

# Research

## Temporal variation in the behavior of *Apis mellifera* (Hymenoptera: Apidae) and *Lycastirrhyncha nitens* (Diptera: Syrphidae) on *Pontederia sagittata* (Commelinales: Pontederiaceae) inflorescences in relation to nectar availability

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### Abstract

Insect-pollinated plants offer nectar as the main reward, which influences the preference of flower visitors. We describe the feeding behavior of the exotic bee *Apis mellifera* (L.) (Hymenoptera: Apidae) and the flower fly *Lycastirrhyncha nitens* (Bigot) (Diptera: Syrphidae) on tristylous flowers of *Pontederia sagittata* (C. Presl) (Commelinales: Pontederiaceae) in relation to temporal nectar availability. The production of this resource was similar between floral morphs but there were temporal variations during the anthesis period, in a coincidence with a higher number of visitors and activity of bees and flies. The dynamics of nectar production could be related to the feeding behavior of these insects despite the similarity in daily nectar volume produced in all the 3 types of flowers. The variations of nectar feeding may affect the transportation of pollen among the 3 floral morphs.

Key Words: *Apis mellifera*; flower fly; tristily; behavior; nectar

### Resumen

Las plantas polinizadas por insectos ofrecen el néctar como la principal recompensa que influye las preferencias de los visitantes florales. Describimos el comportamiento de alimentación de la abeja exótica *Apis mellifera* (L.) (Hymenoptera: Apidae) y la mosca de las flores *Lycastirrhyncha nitens* (Bigot) (Diptera: Syrphidae) en las flores trístilicas de *Pontederia sagittata* (C. Presl) (Commelinales: Pontederiaceae) en relación con la disponibilidad temporal de néctar. La producción de este recurso fue similar entre los morfos florales pero hubo variación temporal durante el periodo de antesis, en coincidencia con un mayor número de visitantes y actividad de abejas y moscas. La dinámica en la producción de néctar podría estar relacionada con el comportamiento de alimentación de estos insectos a pesar de la similitud en los valores de volumen de néctar producido por los tres tipos de flores. Las variaciones en el consumo de néctar podría afectar el transporte de polen entre los tres morfos florales.

Palabras Clave: *Apis mellifera*; mosca de las flores; trístilia; comportamiento; néctar

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Floral nectar is an important reward to pollinators (Stpiczyńska et al. 2012) that modifies the activity and behavior of insects visitors, which in turn affects intra-and interplant pollen flow (Nicolson 2007; Yokoi & Fujisaki 2008). Nectar production varies during anthesis (Pacini & Nepi 2007), due in part to environmental conditions such as temperature (Galetto & Bernardello 2004; Nicolson 2007), among other

factors. Likewise, the amount of nectar available in flowers (standing crop) and consumed by pollinators promotes variations in nectar volume (Corbet 2003; De Alencar et al. 2005).

In tristylous species, the amount and access to pollen and nectar rewards are mediated by the reciprocal disposition in the lengths of anthers and styles (Zomlefer 1994, Fig. 1), a condition that promotes

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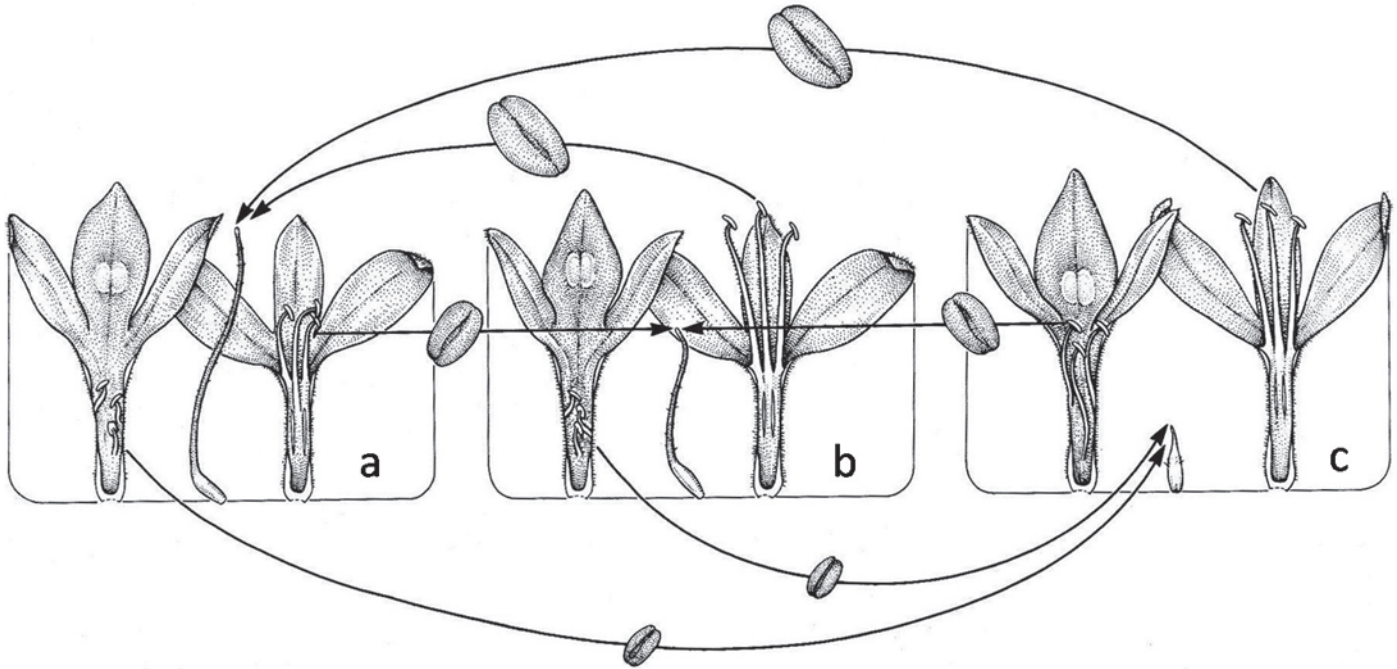
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**Fig. 1.** Position of styles and stamens and differences in pollen size in the three floral morphs of *Pontederia* sp. **a)** long-styled [L], **b)** mid-styled [M] and **c)** short-styled [S] (Zomlefer 1994). Legitimate pollinations are indicated by arrows.

the activity and feeding time of each type of pollinator varies between floral morphs (Wolfe & Barrett 1987; Barrett 1990). As a result, the transport of legitimate pollen between morphs, an indispensable condition for efficient pollination in these species, could be affected (Barrett & Forno 1982; Dos Santos & Wittmann 2000; Barrett & Shore 2008). Therefore a visitor's behavior can directly impact plant reproduction and the adaptive significance of tristylly.

In the Pontederiaceae 4 *Pontederia* species and 3 *Eichhornia* species (Glover & Barrett 1983; Graham et al. 1998) exhibit tristylly. However, few reports describe the preferences of visitors to Pontederiaceae flowers in relation to nectar availability. In *P. cordata*, for example, the quantity, sugar concentration and dynamics of nectar production are similar among the floral morphs (Wolfe & Barrett 1987; Orth & Waddington 1997), but the behavior of *Apis mellifera* (L.) (Hymenoptera: Apidae), *Melissodes apicata* and *Bombus* spp. differ depending on whether they are collecting pollen or nectar (Harder & Barrett 1992). In the same species, *A. mellifera* rarely probes into flowers to feed on nectar, but mostly collects pollen from the everted, long-level stamens (Wolfe & Barrett 1987). In contrast, an unidentified flower fly collected pollen and fed on nectar (Wolfe & Barrett 1988).

The exotic honeybee *A. mellifera* stands out because of its generalist nature (Roubik 1989) and because its behavior can affect the foraging behavior of other flower visitors that compete for resources (Goulson et al. 2002). On the contrary, flies consume nectar and pollen (Gilbert 1981; Proctor et al. 1996; Diaz-Forestier et al. 2009) and are pollinators of several plant species (Ssymank et al. 2008; Zamora-Carrillo et al. 2011). But little is known of the behavior of exotic bees and flower flies when visiting other tristylous species in relation to the amounts of nectar available, such as *Pontederia sagittata* (C. Presl) (Commelinales: Pontederiaceae), whose septal nectaries can only be reached by visitors with long proboscises.

The behaviors and visit preferences of *A. mellifera* and the flower fly *Lycastrirhyncha nitens* (Bigot) (Diptera: Syrphidae) upon arrival at the inflorescences of the 3 morphs of *P. sagittata* were described in a disturbed site during a 3-h anthesis period (Campos-Jiménez et al.

2014). Even though all the morphs produce equivalent nectar volumes, here we hypothesized that potential temporal changes in nectar secretion and availability should be related to the activity and behavior of insect visitors. In this context the aim of this study was to examine if nectar dynamics could be related to the temporal activities and behaviors of bees and flower flies when visiting *P. sagittata* inflorescences.

## Materials and Methods

### STUDY SITE

Field work was conducted at Cansa Burros (19° 32' 08" N 96° 22' 37" W, 10 m asl), Veracruz, Mexico. The *P. sagittata* population occurred along 1 km of "Canal Gallegos" and includes all 3 floral morphs (L, M and S). The site had been disturbed by human activities and was dominated by the introduced grass *Cynodon plectostachyus* ([K. Schum.] Pilg; Cyperales: Poaceae), sugarcane plantations and coastal dunes. The native vegetation was a semi-evergreen tropical forest and during the study period only *P. sagittata* flowered.

### MICROENVIRONMENTAL CONDITIONS

The conditions in the micro-environment occupied by each studied inflorescence were measured with a Kestrel® 4000 Pocket Weather Meter (Nielsen-Kellerman Company, Boothwyn, Pennsylvania, USA) that recorded wind speed (m/s), temperature (C) and humidity (%) before and after each video session. The averages of these values were analyzed with Generalized Linear Models (GLM) with a 2-factor design, with Poisson error distribution to evaluate the effect of the floral morph and the period of observation as fixed factors, and their interaction.

Micro-environmental parameters varied among the 4 sampling periods (wind speed  $\chi^2 = 10.37$ ; df 3;  $P = 0.01$ ; temp  $\chi^2 = 10.01$ ; df 3;  $P = 0.02$ ; humidity  $\chi^2 = 64.96$ ; df 3;  $P < 0.0001$ ), but did not differ significantly among the floral morphs ( $\chi^2 = 8.12$ ; df 2;  $P = 0.2$ ;  $\chi^2 = 1.21$ ; df 2;  $P = 0.61$ ;  $\chi^2 = 4.88$ ; df 2;  $P = 0.9$ ) or with the period  $\times$  morph interaction

(wind speed  $\chi^2 = 24.33$ ; df 2;  $P = 0.6$ ; temperature  $\chi^2 = 2.94$ ; df 2;  $P = 0.8$ ; and humidity  $\chi^2 = 4.12$ ; df 2;  $P = 0.7$ ).

Humidity and temperature varied negatively ( $r = -0.39$ ;  $P < 0.01$ ), indicating that early in the morning the relative humidity was high ( $\bar{x} \pm SE = 65 \pm 0.01\%$ ) and temperature low ( $\bar{x} \pm SE = 25 \pm 0.02$  °C), and the wind speed increased at 13:00 h ( $\bar{x} \pm SE = 0.52 \pm 0.1$  m/s).

## STUDY SPECIES

*Pontederia sagittata* is a perennial aquatic plant, with erect stoloniferous or rhizomatous 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. The inflorescences are racemose, slender, elongated and almost globose, 7-15 cm long, subtended by a modified leaf often reduced to a terminal spathe, with 70-220 zygomorphic, perfect, hypogenous flowers (Glover & Barrett 1983). The flowers include 6 persistent blue lilac tepals fused along half their length into a perianth tube; the androecium consist of 6 stamens inserted 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 (see Campos-Jiménez et al. 2014 for more details). Also an individual inflorescence bears flowers for an average of 6 consecutive days, and from several to many inflorescences may bloom at the same time within a single clone (Glover & Barrett 1983). The flowers remain open for only half a day from approximately 08:30 to 14:30 h.

## SURVEYS OF INSECT ACTIVITY

In late Mar 2011, we videotaped 180 independent inflorescences of *P. sagittata* (60 per morph), to record the activity of insect visitors. For each recording session we selected 3 plants of each morph with similar floral display size and insect activity, to reduce the influence of these factors on visitor behavior. The videos of the 3 morphs were recorded simultaneously during 3-min intervals using a Sony Handycam 40x Optical Zoom DCR-DVD610 (10×) placed approximately 1 m from the inflorescences. Recordings were made each h in four 15-min periods (09:00-09:15, 10:00-10:15, 11:00-11:15 and 12:00-12:15 h) for 5 days.

Videos were analyzed using the Windows Media Player and Inter-Video WinDVD software. We counted the bees and flies visiting the inflorescences of each floral morph, the feeding events (number of times that bees collected pollen or fed on nectar in each flower per inflorescence) and the time spent on these behaviors. To avoid counting the same individual more than once, we registered only the first individual of each species recorded during each video session. To identify the foraging behavior observed in both visitor species we followed the method described by Campos-Jiménez et al. (2014).

We fit generalized linear models (GLM) to analyse the influences of floral morph, hour and insect species on total activity time, the number of feeding events and their durations (the dependent variables). The analyses considered Poisson distributions and used logarithmic link functions (Crawley 1993; Bolker et al. 2009) and there was over dispersion. We modified the analytical model in the case of hierarchically structured data that is when multiple species of insects are visitors to the same inflorescence, because two or more insects that visit the same inflorescence are not independent (Hurlbert 1984). They are not independent because they share the same foraging site, which affects their behaviour, and therefore the conditions of a bee and a fly are nested within an inflorescence (Crawley 1993). Hence the model

was then defined as:  $y = \text{Morph} + \text{Hour} + (\text{Morph}, \text{Hour}_{[\text{Insect species}]}) + \text{error}$  (nesting factor within brackets), where  $y$  is the dependent variable, and the morph and hour are the independent variables. A posteriori analysis of LS means contrasts to the t test for pair-wise comparisons because multiples between means were performed. All analyses were carried out with JMP 9.0.1 (SAS 1999-2010, SAS Institute, Cary, North Carolina, USA).

## NECTAR

Once behavioral observation of floral visitors was completed, we estimated the volume of nectar available in 3 randomly selected flowers of each video recorded inflorescence by removing the liquid accumulated around the base of the ovary with 2  $\mu\text{L}$  micropipettes. The values were averaged and analyzed by fitting a Generalized Linear Model (GLM) with a two-factor design with 3 floral morphs (L, M and S) and 4 sampling periods. We used a normal distribution and identity link function. Nectar volume correlated positively, but weakly with humidity ( $r = 0.26$ ,  $P < 0.05$ ), and the highest availability was recorded at 10:00 h.

We measured accumulated nectar in 15 inflorescences from 5 clones per floral morph. At 08:00 h (prior to anthesis) inflorescences were covered with a fine mesh and the volume was measured by removing the liquid accumulated around the base of the ovary using 2  $\mu\text{L}$  micropipettes in 3 randomly selected flowers of each inflorescence at hourly intervals. Data were analyzed in a manner similar to that used for the nectar standing crop.

We also estimated the cumulative nectar production during anthesis in 10 inflorescences for each morph in independent plants. At 08:00 h inflorescences were excluded and after 5 h nectar was measured by removing the liquid accumulated around the base of the ovary using 2  $\mu\text{L}$  micropipettes on 3 randomly selected flowers per inflorescence. Data were analyzed in a manner similar to that used for the nectar standing crop.

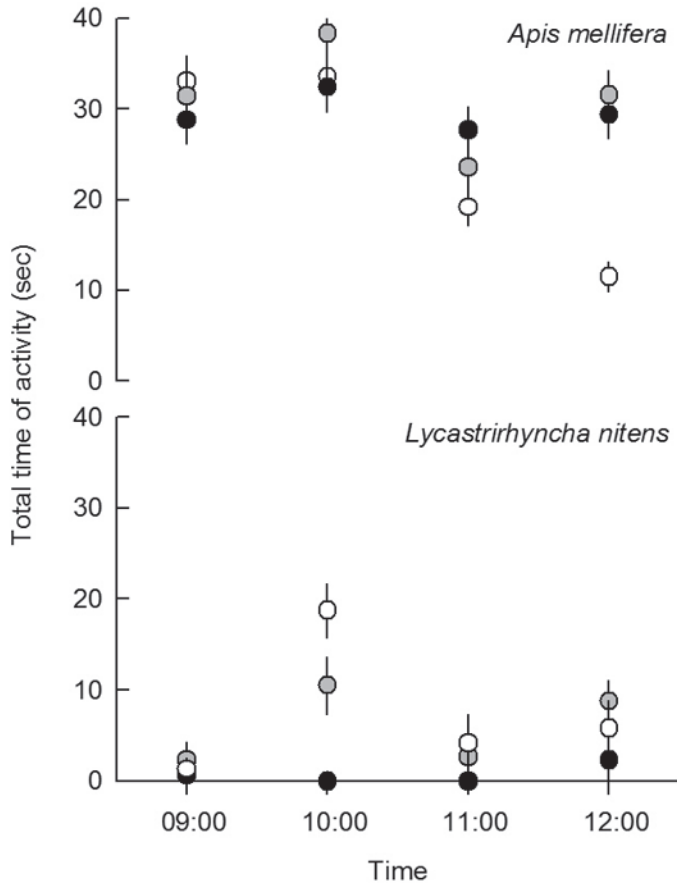
## Results

### VISITOR ACTIVITY

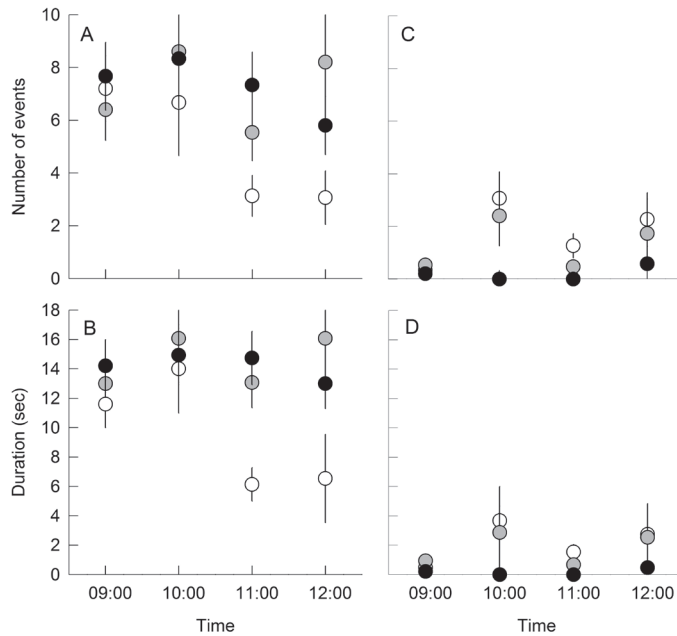
A total of 297 bees and flies were recorded visiting the 180 inflorescences of *P. sagittata*. *A. mellifera* was the most common species (84.5%), whereas *L. nitens* accounted for only 15.5% of the visits. Both species were observed visiting flowers during 1.65 of the 9 h of video recording: 86% corresponding to bees (1.41 h) and 14% to flies (0.24 h). Although the S morph was the most abundant in the study population (Campos-Jiménez et al. 2014), some preferences by visitors were observed. *Apis mellifera* was more active on M and L inflorescences whereas *L. nitens* spent more time visiting the inflorescences of the S and M morphs ( $\chi^2 = 8.11$ ; df 2;  $P = 0.02$ ) mostly at 09:00 and 10:00 h, with the nested species in hour and morph also providing contrasts ( $\chi^2 = 134.61$ ; df 3;  $P < 0.0001$ , Fig. 2).

Most of the time that visitors remained active on inflorescences was spent collecting pollen and/or feeding on nectar, although their behavior and preferences differed. Honey bees spent more time collecting pollen (0.34 h) than feeding on nectar (0.3 h), this being 53.5 and 46.5% of their total foraging time, respectively. In contrast, flies were observed consuming only nectar during 238 s, but spent 320 s in the movements of the proboscis prior to insertion into the corolla.

Bees and flies performed more foraging events at 10:00 and 12:00 h (Fig. 3), although they differed in their morph preferences. While bees mostly visited the M and L morphs, the flower flies preferred M and S inflorescences ( $\chi^2 = 6.24$ ; df 2;  $P = 0.04$ ). This was also demonstrated by the differences provided by the nested species within hour



**Fig. 2.** Total activity time ( $\pm$  95 % CI) of *Apis mellifera* and *Lycastrirhyncha nitens* on inflorescences of L (black circle), M (gray circle) and S (white circle) morphs of *Pontederia sagittata* during daily periods of video-recording.



**Fig. 3.** Mean ( $\pm$  95 % CI) number and duration of the foraging events recorded by *Apis mellifera* (A, B) and *Lycastrirhyncha nitens* (C, D) on inflorescences of L (black circle), M (gray circle) and S (white circle) morphs of *Pontederia sagittata* during daily periods of video-recording.

and morph ( $\chi^2 = 105.83$ ; df 3;  $P < 0.0001$ ), since the S inflorescences received consistently fewer visits by bees (Fig. 3A) and flies foraged less on L inflorescences at 09:00 and 11:00 h (Fig. 3C).

In similar conditions, the average time that bees and flies spent foraging on the inflorescences differed between morphs. Bees stayed longer in M and L inflorescences than flies, which visited M and S inflorescences ( $\chi^2 = 6.31$ ; df 2;  $P = 0.03$ ) in all the recorded hours. This was also confirmed by the nested species within hour and morph results ( $\chi^2 = 158.12$ ; df 3;  $P < 0.0001$ ), which showed that bees made few visits to S morph mainly at 11:00 and 12:00 h (Fig. 3B), whereas flies were more active at 10:00 and 12:00 h (Fig. 3D).

**NECTAR CHARACTERISTICS**

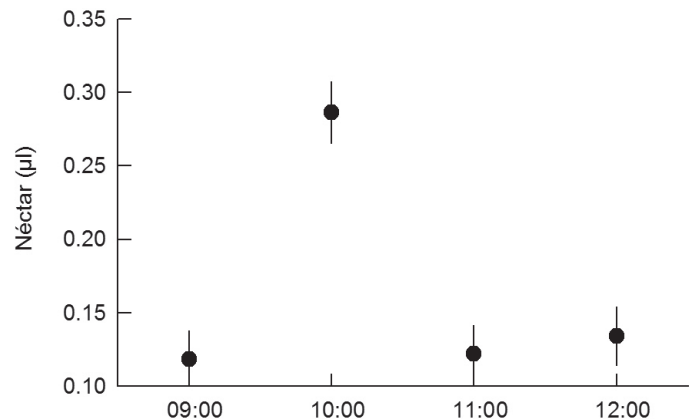
Flowers of the 3 morphs contained similar nectar volumes at the end of each video-recording period ( $\chi^2 = 6.34$ ; df 2;  $P = 0.06$ ;  $\bar{x} \pm SE = 0.16 \pm 0.08 \mu\text{L}$ ). Nectar standing crop differed between the 4 registration periods ( $\chi^2 = 112.76$ ; df 3;  $P < 0.0001$ , Fig. 4), being greatest at 10:00 h.

Nectar volumes accumulated during the hourly sampling periods were similar in the 3 floral morphs ( $\chi^2 = 4.41$ ; df 2;  $P = 0.2$ ;  $\bar{x} \pm SE = 0.22 \pm 0.04 \mu\text{L}$ ; morph x period interaction  $\chi^2 = 6.13$ ; df 2;  $P = 0.04$ ) with differences between hour intervals ( $\chi^2 = 87.42$ ; df 3;  $P < 0.0001$ ). The 3 floral morphs produced similar total nectar volumes by the final 1200 h sample ( $\chi^2 = 1.12$ ; df 2;  $P = 0.53$ ), with an average ( $\pm SE$ ) of  $0.66 \pm 0.05 \mu\text{L}$ .

**Discussion**

This study yielded 2 major results. Firstly, the behavioral patterns of *A. mellifera* and *L. nitens* were found to vary during the anthesis period of *P. sagittata* in accordance with the dynamics in the production and availability of nectar. Secondly, honeybees and flower flies exhibited contrasting preferences for morphs compared to our previous findings in the same population (Campos-Jiménez et al. 2014).

At our study site the higher volume of nectar in *P. sagittata* corresponded to the higher activity of bees and flies that visit the inflorescences, as before and during the peak of highest availability of the nectar both visitors were more active. *Apis mellifera* remained active in 86% of the total time recorded and showed a preference for the L and M inflorescences, spending more time collecting pollen and feeding on nectar even in those periods in which the flowers had less standing crop of that resource, but we found some important contrasts regarding its behavior.



**Fig. 4.** Mean ( $\pm$  95 % CI) standing crop of floral nectar on inflorescences of three floral morphs of *Pontederia sagittata* during daily periods of video-recording.



The presence of long anthers facilitated the collection of pollen by this exotic bee, resulting in less investment of time and therefore lower energy cost. Thus, according to Thorp (2000), *A. mellifera* should visit M and S morph inflorescences at a higher frequency if their preferences were based on pollen availability, as only these morphs present accessible long-level anthers. That hypothesis is consistent with some reports on other tristylous species such as *Lythrum junceum* (Ornduff 1975), *E. crassipes* (Barrett 1980) and *P. cordata* (Wolfe & Barrett 1987, 1989; Husband & Barrett 1992). Despite the temporal variation in their visits and also considering that there were no differences in nectar production among the morphs, our results demonstrate that the preferences of *A. mellifera* may temporarily change even in cases involving the same study population (Campos-Jiménez et al. 2014) since in the present study *A. mellifera* preferred L and M morphs whereas previously they had visited more M and S inflorescences, respectively.

The flower fly *L. nitens* also varied its behavior between hours, and this was related to the standing crop of nectar and cumulative nectar per hour. These flies had large events of feeding on nectar in the inflorescences of M and S morphs and individuals were less active in the inflorescences of the L morph, although these insects spent less time on foraging behavior in relation to the bees. However these flies also changed their preferences towards the floral morphs, and this is demonstrated by the contrast between the present results and those previously reported in the same population (Campos-Jiménez et al. 2014). This behavioral change could be the result of some influencing factors such as the differences in the floral displays in different flowering seasons.

The increase in the number of visits at 10:00 h indicates that *L. nitens* visits *P. sagittata* inflorescences for nectar, so the temporal variation in the availability of this source determines the fly's feeding behavior. We did not evaluate the dynamics on pollen availability, another floral resource that *L. nitens* could be transporting among the floral morphs since hairs are present on its head and thorax. Therefore the feeding behavior and visits of *L. nitens* between morphs could contribute to pollen movement within and between plants.

The temporal variation of *A. mellifera* and *L. nitens* behavior on *P. sagittata* in relation to standing crop of floral nectar and different preferences for the 3 floral morphs could influence the reproductive success of the plants in the study population. As the honeybee mainly fed on the flowers of the L and M morphs, its behavior could affect the legitimate pollen deposition between the floral morphs of *P. sagittata*. Furthermore S morph inflorescences are less visited by bees, which may possibly promote decreases in pollination and seed production as happens in the L morph of *P. cordata* (Wolfe & Barrett 1987). We also do not know the contribution of *L. nitens* as a pollinator, but their behavior indicates a low preference for L inflorescences at different times.

In summary, our results demonstrate correspondence between the behavioral patterns of *A. mellifera* and *L. nitens* and the amount of nectar in *P. sagittata* inflorescences at 10:00 h, although the preferences of both visitors change over the time probably as a result of variations in their abundance and the availability of food resources. Because the nectar volumes of the 3 floral morphs were similar, the preferential visits of these two species to certain flower morphs could influence the pollination services in this *P. sagittata* population. We further assume that others parameters are key in the dynamics of cross-pollination in this species, such as the temporal availability of pollen because pollen is an important reward for bees and flies.

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