

# **ORIGINAL ARTICLE**

# Small sweat bees (Hymenoptera: Halictidae) as potential major pollinators of melon (*Cucumis melo*) in the Mediterranean

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#### **Abstract**

In the current scenario of a general decline of the honeybee worldwide, studies on the potential of alternative bee species in pollinating cultivated plants are important. Although melon, *Cucumis melo*, is a crop with great commercial importance, there is very little information on its pollinating fauna in Europe, and none from the southern Mediterranean area. In a locality in central Spain, using both pantraps and net collections, we found that melon flowers are visited by 31 species of bees spanning four families, though only four were both dominant and constant. These four species belonged to the family Halictidae (sweat bees) and mostly (three species) to the genus *Lasioglossum*. Five other species could be defined as accessory: honeybee, *Apis mellifera*, and four other halictids. Individuals of the dominant species were smaller, on average, than those from all the other species. Observations on the frequency of pollen and nectar foraging and on flower visit duration further suggested *L.malachurum* as the potential key pollinator. Females of this species started to forage on melon early in the flowering season and exhibited two activity peaks in summer, thus covering the whole season. Although in other sites across continents melon seems to be more heavily pollinated by honeybees, this seems to be not the case in the Mediterranean, where sweat bees seem to be the major pollinators of this crop.

Key words: foraging behavior, honeybee, Lasioglossum, pollination, Spain.

# INTRODUCTION

The loss of pollinators is currently a hot topic in ecology and conservation, especially because of its clear relationship with consequent socioeconomic damage in both agricultural and natural areas (Potts *et al.* 2010; Burkle *et al.* 2013). Recent estimates of the total economic benefit provided by pollination in the world total more than 150 billion euros (Blacquiere 2010), mainly because about 35% of world crop production depends on pollinators (Kevan & Viana 2003; Klein *et al.* 2007), and it was estimated that the production value of one

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tonne of crops not depending on insect pollination averages about 20% of the value of pollinator-dependent crops (Gallai *et al.* 2009).

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About 140 species of plants cultivated in the European Union (EU) benefit from insect pollination (Williams 1994), implying an important diversity of the pollinating fauna associated with these crops. However, for most crop species, pollinators are still poorly studied (Williams 2002), with no information available for about one-third of these plant species (Williams 1994, 2002).

The flowers of most crops are visited and pollinated by a variety of insects, which typically include honeybees, *Apis mellifera* L., bumblebees, *Bombus* spp., solitary and social wild bees from a variety of families (Hymenoptera: Apoidea) and some species of flies (Diptera), beetles (Coleoptera), butterflies (Lepidoptera) and thrips (Thysanoptera) (Williams 2002). Honeybee alone acts as the main pollinator of

about 15% of the crops of the world (Buchmann & Nabhan 1996), while for several crop species wild bees were observed as providing the most efficient pollination (Williams 2002; Breeze *et al.* 2011; Garibaldi *et al.* 2013). Wild bees may act as main pollinators of a variety of crops in particular when considering that honeybee numbers are severely decreasing worldwide (Winfree *et al.* 2007).

Among wild bees, members of the family Halictidae (sweat bees) have been shown to efficiently pollinate some crops around the world. For example, *Nomia melanderi* Cockerell is managed for alfalfa, *Medicago sativa* L., pollination in North America (Cane 2008), *Macronomia rufipes* (Smith) is an important pollinator of eggplant, *Solanum melongena* L., in East Africa (Gemmill-Herren & Ochieng 2008), *Lasioglossum villosulum trichopse* (Strand) is crucial for lettuce, *Lactuca sativa* L., pollination in Japan (Goubara & Takasaki 2004) and a number of species from several genera were recorded in both Europe and America to be effective pollinators of tomato, *Solanum lycopersicum* L. (Harter *et al.* 2002; Teppner 2005).

Here, we present a study on the bee fauna associated with melon flowers, Cucumis melo L. (Cucurbitaceae) in Spain, a country considered to be an important secondary diversification center for melon (López-Sesé et al. 2003). The melon is a herbaceous, annual plant with a hairy stem, and hairy and rough leaves that vary in size and shape (three to seven lobes) (Zapata et al. 1989; Stepansky et al. 1999). Most of the cultivated varieties belong to the andromonoic type (male and hermaphrodite flowers) (Maroto 1989). Flowers have a yellow corolla. The pollen grains are large, sticky and heavy, so cannot be transported by wind (Zapata al. 1989). Pollination is thus exclusively entomophilous, with pollinators increasing the production of plant parts that humans consume (reviewed in Klein et al. 2007). Depending on fruit morphology, several different varieties of C. melo were classified (Munger & Robinson 1991): the "toad skin" variety, with fruits of elliptical-oval shape and green skin with dark spots (Fig. 1D), is abundantly cultivated in Spain and was here studied.

Melon crops have experienced, since the end of the 1960s, extraordinary development worldwide (Zapata et al. 1989). Despite its economic importance, little information is available on its pollinators, and as far as we know only one study has been performed in the Mediterranean (in southern France; Carrè et al. 2009), with no studies in southern Europe. In addition, several studies showed important apparent differences in the pollinator fauna associated with melon in different parts of the world, with studies identifying, for example,

honeybees in Arizona (McGregor & Todd 1952), bumblebees in Columbia and South Carolina (Handel 1982), a mix of syrphid flies, thrips and wild bees in Ivory Coast (Kouonon *et al.* 2009) or almost exclusively wild bees in Mexico and France (Meléndez-Ramirez *et al.* 2002; Carrè *et al.* 2009) as potential key pollinators of this crop.

The aims of our study were to investigate the bee species assemblage associated with melon in a southern European area (central Spain), with special reference to the study of foraging behavior of the (more abundant) sweat bees and to its comparison with honeybee foraging behavior.

#### **MATERIALS AND METHODS**

# Study area

Fieldwork was performed in June and July (corresponding with melon flowering period at such locality) of 2011 and 2012. The study melon field was located at the farm "La Entresierra" of the agricultural station "El Chaparrillo", province of Ciudad Real, region of Castilla-La Mancha, central Spain (3°56′W, 39°0′N, 640 m a.s.l.) (Fig. 1A,B). The climate is Mediterranean–continental, subtype temperate, with cold and dry winters and hot and dry summers. Rain is more abundant in autumn and spring. Average annual temperature is about 15°C and average annual precipitation is about 400 mm (Ninyerola *et al.* 2005). The melon at this site is andromonoic in sex expression.

Management of melon at the study site is conventional, with planting occurring in spring (late April–early May) and fruits becoming ready to collect at middle–end of July. The cycle of melon at this site is annual. Melon plants are treated during growth against pathogens by applying lambda-cyhalothrin, imidacloprid and sulfur once a week. Around the melon fields, both honeybee and bumblebee hives are routinely managed to increase the pollination of this and other adjacent crops (including tomato, eggplant and pepper).

The melon field used in our study included a total of 232 plants, arranged in 29 rows (eight plants per row), with 1.5 m between plants (Fig. 1C).

# Bee sampling

During summer in 2011 we aimed to obtain an overview of the bee fauna occurring in the field and their foraging association with melon. To achieve this goal, we used a combination of colored pan-traps, net collection within transects and data on bee behavior during visits on melon

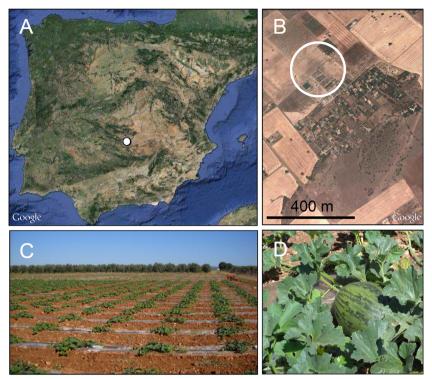


Figure 1 Studied melon crop field. (A) Iberian Peninsula with location of the study site (white point) (courtesy of Google Earth); (B) location of the melon field within the agricultural station "El Chaparillo" in the province of Ciudad Real (white circle) (courtesy of Google Earth); (C) view of the melon field (note plants arranged in rows); (D) melon fruit from the field at the end of the study (end of July).

flowers. Such a combination of techniques was recently highlighted as the most efficient method to describe pollinating bee communities (Cane *et al.* 2000; Roulston *et al.* 2007; Popic *et al.* 2013).

Pan-traps were located in nine rows within the first 18 rows (alternation of one row with traps and the neighboring one without traps), four traps per row in an alternating arrangement (i.e. for each row, one plant had a trap located at the base and the neighboring one had no traps, so that a row had four plants with traps and four without traps). Because pan-trap color can affect the collection of different groups of bees (e.g. Leong & Thorp 1999; Gollan et al. 2011) and the combined use of pan-traps of different colors seems important in collecting bees of a wide taxonomic spectrum (Stephen & Rao 2005; Wilson et al. 2008), we used traps of three colors: yellow, white and blue (12 traps per color), and the arrangement of the three color types followed a random assignment that changed once a week. Thus, overall 36 plants were associated with traps during the study, and they were marked with unique codes. Traps were placed in the morning, between 0800 and 0900 h, removed in the afternoon of the next day, and substituted with new traps. The content of each trap thus had the following associated data: day, plant code and trap color.

Direct sampling with a net was done with linear transects. Such transects were set up from row 19 to row 29, twice a week, across ten plants located in the same column (but in ten different rows). Also in this case the plants were coded with unique identification codes. Each day, six transects, one per hour from 0900 to 1400 h (flowers opened from 0800 to 1500 h, but no bees were recorded before 0900 h in preliminary observations), out of all the possible combinations were performed, with a sequence each day and hour randomly determined. During each transect, we collected with a net, during 10 min, all the bees landing on flowers and foraging on them (either for pollen or nectar). We then recorded the day, hour, plant code, flower sex (male or hermaphrodite) and taxonomic identification of the bees (determined later in the laboratory). The head width of all the collected bees from both sexes was measured with a digital caliper to the nearest 0.02 mm and was used as an indicator of bee body size.

Although the number of visits is not equivalent to the pollination efficiency, which also depends on the deposition of pollen in each visit, the number of visits is an important variable in predicting pollination efficiency ("quantity" of flower visitors to deduce stigmatic pollen deposition; see Ne'eman *et al.* 2009).

#### Bee visits on flowers

In 2012 we aimed to study in more detail the visits of bees on melon flowers. We selected seven plants randomly among all plants of the experimental plot (arranged identically as in 2011). Each of these plants was in a different row and included between row 3 and row 29. Each day of observation, five or six of these plants were randomly selected and, from about 1000 to about 1300 h, we spent 20 min for observations on each of them, recording with a hand recorder the following data for each bee visit on a flower: (i) time the visit started; (ii) time of departure from the flower (for both data, to the nearest second); (iii) type of foraging (nectar, pollen or both); and (iv) sex of the flower. Then, the bee individual was collected by netting and preserved for identification. When possible, two subsequent visits were recorded before collecting the bee. In the case of observations of A. mellifera, it was not necessary to capture individuals because the species was easily recognizable in the field. This method allowed assessing in more detail the potential pollination activity of bees, since species mostly collecting nectar may not be effective pollinators compared to those mostly collecting pollen or both resources. Furthermore, the flower visit duration ("quality" of flower visitors to deduce stigmatic pollen deposition; see Ne'eman et al. 2009), and the time spent in moving from flower to flower (related to visitation rate; Rader et al. 2009) help in predicting pollination efficiency. An effective pollinator would thus spend enough time on a flower to gather an adequate amount of pollen for transfer and would rapidly move between flowers (Rader et al. 2009).

#### Statistical analysis

Species dominance (DOM) was calculated according to Palma (1975) in Buschini (2006). DOM is defined as  $(N_i / N_{total}) \times 100$ , where  $N_i$  is the number of individuals of the species i and  $N_{total}$  is the total number of individuals of all species. If DOM $\geq 5\%$ , the species is termed a dominant species; if  $2.5\% \leq \text{DOM} < 5\%$ , the species is termed an accessory species; if DOM< 2.5%, the species is termed an incidental species. Species occurrence (OCC) was calculated according to Silveira Neto *et al.* (1976). OCC is defined as (number of days the species i was collected i total number of sampling days) i 100. If OCC $\geq 50\%$ , the species is termed a constant (primary) species; if  $2.5\% \leq \text{OCC} < 50\%$ , the species is termed a secondary species; if OCCi 25%, the species is termed an incidental species. To test if the color type of the

pan-trap has an effect on the capture rate of the bees we used a  $\chi^2$ -test. The same test was used to look for differences in the number of bees visiting male or hermaphrodite flowers, for difference in the number of nectar, pollen or both resource-gathering visits, and for difference in the number of visits between plants of different rows. To evaluate whether an inventory of fairly complete and reliable species was achieved, the accumulation curve of species number was constructed with EstimateS v9.1 (Colwell 1999) using the number of samples and the number of species cumulative average (Sobs). We then attempted to adjust the curve to a negative exponential equation, which is recommended if the sampling area is relatively small and the taxonomic group is well known, and evaluated the goodness of this approximation looking at the coefficient of determination  $(R^2)$  and slope at the end of the curve (the smaller it is, the more appropriate was the sampling). The distribution of females' and males' activities across time was compared with a Kolmogorov-Smirnov test. The time spent on flowers during a visit was compared between species with a Kruskal-Wallis test, and the relationship between time spent on flowers and bee head width was tested with a Spearman correlation test. Difference in the average head width between two groups of bees was tested with a Student's t-test. Time spent on flower and time between visits (their sum being the time spent on a "foraging bout" (visit + time to the next visit)), and daily and seasonal activity duration, were calculated for the females of the most abundant species observed foraging on flowers to roughly estimate their difference in the total number of visits per year. Because the bee community associated with melon at the study site was primarily composed of sweat bees (see Results), many of the analyses were performed on these species only, and on A. mellifera for comparison. In the text, mean values are expressed  $\pm$  SE.

# **RESULTS**

#### Bee community

In total, in 2011, 717 individuals of 31 species of bees in four families were collected: Andrenidae (three spp.), Apidae (six spp.), Halictidae (18 spp.) and Megachilidae (four spp.) (Table 1). Halictidae (sweat bees), especially those of the genus *Lasioglossum*, were collected in much higher proportions (>70%) than the other families. In respect of their natural history traits (social organization and nesting substrate), half of the halictids are solitary species nesting in the soil (Table 1). However, the two most abundant species, *Lasioglossum malachurum* (Kirby) and *L. marginatum* (Brullé), are eusocial and nest in the soil.

Table 1 List of bee species collected during the study with pan-traps and netting in transects (2011 data), with biological traits

Family	Species	Number of females <sup>†</sup>	Number of males <sup>†</sup>	DOM <sup>‡</sup>	OCC <sup>‡</sup>	Nesting	Sociality
Andrenidae	Andrena sp.	1	2	Incidental	Incidental	Soil	
Andrenidae	Panurgus calcaratus (Scopoli)	1	0	Incidental	Incidental	Soil	CO
Andrenidae	Panurgus cephalotes Latreille	4	0	Incidental	Incidental	Soil	SO
Apidae	<i>Apis mellifera</i> Linnaeus <sup>§</sup>	28 (25)	0	Accessory	Secondary	Hole	EU
Apidae	Ceratina cucurbitina (Rossi)	1	0	Incidental	Incidental	Hole	SO
Apidae	Ceratina dentiventris Gerstaecker	1	0	Incidental	Incidental	Hole	SO
Apidae	Ceratina nigrolabiata Friese <sup>§</sup>	1(1)	0	Incidental	Incidental	Hole	SO
Apidae	Eucera seminuda Brullé <sup>¶</sup>	3	0	Incidental	Incidental	Soil	SO
Apidae	Nomada sp.§	0	1 (1)	Incidental	Incidental	Clep	Clep
Halictidae	Halictus fulvipes (Klug) <sup>§</sup>	20 (10)	3	Accessory	Primary	Soil	EU
Halictidae	Halictus pollinosus Sichel	5	0	Incidental	Incidental	Soil	EU
Halictidae	Halictus smaragdulus Vachal <sup>§</sup>	2	1 (1)	Incidental	Incidental	Soil	EU
Halictidae	Halictus vestitus Lepeletier§	21 (2)	17	Dominant	Primary	Soil	EU
Halictidae	Halictus sp.	1	5	Incidental	Secondary	Soil	?
Halictidae	Lasioglossum brevicorne (Schenck)	2	0	Incidental	Incidental	Soil	SO
Halictidae	Lasioglossum callizonium (Pérez)§	3	12 (8)	Incidental	Secondary	Soil	SO
Halictidae	Lasioglossum discum (Smith) <sup>§</sup>	39 (10)	4(1)	Dominant	Primary	Soil	SO
Halictidae	<i>Lasioglossum griseolum</i> (Morawitz) <sup>¶</sup>	18	1	Accessory	Secondary	Soil	EU
Halictidae	Lasioglossum leucozonium (Schrank)§	16	6 (4)	Accessory	Primary	Soil	SO
Halictidae	<i>Lasioglossum malachurum</i> (Kirby) <sup>§</sup>	206 (104)	100 (23)	Dominant	Primary	Soil	EU
Halictidae	Lasioglossum marginatum (Brullé)§	110	17 (1)	Dominant	Primary	Soil	EU
Halictidae	Lasioglossum pauperatum (Brullé)	4	0	Incidental	Incidental	Soil	SO
Halictidae	Lasioglossum pauxillum (Schenck)	2	0	Incidental	Incidental	Soil	EU
Halictidae	Lasioglossum puncticolle (Morawitz)	3	0	Incidental	Incidental	Soil	SO
Halictidae	Lasioglossum villosulum (Kirby) <sup>§</sup>	7(1)	5 (3)	Incidental	Secondary	Soil	SO
Halictidae	Lasioglossum sp.§	2(1)	0	Incidental	Incidental	Soil	?
Halictidae	Nomioides minutissimus (Rossi)§	27 (1)	0	Accessory	Incidental	Soil	SO
Megachilidae	Anthidium taeniatum Latreille	1	0	Incidental	Incidental	Hole	SO
Megachilidae	Megachile concinna Smith <sup>§</sup>	3	9 (2)	Incidental	Secondary	Hole	SO
Megachilidae	Megachile dorsalis Pérez <sup>§</sup>	0	1 (1)	Incidental	Incidental	Hole	SO
Megachilidae	Megachile sp.	1	0	Incidental	Incidental	Hole	SO
-	Total	533 (155)	184 (45)				

<sup>†</sup>Data are shown as total number (number collected in transects). <sup>‡</sup>Species dominance (DOM) and species occurrence (OCC) were calculated using females + males of each species. <sup>§</sup>Species collected in 2011 in transects. <sup>¶</sup>Species collected in 2012 during observations on flowers. <sup>††</sup>Frequency ≥10% (n = 123). Soil, ground-nesting; Hole, hole-nesting (e.g. pre-existing tunnels in wood); SO, solitary; CO, communal (i.e. nest-sharing without eusociality); EU, eusocial; Clep, cleptoparasitic on other bees, not nesting or solitary. <sup>?</sup>, unknown information.

Considering DOM, 22 species were incidental, five species were accessory (A. mellifera and four species of sweat bees) and four species were dominant (three from the genus Lasioglossum and one from the genus Halictus) (Table 1). On the other hand, considering OCC, 19 species were incidental, six species were secondary and six species were primary (Table 1). Thus, most species were collected infrequently. The distribution of the curve well fitted a logarithmic model  $(y = -79.98 \ln(x) + 197.71; R^2 = 0.72, n = 17, P < 0.001),$ and the accumulation curve across the 16 sampling days showed a strong fit to a negative exponential curve (Sobs (Mao Tau) =  $31.08 \times (1 - \exp(-0.24 \times \text{day}))$ ;  $R^2 = 0.977$ , n = 16, P < 0.001), suggesting that almost all species in the community were collected (asymptote at 31.08).

Only 16 species of bees, i.e. half the total collected with all methods together, were sampled during observations on flowers in 2011 (in transects) (Table 1). Of these 16 species, nine were very rare (one or two captures only), two rare (four captures) and only five had eight or more captures. Of these five species, however, L. malachurum was predominant and, to a much lesser extent, A. mellifera (127 and 25 captures, respectively). Lasioglossum malachurum was thus by far the species that most visited melon flowers in the study field (104 individuals were females). The other very abundant species collected in the pan-traps, L. marginatum, was collected only once with transects. During 2012, 123 observations with data on flower visit duration were recorded, regarding eight species of bees, and all observed individuals turned out to be females after collection. As in the 2011 data, also in 2012, several species of *Lasioglossum*, particularly *L.malachurum* (22%), were the most abundant, together with *A.mellifera* (28%). Abundance for the remaining six species ranged from 5% to 11% only. *Lasioglossum marginatum* was not observed on flowers in 2012.

Pan-traps of different colors seemed to have different success in attracting bees, with yellow traps having been overall the most attractive to both females and males (females:  $\chi^2 = 98.8$ , df = 2, P < 0.001; males:  $\chi^2 = 34.2$ , df = 2, P < 0.001) (Table 2). This pattern is conservative when the most abundant species were considered separately, with the exception of females of *Nomioides minutissimus* (Rossi), which were collected with similar frequencies in the pan-traps of the three colors. Notably, *A. mellifera* was extremely rarely collected by pan-traps (four individuals, two in yellow traps and two in white traps), with most of samples coming from transects.

Using bees with sufficient sample size it appeared that bees visited more male flowers than hermaphrodite flowers in both years of study (Table 3). In these flowers, bees overall collected more frequently nectar (80 times) than pollen (28 times) or both pollen and nectar (26 times) ( $\chi^2 = 41.99$ , df = 2, P < 0.0001).

#### Sweat bees

The number of individuals and species of sweat bees was higher in mid-June and early July (number of species showed a further peak in mid-July), while few individuals and species were collected in late June and late July (Fig. 2A). Across the day, using only data from transects (since the pan-traps did not provide hourly data), sweat bees were mostly collected in late morning and early afternoon (Fig. 2B). The daily distribution was not statistically different for females and males (Kolmogorov–Smirnov test: D = 0.33, n = 6, P = 0.89), despite the former being apparently mostly active between 1100 and 1200 h, and the latter between 1200 and 1400 h (Fig. 2B).

At the seasonal level, females of L.malachurum showed two peaks of activity (mid-June and early July), while males had only one clear peak at early July (Fig. 3B). The first date of collection of L.malachurum was 14 June, while that of A.mellifera was 21 June; both species were found foraging roughly until the same date (15–18 July). At a daily level, L.malachurum females started the daily activity about 1h earlier than males, but the two sexes did not have different activity distribution (Kolmogorov–Smirnov test: D=0.33, n=6, P=0.93)

Table 2 Number of individuals collected in the pan-traps of different colors, for females and males of the most abundant species, and tests for their differences among trap colors

Species (sex)	Yellow trap	White trap	Blue trap	$\chi^2 (df = 2), P$
L. discum (females)	27	1	1	46.62, < 0.001
L. malachurum (females)	62	23	17	35.11, < 0.001
L. malachurum (males)	49	15	13	31.89, < 0.001
L. marginatum (females)	68	18	24	40.65, < 0.001
N. minutissimus (females)	8	10	8	0.31, 0.86

Table 3 Number of bee individuals of the most abundant species (≥10 individuals) collected on male and hermaphrodite flowers of melon, with test for difference in frequency between the two types of flowers

Year	Species (sex)	Male flowers	Hermaphrodite flowers	$\chi^2$ (df = 1), P (Yate's correction)
2011	A. mellifera (females)	22	3	12.9, < 0.001
2011	H. fulvipes (females)	9	1	4.9, 0.02
2011	L. discum (females)	7	3	0.9, 0.34
2011	L. malachurum (females)	92	12	60, < 0.0001
2011	L. malachurum (males)	21	2	14.1, < 0.001
2012	A. mellifera (females)	30	5	8.2, 0.004
2012	L. discum (females)	10	0	4, 0.045
2012	L. leucozonium (females)	13	1	4.3, 0.038
2012	L. malachurum (females)	26	1	10.6, 0.001
2012	M. concinna (females)	12	0	5, 0.025

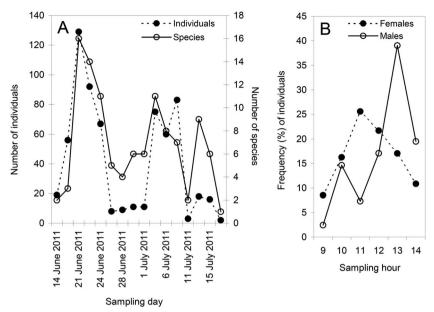


Figure 2 Number of individuals and number of species of sweat bees (Halictidae) collected across the days of sampling (2011 data of pan-traps + transects) (A), and frequency of female and male individuals of sweat bees collected across the hours of sampling (2011 data of transects) (B).

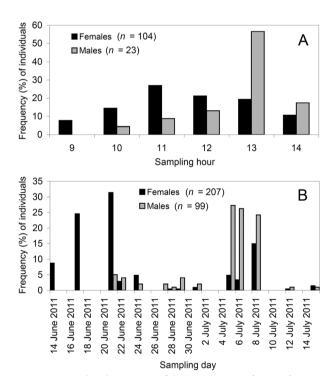


Figure 3 Graphical summary of data on activity of *Lasioglossum malachurum*. (A) Frequency of female and male individuals across the hours of sampling (2011 data of transects); (B) frequency of female and male individuals across the days of the sampling period (2011 data of pan-traps + transects).

(Fig. 3A). *Apis mellifera* was collected more often between 1200 and 1300 h, thus not much differently from *L. malachurum*.

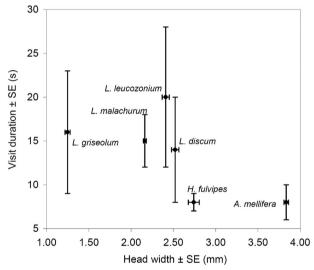


Figure 4 Relationship between head width of bee species and duration of visits on the melon flowers (2012 observation data). Mean values are expressed  $\pm$  SE.

Lasioglossum malachurum gathered equally pollen (11 times), nectar (eight times) or both (eight times) during a visit ( $\chi^2$  = 1, df = 2, P = 0.6), while A. mellifera used primarily the melon flowers as a source of nectar (23 times) and rarely to gather pollen (three times) or both resources (four times) during a visit ( $\chi^2$  = 28.5, df = 2, P < 0.001). Lasioglossum malachurum did not show any preferences for plants of certain rows within those sampled ( $\chi^2$  = 8.5, df = 9, P = 0.48).

Table 4 Presence/absence data for bee taxa collected on flowers of melon, Cucumis melo (all varieties considered) from 17 different studies across five continents

Country or state	Am	Bom	Xyl	Cer	Ant	Melip	Euc	Hal	Melit	And	Meg	References
Arizona, USA	+	_	_	_	_	_	_	_	_	_	_	McGregor and Todd 1952
Brazil	+	_	_	_	_	_	_	_	_	_	_	Souza 2003
Brazil	+	_	_	_	_	_	_	_	_	_	_	Siqueira et al. 2011
Brazil	+	_	_	_	_	_	_	_	_	_	_	Kiill et al. 2012
Columbia and South Carolina, USA	_	+	_	_	_	_	_	_	_	_	_	Handel 1982
Costa Rica	+	_	_	_	_	_	_	_	_	_	_	Danka et al. 1993
France	+	+	_	_	_	_	_	+	+	_	_	Carrè et al. 2009
India	+	_	_	_	_	_	_	+	_	_	_	Grewal and Sidhu 1978
Israel	+	_	_	_	_	_	_	_	_	_	_	Orr and Eisikowitch 1988
Israel	+	_	_	_	_	_	_	_	_	_	_	Dag and Eisikowitch 1999
Israel	+	_	+	_	_	_	_	_	_	_	_	Sadeh et al. 2007
Ivory Cost	+	_	_	+	+	+	_	+	_	_	_	Kouonon et al. 2009
Mexico	_	_	_	+	_	+	_	+	_	_	_	Meléndez-Ramirez et al. 2002
Panama	+	_	_	_	_	_	_	_	_	_	_	Di Trani de la Hoz 2007
Spain	+	_	_	+	_	_	+	+	_	+	+	Present study
Turkey	+	+	_	_	_	_	_	_	_	_	_	Daşgan et al. 1999
Virginia, USA	_	+	+	_	_	_	+	+	_	_	_	Adamson et al. 2012

+ and – denote presence and absence, respectively. Am, *Apis mellifera*; Bom, *Bombus* spp.; Xyl, *Xylocopa* spp.; Cer, Ceratini spp.; Ant, Anthophoridae spp.; Melip, Meliponini spp.; Euc, Eucerini spp.; Hal, Halictidae spp.; Mel, Melittidae spp.; And, Andrenidae spp.; Meg, Megachilidae spp.

The smallest bee individual collected had a head width of 0.91 mm and belonged to N. minutissimus, and the largest had a head width of 4.05 mm and belonged to A. mellifera. Individuals of the four dominant halictid species were smaller (head width: 2.12 ± 0.012 mm), on average, than those from all the other species (head width:  $2.38 \pm 0.09 \,\mathrm{mm}$ ) (Student's *t*-test: t = -2.85, df = 141, P = 0.005). The mean times spent on flowers by female bees during a visit ranged from 8s (Halicuts fulvipes (Rossi) and A. mellifera) to 20 s (Lasioglossum leucozonium (Schrank)) and was very variable within species (Fig. 4). A Kruskal-Wallis test showed no overall difference among species (K = 6.43, n = 102, P = 0.26). The time spent on flowers did weakly and negatively correlate (with a marginal P value) with bee species head width (Spearman correlation test:  $\rho = -0.81$ , n = 6, P = 0.058) (Fig. 4). Records of individual bees foraging on two subsequent flowers were rare during 2012 observations and concerned with only A. mellifera and L. malachurum, which on average spent, respectively, 4s (range: 1-12, n = 14) and 2 s (range: 1-4, n = 5) between visits (statistics was not performed due to the small sample size).

The time spent in a foraging bout is roughly calculated as 6s for *A. mellifera* and 4s for *L. malachurum*. Considering that the length of the daily foraging activity for the two species is the same (6h), *L. malachurum* would perform approximately 30% more visits than *A. mellifera* in a day. This value would even increase to 50% at a seasonal level (e.g. in a year) if considering that the number of days females were collected (i.e. while foraging) was a bit greater for *L. malachurum* (31 vs 27).

# Comparison of data with previous studies on melon pollinators

As shown in Table 4, it seems that in most of the studied areas melon is heavily, possibly exclusively, pollinated by honeybee (14 of 17 reviewed studies). On the other side, in a few studies honeybee was recorded together with other bee species, often from a great range of families. For example, in Ivory Coast, melon is pollinated by honeybee together with species of Ceratini, Anthophoridae, Meliponini and Halictidae (Table 4). At an Indian locality, Grewal and Sidhu (1978) collected a few A. mellifera but most abundant were halicted bees in the genus Nomioides. In very few localities, honeybee seems to be absent in melon crops, and pollination is carried out only or mainly by Bombus (three localities in USA), Halictidae (one locality in India) or a mixture of species from various groups (one locality in Mexico). Carrè et al. (2009) recorded a great diversity of bee species spanning 37 subgenera (including species of Lasioglossum) in France. Species of Lasioglossum were also previously observed on melon crops in Mexico (Table 4).

# **DISCUSSION**

The value of bees as pollinators of melons has been known from long ago (Beattie 1926; Rosa 1927) but still needs to be investigated in detail in many parts of the world. The present study is the first to analyze the bee assemblage associated with melon crops in southern Europe, while

the only other study performed in the continent is that of Carrè et al. (2009) at a locality in southern France. In this locality, the diversity of bee species visiting melon was as high as in our study, possibly due to the great bee diversity typical of the Mediterranean areas (Michener 2000; Vamosi et al. 2006) and also because of the efficient mixture of sampling techniques used in both studies. These include integrating pan-traps with transects, but also the use of pan-traps of different colors. In our study, for example, yellow traps were the most attractive to both females and males of most sweat bee species (Table 2), in accordance with many previous studies (Abrahamczyk et al. 2010; Gollan et al. 2011; Munyuli 2013). However, the abundance of one species, N. minutissimus, did not show a bias among pan-trap colors, confirming that attractiveness to a certain color can also change among species and thus that the use of different trap colors should be preferred in sampling protocols (e.g. Gollan et al. 2011).

Although it seems that in some of the previously studied areas melon is at least mainly pollinated by honeybee (Table 4), in other areas honeybee was recorded together with other bee species or seems to be even absent on melon crops. This suggests that the efficiency of A. mellifera as a pollen vector for many cultivated species should be evaluated before the introduction of hives into cultivated fields (a common practice), since in some cases other pollinator species may do better than this domestic bee. This is particularly important when considering the current pandemic among honeybees (Oldroyd 1999), highlighting the need to find alternative species as managed crop pollinators (e.g. Canto-Aguilar & Parra-Tabla 2000). For example, Adamson et al. (2012) and Carrè et al. (2009) showed that various species of wild bees were as important as honeybee in melon flower visit frequency in Virginia and France, respectively.

In our case, honeybee was sampled with appreciable frequency and thus possibly has a role in pollinating melon; however, different considerations suggest that *L. malachurum* is potentially a better pollinator of melon than honeybee at our study locality.

First, honeybee seemed to use melon flowers mainly as a source of nectar, while *L. malachurum* also abundantly collected pollen from flowers. Most of the visits occurred on male flowers (Table 3). Thus, the latter species would be more prone to leave a higher amount of pollen grains in hermaphrodite flowers, while honeybees would mainly transport pollen grains accidentally gluing to their body during nectar collection. In a study carried out on sunflower, *A. mellifera* was also a major nectar-forager and *L. malachurum* a major pollen-forager (Pisanty *et al.* 2014). However, in that case, the halictid bee did not visit

female flowers as much as honeybee, resulting in a higher pollen deposition in the stigma by the latter species (Pisanty *et al.* 2014). The main use of melon flowers as a nectar source by honeybee was also reported by Reyes-Carrillo *et al.* (2007) in Mexico.

Second, calculations that included time spent on a flower, time between visits, daily activity duration and seasonal activity duration for L. malachurum and A. mellifera females help suggest an overall higher number of visits on flowers per year for the former species. Values retrieved from the literature suggest that the average time spent in the nest after a foraging trip may be roughly similar in A. mellifera (about 5 min) (Weidenmüller & Tautz 2002) and Lasioglossum spp. (about 8 min) (Michener & Wille 1961; Sakagami & Hayashida 1968; C Polidori, unpubl. data, 2005), thus likely not affecting the estimated difference. Third, the mean number of L. malachurum females collected from transects per day was about six times the mean number of A. mellifera females (26 vs 4.1). Fourth, phenological data revealed that L. malachurum start visiting melon flowers earlier than honeybee (about 1 week), and that females exhibit two peaks of activity during the summer (as it is the rule for temperate social sweat bees; Mitesser et al. 2006; Polidori et al. 2009), suggesting that this species is among the first to forage on melon flowers and that it provides pollination up to the end of the flowering period. We cannot exclude, however, that such variation in activity was partially due also to pesticide application to the crop field at regular intervals during the summer (three times in total). For example, one application was done on 8 July 2011, and during the following 3-4 days bee activity was very poor.

Future studies may further disclose the pollination role of *L. malachurum* by studying new aspects such as nectar and pollen production in flowers across the day and the season, pollen load on bee body, and pollen movement distance via these bees (Ne'eman *et al.* 2009).

In conclusion, melon in central Spain seems to be mainly pollinated by small bees in the family Halictidae, and in particular by *Lasioglossum* species. The weaker, though not negligible, activity of honeybees on this crop may suggest that increasing hives may only partially lead to an increase of fruit set. Even more importantly, the complete absence of *Bombus* spp. on our studied crop (contrary to that observed in other melon crops, e.g. in USA by Handel 1982) suggests that placing nest boxes nearby would not be useful to increase melon production. Instead, we suggest that it could be useful to attract nestfounding *Lasioglossum* females around the crop by providing soil patches with characteristics adequate for nesting (e.g. compact soil almost avoid of vegetation;

Polidori et al. 2010). Severns (2004) successfully attracted Lasioglossum females to nests in artificially created plots of bare ground while planting seeds of a lupine species at a site in Oregon, with more than 300 nests established in 3 years. As the vast majority of Lasioglossum species are polylectic, planting diverse plants normally used by these bees around the crops would also help establishing and sustaining the populations.

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