Vela Blanca)	Site 1 (Colmenar Viejo)	Site 2 (San Pedro hill)
Seed set (%)	79.9% <i>n</i> = 10	98.4% <i>n</i> = 8	92% <i>n</i> = 10	92% <i>n</i> = 9	75.4% <i>n</i> = 10	90.2% <i>n</i> = 10
Fruit set (%)	Plot 1 46.3% <i>n</i> = 123	Plot 1 66.6% <i>n</i> = 15	Plot 1 67.6% <i>n</i> = 108	Plot 1 60.8% <i>n</i> = 79	Plot 1 78.8% <i>n</i> = 118	Plot 1 39.0% <i>n</i> = 118
	Plot 2 53.3% <i>n</i> = 30	Plot 2 40.0% <i>n</i> = 30	Plot 2 64.1% <i>n</i> = 78	Plot 2 50.9% <i>n</i> = 163	Plot 2 90.2% <i>n</i> = 92	Plot 2 60.0% <i>n</i> = 197

Note: Localities as described in Material and methods.

species was *Ceratina cucurbitina* (Tables 1–3). Interestingly, the territorial behaviour pattern of the *Anthidium* species was noted in the three *Antirrhinum* species: *Anthidium manicatum* in *A. braun-blanquetii* (Bielva); *Anthidium cingulatum* in *A. graniticum* (San Pedro hill); and *Anthidium sticticum* in *A. charidemi* (Barranco Dragoncillo Blanco). Apart from *Anthidium* species, no significant differences of the pollinator fauna were observed between the two subpopulations of *A. braun-blanquetii* or those of *A. graniticum* over the two census periods. In contrast, *A. charidemi* had a more diverse pollinator fauna during the flowering period. These differences were greater when accounting for pollinator visit frequency. Hence, pollinator diversity (Shannon–Wiener index;  $H'$ ) in *A. charidemi* was 1.40, which is about three-fold higher than those of *A. braun-blanquetii* ( $H' = 0.57$ ) and *A. graniticum* ( $H' = 0.46$ ).

### Flower – insect volumes and reproductive success

Estimates of body volumes of pollinators are given in Tables 1–3. Although bee/corolla tube differences have been hypothesized as imposing important size constraints on the pollinators entering the flowers, we did not observe full congruence. Predominant pollinators had smaller body sizes than corolla tubes in all cases. However, volumes of *Xylocopa violacea* ( $1218.9 \text{ mm}^3$ )/*A. graniticum* ( $1399.6 \text{ mm}^3$ ) were comparable, whereas those of *Bombus hortorum* ( $569.4 \text{ mm}^3$ )/*A. braun-blanquetii* ( $1908.5 \text{ mm}^3$ ), and *Chalicodoma lefebvrei* ( $165.3 \text{ mm}^3$ ) or *Anthidium sticticum* ( $331.7 \text{ mm}^3$ )/*A. charidemi* ( $537.2 \text{ mm}^3$ ) were clearly dissimilar. On the other hand, the relationship between insect size and number of visitors was significant for *A. braun-blanquetii* ( $r = 0.847$ ,  $P < 0.05$ ,  $n = 5$ ) and *A. graniticum* ( $r = 0.882$ ,  $P < 0.025$ ,  $n = 5$ ), but not significant for *A. charidemi* ( $r = 0.213$ ,  $P = 0.25$ ,  $n = 10$ ) despite the higher sample size. Although these results should be further tested with more data, differences between species are clear.

Reproductive success at the population level was high in the three species in terms of fruit set (average 57.6%; range 39.0–90.2%;  $n = 11$  plots) (Table 4). Estimates of seed set reached even higher values in all cases (average of seed/ovule ratio of 88.0%; range 75.4–98.4%;  $n = 6$  sites). Differences for both fruit and seed sets were higher between sites than between species. Although a positive correlation was observed in differences of fruit set, they were not significantly correlated with pollinator frequency taking into account all pollinators and plots together ( $r = 0.247$ ) or separating them into size classes ( $> 1.4 \text{ cm long}$ :  $r = 0.127$ ;  $< 1.4 \text{ cm long}$ :  $r = 0.336$ ;  $P > 0.2$  in all cases,  $n = 11$  plots). Values of correlations for seed set were lower ( $> 1.4 \text{ cm long}$ :  $r = -0.146$ ;  $< 1.4 \text{ cm long}$ :  $r = -0.200$ ,  $P = > 0.5$  for the three insect size classes).

## Discussion

This study is the first attempt to demonstrate that bees are the principal pollinators in natural areas of *Antirrhinum* species. A time effort of 3225 observation minutes on plots of three *Antirrhinum* species recorded a total of 2018 visits only performed by bees.

### Bee species and pollination

Twenty-two bee species of different sizes, body structures, behaviour patterns and taxonomic assignments were observed pollinating the three *Antirrhinum* species

(Tables 1–3). However, this figure is low in comparison with the number of bees suitable for pollination of *Antirrhinum* flowers in the three study regions (south-east, central and northern Iberia) (García-González and Ornos 1998; J Herrera 1988; Ortiz-Sánchez and Belda 1994; Ornos and Ortiz-Sánchez 2004). On the plant side, few pollinators appear to be responsible for *Antirrhinum* pollination. Specificity is also supported because a single bee species (*Xylocopa violacea*) accounted for most flower visits in *A. graniticum* (Table 3). The same is true for *A. braun-blanquetii*, for which *Bombus hortorum* had a similar visit rate (percentage), but to a remarkably lower number of flowers (Table 1), and also a lower flower visitation index. A higher number of bee species as pollinators in *A. charidemi* may be interpreted as the result of a longer flower phenology and of the distribution of this species in an area with rich diversity of bee species in south-eastern Iberia (Ornos and Ortiz-Sánchez 2004). High specificity of the occluded corolla in terms of pollinator guilds and principal pollinators for each *Antirrhinum* species agrees with preliminary results for the central Iberian *A. microphyllum*, in which the predominant role of *Anthidium sticticum* was also reported (Torres et al. 2003). In contrast to bee constancy in visiting particular *Antirrhinum* populations, estimates of flower tube volumes of *Antirrhinum* species do not parallel the volumes of their most frequent bee pollinators. It is particularly intriguing that there is a considerable mismatch of corolla tube sizes of *A. braun-blanquetii* and *A. charidemi* with the volumes of their three most frequent pollinators (*Bombus hortorum*, and *Chalicodoma lefebvrei* and *Anthidium sticticum*, respectively) (Tables 1–3). In contrast, there was a close relationship between flower volumes of *A. graniticum* and the body size of *Xylocopa violacea*. Similar to *A. braun-blanquetii* and *A. charidemi*, the personate flower of *Linaria* (tribe Antirrhineae) has both a limited number of principal pollinators and dissimilar volume relationships in natural populations: *L. lilacina* is visited by *Apis mellifera* (61.4%) and *Anthophora dispar* (21.2%) in Andalusia (Spain) (Sánchez-Lafuente 2007); *L. vulgaris* is pollinated by *Bombus pascuorum* (c. 90%) and *Bombus hortorum* (10%) in England (Stout et al. 2000). Whether a mismatch between principal pollinators and corolla sizes is a general pattern in personate flowers deserves further investigation.

Pollinator diversity in *Antirrhinum* species is interpreted as being low because of their restrictive occluded corolla, at least in comparison with the pollinator diversities of less restrictive flowers of the Mediterranean flora: e.g. *Lavandula latifolia* (CM Herrera 1990), *Cistus ladanifer* (Talavera et al. 1993), *Erysimum mediohispanicum* (Gómez et al. 2007). Indeed, Gómez et al. (2007) reported Shannon index values of  $H' = 2.83\text{--}3.10$  in populations of the Mediterranean crucifer *E. mediohispanicum*, which is visited by 112 insect species. In contrast, this index in *Antirrhinum* ( $H' = 0.46\text{--}1.40$ ) resembles those of other restrictive flowers and their pollinators in the Mediterranean floristic region, in which low pollinator diversity is observed. An extreme case is the long-spurred *Viola cazorlensis* and its exclusive pollination by *Macroglossum stellatarum* (CM Herrera 1993).

Specificity of the bees was not assessed in this study. In any case, it appears to be low, given that *Xylocopa violacea* and *Bombus hortorum* are shared as pollinators by unrelated plant species. *Xylocopa violacea* was recorded as one of the pollinators of *Lavandula latifolia* (CM Herrera 1988). Among many available studies, *Bombus hortorum* has been recorded on *Delphinium montanum* (Simon et al. 2001), *Silene acutifolia* (Buide 2006) and *Antirrhinum majus* (Vargas and Ornos unpublished results). *Bombus*, particularly *Bombus terrestris*, has been employed in experimental studies to

describe its preference for different flowers of *A. majus* (Glover and Martin 1998; Comba et al. 2000; Dyer et al. 2007). In nature, the *Antirrhinum*–*Bombus* system does not appear to be the only specialized interaction, at least in two of the three *Antirrhinum* species herein studied. In addition, not only *Bombus* and other genera of the family Apidae are involved in *Antirrhinum* pollination. Indeed, our three-species approach enabled us to determine that four of the eight bee families (Michener 2007; see also Ornosá and Ortiz-Sánchez 2004) were linked to *Antirrhinum* pollination, although with remarkably different visitation rates (Tables 1–3). All this reveals that incorrect inferences are made concerning actual pollinators in *Antirrhinum* by studying non-natural systems (only Apidae species were predicted by Macior 1967). In contrast, bee families, body sizes and complex behaviour patterns of bees in natural areas appear to be a more determinant factor, including their ability to enter the occluded flower of *Antirrhinum* species. Video records also obtained in the present study revealed that the weight of the four main pollinators found in the three species of *Antirrhinum* primarily enabled opening of the corolla, whereas smaller bees (< 8 mm long) combined different manipulation strategies to access the flower through the corolla mouth (Vargas unpublished data).

#### **Pollen transfer and reproductive success in *Antirrhinum***

The high reproductive output observed in the three *Antirrhinum* species indicates that their pollinator guilds are highly efficient, particularly for seed set (Table 4). The medium value of the flower visitation index (0.0126–0.032) in *Antirrhinum* does not support *per se* this result (cm Herrera 1989). We interpret that once a flower is visited by bees many ovules become fecund, in spite of the high number of ovules (15–471,  $n = 57$ ) per flower of the three species, which may indicate a high capacity for pollen transport by large bees. This statement is supported by high seed-set values rendered by a single hand pollination in the field for *A. braun-blanquetii* (58%, this study) and other species (Vargas unpublished data). The values of fruit set for *Antirrhinum* fit those reported for other Mediterranean plants (CM Herrera 1988), although they may be subject to high spatial and temporal variation.

The fact that the bees with large body sizes and suitable structures (hairy scutum) for loading high amounts of pollen, such as *Bombus*, *Xylocopa*, *Chalicodoma* and *Anthidium*, are the ones with higher visit rates in *Antirrhinum* species (Tables 1–3) indicates that this bee type may fit the floral characteristics of the personate flower. Indeed, records of pollen-foraging bees on nototribic flowers, i.e. those brushing the dorsal side of an insect thorax with their essential parts, of *Antirrhinum* support this statement (Macior 1967). Given the major role played by a few bee species observed in this study, we hypothesize that wide-ranging bee species, such as *Bombus hortorum*, *Xylocopa violacea*, *Chalicodoma lefebvrei* and *Anthidium sticticum*, or similar species in different areas, are effective pollinators in any *Antirrhinum* species. In contrast, bees displaying a pattern of flower constancy, such as *Ceratina* and *Lasioglossum*, have lower visitation rates, smaller body sizes unadjusted to nototribic flowers, and considerably less hair. Irrespective of the net contribution of each pollinator species, our results suggest that a narrow range of bees are involved in the high levels of reproductive success and that bees have to be large enough on nototribic flowers of *Antirrhinum*. Indeed, a significant and positive correlation was observed between the high number of visits and the size of bees of the pollinator guilds in *A. braun-blanquetii* and

*A. graniticum*, but not in *A. charidemi*. When the correlations were calculated between the insect visit rate and reproductive success (fruit and seed sets) the trend was as expected: the higher the visit rate the higher the reproductive success, but the correlation coefficient was not significant. There is a need for further studies involving pollinator exclusion, single-pollinator efficiency on pollen delivery and receipt, pollinator-mediated mating systems, pollen competition, seed viability assessment, and seedling survival and performance to establish whether the bees observed in these studies support the controversial concept of the most pollinator-effective principle (Aigner 2001). In particular, fine-scale studies of reproductive success are needed to investigate the relationships between plant species and bee roles, which have been invoked to be determinant in the evolution of *Antirrhinum* species (Schwarz-Sommer et al. 2003; Whibley et al. 2006).

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