Foraging Behavior of Apis Mellifera (Hymenoptera: Apidae)and Lycastrirhyncha Nitens (Diptera: Syrphidae) on Pontederia sagittata (Commelinales: Pontederiaceae) on aDisturbed Site

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Abstract

By influencing the exchange of pollen, floral visitor behavior largely promotes the reproductive success of the plants. *Pontederia sagittata* (C. Presl) (Commelinales: Pontederiaceae) is a tristylous species whose morphs (long-styled L, mid-styled M and short-styled S) differ in the arrangement of reproductive organs and the amounts and accessibility for food for pollinating insects. We evaluated the behavior of 2 common contemporary visitors to inflorescences, the exotic bee *Apis mellifera* (L.) (Apidae), a not historical pollinator, and the flower fly *Lycastrirhyncha nitens* (Bigot) (Syrphidae), a frequent visitor already reported on this aquatic plant, based on HD video records of the number of individuals and the frequency and duration of their visits to 300 inflorescences. Both species of insects preferred to visit S-morph inflorescences. Pollen collection and nectar feeding were the most important activities of the bees, whereas flower flies were observed fed only on nectar. Thus, these behaviors could play an important role in reproduction in the study population of *P. sagittata*.

Key Words: insect behavior, floral visitors, *Apis mellifera,* syrphid, heterostyly

Resumen

El comportamiento de los visitantes florales influye en el intercambio de polen y define en gran medida el éxito reproductivo de las plantas. *Pontederia sagittata* (C. Presl) es una especie tristílica cuyos morfos (estilo largo L, estilo mediano M y estilo corto S) difieren en la disposición de los órganos reproductivos así como en la cantidad y acceso a la recompensa alimenticia para los insectos. Evaluamos el comportamiento de dos visitantes contemporáneos comunes a las inflorescencias, la abeja exótica *Apis mellifera* (L.) que no es un polinizador histórico, y la mosca de las flores *Lycastrirhyncha nitens* (Bigot), un visitante frecuente ya reportado en esta planta acuática, con base en video grabaciones de alta definición del número de individuos, frecuencia y duración de sus visitas a 300 inflorescencias. Ambas especies de insectos prefirieron visitar las inflorescencias del morfo S. La colecta de polen y la alimentación de néctar fueron las actividades más importantes de las abejas, mientras que las moscas sólo fueron observadas alimentándose de néctar. Estos comportamientos podrían desempeñar un papel importante en la reproducción de la población estudiada de *P. sagittata*.

Palabras Clave: comportamiento de insectos, visitantes florales, *Apis mellifera,* sírfido, heterostilia

Floral traits play an important role in the visual search patterns of floral visitors (Chittka & Spaethe 2007; Glaettli & Barrett 2008), and provide significant cues that are used to identify the type of reward offered (Chittka et al. 1999), directly influencing the behavior of the visitors (Sapir 2009). Thus, the number of visits may vary in relation to several floral design features, such as color (Waser & Price 1981), size (Conner & Rush 1996), nectar production (Mitchell 1994) and morphs (Husband & Barrett 1992), many of which have not been extensively studied in reproductive systems such as tristyly, in which populations are composed of 3 floral morphs that already mentioned, can influence the behavior of their visitors.

In these insect-pollinated systems, the reciprocal positioning of the anthers level with respect to the stigma increases the efficiency of legitimate pollen transfer among morphs (Glover & Barrett 1986; Dos Santos & Wittmann 2000). However, due to the differences in the amount and size of pollen grains, such polymorphism can result in differences of floral rewards and time that visitors spend feeding on nectar or collecting pollen (Barrett 1990). Foraging behaviors and morphological features of potential pollinators are also critical in determining their efficiencies during pollination. Hence the behaviors of floral visitors can impose constraints on the reproductive biology of a tristylous system.

In the Pontederiaceae, tristyly occurs in 4 *Pontederia* species and 3 *Eichhornia* species (Glover & Barrett 1983) and several studies on the diversity and behaviors of insect visitors have been made on populations of *P. cordata* (Harder & Barrett 1992; Orth & Waddington 1997; Wolfe & Barrett 1989)*, E. crassipes* (Barrett 1980)*, E. paniculata* (Husband & Barrett 1992) and *E. azurea* (Dos Santos & Wittmann 2000). For example, some Canadian populations of *P. cordata* were visited by several species of hymenopterans, butterflies, flies and birds (Wolfe & Barrett 1988) whose preferences in collecting pollen or feeding on nectar among the floral morphs differed (Wolfe & Barrett 1987, 1989; Harder & Barrett 1993; Orth & Waddington 1997), even though the amount of nectar produced did not differ between morphs (Wolfe & Barrett 1987). Nevertheless, to our knowledge there are very few published reports on the pollinators of other *Pontederia* species.

In particular, Glover & Barrett (1983) reported that in 7 of 8 studied populations of *P. sagittata* C. Presl (Commelinales: Pontederiaceae), occurring along 500 km of highway from Xalapa to Villahermosa, in the lowland coastal plain of Veracruz state, the inflorescences were consistently visited by the flower fly, *Lycastrirhyncha willistoni* Coquillett (currently a synonym of *L. nitens* Bigot, the accepted name for this species [Pape & Thompson 2013]) (Diptera: Syrphidae),

and the solitary bee, *Florilegus condignus* Cresson (Hymenoptera: Apidae), although they did not evaluate these insects' behaviors. No other studies of natural populations of *P. sagittata* have been published.

Based on this evidence we hypothesized that tristyly in inflorescences of *P. sagittata* plays a key role in the foraging behavior of its pollinators, as also occurs with other tristylous species of *Pontederia*, promoting interactions that produce different effects on their abundance and behavior. Thus, our aims were to determine the floral morph preference exhibited by the honey bee (*Apis mellifera* L.; Apidae) and the flower fly (*L. nitens*) observed visiting *P. sagittata* inflorescences, by comparing the numbers of visits and durations of foraging behaviors.

Materials and Methods

Pontederia sagittata is a perennial aquatic plant, with erect, floating or creeping, stoloniferous or rizhomatous stems, that occurs commonly along the coastal plains of Mexico, Guatemala and Honduras (Lowden 1973). The leaves are simple, entire, alternate and distichous, with parallel venation, petiolate lanceolate to broadly ovate. The inflorescences are racemose, slender, elongated and almost globose, 7-15 cm long, sustained by a modified leaf often reduced to a terminal spathe with 70-220 zygomorphic, perfect, hypogenous flowers (Glover & Barrett 1983). The flowers are composed of 6 blue lilac tepals, persistent tepals fused along half their length into a perianth tube; the androecium consist of six stamens inserts at different levels, with long-, mid- and short-styled morphs (hereafter referred to as the L, M and S morphs, respectively), and a yellow mark or nectar guide on the upper middle lobe.

The flowers bloom sequentially from bottom to top and cover 360° around the vertical axis of the inflorescence, in a pattern similar to that in *P. cordata* (Orth & Waddington 1997), which results in the presence of mature open flowers along the entire length of the inflorescence. Thus an individual inflorescence bears flowers for an average of 6 consecutive days, and various inflorescences may be blooming simultaneously within the same clone (Glover & Barrett 1983). The flowers remain open only for half a day, approximately from 0830 to 1430.

Study site

Field work was conducted at Cansa Burros (N 19° 32' W 96° 22', 10 m asl), Veracruz, Mexico during Feb 2010. The *P. sagittata* population occurred along 1 km of a canal ("Canal Gallegos") and includes all 3 floral morphs (L, M and S). The site is highly disturbed and is surrounded by ag-

ricultural areas, flooded pastures dominated by *Cynodon plectostachyus* ([K. Schum.] Pilg.; Cyperales: Poaceae) and coastal dunes on the western side. Relicts of the original vegetation correspond to a tropical semi-evergreen forest. During the study period, only *P. sagittata* was flowering.

Nectar Production

To estimate the amount of nectar available in flowers for a full period of anthesis, we measured the volume by removing the liquid accumulated around the base of the ovary. Observations were conducted on a single day when 90 inflorescences were randomly chosen (30 from each morph, from different plants) and covered with a fine mesh bag at between 0700 h and 0800 h to exclude pollinators. Five hours later we removed the bags and sampled 4 flowers (2 from the bottom and 2 from the top of each inflorescence) with 2 μL micropipettes. Nectar was extracted only once per flower, because complete removal of nectar permanently damages the flower.

Micro-Environmental Variables

We recorded 3 micro-environmental variables (wind speed [m/s], temperature [°C], and relative humidity [%]) at the beginning and end of each monitoring session on flowers with a Kestrel® 4000 Pocket Weather Meter (Nielsen-Kellerman Company, Boothwyn, Pennsylvania, USA). Thus, the micro-environmental conditions were similar between the times of the records on L, M and S morphs and were not influence insect behavior (wind speed $\bar{x}$ = 0.33 ± 0.02 m/s; χ^2 = 0.86, P = 0.65; temperature $\bar{x}$ = 24 ± 0.2 °C; χ^2 = 4, P = 0.15; and humidity $\bar{x}$ = 63 ± 0.2%; χ^2 = 0.12, P = 0.94).

Surveys of Insect Activity

Three observers recorded the behavior of bees and flower flies during 12 days between Feb and Mar 2010 along the edge of the *P. sagittata* population. Floral visitors began their activity around 0900 when some flowers started to open, and remained active until about 1300 or when wind conditions began to change. Observations were made between 0900 and 1200 h, when the flowers were in full bloom and weather conditions were favourable for insect activity.

The canal was divided into 3 segments (separated 2 m) and in each segment an observer video-recorded individual inflorescences with a Sony Handycam 40 × Optical Zoom DCR-DVD610 for 3-min per inflorescence. A camera was placed 1 m from an inflorescence to minimise physical interference and to allow the entire inflorescence to be filmed, ensuring better behavioral observations. After filming an inflorescence, the observer selected another inflorescence at least 2 m away to prevent filming the same genet. Recording sessions occurred simultaneously in the 3 segments and the sessions were finished when each observer had observed 100 inflorescences).

We analysed videotapes using image-editing software (Windows Media Player, InterVideo WinDVD). To avoid counting the same individual more than once, we registered only in the first individual recorded in each video session. We counted the number of visitors as well as the number and durations of their behaviors on each filmed inflorescence and per morph. To facilitate analysis, the only activities by visitors that we considered were feeding on nectar and collecting pollen. For bees we identified 2 foraging methods: the most frequent was hovering near flowers and sometimes landing on the long-level anthers to collect pollen without feeding on nectar; the second and less frequent method involved individuals landing on either the nectar guide or on the mid-style, in which case the bees probed for nectar. For flies we quantified the handling time, defined by Gilbert (1981) as the time taken to insert the proboscis, suck up nectar and withdraw the proboscis.

Taxonomy

The flower fly, *L. nitens* Bigot (1859: 307) was identify by Dr. Segio Ibañez Bernal and voucher specimens were deposited in the Colección Entomológica IEXA, SEMANAT: VER.IN.048.0198, Instituto de Ecología A.C. Xalapa, Veracruz, Mexico.

Statistical Analyses

In analyzing nectar production and micro-environmental conditions, we considered normal distributions and identity link functions. To test for differences in these variables, we fit unifactorial designs with the Generalised Linear Model (GLM). We fit nested designs in which the floral morphs and insect species were the independent variables, and data on number of visitors per inflorescence, the number of feeding events and their durations (the dependent variables) were Poisson error distributions (which assumes that the variance is equal to the mean). Also we used log link with GLM (Crawley 1993; Bolker et al. 2009) to compare the relationships of morphs to insect species behavior. The model was then defined by: y = Morph + Morph $_{[Insect\ species]}$ + ε (nesting factor within brackets), where y is the dependent variable, and the morph and the insect species are the independent variables. This model includes an adjustment for overdispersion correction using scaled Pearson chi-square. A posteriori analysis of multiple comparisons to test for pair-wise comparisons between

means, and all analyses were performed in JMP 6.0 (SAS Institute, Inc. Cary NC 1989-2007).

RESULTS

The 3 floral morphs of *P. sagittata* were represented at Cansa Burros albeit at different frequencies among the 300 surveyed inflorescences. In particular, the 166 S-morph inflorescences outnumbered M-(90) and L-morphs (44) inflorescences combined ($\chi^2_2 = 76$, $P < 0.001$). Such S-morph surplus has been reported for other Pontederiaceae populations (Wolfe & Barrett 1989).

The 3 morphs produced equivalent nectar volumes during 5 h ($\bar{x} = 0.37 \pm 0.02$ µL (SE); $F_{2,87} = 0.5$, $P = 0.7$).

Visitor Preferences

Five orders of insects visiting flowers were identified, Hymenoptera, Coleoptera, Diptera, Lepidoptera, Orthoptera and Hemiptera (unpublished data). The 867 recorded insect visitors included 604 individuals of *A. mellifera*, 10 of *F. condignus* and 131 of the fly *L. nitens*. Because *A. mellifera* and *L. nitens* were the most abundant visitors, we focused our analyses on their behavior. Honey bees strongly preferred the S over the L and M-morphs whereas the flower flies selected S and L equally over M-morph ($\chi^2_2 = 18$, $P < 0.0001$), with the nested species in morph also provided contrast ($\chi^2_3 = 224$, $P < 0.0001$, Fig. 1).

Feeding for bees and flower flies

Honey bees and flower flies were active on inflorescences during 3.73 of the 15 h of video-recording. *A. mellifera* represented approximately 79% of the total duration of activity (2.96 h), with feeding on nectar and collecting pollen as the most common activities (1.51 h). They spent the rest of the time moving or flying on/over inflorescences in their search for food. In contrast, *L. nitens* was active during 0.77 h (21% of the total duration of activity recorded) of which 87.4% was spent handling flowers. The flower flies spent a total of 240 s ingesting nectar and 2,191 s moving the proboscis before inserting it into the corolla.

Bees visited *P. sagittata* in search of both pollen and nectar whereas the flies were observed consuming only nectar; however it is possible that *L. nitens* also consumed pollen, although this could not be confirmed. Both species of visitors had different behaviors on the inflorescences.

Although the honey bees were more active than flies, the 2 insects preferred the S inflorescences as a food resource, as demonstrated by more feeding events on this morph ($\chi^2_2 = 15$, $P < 0.0001$), whereas the second election by bees was the M-morph and by flower flies was the L-morph as demonstrated by the differences provided by the nested within morph the species factors ($\chi^2_2 = 240$, $P < 0.0001$, Fig. 2A).

The duration of these events was also clearly longer for *A. mellifera*, which showed well-defined preferences for the S-morph ($\chi^2_2 = 27$, $P < 0.0001$) whereas *L. nitens* spent more time feeding on L and S than on M flowers ($\chi^2_2 = 436$, $P < 0.0001$, Fig. 2B).

Feeding on nectar represented 3094 s (*A. mellifera*) and 240 s (*L. nitens*) and differences were observed as a result of the particular behavior of each species and in terms of their preferences between, with the S morph preferred by bees and flower flies ($\chi^2_2 = 25$, $P < 0.0001$). The nested within morph the species factors also showed differences ($\chi^2_3 = 119$, $P = 0.0001$). The S morph was the most frequently visited for *A. mellifera* ($\bar{x} = 5 \pm 0.6$ SE), followed by the M ($\bar{x} = 3 \pm 0.4$ SE) and L morph ($\bar{x} = 2 \pm 0.6$ SE), and flowers flies more collected nectar in S ($\bar{x} = 1 \pm 0.2$ SE), followed by the L ($\bar{x} = 0.7 \pm 0.03$ SE) and M ($\bar{x} = 0.2 \pm 0.01$ SE).

Pollen collection was observed in bees during 2331 s, with significant differences between the 3 floral morphs ($\chi^2_2 = 29$, $P < 0.0001$) and by the differences the nested within morph the species factors ($\chi^2_3 = 386$, $P < 0.0001$). The M morph was the most frequently visited for *A. mellifera* ($\bar{x} = 7 \pm 0.04$ SE), followed by the S ($\bar{x} = 5 \pm 0.03$ SE) and L morph ($\bar{x} = 2 \pm 0.11$ SE), and flowers flies not collected pollen.
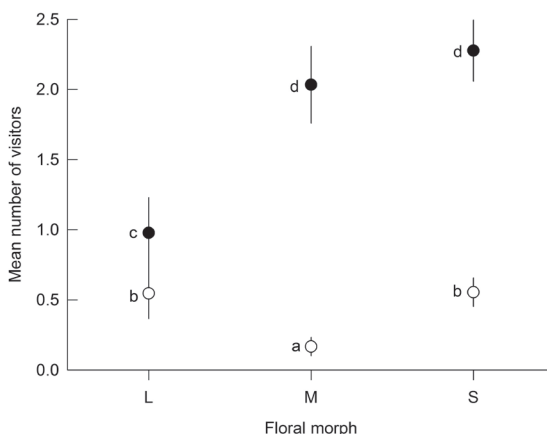

Fig. 1. Mean (± 95% CI) number of *Apis mellifera* (black circles) and *Lycastrirhyncha nitens* (white circles) observed on *Pontederia sagittata* inflorescences (long-styled L, mid-styled M and short-styled S). The different letters were significantly different (α = 0.05).

DISCUSSION

At our study site, inflorescences of *P. sagittata* were visited by the honey bee *A. mellifera* as a food resource, whereas we observed very few *F. condignus* (Glover & Barrett 1983) described as
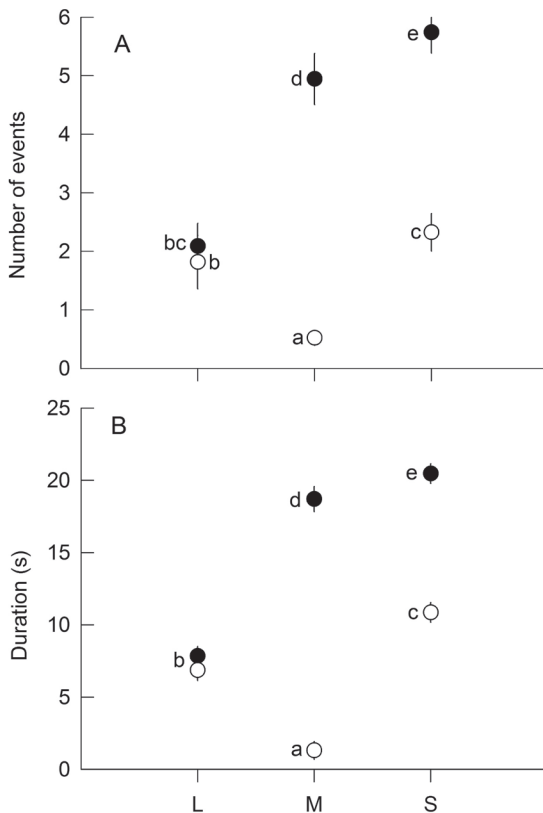
Fig. 2. Mean (± 95% CI) A) number and B) duration of feeding events by *Apis mellifera* (black circles) and *Lycastrirhyncha nitens* (white circles) on *Pontederia sagittata* inflorescences (long-styled L, mid-styled M, and short-styled S). The different letters were significantly different (α = 0.05).

the primary pollinator of *P. sagittata*. The scarcity of native bees likely reflects the disturbed state of the study site, making it amenable to occupancy by *A. mellifera* (whose presence was not reported by Glover & Barrett). In a wetland 8 km away *F. condignus* was recorded as the most abundant native bee (González 2011) so it is very likely that the native bee has been displaced by *A. mellifera* or has been isolated from the populations of *P. sagittata* by the surrounding disturbed landscape. The continued occurrence of the syrphid fly *L. nitens* on the Mexican *P. sagittata* population, which was studied by Glover & Barrett 27 years ago, indicates that the entomofaunal composition has been partially maintained, despite habitat disturbance. However, pollen transfer may not be fully functional and must be evaluated as the fly collected only nectar, and as far as we know, did not transport pollen.

The preference of *A. mellifera* for flowers of the M and S morphs could be related to pollen accessibility and quantity, as has been reported for other Pontederiaceae (Wolfe & Barrett 1989; Husband & Barrett 1992). These bees select flowers that offer a good reward of nectar and/or pollen. As the amount of nectar did not differ among the 3 morphs, pollen could be the resource that determines bee preference. Unlike other insects, honey bees probe flowers of *P. cordata* primarily for pollen, and prefer the long anthers of the M and S morphs (Wolfe & Barrett 1989) and even avoid visiting L flowers (Husband & Barrett 1992).

Like other tristylous species, the floral morphs of *P. sagittata* differ not only in the arrangement of their reproductive organs but also in the number and size of pollen grains: tall stamens produce few but large pollen grains, intermediate length stamens produce pollen grains of intermediate size and number, and short stamens produce large numbers of small pollen grains (Glover & Barrett 1983). Thus from the perspective of different pollen-collecting insect species, the flowers of the 3 morphs offer pollen in varying sizes, amounts and accessibility. For *P. sagittata* the possible explanations for the observed preference of honey bees for S flowers may include the greater accessibility to more exposed tall anthers, which reduces the search time for food and lowers the associated energy requirement (Wolfe & Barrett 1987). This could be also supported by the behavior of bees who visit all the flowers from the base to the top of inflorescences, which provides further evidence of the lack of differences in nectar production among style morphs (Orth & Waddington 1997).

Although *P. sagittata* was the sole floral resource available during the study period, we should also take into consideration that the S morph is the most abundant in the population, so our findings may be a consequence of the predominance of the S morph and not evidence of preference of one morph over another. According to Thompson (2001) insect visitation may be influenced by spatial and temporal floral displays, and it is also important to consider that constancy exhibited by insects in visiting heterostylous populations could be a result of spatial aggregation by clonal growth and near-neighbor foraging (Husband & Barrett 1992).

At our study site, the European honey bee exhibited a wide repertoire of behaviors, that representing 79% of the total duration of all recorded activity. Unlike Wolfe & Barrett's (1987) observations that *A. mellifera* preferentially collected pollen from *P. cordata*, our results show that the honey bee spent more time feeding on nectar than collecting pollen from *P. sagittata*, perhaps because of limited competition for nectar with other visitors. These behaviors could play an important role in the reproductive biology of this aquatic plant. Barrett (1980) described 2 methods of for-

aging by *A. mellifera* on *E. crassipes* that we also observed on *P. sagittata* inflorescences. The most frequent method was hovering near flowers and sometimes landing on the tall anthers to collect pollen without feeding on nectar. A second, less frequent behavior involved individuals landing on either the nectar guide or on the mid-style. In this case, the bees probed for nectar. In our study, the time devoted to collect pollen was substantial (4,244 s), whereas landing directly on flowers to forage for nectar was less common (983 s). Although there are no previous data on nectar production in *P. sagittata*, the low values found in our samples are similar to those described by Wolfe & Barrett (1987) in *P. cordata,* in which also no differences in nectar production was found among the 3 style morphs.

We found that dipterans were represented by the syrphid fly *L. nitens* (the same species reported by Glover & Barrett in 1983 as *L. willistoni*). Although the visits were related to the consumption of nectar, it could be that, like other flower flies, *L. nitens* also fed on pollen (Lunau & Maier 1995), because adult require pollen for the maturation of their reproductive system (Chambers 1988). The exclusive nectar-collecting behavior is consistent whit that reported by Barrett (1980) and Wolfe & Barrett (1988) for *P. cordata* in North America, where only a few syrphids flies were observed to collected pollen. Other long-proboscis visitors of Pontederiaceae inflorescences had a high number of pollen grains from short anthers on their body (Wolfe & Barrett 1989; Harder & Barrett 1993; Dos Santos & Wittmann 2000). This suggested that although *L. nitens* did not show preference for any floral morph, the presence of hairs on the proboscis may allow the adherence pollen.

Tristyly in *P. sagittata* affects the behavior of bees and flower flies, with a bias towards certain morphs. Given that *P. sagittata* is self-incompatible (Glover & Barrett 1983) and is therefore completely dependent on pollinators for reproduction, *A. mellifera* may not be an effective pollinator, because of its low preference for L-morph inflorescences. Further study is needed to assess whether bees are equally effective as pollinators of *P. sagittata* flowers. However, as *L. nitens* entered the floral tube to collect nectar from the *P. sagittata* flowers at our study site, pollen from all 3-anther levels may have adhered to its body. Therefore, it may be a vector for pollen transportation among morphs, although further studies are required to evaluate the pollination efficiency of *L. nitens*.

Acknowledgments

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