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Temporal activity patterns in a flower visitor community of *Dictamnus albus* in relation to some biotic and abiotic factors

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Abstract

Several biotic and abiotic factors affect the seasonal and daily activities of flower-visiting insects, which, ultimately, influence the composition and abundance of a pollinator community and the extent of their pollination service. The aim of this four-year study was to assess the effect of some abiotic and biotic factors on the abundance and activity pattern of flower-visiting insects throughout the blooming of a locally rare plant species, *Dictamnus albus* L. (Rutaceae). We found a large inter-annual variation in the abundance and activity of flower visitors, with up to a six-fold variation in the total number of individual insects observed. Moreover, a similar fluctuation among years was also highlighted by biodiversity indices and by the changes in the relative frequency of each flower-visiting taxon. Annual, seasonal and daily variations were explained by a certain level of "response diversity" between flower-visiting taxa in relation to different environmental conditions during *D. albus* blooming. The foraging activity was related to ambient temperature in the tribe of Halictini and to relative humidity in four flower-visiting taxa (positive relation in the tribe Anthophorini; negative relation in the taxa Megachilini, Halictini and Syrphidae). The climate conditions during the winter might have also affected the phenology and the abundance of flower-visiting taxa with a large number of insects observed in warmer years. Among the biotic factors, flower availability did not affect the abundance of insects observed in the patch. Moreover, we did not find any significant negative associations (e.g. possible competition) in the co-occurrence of different flower-visiting taxa, instead we found six significant positive associations probably related to similar preferences towards specific environmental conditions. In conclusion, our results show that the flower visitor community of *D. albus* is quite variable among years, seasons and daily hours, but the relative high visitor diversity and the different patterns of activity related to different environmental responses might guarantee a stable pollination service of this plant species in years with extreme environmental conditions.

Key words: flower visitors, Hymenoptera Apoidea, bees, plant-pollinator interactions, climate, weather conditions.

Introduction

Pollinator behaviour depends on several biotic (e.g. predation and competition interactions, pollen and nectar availability) and abiotic (e.g. ambient temperature, solar radiation, relative humidity and wind speed) factors that determine the variation in seasonal and daily patterns of activity among species. The presence of different pollinator species visiting the flowers of a given plant species in the course of a day and during the flowering period depends on the interspecific differences in temporal activity (e.g. Giovanetti *et al.*, 2006). Moreover, the climatic conditions during the winter period and early spring can affect the timing of pollinator emergence in diapausing insects and thus the synchronization with the flowering period (Schweiger *et al.*, 2008; Forrest and Thomson, 2011). In fact, some studies on bumblebees and solitary bees have shown that the temperature during overwintering and the accumulation of degree-day in early spring can affect their survival and phenology (Gosterit and Gurel, 2009; White *et al.*, 2009; Jiang *et al.*, 2010; Sgolastra *et al.*, 2010; Bartomeus *et al.*, 2011).

The daily activity of pollinators can vary considerably among taxa. For example, Herrera (1990) observed variation in the timing of foraging on *Lavandula latifolia* Medicus, both among major taxa (hymenopterans, dipterans and lepidopterans) and among species within taxa. In these cases, weather is often one of the main abiotic factors affecting pollinator activity. Tuell and Isaacs (2010) showed an effect of the ambient temperature, wind speed, humidity and solar radiation on the flower-visitor community composition on *Vaccinium corymbosum* L. (high bush blueberry). In the aforementioned study, honey bees were more abundant during good weather conditions (high temperature and solar radiation, low wind speed and humidity), whereas bumble bees dominated during poor weather. Similar results were also observed in an apple orchard where *Apis mellifera* L. showed a peak of activity in late morning and early afternoon, when the ambient temperatures were higher; in contrast, *Osmia cornuta* (Latreille) was equally abundant throughout the day (Vicens and Bosch, 2000). Considering the large thermal range across which insects can be active, species with highly efficient individual thermoregulation (i.e. bumblebees) should show a more stable temporal foraging activity (Heinrich, 1974; Bishop and Armbruster, 1999).

Since the weather conditions can vary significantly between years, the abundance of pollinators during the flowering season of a given plant can show annual variations with possible effects on the pollination success and the percentage of fruit-set (Ivey *et al.*, 2003).

Although the pollinator seasonal activity is usually associated to the flowering period of pollinated plants, in particular in oligolectic species, the recent climate changes could potentially lead to phenological mismatches in plant-pollinator interactions (Bartomeus *et al.*, 2011). Over the period 1880 to 2012, the global surface temperature calculated as linear trend shows a warming of 0.85 (0.65 to 1.06) °C and some ecological responses to climate change are already evident (Walther *et al.*, 2002; IPCC, 2013). The animal and plant phenology (i.e. the timing of seasonal activities) is probably the simplest process in which the climate changes can affect the ecology of animal and plant species in a way that is observable in relatively few years (Rosenzweig *et al.*, 2007; Bartomeus *et al.*, 2011). Walther *et al.* (2002) reported that spring activities, including early blooming, bird migration, amphibian breeding and butterfly appearance, have occurred progressively earlier since the 1960s both in Europe and in North America. Because not all taxonomic taxa respond similarly to the temperature variations, differences in the magnitude of phenological responses may affect food-web interactions with important ecological consequences (Winder and Schindler, 2004). Shifts in seasonal events due to climate change have also been observed in several plant-pollinator systems (Kudo and Ida, 2013; Robbirt *et al.*, 2014; Forrest, 2015).

Several biotic factors such as predation, competition, nectar and pollen availability can affect the abundance of flower-visiting insects. For example, Dauber *et al.* (2010) showed that increasing flower availability in terms of plant population size and density might increase the abundance of pollinators, as a direct consequence of increased attraction. In addition, the possible competition for pollen and nectar sources between different pollinator species can affect their relative abundance (Paini, 2004).

The identification of the abiotic and biotic factors that affect the plant-pollinator interaction is essential to understand and mitigate the decline of threatened plant species. In Italy, *Dictamnus albus* L. (Rutaceae) reaches its southern distribution limit and is protected at regional level. The species is locally protected across Europe and has been designated as 'vulnerable' in several European countries (Schnittler and Günther, 1999). Previous studies reported an occasional pollination deficit that, in isolated population, might lead to fitness reduction (Fisogni, 2011; Fisogni *et al.*, 2016). This study was performed in the framework of the PP-ICON project (Plant Pollinator Integrated CONservation approach: a demonstrative proposal), a Life+ European Project, with the aim to ensure the persistence of an isolated population of *D. albus* located in a protected area near Bologna (North Italy) and to restore the community of its natural pollinators. Here, in particular, we addressed the following questions: 1) What is the annual, seasonal and daily activity pattern of each flower-visiting taxon? 2) Do weather conditions (ambient temperature and relative humidity) and flower availability during the blooming affect the abundance of the visitor community of *D. albus*? 3) Do the climatic conditions (mean temperature and degree-day) during wintering affect the annual abundance of each flower-visiting taxon? 4) Are there associations of flower-visiting insect occurrences during the same units of observation?

Materials and methods

Study system

Pollinator surveys were performed on *D. albus*, a perennial entomophilous herb that produces flowering racemes bearing numerous white-purple slightly zygomorphic flowers. Single flowers show marked herkogamy and protandry that strongly reduce the possibility of autonomous self-pollination; flowering proceeds from the bottom to the top of the raceme (Fisogni *et al.*, 2011). Observations were carried out in a natural population in the SCI-SPA IT4050001 - Natura 2000 site, situated within the Regional Park "Parco dei Gessi Bolognesi e Calanchi dell'Abbadessa", on the hills nearby the city of Bologna, Italy (168 m a.s.l.; 44°25'11"N 11°23'54"E). The study population is found on the fringe of a xerothermic wood mainly composed of downy oak (*Quercus pubescens* Willd.) and ash (*Fraxinus ornus* L.).

Insect observations

We performed insect observations in a randomly chosen patch of *D. albus* plants in four consecutive years (2011-2014). From 2011 to 2013 the patch consisted of eight plants, while in 2014 it included five plants due to the scarcity of flowering in the area. All the plants in the patch were followed throughout the flowering season. We carried out six days of observation per year: two at the beginning of the flowering (14-73% of open flowers), two in full blooming (61-92% of open flowers) and two at the end of the flowering season (24-76% of open flowers). Discrepancies in flowering times among plants in the patch were negligible. Each day we performed 4 observation units, two in the morning (9:00 and 12:00 h) and two in the afternoon (15:00 and 18:00 h). Each observation unit consisted of two 15-minute surveys, separated by an interval of 10 minutes, for a cumulative observation time of 2 hours per day and a total of 12 hours per year. Observations of flower visitors were performed in favourable weather conditions (i.e., low cloud cover, dry weather and low wind speed). During each survey, we recorded the number of approaches to the patch and the number of flowers probed for nectar and/or pollen by each visiting insect. Insect visitors were identified at the lower taxonomic level allowed by visual recognition (i.e. family, genus or species) but analysed at tribe level (with the exclusion of syrphids, bee flies and wasps that were grouped at family level). We did not capture insects for the purpose of this study. However, in previous studies (Fisogni *et al.*, 2011; 2016) flower-visiting insects were sampled in the whole population of *D. albus* and the results are in accordance with those from this study, thus our observations on the selected patch can be considered a good representation of the flower visitors in the area. In order to correlate the number of available flowers to visitor abundance, prior to each observation unit we counted the number of open flowers in each plant of the observed patch.

Climatic factors

Each year of the study we performed two types of climatic data collection. During daily surveys, at the beginning and at the end of each observation unit we recorded air temperature above ground (°C) and relative humidity (RH%), by means of a LCD thermo-hygrometer (Cornwall Electronics) placed in the shade adjacent to the observed patch. Mean values for every unit were further considered for data analysis.

In addition, in order to take into account the effect of overwinter climatic conditions on the abundance of pollinators during the flowering period of *D. albus*, for each year we calculated the degree-hour (DH) accumulated during wintering and the hourly mean temperature recorded from January 1st to the observation dates. The DH was calculated with the equation:

$$\sum_{1}^{n}(T - Tt)$$

where T was the recorded hour temperature and Tt was the baseline temperature. We calculated the accumulation of heat units when the hourly temperature was higher than 7 ($Tt > 7$ °C) since the thermal thresholds required to emerge after winter diapause in several spring-flying bees are usually around 7 °C (White *et al.*, 2009; Sgolastra *et al.*, 2010; Fründ *et al.*, 2013; Ahn *et al.*, 2014). We started the DH accumulation on January 1st of each year because this is considered the end of the diapause and the beginning of the post-diapause in several insect species (Hodek, 1996). For example, Sgolastra *et al.* (2010) showed that the winter diapause in the solitary bee *Osmia lignaria* Say (Hymenoptera Megachilidae) can be divided in two phases. In the first phase (from September to December), diapause intensity remained stable independently of temperatures while, in the second phase (from December to the spring-emergence, also called post-diapause), diapause intensity decreased with increasing temperatures. Since these two phases in winter diapause have been also observed in other temperate-zone insects (Hodek, 1996) and species can differ in their response to environmental conditions during winter (Fründ *et al.*, 2013), we would expected that the winter-spring temperatures might affect phenology in a different way among pollinators, when they are in post-diapause. Data were downloaded from the Regional Agency for the Environment Protection (ARPA Emilia-Romagna, www.arpa.emr.it; permanent weather station of Settefonti, Ozzano dell'Emilia 44°24'09"N 11°27'42"E, 4 km south-east of the study site).

Data analysis

For each year, we counted the total number of insects and the abundance of each flower-visiting taxon. We also calculated for the observed insects belonging to the superfamily Apoidea the Shannon's diversity (expH'), Simpson's diversity (1/D) for a finite community and Berger-Parker dominance (1/d) biodiversity indices (Magurran, 2004).

The number of each flower-visiting taxon during the flowering period of each year was reported graphically. Moreover, in order to assess the activity of each flower-visiting taxon during the day, the mean abundance values were obtained for each observation unit and expressed as percentages of the mean abundance value at its daily peak of activity (i.e. period with maximum abundance).

Relationships between the abundance of each visitor taxon on *D. albus* with weather conditions (hourly temperature and relative humidity) and flower availability (total number of open flowers in the patch) during each observation unit were tested by means of Spearman's rank correlations. Only taxa with at least 20 individuals recorded during the four-year study have been considered in the statistical analysis.

The associations between two flower-visiting insects in the same unit of observation were measured using presence and absence data with a phi coefficient (φ2) for dichotomous nominal-scale data and its significance was tested with a contingency table with a Bonferroni correction for multiple tests. The phi coefficient is used to measure the association between two binary variables (Zar, 1999).

Analyses were performed using STATISTICA version 7.1 software (Statsoft Inc.).

Results

Diversity and activity patterns of flower-visiting taxa

We observed 14 taxonomic categories that visited flowers of *D. albus* in the study period and we found a strong fluctuation among years in the total number of observed insects (table 1). The three biodiversity indices calculated on the abundance of bees (superfamily Apoidea) showed similar trends among years, with the minimum values in 2012 and the maximum values in 2014, except for the Shannon index that showed the maximum value in 2011. Halictini were the most abundant flower-visiting taxon in three out of the four study years (except in 2013). Anthophorini were the second most abundant visitor taxon with a large contribution of the species *Habropoda tarsata* (Spinola).

In the observation patch, we also found a strong variation in the number of insects of each flower-visiting taxon during the flowering season of *D. albus* (figure 1). Peaks in the total number of insects observed were recorded toward the end of flowering period (in 2011 and 2014) or in the first half (in 2012 and 2013).

The relative daily abundance of each flower-visiting taxon showed a large variation among observation units and taxonomic groups (figure 2). Several taxa displayed a unimodal activity pattern, with maximum abundance occurring in the morning (Halictini, Osmiini, Megachilini, Anthidiini, Anthophorini, Nomadini, Syrphidae, Bombyliidae), or in the afternoon (Vespidae and Xylocopini), or in the middle of the day (Apini). A bimodal activity pattern was observed only in Bombini whereas Eucerini showed a homogeneous activity for most of the day (figure 2).

Abiotic factors

The minimum, maximum and mean temperatures and relative humidity recorded during the observation units

Table 1. List of flower visitors, total and yearly number of individuals recorded, and (in brackets) relative frequencies. Final lines report total number of insect per year and biodiversity indices.

Flower-visiting taxon	Genera or species of insects visually identified	2011	2012	2013	2014	Total
Apini	*Apis mellifera*	3 (4.2)	0 (0.0)	8 (6.1)	78 (19.3)	89 (8.6)
Andrenini	*Andrena* spp.	2 (2.8)	0 (0.0)	0 (0.0)	2 (0.5)	4 (0.4)
Anthidiini	*Anthidium* spp.	0 (0.0)	2 (0.5)	2 (1.5)	3 (0.7)	7 (0.7)
Anthophorini	*Anthophora plumipes*; *Habropoda tarsata*	7 (9.9)	134 (31.4)	59 (44.7)	12 (3.0)	212 (20.5)
Bombini	*Bombus* spp.	7 (9.9)	34 (8.0)	7 (5.3)	17 (4.2)	65 (6.3)
Eucerini	*Eucera* spp.	7 (9.9)	7 (1.6)	7 (5.3)	5 (1.2)	26 (2.5)
Megachilini	*Megachile* spp.	0 (0.0)	7 (1.6)	2 (1.5)	88 (21.8)	97 (9.4)
Osmiini	*Osmia* spp.	2 (2.8)	21 (4.9)	6 (4.5)	35 (8.7)	64 (6.2)
Xylocopini	*Xylocopa* spp.	4 (5.6)	1 (0.2)	4 (3.0)	3 (0.7)	12 (1.2)
Halictini	*Halictus* spp.; *Lasioglossum* spp.	28 (39.4)	211 (49.5)	35 (26.5)	141 (34.9)	415 (40.2)
Nomadini	*Nomada* spp.	0 (0.0)	4 (0.9)	0 (0.0)	0 (0.0)	4 (0.4)
Syrphidae		1 (1.4)	0 (0.0)	1 (0.8)	20 (5.0)	22 (2.1)
Vespidae		5 (7.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (0.5)
Bombyliidae	*Bombylius* spp.	5 (7.0)	5 (1.2)	1 (0.8)	0 (0.0)	11 (1.1)
Total insects		71	426	132	404	1033
Shannon Index (expH')*		5.28	3.15	4.25	3.59	5.58
Simpson Index (1/D)*		3.92	2.77	3.50	4.20	4.06
Berger-Parker Index (1/d)*		2.14	2.00	2.20	2.72	2.40

*the biodiversity indices were calculated considering only the tribes in the superfamily Apoidea (Syrphidae, Vespidae and Bombyliidae were not included).

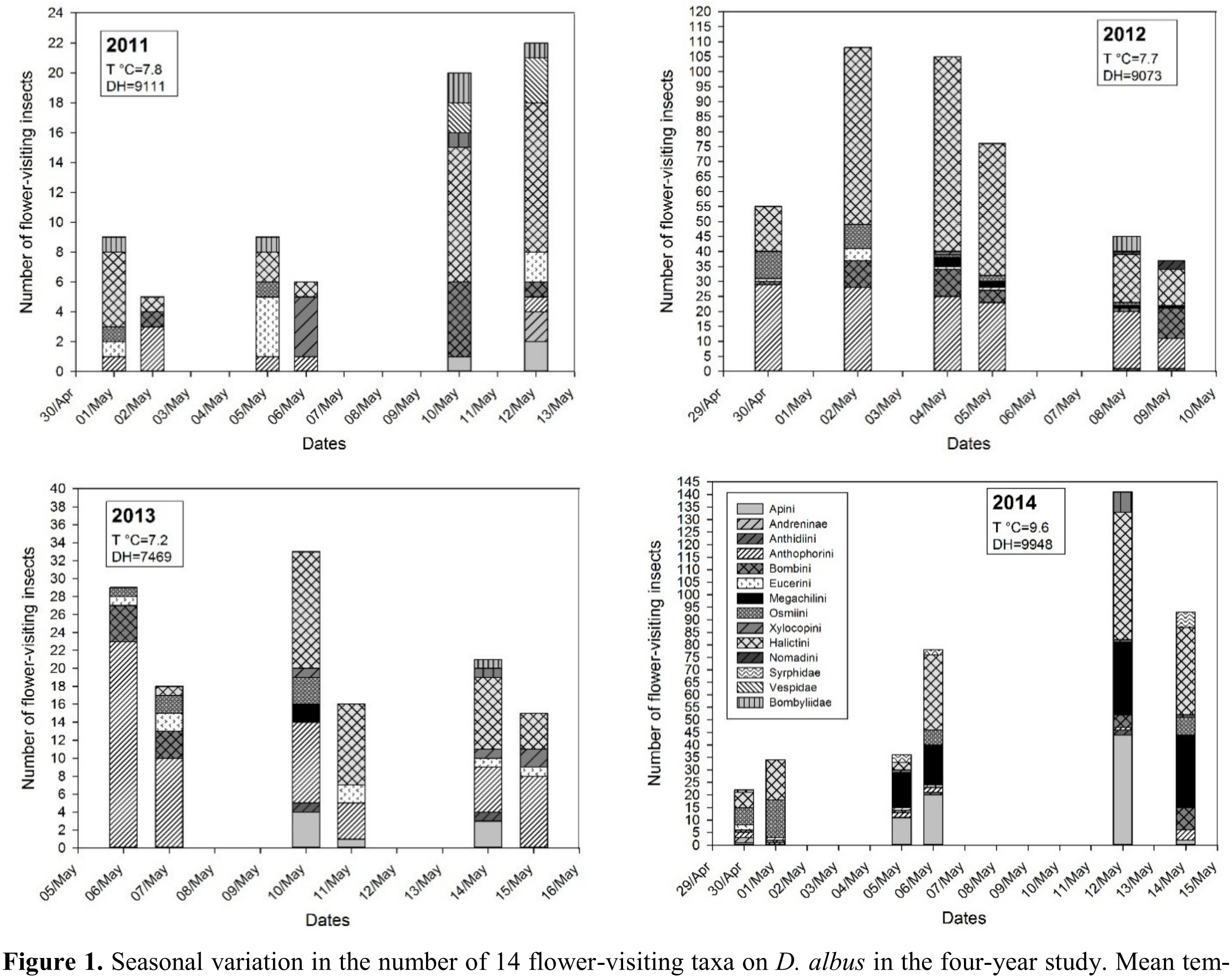
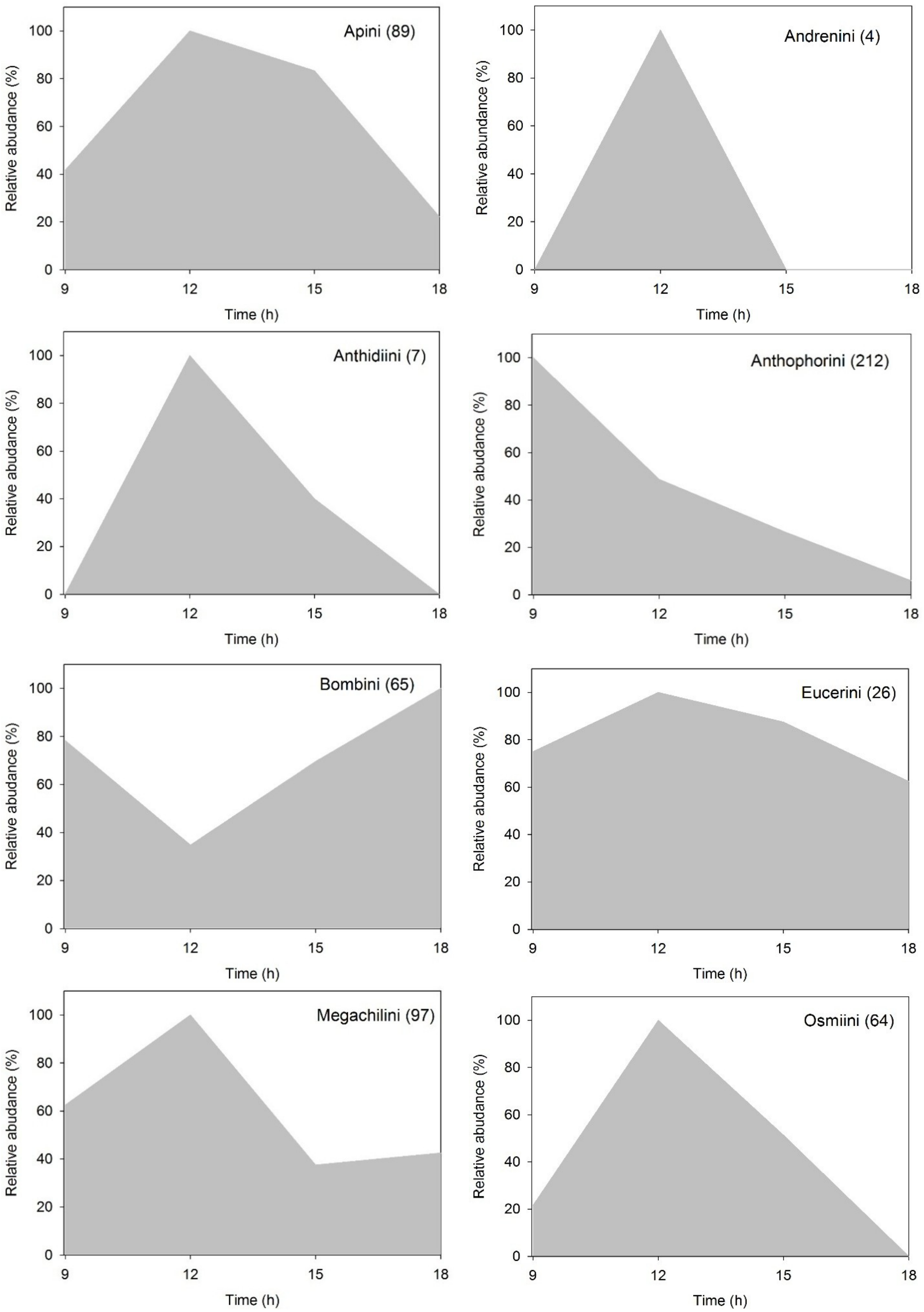


Figure 1. Seasonal variation in the number of 14 flower-visiting taxa on *D. albus* in the four-year study. Mean temperature (T °C) and degree-hour (DH) accumulated during winter are reported for each year.



(continued)

Figure 2. Daily variation in the abundance of 14 flower-visiting taxa on *D. albus*. For each taxon, abundance at a given hourly period is expressed as the percentage of its abundance in relation to the daily peak. Sample size within brackets.

(Figure 2 continued)

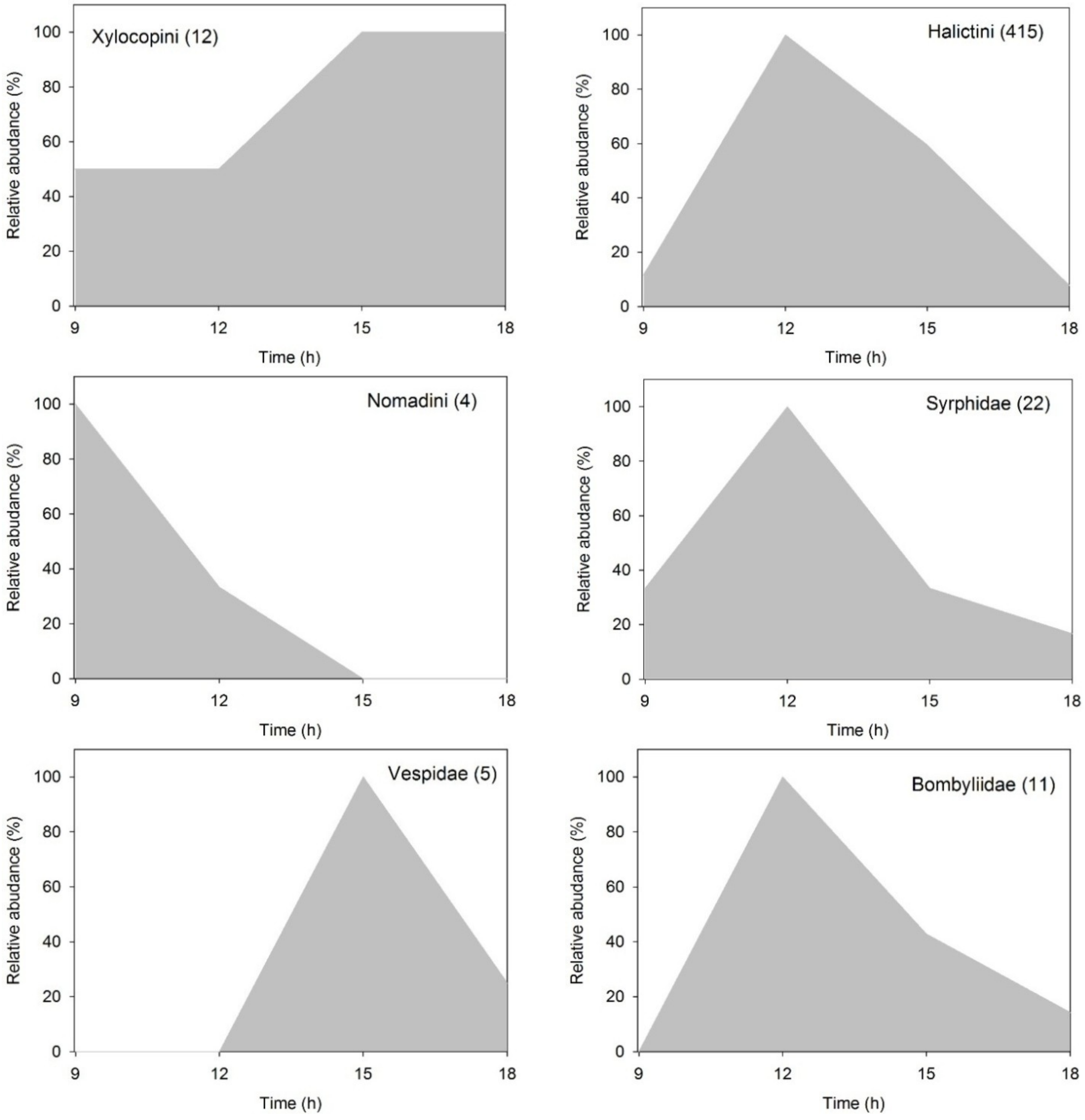


Figure 2. Daily variation in the abundance of 14 flower-visiting taxa on *D. albus.* For each taxon, abundance at a given hourly period is expressed as the percentage of its abundance in relation to the daily peak. Sample size within brackets.

for each year are reported in table 2. Overall, the temperatures ranged from 15 to 31 °C and the abundance of only one taxon (Halictini) was positively related with this factor (table 3). The relative humidity recorded in the four-year study ranged from 22 to 76.5%. Our data showed that the abundance of Anthophorini was positively correlated with the relative humidity, whereas the abundance of Megachilini, Halictini and Syrphidae decreased with the increase of this factor (table 3).

The mean temperature and the degree-hour accumulated during winters 2011-2014 ranged, respectively, from 7.2 to 9.6 and from 7469 to 9947 (figure 1). The warmest winter (higher mean temperature and degree-hour values) was 2014, which was the second year with the highest number of insects recorded and the year with the highest biodiversity indices (except Shannon index) (table 1). The coldest winter was 2013, while the other years showed similar values of mean temperature and degree-hour values (figure 1).

Biotic factors

During the four-year study, the number of open flowers available in the patch ranged from 20 to 135 (table 2) but we found no significant correlation between this biotic factor and the abundance of each flower-visiting taxon (table 3).

Table 2. Minimum, maximum and mean values of temperature, relative humidity and flower availability in each observation unit per year.

Year	Observation unit	Temperature (°C)			Relative Humidity (%)			Flower availability (total number of open flowers)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
2011	09:00	15	22	17.9	39	55	47.4	31	86	64.7
	12:00	18.5	26	21.2	35	53.5	42.1	33	89	64.8
	15:00	20.5	31	23.7	30.5	45	36.5	36	95	65.0
	18:00	19.5	31	23.6	25.5	52.5	35.0	39	94	66.3
2012	09:00	14.5	26	19.0	40.5	55.5	48.6	40	107	83.5
	12:00	21	26.5	24.4	30.5	39.5	35.8	40	106	82.5
	15:00	22.5	26.5	24.2	31	43	37.4	46	108	84.8
	18:00	17.5	22.5	20.7	33	63	46.8	49	106	84.3
2013	09:00	17	20.5	18.9	40	68	58.5	21	133	89.0
	12:00	17	26	21.9	25	76.5	52.6	25	127	87.3
	15:00	18.5	24	21.3	32	71	52.2	31	135	90.5
	18:00	19.5	24	21.3	36	64	51.4	36	135	91.8
2014	09:00	16	20	17.7	36.5	60	44.8	20	68	40.7
	12:00	20.5	24.5	21.9	30	43	36.9	20	69	41.3
	15:00	21	24	22.5	23	40	34.2	21	76	44.0
	18:00	18.5	23.5	20.3	22	48	39.0	21	74	43.2

Table 3. Spearman correlation values (r_s) between the abundance of each flower-visiting taxon and the ambient temperature (°C), the relative humidity (RH) and the flower availability during the flowering period of *D. albus*. Only taxa with at least 20 individuals were considered.

Flower-visiting taxon	Temperature	RH	Flower availability
Apini	0.19	-0.19	-0.02
Anthophorini	-0.23	0.37*	0.04
Bombini	-0.03	-0.06	-0.15
Eucerini	-0.08	0.11	0.06
Megachilini	0.13	-0.35*	-0.06
Osmiini	0.11	0.07	-0.12
Halictini	0.63*	-0.47*	0.13
Syrphidae	0.12	-0.32*	-0.23

* indicates a statistically significant correlation with Bonferroni correction ($P < 0.017$).

The test for association between flower-visiting insects showed a positive correlation between the presence of Apini and the presence of Andrenini, Megachilini and Syrphidae. The presence of Megachilini was positively correlated with Halictini and Syrphidae occurrences, whereas the presence of Bombyliini was positively associated with Vespidae. No significant negative associations were observed (table 4).

Discussion and conclusions

Diversity and activity patterns of flower visitors

Inter-annual variation in the abundance and activity of pollinator guilds is a common trend in natural communities (Petanidou *et al.*, 2008; Couvillon *et al.*, 2015). In our study, the insect visitor spectrum did not change dramatically among years with 6 out of 14 flower-visiting taxa constantly present every year. However, we found marked fluctuations in the insect abundance within taxa among years. The total number of individual insects observed showed a six-fold increase from 71 in 2011 to 426 in 2012. The lowest insect abundance observed in 2011 corresponded to the highest Shannon's index calculated in the same year for the tribes belonging to the superfamily Apoidea. On the contrary, the highest insect abundance in 2012 corresponded to the lowest diversity values for the Shannon's, Simpson's and Berger-Parker indices. In fact, in 2012 we observed a striking dominance of both Anthophorini and Halictini, while in the other years the abundance was more equally distributed among taxa. Overall, *H. tarsata*, the most important pollinator of *D. albus* (Fisogni *et al.*, 2016), was the most abundant species in the tribe Anthophorini. However, Halictini offered the highest contribution in terms of total insect abundance during the whole four-year study (frequency range: 26.5-49.5%).

We also found some differences in the distribution of the total number of insects observed in the patch during the flowering period and during the day. The tribes Apini and Megachilini tended to be more abundant toward the end of the flowering period and during the middle of the day or in the morning; bees belonging to the tribe Osmiini were more abundant toward the beginning of the flowering period. The other major taxa were equally abundant during the flowering season with differences in

Table 4. The associations between pairs of flower-visiting insects in the same units of observation.

	Apini	Andrenini	Anthidiini	Anthophorini	Bombini	Eucerini	Megachilini	Osmiini	Xylocopini	Halictini	Nomadini	Syrphidae	Vespidae
Andrenini	**φ=0.3144** **p=0.002**												
Anthidiini	φ=0.2897 p=0.004	φ=−0.0409 p=0.692											
Anthophorini	φ=−0.181 p=0.077	φ=−0.143 p=0.165	φ=−0.034 p=0.741										
Bombini	φ=−0.1361 p=0.186	φ=0.0590 p=0.568	φ=−0.1026 p=0.320	φ=0.014 p=0.892									
Eucerini	φ=−0.1231 p=0.232	φ=0.0940 p=0.362	φ=−0.0576 p=0.577	φ=0.0609 p=0.555	φ=−0.1537 p=0.135								
Megachilini	**φ=0.3314** **p=0.001**	φ=−0.0795 p=0.441	φ=0.2284 p=0.025	φ=−0.038 p=0.712	φ=0.0602 p=0.560	φ=−0.0614 p=0.552							
Osmiini	φ=0.0308 p=0.766	φ=−0.0748 p=0.469	φ=−0.0452 p=0.662	φ=0.0609 p=0.555	φ=0.0415 p=0.688	φ=0.1475 p=0.152	φ=0.565 p=0.584						
Xylocopini	φ=0.1991 p=0.052	φ=−0.0497 p=0.630	φ=0.0355 p=0.731	φ=−0.129 p=0.209	φ=−0.0828 p=0.423	φ=−0.1048 p=0.310	φ=0.1386 p=0.178	φ=−0.0070 p=0.946					
Halictini	φ=0.2077 p=0.042	φ=0.1342 p=0.192	φ=0.2580 p=0.011	φ=−0.103 p=0.319	φ=−0.0564 p=0.585	φ=0.1534 p=0.136	**φ=0.3026** **p=0.003**	φ=0.1115 p=0.279	φ=0.1084 p=0.293				
Nomadini	φ=−0.0677 p=0.512	φ=−0.0213 p=0.837	φ=0.2396 p=0.019	φ=0.149 p=0.148	φ=0.0590 p=0.568	φ=−0.0795 p=0.441	φ=0.0940 p=0.362	φ=−0.0748 p=0.469	φ=−0.0497 p=0.630	φ=0.1342 p=0.192			
Syrphidae	**φ=0.4328** **p<0.001**	φ=0.1765 p=0.085	φ=0.1507 p=0.143	φ=−0.221 p=0.030	φ=0.1102 p=0.285	φ=−0.1183 p=0.251	**φ=0.4263** **p<0.001**	φ=−0.0235 p=0.820	φ=0.0914 p=0.376	φ=0.1340 p=0.193	φ=−0.0525 p=0.612		
Vespidae	φ=0.2303 p=0.024	φ=−0.0262 p=0.800	φ=−0.0504 p=0.626	φ=−0.0561 p=0.587	φ=0.0081 p=0.938	φ=−0.0979 p=0.343	φ=−0.0979 p=0.343	φ=−0.0921 p=0.372	φ=−0.0612 p=0.553	φ=0.1652 p=0.108	φ=−0.0262 p=0.800	φ=0.1233 p=0.231	
Bombyliidae	φ=0.0798 p=0.440	φ=−0.0409 p=0.692	φ=0.2295 p=0.024	φ=−0.0342 p=0.741	φ=−0.1026 p=0.320	φ=0.0377 p=0.715	φ=−0.1529 p=0.137	φ=−0.0452 p=0.662	φ=−0.0956 p=0.354	φ=0.1776 p=0.083	φ=0.2396 p=0.019	φ=0.0249 p=0.810	**φ=0.4102** **p<0.001**

Statistically significant correlations using Bonferroni correction ($P < 0.003$) are indicated in bold.

their daily activity. These trends likely reflect the effect of both the insect phenology and the abiotic and biotic factors, discussed below.

Abiotic factors

Halictini, the most abundant taxon, showed a positive correlation with the ambient temperature and a negative correlation with the relative humidity during the flowering period. This response was also in accordance with the daily pattern of activity of this taxon with a peak in the middle of the day that corresponded to the warmest period of the day. Despite the higher frequency of Halictini, their presence was not positively connected to the pollination of *D. albus* due to behavioural and morphological constraints that exclude pollen deposition on receptive stigmas (Fisogni *et al.*, 2011; 2016). These small bees may act as pollen or nectar robbers and have therefore potential negative effects on plant male and female fitness. In fact, a reduction of flower nectar might reduce the attractiveness towards the main pollinators and the pollen collected by Halictini is no longer available for pollination.

Variation in responses to environmental conditions among pollinator taxa represents an “environmental complementarity”, which is an important component of the insurance hypothesis (Yachi and Loreau, 1999). This hypothesis implies that two pollinator species can show long-term complementarity in the pollination service when they present different response to stress and environmental changes. Our data showed a certain level of “response diversity” between flower-visiting taxa in relation to different environmental conditions during *D. albus* blooming. In fact, the activity of flower-visiting taxa was related to ambient temperature in Halictini and to relative humidity in 4 taxa (positive relation in Anthophorini and negative relations in Megachilini, Halictini and Syrphidae), leading to different patterns of daily and seasonal activity.

The differences in the hourly activity pattern observed in our study can be therefore partially explained by the different specific temperature and humidity responses, although some species (i.e. bumblebees) did not show any significant correlations with these factors. Pollinator daily activity, however, can depend directly, on their temperature and humidity tolerance, and indirectly, when the caloric reward offered by their host plants is affected by weather conditions. In fact, sugar concentration in nectar varies daily and seasonally depending on the equilibrium with the humidity of the air (evaporation and condensation) other than on the activity of nectaries (secretion or re-absorption) and on the removal of nectar by flower visitors. Indeed, it has been showed that some pollinators can adjust their collection activities to the rhythms of nectar production (Nicolson *et al.*, 2007). These variations may affect the spectrum of flower visitors. Honey bees, for example, stopped to visit almond flowers following intermittent rains because the nectar concentration dropped to 9-15% (Abrol, 2012). In our study we found mainly significant negative effects of the relative humidity on the daily pollinator activity. High humidity can increase the nectar availability while decreasing its sugar concentration, thus potentially negatively affecting the visits of those insects with a preference for high-sugar concentration (Corbet *et al.*, 1979).

Climate conditions during the winter can also affect the phenology of flower-visiting taxa, thus causing possible mismatches between the presence and/or abundance of different pollinator taxa and the flowering period of *D. albus*. Overall, we observed a higher abundance of Apini, Megachilini and Syrphidae in the year with the highest value of mean temperature and degree-hours accumulated during winter (i.e. 2014). On the contrary, Anthophorini showed the highest relative abundance in the year with the lowest values of DH and mean temperature during winter (i.e. 2013). Although the only four data points (years) did not allow us to perform a robust statistical analysis, this result might suggest a relationship between the annual abundance of some flower-visiting taxa and the climatic conditions during wintering. Thus, we should expect a higher number of Apini (i.e. honey bees) and Megachilini on *D. albus* in years with a major heat accumulation during winter and this situation could be more frequent under a scenario of global warming.

Biotic factors

The abundance of flower availability and the association among pollinators during blooming of *D. albus* seem to play a marginal role in shaping the pollinator community composition of this plant species. No taxon showed any correlation between their abundance and the flower abundance in the patch. These results confirm that high flower density is not necessarily related with pollinator abundance (Filella *et al.*, 2013). Overall, only significant positive associations were observed between flower-visiting taxa, probably related to similar preferences towards specific environmental conditions. For example, the presence of Megachilini resulted positively related with the presence of Halictini and Syrphidae. All of them showed a negative correlation with the relative humidity.

No significant negative associations were observed in our study, suggesting the absence of among-taxon competition for flower resources. Although some studies have demonstrated that honey bees may compete for food resources with wild pollinators and reduce their abundance or flower visits (Shavit *et al.*, 2009; Hudewenz and Klein, 2013; 2015), other studies showed that interspecific competition did not negatively affect the abundance and species richness of wild bees (Steffan-Dewenter and Tscharnke, 2000; Roubik and Wolda, 2001). Our data showed that honey bees, when present, did not negatively affect the presence of other foraging insects. On the contrary, we found some positive relations between honey bee abundance and that of some pollinators (Andrenini, Megachilini, Syrphidae). This finding suggests the absence of competition between domestic and wild bees on *D. albus* under the observed environmental conditions. It is possible that the abundance of *A. mellifera* in the site is not detrimental for wild bees, due to the reduced beekeeping activities in the surroundings (AF, personal observation). The absence of significant negative associations between the main pollinators of *D. albus* also suggests the overlap in the diet breadth of wild bees when feeding on this early blooming species, likely as a consequence of the abundant display of nectar and pollen throughout its flowering period. However, further studies on pollen loads found on insect bodies would help to clarify the actual feeding choices and possible niche overlap between taxa, and possible competition among plants for pollinator services.

In conclusion, our results show that the flower visitor community of *D. albus* is quite variable among years and during the days of blooming. This variation could be partially explained by different environmental responses among flower-visiting taxa; however, the relatively high pollinator diversity (excluding Halictini) observed on *D. albus* and the different patterns of activity among taxa might guarantee a stable pollination service of this plant species. In fact, a more diverse community of pollinators is better able to buffer environmental changes than a simple community (Hooper *et al.*, 2005).

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