



SPATIAL AND TEMPORAL DISTRIBUTION OF FLORAL REWARDS WITHIN THE CAPITULA: THE CASE OF *HYALIS ARGENTEA* (ASTERACEAE)

DISTRIBUCIÓN ESPACIAL Y TEMPORAL DE LAS RECOMPENSAS FLORALES DENTRO DE LOS CAPÍTULOS: EL CASO DE *HYALIS ARGENTEA* (ASTERACEAE)

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SUMMARY

Background and aims: Differential resource allocation to attraction, reward, and sexual functions has been observed at capitula level of derived species of Asteraceae. Heterogamous capitula contain a combination of pistillate, staminate or sterile and perfect florets, thus rewarding and sexual functions are performed by different kinds of florets and at different times. Such spatial and temporal distribution of rewards within the capitula is not as clearly established in species with homogamous capitula, where all florets are perfect and produce pollen and nectar. We evaluated the spatial and temporal distribution of floral rewards in homogamous capitula of *Hyalis argentea*.

M&M: We compared the floral phenology, the number of pollen grains and nectar volume and concentration between ray and disk florets, and registered the foraging behaviour of floral visitors.

Results: Capitula have a centripetal and alternate flowering pattern and they were visited by bees, ants, butterflies, moths, beetles and thrips, but pollinated mainly by *Apis mellifera*. We did not find a temporal pattern in the offer of rewards within the capitula, but we do find a spatial pattern in nectar volume increasing from outer to inner florets.

Conclusions: This spatial variability in nectar quantity could impact pollinator behavior and thus enhance outcrossing likelihoods improving sexual reproduction in this self-incompatible species.

KEY WORDS

Asteraceae, capitulum maturation, *Hyalis argentea*, nectar, pollen.

RESUMEN

Introducción y objetivos: Una asignación diferencial de recursos entre la función de atracción, recompensa y sexual ha sido observada en capítulos de especies derivadas de Asteraceae. Los capítulos heterógamos están compuestos por flores pistiladas, estaminadas o estériles y perfectas, con lo cual las funciones sexual y de recompensa son realizadas por diferentes tipos de flores y en distintos momentos. Esta distribución espacial y temporal de las recompensas dentro del capítulo no es tan clara en especies con capítulos homogámicos, donde todas las flores son perfectas y producen polen y néctar. Aquí evaluamos la distribución espacial y temporal de las recompensas florales en los capítulos homogámicos de *Hyalis argentea*.

M&M: Comparamos la fenología floral, el número de granos de polen y la concentración y volumen de néctar entre las flores marginales y centrales, y registramos el comportamiento de forrajeo de los visitantes florales.

Resultados: Los capítulos tienen un patrón de floración centrípeta y también alterno y son visitados por abejas, hormigas, mariposas, polillas, escarabajos y trips, siendo *Apis mellifera* su principal polinizador. No encontramos un patrón temporal en la oferta de recompensas dentro de los capítulos, pero sí un patrón espacial en el volumen de néctar que aumenta desde las flores marginales hacia las del centro del capítulo.

Conclusiones: Dicha variabilidad espacial en la cantidad de néctar podría afectar el comportamiento de forrajeo de los polinizadores y así aumentar las probabilidades de polinización cruzada, mejorando la reproducción sexual de esta especie auto-incompatible.

PALABRAS CLAVE

Asteraceae, maduración de capítulos, *Hyalis argentea*, néctar, polen.

INTRODUCTION

Angiosperms have been successful colonizers of different habitats worldwide and their great species diversity is closely related to its interaction with animal pollinators and seed dispersers (Regal, 1977; Wilmer, 2011; Van der Niet & Johnson, 2012). Given that plants are sessile organisms, they depend on vectors such as animals to ensure the movement of pollen grains to a conspecific receptive stigma and thus to achieving successful sexual reproduction. Indeed, the role of the flower is crucial because they not only contain and protect the female gametes, but also develop attracting and rewarding structures to ensure pollinator visitation. Flowers are one of the classic examples of food-reproductive exchange; they offer food to pollinators (usually pollen and nectar) in order to move male gametes and enhance outcrossing (Wilmer, 2011).

As rewards usually cannot be directly perceived by pollinators, flowers are informative structures with visual and olfactory advertising signals that generally reveal the amount and quality of rewards offered. Usually larger flowers offer more nectar (e.g. Blarer *et al.*, 2002; Fenster *et al.*, 2006; Gómez *et al.*, 2008), flowers with wider corollas have greater pollen production (Gómez *et al.*, 2008) and flower scent can advertise on nectar quality and its specific location (von Helversen *et al.*, 2000). A successful strategy for dealing with resource allocation to multiple functions relative to pollinator attraction, floral rewards, breeding, gamete protection and seed dispersal is that developed by the cosmopolitan Asteraceae family. Asteraceae species have flowers consolidated in *capitula* (inflorescences), which are very versatile structures that contain and protect reproductive organs. The *capitulum* is the pollinator attraction unit that balances morphological and physiological demands of the florets to improve reproduction (Jeffrey, 2009). *Capitula* are classified according to their morphology as homomorphic (discoid) and heteromorphic (radiate) containing only one or two morphologic type of florets, respectively (Mani & Saravanan, 1999). Moreover, *capitula* are also classified according to their sexuality as homogamous containing only perfect florets and heterogamous containing a combination of pistillate, staminate or sterile and perfect florets

(Panero & Funk, 2008). In evolutionary derived heteromorphic and heterogamous *capitula* the attracting, rewarding, and sexual functions are performed by different kinds of florets. The outer whorl of the *capitulum* (ray florets) has usually pistillate or sterile florets with large bilabiated, pseudolabiated or ligulated corollas much showier than inner whorls with tubular corollas (disk florets). Therefore ray florets attract pollinators (Stuessy *et al.*, 1996; Mani & Saravanan, 1999), and are usually non-rewarding, while inconspicuous disk florets are perfect and produce both types of reward (nectar and pollen) preserving both rewarding and sexual functions (Burt, 1961; Stuessy *et al.*, 1996; Mani & Saravanan, 1999). This spatial separation of rewarding functions among florets of the inflorescence is not clearly established in basal species of Asteraceae, which usually have homogamous *capitula* (i.e. all florets are sexually similar and usually produce pollen and nectar as rewards, Lane, 1996; Mani & Saravanan, 1999; Stuessy & Urtubey, 2006; Torices *et al.*, 2006).

Asteraceae species have a centripetal maturation of the inflorescences; it means that the outer whorls open first than the inner ones. This phenological pattern would imply a temporal separation in the offer of rewards among florets from heterogamous *capitula*, but the lack of temporal separation among florets from homogamous *capitula*. In heteromorphic-heterogamous *capitula* the showy female or sterile florets open first and they do not produce rewards, then, as the *capitulum* mature, perfect rewarding florets open offering both pollen and nectar (Wist, 2005; Lelis, 2008). In this type of *capitula* the distribution of rewards varies temporally: rewards are absent at the beginning of the *capitula* maturation but they increase later with the anthesis of disk florets. Similarly, in such *capitula* there is also a spatial distribution of rewards, increasing from the outer ray to the inner disk florets (Wist, 2005; Chambó *et al.*, 2011). In heteromorphic-homogamous *capitula*, all the florets are perfect, thus, beside the centripetal maturation, the temporal separation of rewards within the *capitulum* is unlikely since pollen and nectar are offered from the first floret anthesis to the last one. However, if in such *capitula* the quantity of rewards increases from the outer to the inner florets, like in heteromorphic-heterogamous *capitula*, it is expected a spatial gradient in reward quantity.

Outer ray florets will produce less pollen and nectar than the inner disk florets. Interestingly, as far as we know, no studies have been conducted to evaluate whether differential reward quantity do exist among florets in Asteraceae species within heteromorphic-homogamous *capitula*. To test this hypothesis we worked with *Hyalis argentea* D. Don ex Hook. & Arn. var. *latisquama* Cabrera, a self-incompatible specie (Camina, 2011), belonging to the basal Tribu Hyalidae, subfamily Wunderlichioideae (Panero & Funk, 2008). *Hyalis argentea* has heteromorphic-homogamous *capitula* composed mainly of six perfect florets, five of which are ray bilabiated and one disk tubular (Cabrera, 1963). In this study, we compared the floral rewards and floral phenology between the ray and disk florets and registered the foraging behavior of floral visitors.

MATERIALS AND METHODS

Species Studied

Hyalis argentea is an endemic species from Argentina; its distribution is limited to sandy soils in the provinces of La Pampa, Córdoba, San Luis, Mendoza, Neuquén, Río Negro, Buenos Aires and Chubut (Zuloaga *et al.*, 2008). It is a perennial rhizomatous sub-shrub of *ca.* 1 m height used in environment restoration (Dalmasso, 2010). *Hyalis argentea* has *capitula* (inflorescences) arranged in terminal pseudocorymbes (Freire *et al.*, 2002). Each *capitulum* has five to six violet florets: four or five bilabiated ray florets and one tubular pentasect disk floret (Cabrera, 1963; Fig. 1). The style is bilobed, without sweeping hairs and the achene has a *pappus* with heterogeneous bristles (Cabrera, 1963; Freire *et al.*, 2002; Torres & Galetto, 2007; Roque & Funk, 2013). *Hyalis argentea* is a non-dormant species that germinates during autumn and blooms from December to February (Camina *et al.*, 2013; Forcone & Andrada, 2006), and its inflorescences are visited mainly by insects (Camina, 2011). This study was performed in a natural population at the Agronomic Department of the Universidad Nacional del Sur-UNS (38°41'S, 62°15'O, Bahía Blanca, Buenos Aires, Argentina) from December 2009 to February 2010. The reference plant specimen (Camina 5497) was deposited in Bahía Blanca Regional Herbarium (BB), in the Agronomy Department (UNS).

Phenology

In order to evaluate whether in *H. argentea* *capitulum* maturation follows the centripetal opening pattern of Asteraceae and to assess whether the florets expose pollen and nectar simultaneously, we tagged 50 unopened *capitula* (composed by five ray and one disk florets) in 25 plants separated among them by 10 m. For these observations all the *capitula* were tagged the same day, observations started at 20:00 hs and phenological stages were checked every hour until the senescence (approximately at midnight) of the last floret occurred. All the process took three days (total 72 hours of observations per *capitulum*). For individual florets (ray and disk) we defined five phenological stages (Fig. 1): I- from the beginning of anthesis (Fig. 1A) to corolla fully extended (Fig. 1B); II- from the beginning of pollen exposure (Fig. 1C) to the beginning of stigma tip aperture (male phase, Fig. 1D); III- from the beginning of stigmatic lobes aperture to total stigma aperture at 180° (stigmatic aperture phase, Fig. 1D, E); IV- from stigmatic lobes total aperture to first signs of corolla senescence (female phase, Fig. 1D); and V-senescence, from corolla starting gets pale and oxidized to the drop of it (Fig. 1E). The phenological stage I was used to define the centripetality of the *capitula* maturation. In order to compare the initiation time of more representative functional phenological stages between ray and disk florets, anthesis (I), male phase (II), female phase (IV) and total floret lifetime (from I to V) were used. The stigma receptivity was checked by the hydrogen peroxide technique from III to V phenological stages (Zeisler, 1938).

Floral Visitors

In order to determine the rewards foraged by pollinators (pollen or/and nectar), observations of floral visitors were made during the bloom peak of *H. argentea* (January 2010). Four plots of 1 m² separated by at least 5 m were randomly chosen. Each plot had approximately 12 plants and on average 55 inflorescences (Camina, 2011). Observations were made during 15 minutes per plot and every two hours, from 8:00 to 20:00, at two different days (seven measures per day per plot). The total observation period was 840 minutes. The floral visitors were captured, identified and deposited at Laboratorio de Estudios Apícolas (LabEA-UNS). The frequency of visits was calculated as the

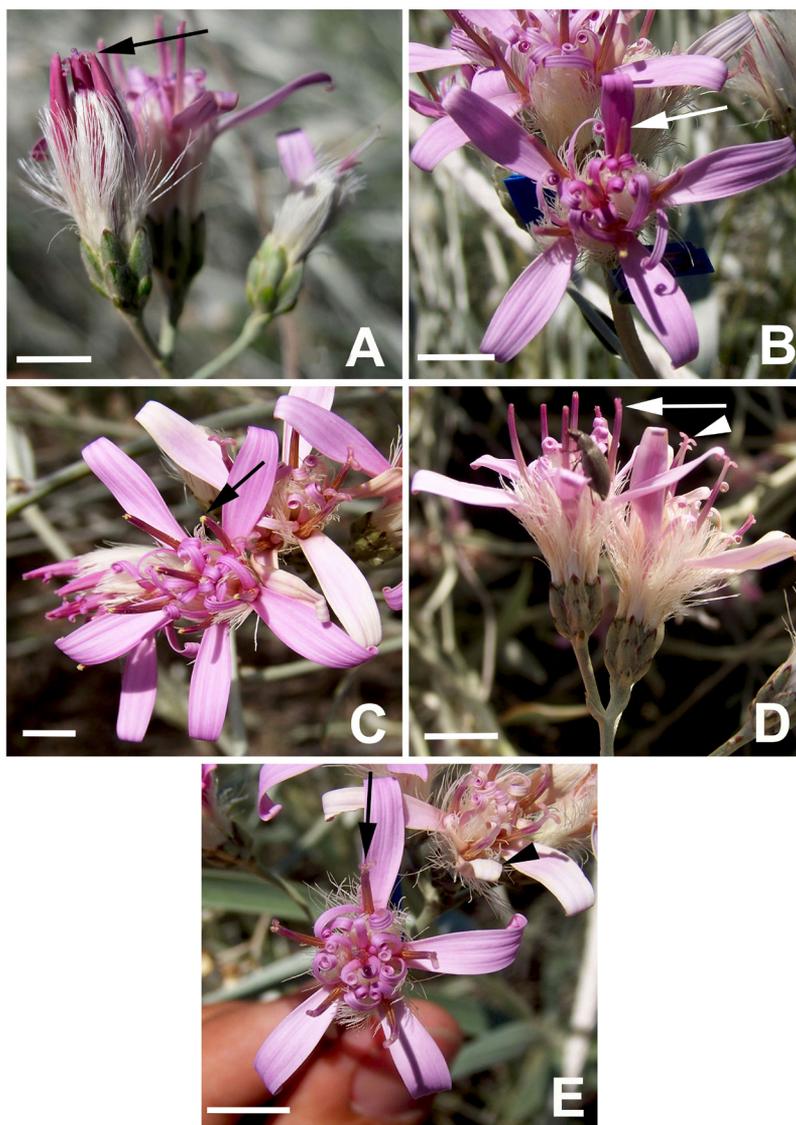


Fig. 1. Floret phenological stages of *Hyalis argentea*. **A:** beginning of corolla aperture (arrow). **B:** corolla opening (arrow) and corolla full expanded (the others four ray florets in the *capitulum*). **C:** pollen exposure (arrow). **D:** stigma tip aperture (arrow) and stigma lobes at 180° (arrowhead). **E:** stigma lobes at 45° (arrow) and floret senescence (arrowhead). Scale bar = 6 mm.

number of *capitula* visited/number of available *capitula* per plot*15 minutes (Dafni, 2005).

Floral Rewards

In *Hyalis argentea*, like in all the Asteraceae, the androecium is a synantherium with anthers forming a tubular structure, *i.e.* an anther tube. The number of pollen grains was calculated in 45

indehiscent anther tubes, from ray (three florets per *capitula*, three *capitula* per plant, five plants) and disk florets (one floret per *capitula*, five *capitula* per plant, nine plants). Anther tubes were stored in glacial acetic acid at room temperature. Pollen grains were counted from each anther tube with a haemocytometer slide in 50% glycerin (Neubauer Improved Double; Lloyd, 1972). The estimation

of pollen grains number per floret was made from three aliquots (1 µl each) per sample (one anther tube in 1 ml of glycerin 50%), in which the amount of pollen grains within the grid pattern was counted, averaged and extrapolated.

To quantify nectar volume and concentration, *capitula* from 18 plants were bagged with a mesh 48 hours before florets opened. Nectar was collected from 27 disk and 27 ray florets (three *capitula* per plant, nine plants) with microcapilar tubes during the male phase (II), between 11:00 and 16:00 hs. The volume was measured with a digital caliper. Nectar concentration was estimated with a table refractometer with sodium light at 20°C; original measures were in °Brix (weight/weight) and transformed to obtain the concentration in solute mass/solution volume (w/v, Bolten *et al.*, 1979).

Statistical analysis

In order to compare the floret phenology among ray and disk florets, the total floret lifetime (from I to V) and the initiation time of the most representative

floret phenological stages (I, II and IV) were used. A nested ANOVA with floret type (ray vs. disk) as the fixed factor and *capitulum* nested within plants as random factors was used. As data are small ranges of hours and we evaluate the most frequent initiation hour, the mode was used as a central tendency measure (Zar, 1999). The amount of pollen and nectar volume and concentration were compared between ray and disk florets by one way ANOVA. For data analysis the statistical software InfoStat (free version) was used.

RESULTS

Phenology

Total floret lifetime was *ca.* 53 ± 0.51 (SE) hours and differences were not significant between ray and disk florets ($F_{1,49} = 0.10$ $P = 0.758$; Fig. 2). The anthesis of ray florets began at night, one hour before disk florets ($20:00 \pm 0.16$ vs. $21:00 \pm 0.16$, respectively; $F_{1,49} = 23.4$, $P < 0.001$; Fig. 2). The initiation time of male (II) and female (IV)

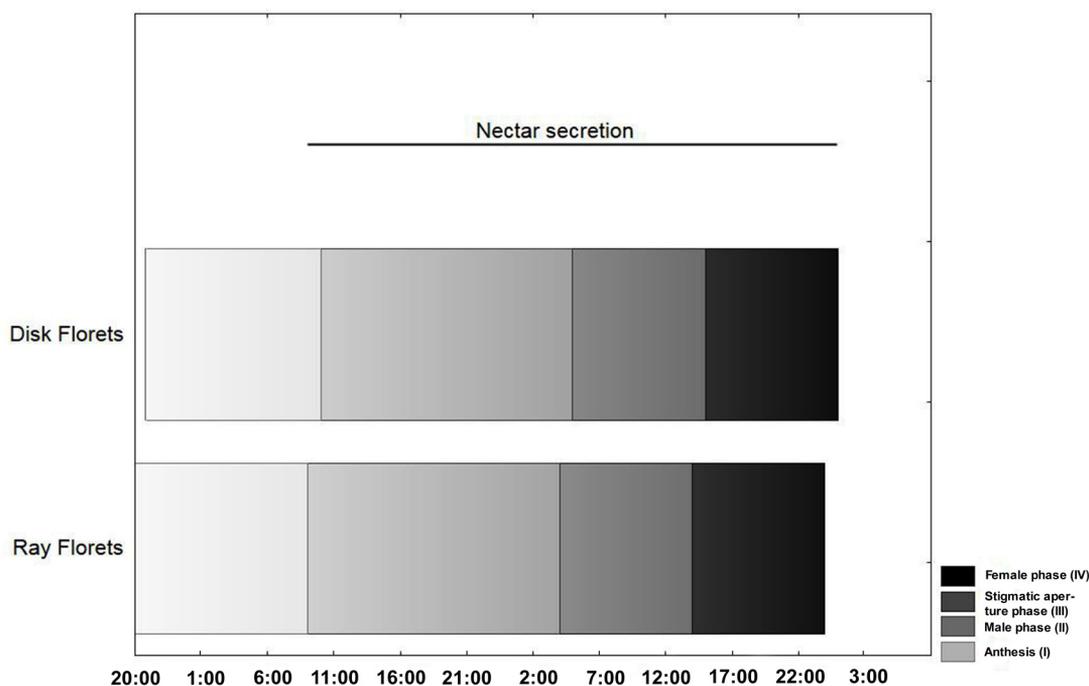


Fig. 2. Duration of floret phenological phases, total floret lifetime and nectar secretion period in ray and disk florets of *Hyalis argentea*. I: anthesis. II: male phase. III: stigmatic aperture phase. IV: female phase.

phases did not differ significantly between ray and disk florets ($F_{1, 49} = 2.95, P = 0.096$; $F_{1, 49} = 0.39, P = 0.537$; respectively). Therefore, none of the floret phenological stages differed in duration time between floret types (Fig. 2). Corolla opening extends around 14 hours (all night and part of the morning, anthesis in Fig. 2), then, around 10:00 of the second day, pollen exposure occurs and extends on average 19 hours (male phase, Fig. 2). From the early morning of the third day styles started to elongate and stigmatic lobes to open, and they continued opening around 10 hours (stigmatic aperture phase, Fig. 2). Stigmas were receptive when the aperture of the lobes was 180° , (after midday) and remained so for 10 hours during the afternoon and night (female phase, Fig. 2). Both kinds of florets were completely protandrous, male phase preceded female phase (Fig. 2) and these phases were generally not overlapped within a *capitulum*.

Capitula of *H. argentea* followed partly the centripetal flowering pattern of the Asteraceae. Although 75% of the *capitula* began opening the outer ray florets before the disk floret, the remaining 25% opened one, two, or three ray florets first and then the disk floret opened one hour later in

conjunction with the remaining ray florets (an alternate/non-strictly centripetal maturation, Fig. 3). Nevertheless, disk florets never opened before ray florets or alone, thus, the opening of florets did not follow a random pattern. It is noteworthy that *H. argentea capitula* usually have four to five ray florets and only one disk floret, but in the studied population the presence and quantity of disk florets was variable, *i.e.* we found *capitula* that had none (19%), 1 (76%) or 2 (5%) disk florets.

Floral Visitors

Ten species of insects were observed visiting the *capitula* of *H. argentea*. The domestic bee (*Apis mellifera* L.), three butterflies (*Dione vanillae* L., *Tatochila autodice* H. and *Helicoverpa zea* B.) and one moth species (*Plusia* sp.) were considered pollinators, because they made contact with the fertile organs of the florets. Two species of thrips (Tubulifera and Terebrantia), one species of beetle (Curculionidae) and two species of ants (Formicidae) were also found visiting *capitula*, but they are probably inefficient pollinators due to their low mobility between plants and to the self-incompatibility of *H. argentea*. The frequency of visits was 0.129 ± 0.022 *capitula* visited/15 minutes.

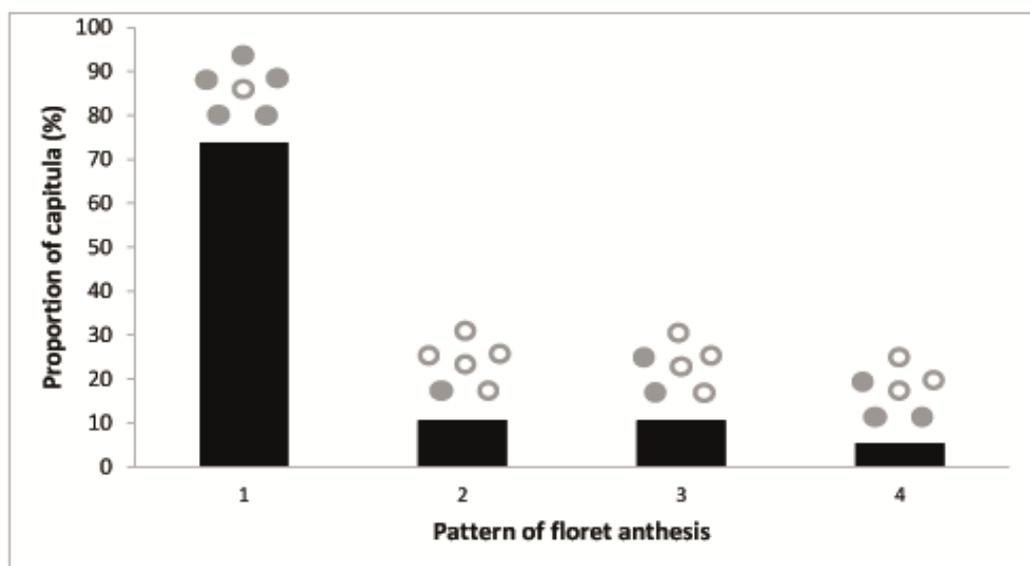


Fig. 3. Proportion of *capitula* of *Hyalis argentea* with different patterns of maturation. **1:** centripetal maturation where ray florets open before disk one. **2, 3 and 4:** options of alternate maturation where some ray florets open simultaneously with disk florets one hour later. Full circles represent florets that open first and empty circle represent florets that open one hour later.

Most of the visits (92%) were made by *A. mellifera* while the remaining 8% were made by Lepidoptera species. Throughout the studied period, *A. mellifera* foraged by pollen mainly in the morning (8:00 to 12:00) and nectar throughout the day but mostly at midday (11:00 to 14:00). Lepidoptera species foraged for nectar between 11:00 to 14:00 hs.

Floral rewards

There was no significant difference in average number of pollen grains per anther tube between ray and disk florets ($F_{1,88} = 0.06$, $P = 0.807$), therefore each floret had on average $3,128.6 \pm 135.5$ pollen grains. In both types of florets, nectar was produced from the moment of pollen release (male phase) until corolla senescence (Fig. 2). Nectar concentration was $41.58 \% \pm 3.06$ w/v and there was no significant difference between ray and disk florets ($F_{1,22} = 3.06 \cdot 10^{-5}$, $P = 0.99$). Nectar volume differed significantly among disk and ray florets ($F_{1,22} = 5.86$, $P = 0.036$), disk florets had more nectar ($0.522 \mu\text{l} \pm 0.173$) than ray florets ($0.378 \mu\text{l} \pm 0.074$). As both kinds of florets produced pollen and nectar, there was no a period of absence of rewards during the flowering of the *capitulum*.

DISCUSSION

Plants call the attention of pollinators by floral signals that advertise rewards (Schaefer *et al.*, 2004; Benítez-Vieyra *et al.*, 2010), and in Asteraceae species the *capitulum* is the advertising unit (Jeffrey, 2009). As we expected, there was no temporal separation in the offer of rewards during the flowering of the *capitulum*. Both types of florets produced pollen and nectar, so both rewards were offered from the anthesis of the first to the last floret in the *capitulum*. Phenological stages did not overlap temporally among florets within the *capitulum* in *H. argentea*, therefore it is possible to find entirely male or entirely female *capitula* in a plant. This complete protandry at the *capitulum* level implies that all the florets within a *capitulum* offer pollen at the same time and simultaneously with nectar. This offer of rewards may improve pollination and sexual reproduction. It would maximize pollinator attraction by catching the attention of both, pollen (*A. mellifera*) and nectar feeders (*A. mellifera* and Lepidoptera species). We

found that *H. argentea* has a complete protandry within the *capitulum*, diminishing the selfing likelihoods. Moreover we showed that the *capitula* of *H. argentea*, although homogamous, have a spatial pattern in the distribution of rewards evidenced by nectar volume. Nectar quantity increased from outer to inner florets. This pattern, although less evident, resemble that found in heterogamous *capitula* where outer florets are not rewarding (Wist, 2005; Chambó *et al.*, 2011). We did not find differences in nectar concentration and pollen quantity. Adjustments in pollen quantity and nectar concentration are probably reproductively riskier compared to adjustments in nectar volume. In *H. argentea* the pollen is actively collected by bees and because pollen grains contain the male gametes, a great variability in pollen production could seriously jeopardize the sexual reproduction of this self-incompatible species (Camina, 2011). On the other hand, nectar concentration of *H. argentea* matched that reported for other Asteraceae species (Cabrera & Dieringer, 1992; Wist, 2005; Willmer, 2011) and for species that are usually pollinated by bees and butterflies (Baker & Baker, 1983). Compared to volume, nectar concentration has higher impact in the foraging decisions of bees (Cnaani *et al.*, 2006; Konzmann & Lunau, 2014). Thus, similar nectar concentration among florets within a *capitulum* may enhance the constancy of pollinator visitation and thus reproductive success. The fact that both kinds of florets had the same concentration of nectar but higher volume in inner florets indicates that inner florets produce also more sugar than outer florets.

Most *capitula* of *H. argentea* followed the centripetal maturation pattern common to Asteraceae (*e.g.* Gross & Weerner, 1983; Andersson, 2001; Grombone-Guaratini *et al.*, 2004), but a considerable proportion of them did not fit this pattern. The alternate (non centripetal) flowering pattern in a quarter of the sampled *capitula* of *H. argentea*, is not common in Asteraceae (Cronquist, 1955, Dadpour *et al.*, 2011).

The pollinator assemblage of *H. argentea* was less diverse than the assemblage of many other species of the family (*e.g.*, Andersson, 1996; Wist, 2005; Lelis, 2008) and it was dominated by the exotic bee *A. mellifera*. Probably this assemblage is consequence of the anthropogenic condition where this study was conducted. It is likely that in

less disturbed sites *H. argentea* has more diverse pollinator assemblages which even may include nocturnal pollinators. We did not do nocturnal observations of floral visitors, but given that florets expose pollen and stigmatic lobes at night, it is likely that *H. argentea* have nocturnal pollinators as observed in other species of the family (Fagua & Gonzalez, 2007; Macgregor *et al.*, 2015). Moreover, we observed an overlapping in the foraging period for nectar among *A. mellifera* and species of Lepidoptera at midday. This suggests that the peak of nectar production could occur at this time and may vary through the day. We did not evaluate the availability of nectar and pollen through the floret lifetime, but it is likely that both rewards decrease with floral visitation. Several plant species can replenish floral nectar but not pollen (*e.g.* Ashworth & Galetto, 2002). Given the high visitation frequency of *A. mellifera*, which collect pollen intentionally in *H. argentea*, it is likely that pollen offer decreases from male to female floral phases. Thus, it is likely that pollen offer occurs during male phase only, but nectar availability occurs throughout all the floral phases. To test these ideas it is needed to evaluate production and availability of rewards throughout the floret lifetime.

Curiously, in the studied population of *H. argentea* around the 20% of *capitula* did not have the disk floret and this condition was highly variable within and among plants, ranging from plants with the majority of *capitula* with the disk floret to plants with the majority of *capitula* without the disk floret. That variability in the quantity of disk florets per *capitulum* was also observed in other natural populations of this species (Camina J. pers. obs.). The absence of the disk floret contrasts with the description made by Cabrera (1963) for this species. It is common that in Asteraceae the proportion of florets per *capitulum* usually vary (Freire *et al.*, 2002), but the variability observed here had not been previously reported for *H. argentea*. Notably, the morphological traits studied here at the *capitulum* level have been little investigated in other Asteraceae species with homogamous *capitula* (but see Lane, 1996). Our results show that some of the observed traits in *H. argentea* do not match those commonly described for derived species of Asteraceae with heterogamous *capitula*, but they interestingly

resemble traits of species from the Calyceraceae family, the sister group of Asteraceae, and might support the hypothesis about the evolutionary origin of Asteraceae and Calyceraceae inflorescences from the same common ancestor (Pozner *et al.*, 2012). The evolutionary origin of the Asteraceae *capitulum* would imply the loss of the terminal flower of the ancestral thyrse/thyrroid structure (inflorescence) that shares the MCGA clade (Menyanthaceae, Calyceraceae, Goodeniaceae and Asteraceae, Pozner *et al.*, 2012). The presence of the only central disk floret in *capitula* of *H. argentea* might suggest a remnant of the terminal flower of this ancestral inflorescence, as proposed by Pozner *et al.*, (2012) for the central floret of some basal *Barnadesia* species. Nevertheless, studies on the *capitulum* development of this and another related species are needed to corroborate this speculation.

In summary, a spatial distribution of floral nectar was found within the *capitulum* of *H. argentea*, inner disk florets had significantly higher volume than outer ray florets. We suggest that such spatial distribution of resources among florets within the *capitulum* could impact pollinator attraction and behavior. This strategy would enhance outcrossing likelihood, which is essential to warranty sexual reproduction in this self-incompatible species.

AUTHOR CONTRIBUTION

JLC, LA and ET conceived the idea and designed the study. JLC and ET conducted fieldwork. RC performed statistical analyses. EG contributed to visualization and data presentation. ACA, CP and LA provided the financial support for the project leading to this publication. JLC and LA wrote the first draft of the manuscript and all authors contributed to the manuscript.

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