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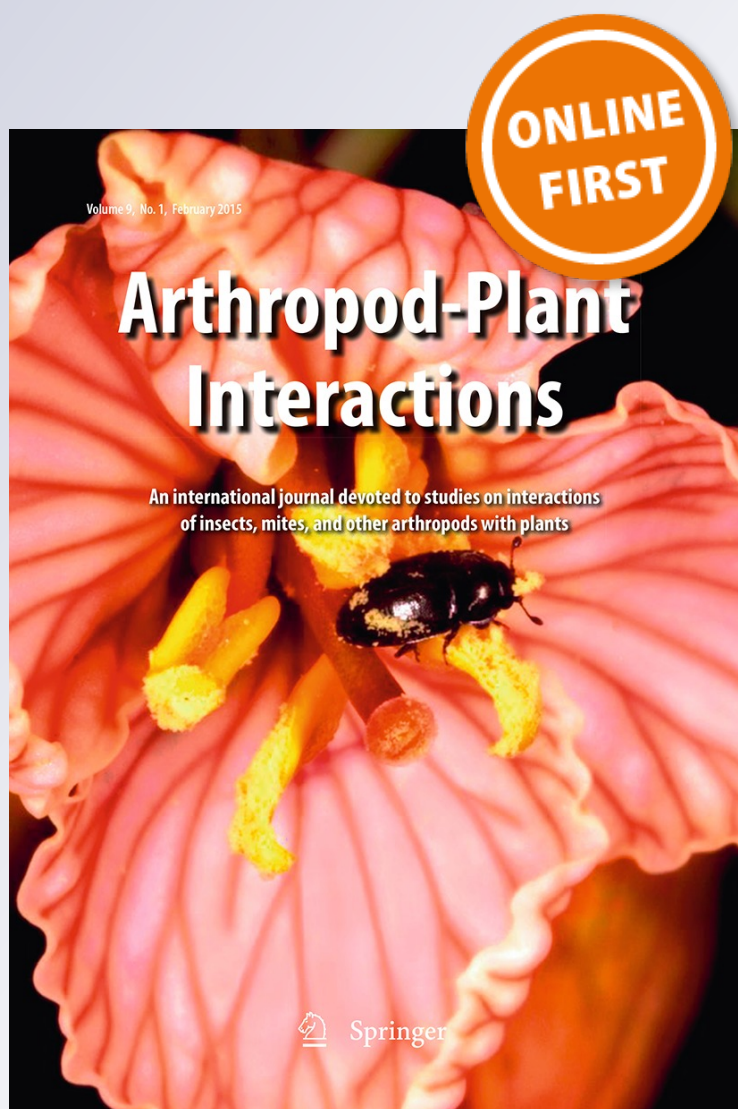
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Pollen host selection by a broadly polylectic halictid bee in relation to resource availability

Milagros Dalmazzo · Favio Gerardo Vossler

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Abstract The relationship between utilized and available resources in the same environment has not yet been studied in most wild bees. In a riparian forest, a total of 15 pollen types from 10 plant families were identified from pollen provisions of 17 nests of the wood-dwelling *Augochlora amphitrite* (Hymenoptera: Halictidae) for which only floral records have been known. During the foraging period, only five out of 108 available flowering species were visited intensively (more than 10 %) for pollen. As more than three families and 32.2 % of the available plant families were visited for pollen, the *broad polylecty* category was inferred. Like in other *Augochlora* species, a variety of floral types was visited by *A. amphitrite*, in agreement with its broadly polylectic foraging habit. The selection index was positive for the highly abundant *Ludwigia* (Onagraceae) and the rare *Gymnocoronis spilanthoides* (Asteraceae). The intensive foraging on *Ludwigia* could indicate

temporary specialization for this abundant pollen resource. The tendency of this bee to visit intensively one flower type for pollen suggests that bee individuals had to learn to handle this abundant flower type. Pollen analysis complemented with field observations on floral resource availability proved to be helpful tools to provide information on the foraging behaviour of a wild bee in its natural environment.

Keywords *Augochlora amphitrite* · Halictidae · Nest provision · Pollen analysis · Wild bee

Introduction

The relationship between flowers and bees has received much attention, mainly because pollination represents a critical service for both natural and agricultural ecosystems (Buchmann and Nabhan 1996; Proctor et al. 1996; Costanza et al. 1997; Waser and Ollerton 2006; Peters et al. 2013). This relationship can be identified and measured by means of field observation of floral visitation and pollen analysis of pollen resources foraged, as shown in Pedro and Camargo (1991) and Cortopassi-Laurino and Ramalho (1988), respectively.

Specialization for pollen hosts by bees can vary from monolecty (in which only one pollen host species is foraged for brood provisioning) to broad polylecty (in which a broad spectrum of pollen hosts belonging to diverse Angiosperm clades is commonly foraged) (Cane and Sipes 2006; Müller and Kuhlmann 2008). Both factors intrinsic to the forager, e.g. learning and memory dynamics, and factors extrinsic to the forager, e.g. floral morphology, affect flower choice behaviour in bees (Chittka et al. 1999; Chittka and Raine 2006; Raine and Chittka 2007).

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In the present study, the bee–plant interaction was studied for the native wild bee *Augochlora amphitrite* (Schrottky) (Halictidae, Augochlorini) whose nests were found in a riparian forest.

Studies of the feeding habits of Augochlorini are scarce (Smith et al. 2012), and there are no recorded cases of oligolecty. The high diversity of species and families visited by *Augochlora* species suggests that these bees have no fixed, innate preferences for certain plant hosts and/or flower attributes. Studies on pollen resources visited by *Augochlora* species (*A. alexanderi* Engel, *A. esox* (Vachal) and *A. isthmii* Schwarz) stated that they are polylectic (Zillikens et al. 2001; Wcislo et al. 2003). However, the relationship between the pollen resources foraged and the available resources in a same environment is unknown for *Augochlora* bees.

The genus *Augochlora* s. l. includes ca. 130 species (Moure and Hurd 1987), and it is broadly distributed from southern Canada to the Buenos Aires province in Argentina (Michener 2007). *Augochlora amphitrite* is one of the five species found in the Pampean region (Dalmazzo and Roig-Alsina 2011). This bee nests in wood of decaying logs; the nest presents two types of structure (namely tunnel and cluster) according to the substrate where it was built. Its annual cycle at this latitude is typical for most halictids in temperate regions. Activity begins in spring (September–October) when post-hibernating foundress females start visiting flowers and building nests. The activity continues until mid-March, when nests become inactive and last generation females look for hibernacula. Foundresses of *A. amphitrite* produce at least two broods during the activity cycle, but each cell is used only once (Dalmazzo and Roig-Alsina 2012). Thus, pollen reserves and/or faeces found in nests during February–April could have been collected at any time from September to March. *Augochlora amphitrite* is of particular interest because of the intraspecific variation of some of its biological traits such as nesting biology and social behaviour (Dalmazzo and Roig-Alsina 2012). On the other hand, only floral records had been known for this augochlorini bee, as nests are quite difficult to find. The aims of this study were to elucidate the pollen usage of the wild bee *Augochlora amphitrite* and assess host selection to understand its foraging behaviour in natural environments.

By considering that *Augochlora* species visit a broad spectrum of floral hosts, it is expected that *A. amphitrite* is polylectic and forages on plants with different flower attributes. It is also hypothesized that *A. amphitrite* has no fixed preference for any particular pollen host, gathering pollen according to availability in the field. To test selection of pollen hosts, Chi-square goodness of fit test and selection index will be calculated.

Materials and methods

Study site and nesting area

This study was carried out in the reserve Refugio Natural Educativo “Ribera Norte” (34°28′10″S, 58°29′40″W), located in the north-east of Buenos Aires province, Argentina. This reserve consists of a 10 Ha fragment of riparian forest located on the banks of the Río de la Plata. The soil is waterlogged during certain periods of the year. In this reserve, the riparian forest contains a tree stratum of 12–15 m in height, shrubby and herbaceous strata, and abundant lianas and epiphytes (Cabrera and Zardini 1978). According to Cabrera (1971) and Cabrera and Willink (1973), the dominant tree species of this phytogeographical area at this latitude are “laureles” (*Ocotea acutifolia* (Nees) Mez, *Nectandra angustifolia* (Schrad.) Nees) (Lauraceae), “mata-ojo” (*Pouteria salicifolia* (Spreng.) Radlk.) (Sapotaceae), “chal-chal” (*Allophylus edulis* (A. St.-Hil., A. Juss. et Cambess.) Niederl.) (Sapindaceae), “blanquillo” (*Sebastiania brasiliensis* Spreng.) (Euphorbiaceae), “lecherón” (*Sapium haematospermum* Müll. Arg.) (Euphorbiaceae), “anacahuita” (*Blepharocalyx salicifolius* (Kunth) O. Berg) (Myrtaceae), “sauce criollo” (*Salix humboldtiana*) (Salicaceae). This forest has been invaded by several alien plant species, such as “ligustrina” (*Ligustrum sinense* Lour.) (Oleaceae) and “lirio amarillo” (*Iris pseudacorus* L.) (Iridaceae) (personal observation).

A nesting site (a total of 17 nests in two aggregations) of *Augochlora amphitrite* was found by direct observation. The nest aggregations were found one metre apart from each other and 80 m away from the river. One of them was found in a fallen and decomposed tree trunk of “sauce criollo” (*Salix humboldtiana* Willd., Salicaceae) and composed of 14 inactive nests with only faeces. The other one was found in a decomposed railroad sleeper of “quebracho colorado” (*Schinopsis* sp., Anacardiaceae) and composed of three nests with both faeces and provisions. The structure of these nests was described by Dalmazzo and Roig-Alsina (2012).

Pollen analysis of nest provisions

During autumn (April 2008), 14 nests (N1–N14), and during summer (February 2009), 3 nests (N15–N17) were extracted, and pollen masses or faeces were analysed. To obtain pollen sediment, provisions and faeces from each cell (number of cells per nest ranged from 1 to 11) were dissolved in water at 80–90 °C for 10 min; this mixture was then handshaken and centrifuged at 472×g for 10 min. Pollen sediment from nests and plant pollen reference was mounted on slides and dyed fuchsia following the

Wodehouse (1935) technique. Pollen identification was carried out by comparing pollen provision slides with the pollen reference of plants that grow in the reserve, using a Nikon Eclipse E200 light microscope at 1000× magnification. A total of 300 pollen grains per slide were counted. Pollen types occurring in >10 % of representativity were considered as the most important pollen resources, following Ramalho et al. (1985) and Cortopassi-Laurino and Ramalho (1988). *Ludwigia* tetrads were counted as individual grains. Pollen-type nomenclature follows the recommendations of Joosten and De Klerk (2002) and De Klerk and Joosten (2007). In the present study, the pollen-type *Ludwigia* included all *Ludwigia* species that have their pollen grains aggregated as tetrads, the type *Baccharis* included *Baccharis* species and *Solidago chilensis*, the type *Pavonia* included two *Pavonia* species. Due to the fact that only a few grains of the types Lamiaceae and *Solanum* were found in samples, their association with the available plant species is uncertain. However, four species in three genera of Lamiaceae and three *Solanum* species were found in the reserve. The type *Croton* included *Croton* species and *Manihot grahamii*, but they were not found there. The reference pollen plants were collected between September 2009 and March 2010 in this riparian forest, pressed, dried, identified by F.G. Vossler and deposited in the Herbarium of La Plata (LP), Buenos Aires, Argentina. The classification of host-plant specialization by bees follows Cane and Sipes (2006) and Müller and Kuhlmann (2008), in which each category is based on the number of species, genera and plant families used for pollen gathering by a bee species throughout its geographical range.

Floral availability around the nesting area

To quantify flower availability surrounding the nesting area, both flowering phenology and flower abundance were sampled once per month during the foraging period of *A. amphitrite* between September 2009 and March 2010. This sampling was made for a distance of 200 m around the nests (the limits of this reserve but not necessarily the flight

range of the bee). Due to climate impediments (river floods and severe storms), flower abundance had to be visually measured with an ordinal scale (semiquantitative) along three linear transects within a 200-m radius circular area surrounding the nesting site. Therefore, as shown in Table 1, the relative abundance of flowered individuals (measured using a cover scale of 1, 2 and 3) and flower phenology (estimated as 1 and 2) was multiplied to obtain flower cover values from 1 to 6. These values were monthly measured (see Table 1). These subjective measures of abundance were modified from Kent and Coker (1992, p. 45).

Since bees and humans perceive colours differently, to analyse plant–bee interactions flower colours should be recorded as seen by bees (Chittka 1996; Kevan et al. 1996; Chittka and Waser 1997; Chittka et al. 1999; Arnold et al. 2009a, b). However, data on floral reflectance are available for *Ludwigia elegans* but not for the other pollen types here analysed (after checking the floral reflectance database (FRd) from Arnold et al. 2010), and therefore, colours had to be considered based on human perception. Human colour of flowers, shape of the flowering unit and symmetry were recorded for each plant species. To investigate whether the distribution of frequencies of these three flower attributes differed in nest provisions and forest, the null hypothesis of no difference between the two distributions was tested using a Chi-square goodness of fit test at 5 % significance level. The categories having expected frequency counts of less than five were summed, as required by this test. For this statistical analysis, the program package Statgraphics® Centurion XVI (StatPoint Technologies 2010) was used.

Selectivity of pollen resources

To estimate preference of each pollen host by the bee *Augochlora amphitrite*, the *selection ratio* was used as index of selectivity, being $w_i = o_i/\pi_i$ (Manly et al. 1993). According to Manly et al. (1993), w_i is the proportion of the population of available units in category i that are used.

Table 1 Values of cover scale of flowers according to their availability in the environment

Abundance of flowered individuals (partial cover value)	Flowering phenology (partial cover value)	Flower cover calculation	Example (see Fig. 2)
1 (few individuals flowered)	1 (beginning or end of flowering period)	$1 \times 1 = 1$	<i>Commelina diffusa</i> in December
1	2 (flowering peak)	$1 \times 2 = 2$	<i>Baccharis</i> sp. in November
2 (a moderate number of individuals with flowers)	1	$2 \times 1 = 2$	<i>Pavonia sepium</i> in December
2	2	$2 \times 2 = 4$	<i>Pavonia sepium</i> in January
3 (most individuals with flowers)	1	$3 \times 1 = 3$	none for the present survey
3	2	$3 \times 2 = 6$	<i>Ludwigia elegans</i> in February

Partial cover values were multiplied to obtain the total flower cover. Examples show plant species for each flower cover value

In the present study, o_i is the addition of frequency values of each pollen type from the 17 nests and π_i is the addition of flower cover values of each species foraged by bees for the 7 months around 200 m of the nesting area. The values of the selection ratio range from 0 to infinite. Positive selection ranges from +1 to infinite. The relationship between the pollen used and the flowering available was calculated for all pollen types identified in the samples.

To investigate whether the distribution of frequencies of pollen types foraged differed from those of cover scale values (availability), the null hypothesis that there is no difference between the two distributions (i.e. the bee *A. amphitrite* is choosing pollen hosts in proportion to availability) was tested using a Chi-square goodness of fit test,

at 5 % significance level. The categories having an expected frequency count of less than five were added, as required by this test. Chi-square test was analysed using the program package Statgraphics® Centurion XVI (StatPoint Technologies 2010).

Results

Pollen composition of provisions and faeces from nests

A total of 15 pollen types belonging to 10 plant families constituted the diet of *A. amphitrite* (Fig. 1): *Ludwigia* (Onagraceae) (SD = 0.9–47.9 %), *Syagrus romanzoffiana*

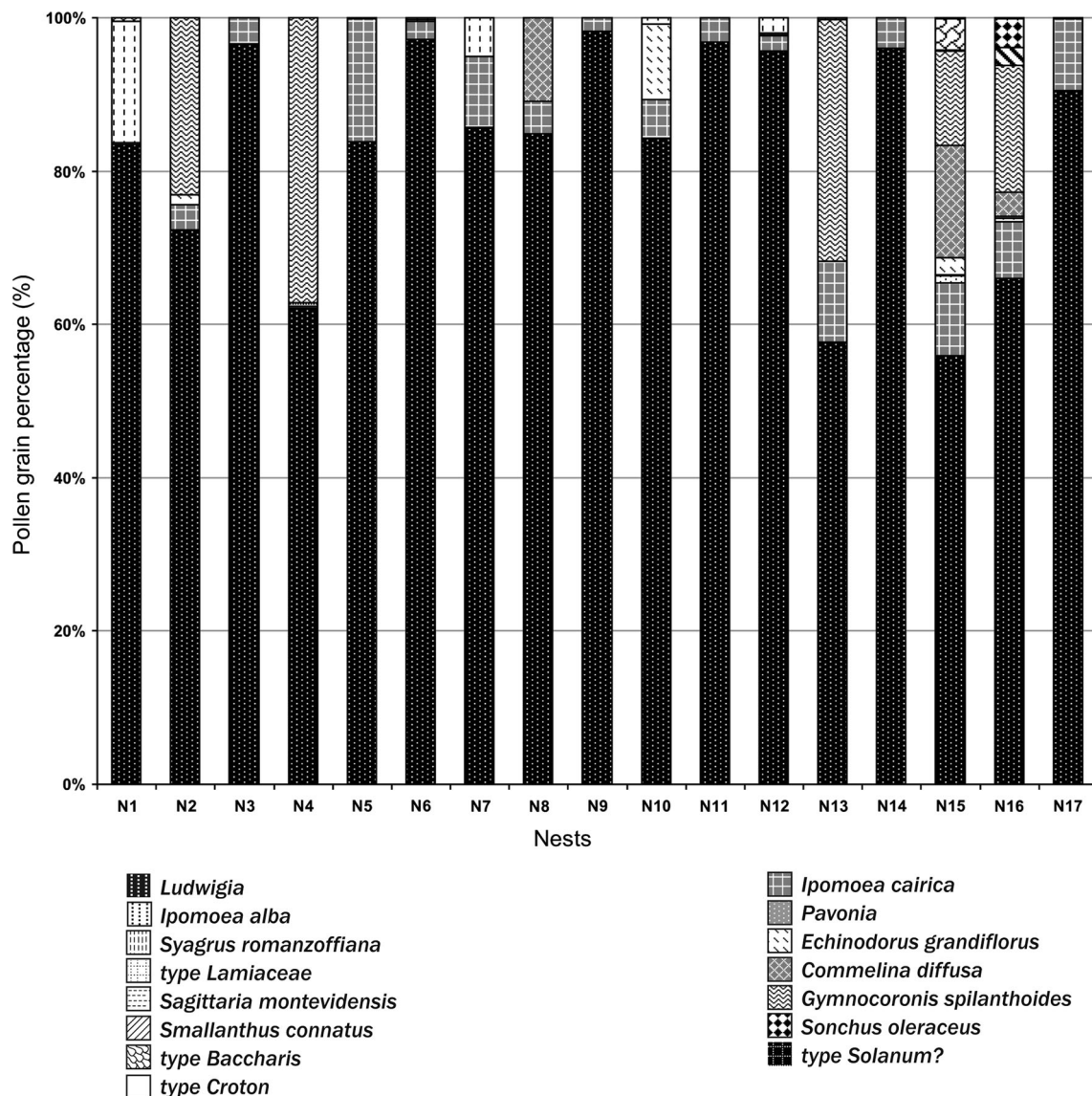


Fig. 1 Abundance of pollen types found in 17 nests of *Augochlora amphitrite*. It is remarkable the dominance of *Ludwigia* in all nests. Pollen types highly abundant in the environment are in *black*

(*Ludwigia*), common in *grey* (such as *Ipomoea cairica*) and rare in *white* (such as *Syagrus romanzoffiana*)

(Cham.) Glassman (Arecaceae) (SD = 0.1–31 %), *Sagittaria montevidensis* Cham. et Schldtl. (Alismataceae) (SD = 0.09–0.1 %), *Baccharis* (Asteraceae) (SD = 0.09 to 13 %), *Ipomoea cairica* (L.) Sweet (Convolvulaceae) (SD = 0.4–25.2 %), *Echinodorus grandiflorus* (Cham. et Schldtl.) Micheli (Alismataceae) (SD = 0.05–22 %), *Gymnocoronis spilanthoides* (Hook. et Arn.) DC. (Asteraceae) (SD = 0.9–54.6 %), *Ipomoea alba* L. (Convolvulaceae) (SD = 0.2–1.6 %), *Smallanthus connatus* (Spreng.) H. Rob. (Asteraceae) (SD = 7.8 %), *Croton* (Euphorbiaceae) (SD = 0.3 %), *Pavonia* (Malvaceae) (SD = 0.4–0.6 %), *Commelina diffusa* Burm. f. (Commelinaceae) (SD = 0.4–22 %), *Sonchus oleraceus* L. (Asteraceae) (SD = 0.08–12.2 %), Lamiaceae and *Solanum* (Solanaceae) (SD = 0.3 %). This bee species used large amounts of pollen (>10 % per nest) from five species of five families: *Ludwigia* (Onagraceae), followed by *Gymnocoronis spilanthoides* (Asteraceae), *Ipomoea cairica* (Convolvulaceae), *Commelina diffusa* (Commelinaceae) and *Syagrus romanzoffiana* (Arecaceae). Fifty nests were composed of between two and five pollen types, while only

two of them (nests 15 and 16) were composed of 10 and 11 pollen types (Fig. 1). All nests contained at least 56 % of *Ludwigia* pollen. The 15 pollen types found in nest samples were ascribed to 24 plant species (see Fig. 2). Most utilized pollen hosts were found in a radius of 30 m from the nests.

Availability of floral resources surrounding the nesting area

A total of 108 plant species belonging to 50 families were recorded in a radius of 200 m around the nesting area (Online Resource 1), which is 13.88 % of the available species and 20 % of the available families were foraged. Most species were herbs (69), followed by climbers (19), shrubs (10), trees (9) and one epiphyte (the orchid *Oncidium bifolium* Sims). This site characterized by the dominance of flowers (mainly from herbs and shrubs) of *Iris pseudacorus* L. (Iridaceae), *Cestrum euanthes* Schldtl. (Solanaceae), *Salix* sp. (Salicaceae), *Ligustrum sinense* Lour. (Oleaceae), *Tradescantia fluminensis* Vell. (Commelinaceae), *Galium aparine* L. (Rubiaceae) and *Fumaria*

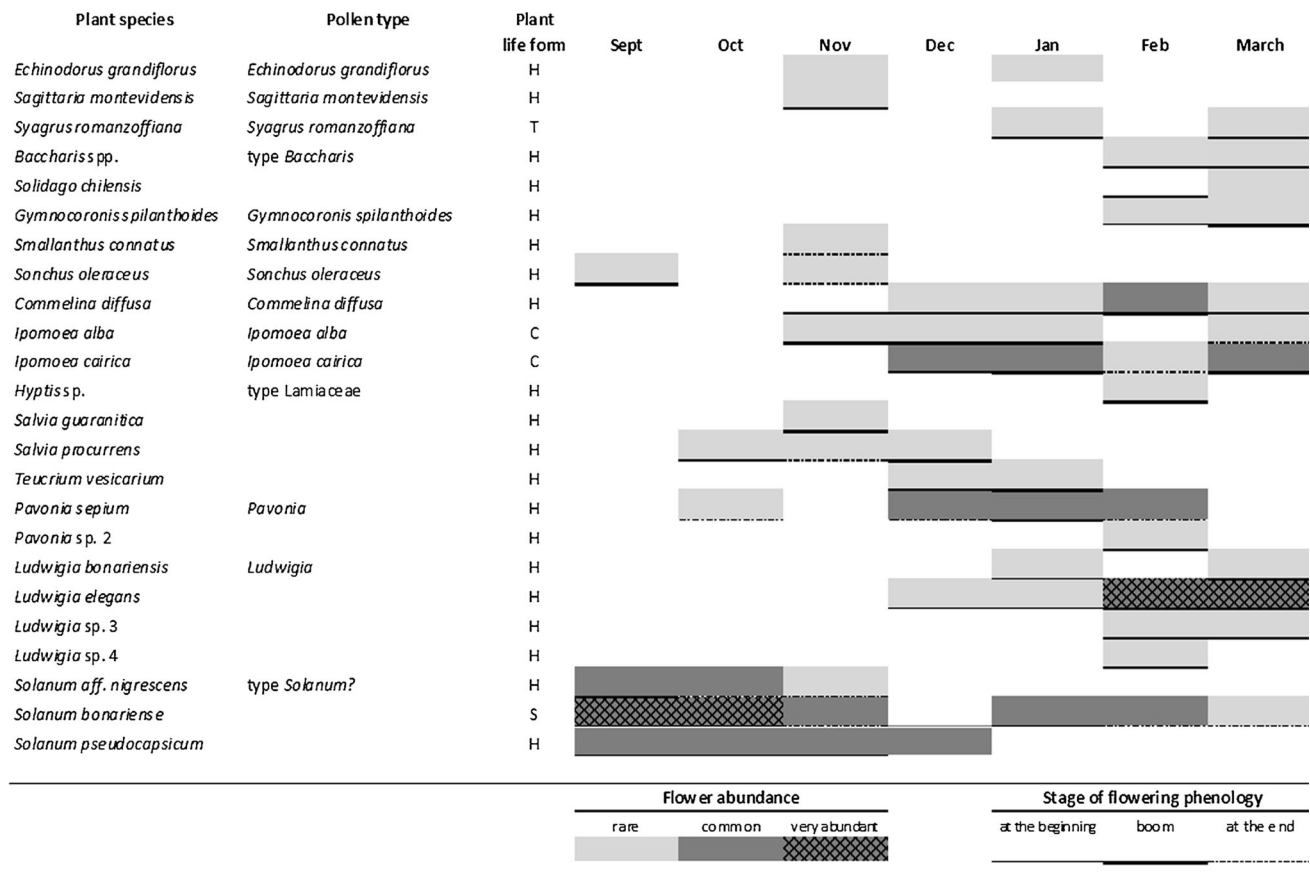


Fig. 2 Flowering phenology of the plant species found in the nests, showing the abundance of flowers and individuals and the flowering stage (for reference see at the bottom) from September to March in

200 m around of the nesting area. Pollen types belonging to each plant species are also provided. H herb, C climber, T tree, S shrub

capreolata L. (Fumariaceae) in winter–spring, of *Ludwigia elegans* (Cambess.) H. Hara (Onagraceae), *Humulus scandens* (Lour.) Merr. (Cannabinaceae), *Commelina diffusa* Burm. f. (Commelinaceae), *Tripogandra diuretica* (Mart.) Handlos (Commelinaceae), *Ipomoea cairica* (L.) Sweet (Convolvulaceae), *Pavonia sepium* A. St.-Hil. (Malvaceae) and *Phyllanthus niruri* L. (Phyllanthaceae) in summer–autumn, and of *Solanum bonariense* L. (Solanaceae), *Calyptocarpus biaristatus* (DC.) H. Rob. (Asteraceae), *Galianthe brasiliensis* (Spreng.) E.L. Cabral & Bacigalupo (Rubiaceae), *Solanum pseudocapsicum* L. (Solanaceae) and *Urtica* sp. (Urticaceae) throughout a great part of the sampling period (species of long flowering period). Considering flower colour as perceived by humans, white and yellow flowers were dominant while green, pink, lilac and red were less represented during the whole flowering period (September–March), violet was recorded from September to February, orange and blue from October to March, and brown only in September (Fig. 3). From eight to nine floral colours were present every month, in the present study, the total number of colours recorded was ten.

Pollen samples revealed that most of the foraged plants (14 pollen types) were found in a radius of 30 m from the nests. In this area, 60 species from 31 families were recorded which represented 25 % of the available species and 32.26 % of the available families foraged. These 14 types were also found within the 200 m radius. *Croton* was the only pollen type not found within the 200 m radius, although it was scarcely represented in pollen stores.

According to flower abundance, during the 7 months and around 200 m of the nesting area, it was possible to identify a total of 14 highly abundant, 24 common and 70 rare species (Online Resource 1). In nest provisions *Ludwigia* and type *Solanum* with highly abundant flowers, *C. diffusa*, *I. cairica* and *Pavonia* with common flowers and

the remaining species with rare flowers were found (Fig. 2).

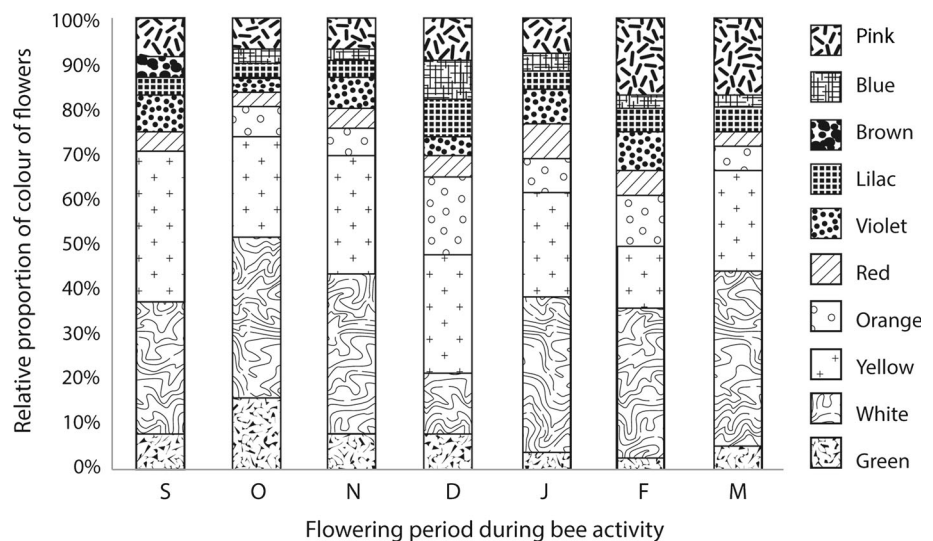
The available plants differed in their flower colour (32.4 % white, 25 % yellow, 11.1 % pink, 8.3 % green, 7.4 % orange, 5.6 % lilac, 3.7 % red, 3.7 % violet, 1.9 % light blue and blue and 0.9 % brown), flower symmetry (68.5 % radial, 24.1 % markedly bilateral and 7.4 % asymmetric and slightly bilateral) and shape of the flowering unit (56.5 % dish, 13 % tube, 12 % ball, 12 % bell, 4.6 % pea-like and 1.9 % brush). The pollen hosts foraged also had varied flower attributes. They were mainly white (46.66 %) and yellow (26.66 %) followed by similar percentages (6.66 %) of orange, violet, blue and lilac. Flower symmetry was mainly radial (80 %), and only 20 % were markedly bilateral. The shapes of the flowering unit varied from dish (66.66 %) to ball (13.33 %), tube (13.33 %) and bell (6.66 %).

The Chi-square goodness of fit test did not show significant difference within each one of the three flower attributes (colour, symmetry and shape) from nest provisions versus the riparian forest ($\chi^2 = 1.93$; $p = 0.38$ for flower colour; $\chi^2 = 0.92$; $p = 0.34$ for symmetry; $\chi^2 = 0.63$; $p = 0.43$ for shape of the flowering unit).

The pollen specialization of *Augochlora amphitrite*

As more than three families and 32.26 % of the available families in a radius of 30 m around the nesting area were foraged, the *broad polylecty* category was inferred, according to Cane and Sipes (2006). Moreover, this bee species foraged on more than 10 % of the available species both in a radius of 30 m (25 %) and 200 m (13.88 %) around the two nesting aggregations, indicating that it is broadly polylectic, as stressed by Cane and Sipes (2006).

Fig. 3 Relative percentages of flower colours as perceived by human from September to March in the study site. From eight to nine floral colours were present every month, in the present study, the total number of colours recorded was ten



On the other hand, according to Müller and Kuhlmann (2008), *A. amphitrite* can be classified as *polylectic s.s.* or *polylectic with strong preference*. The former category includes pollen collection from various genera belonging to at least four plant families, while the latter from several plant families, but one plant clade (family, subfamily, tribe, genus or species) predominates.

Selectivity of pollen resources

For the periods September–March (the whole bee flight period), November–March (months when all plants whose ascribed pollen types were found in the stores were bloomed) and December–March (the summer season), the Chi-square test rejected the null hypothesis which states that the bee *A. amphitrite* was choosing pollen hosts in proportion to availability ($\chi^2 = 53\,138$ for September–March; $\chi^2 = 41\,860$ for November–March; $\chi^2 = 33\,842$ for December–March; $p < 0.0001$). From the 15 pollen types stored in the nests, only two (*Ludwigia* and *G. spilanthoides*) were positively selected by *A. amphitrite* (Fig. 4) being 5.01 for September–March, 3.67 for November–March and 3.26 for December–March for *Ludwigia* while 4.83, 1.47 and 1.31 for *G. spilanthoides*. *Ludwigia* was highly abundant in the environment, while *G. spilanthoides* was rare. The types *I. cairica*, *C. diffusa* and *S. romanzoffiana* were not positively selected but were gathered in high proportion. The former two were common and the latter rare in the field. The remaining types show low values of selection ratio (near zero).

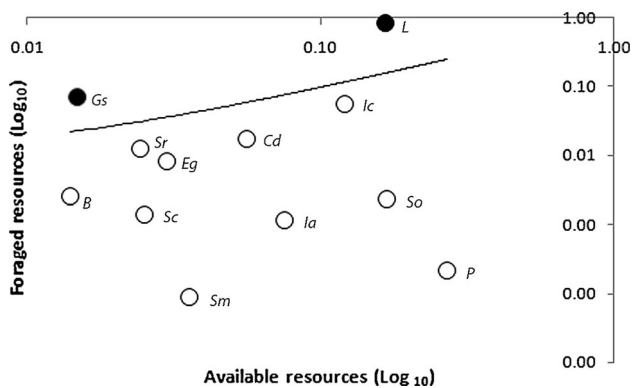


Fig. 4 Pollen types foraged in greater proportion than their availability (positively selected) are shown above the line (full dots: *Ludwigia* and *Gymnocoronis spilanthoides*), while those foraged in lower proportion to their availability are below this line (empty dots: the remaining pollen types). Pollen types references: *Pavonia* P, *Sonchus oleraceus* So, *Ludwigia* L, *Ipomoea cairica* Ic, *Ipomoea alba* Ia, *Commelina diffusa* Cd, *Sagittaria montevidensis* Sm, *Echinodorus grandiflorus* Eg, *Smallanthus connatus* Sc, *Syagrus romanzoffiana* Sr, *Gymnocoronis spilanthoides* Gs, type *Baccharis* B

From the 14 species with highly abundant flowers (11 of them were melitophilous), only *Ludwigia* was visited by *A. amphitrite*.

Discussion

The pollen specialization of *Augochlora amphitrite*

As stressed by Cane and Sipes (2006), taxonomic versatility in pollen foraging is the hallmark of *broad polyleges*. This term also considers the available melitophilous flora at a local community, a fact that was taken into account in the present survey. This *Augochlora* species foraged on 25 % of the available melitophilous flora in this riparian forest near 30 m the nest and 13.88 % in a ratio of 200 m. When comparing with other broadly polylectic bees, these values are lower than those recorded for the highly eusocial bees *Apis mellifera* L. (38.22 % in UK, 54.73 % in Brazil) (Percival 1947; Cortopassi-Laurino 1982) and *Trigona spinipes* (55.26 % in Brazil) (Cortopassi-Laurino 1982). However, they are slightly lower or similar to those from other stingless bees such as *Plebeia remota* (33.68 %) and *Melipona quadrifasciata* (18.94 %) (Ramalho et al. 1985; Cortopassi-Laurino and Ramalho 1988).

Because few flower species are in bloom for long periods of time, it is impractical to the specialization for bees such as *A. amphitrite* whose activity period extends at least 6 months and has multivoltine life cycle (Michener 2007, p. 17). Thus, *broad polylecty* is likely a common trait in *Augochlora* bees.

In this study, the intensive use of five genera in five families and the dominance of *Ludwigia* in high percentage (>56 %) in all nests would also indicate *polylecty with strong preference* on *Ludwigia* (sensu Müller and Kuhlmann 2008). However, it is necessary to sample a larger number of sites to prove this specialization category.

Host selection

The foraging pattern found in *A. amphitrite*, i.e. only a few of the flowering species at a location being visited extensively for pollen, has also been recorded for other polylectic bees, such as honeybees (Free 1963; Pernal and Currie 2002), bumblebees (Free 1970; Raine and Chittka 2007), *Megachile rotundata* (Small et al. 1997; O'Neill et al. 2004; Cane et al. 2011), *Osmia* (Raw 1974; Cripps and Rust 1989; Rust 1990; Stubbs et al. 1994), *Megalopta* (Smith et al. 2012) and stingless bees (Ramalho et al. 1985; Kleinert-Giovannini and Imperatriz-Fonseca 1987; Vossler et al. 2010, 2014) and represents a phenomenon of general occurrence related to an optimal foraging behaviour. These studies also suggest that this bias in pollen choice is time

and site specific (i.e. temporary and local specialization). For instance, the foraging decisions of two polylectic *Osmia* species were influenced by urbanization in a Canadian city as they foraged pollen mainly on *Trifolium repens* and anemophilous trees *Quercus* and *Betula* that were widespread in this landscape (MacIvor et al. 2014). Although *Ludwigia* was only available towards the end of the foraging period, it dominated the composition of all nests (Fig. 2). This could indicate temporary specialization for this abundant pollen resource. The content of some nests (3, 6, 9, 11, 12 and 14) had between 95.7 and 98.3 % of *Ludwigia* pollen (Fig. 1), indicating that they are composed of relatively pure loads (Free 1970). The purity of the load indicates the tendency of the individual bees to visit one type of flower for pollen. It is probable that bee individuals had been stimulated to learn to handle this abundant flower type (Chittka et al. 1999). On the other hand, not all provisions came from flowerings available during summer as the whole spectrum of pollen types found were from September to March flowerings (Fig. 2). The fact that two or more generations of *A. amphitrite* live in a same nest could indicate that the earlier flowerings (*Sonchus oleraceus*, *Solanum*, *Pavonia* and *Salvia procurrens*) were foraged by the first generation while the summer flowerings by the later ones.

Different to *Ludwigia*, the other positively selected host (*G. spilanthoides*) could have been chosen for an attribute other than abundance (e.g. nutritional quality). The selection for a superior reward was tested in individuals of *A. mellifera* whose choice resources were by relative mean caloric values of nectar and not by flower abundance (Waddington and Holden 1979). Moreover, it is known that some species of Asteraceae have copious pollenkitt lipids coating their pollen grains (Williams 2003) which could explain the great percentage of *G. spilanthoides* in some nests. On the other hand, Williams and Tepedino (2003) found that the need to collect both pollen and nectar concurrently by the solitary bee *Osmia lignaria* favours the visiting on more than one species in most foraging trips. They found that the pollen harvesting from the high-nectar low-pollen species is more efficient than collecting nectar only from one species and pollen from another. The constant presence of a few grains from species other than *Ludwigia* in all *A. amphitrite* nests could be related to its need for visiting nectar hosts.

The phenological data showed that most pollen types were available during the last months of the foraging period. For this reason, to avoid misinterpretation on host selection, calculation was based on three time periods: September–March (the whole bee flight period), November–March (months when all pollen types identified in the samples were bloomed) and December–March (summer season). In all cases, this index of selectivity indicated that

pollen sources were not chosen in relation to their availability and showed high values for *Ludwigia*. As stored pollen is the pollen foraged during the whole foraging period, it was not possible to determine when each cell was provisioned.

Ludwigia has an attractive melitophilous flower (large, yellow, dish shaped and actinomorphic). Other four pollen types with different flower attributes were also intensively chosen by *A. amphitrite*. From them, it is remarkable the positive selection of *G. spilanthoides*, which has small, white, ball-shaped and actinomorphic flowers. Also *I. cairica* (large, lilac, bell-shaped and actinomorphic flower), *C. diffusa* (medium-sized, blue, dish-shaped and markedly zygomorphic flowers) and *S. romanzoffiana* (small, yellow, dish-shaped and actinomorphic flowers) were chosen in high proportion (Fig. 4). *Augochlora amphitrite* did not select pollen hosts by none of the flower attributes analysed (human colour of flowers, shape of the flowering unit and symmetry).

When considering the human flower colours available throughout the bee flight period in this riparian forest (Fig. 3), it can be seen that white and yellow flowers are dominant and the proportions of the different colours remain constant along the year. This observation is similar to those taken from five environments of grassland and woodland in Germany except that during spring months (March and April) purple dominated and white was less common (Arnold et al. 2009a). However, pink/purple was the dominant colour followed by an important number of white and yellow flowers along three levels on an alpine altitude gradient in Norway (Arnold et al. 2009b).

Ludwigia elegans flowers are perceived as UV green by bees, as shown in FReD database (Arnold et al. 2010). We hope that data on flower colours as seen by bees could be available for the plant species sampled in this South American riparian site. This would allow us to determine the importance of each colour throughout the months of bee flight activity and to compare with other ecosystems.

Conclusion

It is the first time when availability of floral resources was taken into account to evaluate pollen resources foraged by *Augochlora* bees. It was determined that *Augochlora amphitrite* is a broadly polylectic bee, considering that it used 15 pollen types from 10 plant families having diverse flower attributes. Most of the foraged resources (14 pollen types) were recorded in a radius of 30 m around the nests, which is approximately 25 % of the available species (60 species from 31 families), showing a high use of the available flora in a similar way to other eusocial bees such as *Apis mellifera* and stingless bees.

The foraging pattern of *Augochlora amphitrite* showed a notable selection for only two pollen hosts *Ludwigia* (Onagraceae) and *Gymnocroronis spilanthoides* (Asteraceae), being the flowering of the former highly abundant in the environment, while the latter was rare. Although *Ludwigia* was only available towards the end of the foraging period, it dominated the composition of all nests. This could indicate temporary specialization for this abundant pollen resource. Pollen analysis complemented with field observations on floral resource availability proved to be helpful tools to provide information on the foraging behaviour of a wild bee in its natural environment.

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