

Cleistogamy in the rare high Andean perennial herb *Cryptantha capituliflora* (Boraginaceae)

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Received May 22, 2002; accepted November 14, 2002

Published online: March 20, 2003

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Abstract. Axial and apical flowers of *Cryptantha capituliflora* were analyzed with regard to morphology and pollen tube growth to assess the occurrence of cleistogamy. Although intermediate floral forms do occur, cleistogamous flowers were significantly smaller than chasmogamous flowers, had fewer anthers, and showed a distinctive stigmatic surface. Chasmogamous flowers can be cross-pollinated. Nevertheless, the growth of self-pollen tubes in few chasmogamous buds jointly with flower characters suggests that these flowers can probably produce fruits through autonomous selfing. The mean seed number per fruit did not differ between fruits from chasmogamous and cleistogamous flowers. Cleistogamous flowers were only observed in axial inflorescences, which are completely covered by the leaf. Other species of section *Cryptantha* also show the same trend, with cleistogamous flowers located in the lower half of the stems. This pattern is discussed in relation to dissimilarities in the outcrossing opportunities between flower types within the plant.

Key words: *Cryptantha capituliflora*, flower dimorphism, prior selfing, fruit set, pollen tube growth.

Self-pollination is now considered a complex process that occurs through several pathways (Lloyd and Schoen 1992). The different modes of selfing primarily vary as a function of their dependence on animal vectors for pollen

deposition. Thus, it is not difficult to distinguish autonomous self-pollination from a pollinator-mediated way of selfing that may result in either within- or between-flower self-fertilization. Self-pollination that only occurs through pollinator activity is known as facilitated selfing (Lloyd and Schoen 1992). The other ways of self-pollination involve autonomous self-pollen deposition independent of pollinator activity, differing in the time of pollen deposition during the lifetime (prior, competing, and delayed selfing) (Lloyd and Schoen 1992). Although these self-pollination mechanisms promote spontaneous autogamy, outcrossing is not completely excluded. The fact that self-pollen deposition is not always followed by self-fertilization, relies on factors like pollen prepotency, time of pollen deposition, dichogamy, among many others (Lloyd 1992, Lloyd and Schoen 1992, Ramsey 1995).

Autonomous self-pollination in cleistogamous flowers (i.e. closed flowers that do not allow any pollinator interaction) is the only self-pollination mode that lets no chances of outcrossing. This mode of selfing mainly differs from the other ways of autonomous self-pollen deposition in that it occurs in structurally modified flowers (Lord 1981).

Several floral traits appear associated with cleistogamous (CL hereafter) flowers. In general, these flowers are characterized by a reduction of the androecium and the size of the corolla (Lord 1981, Campbell et al. 1983, Porrás and Álvarez 1999). Other characters such as pollen size and number, and even the shape of the stigmatic papillae, have been observed to differ between chasmogamous (CH hereafter) and CL flowers within species (Lord 1981, Campbell et al. 1983, Lord and Eckard 1986, Ruiz de Clavijo and Jiménez 1993, Porrás and Álvarez 1999). In addition, the size reduction of CL flowers also affects the nectary structure and the production of floral rewards (Lord 1981).

Most cleistogamous species are described with distinctly dimorphic flowers, but species with less differentiated flower types do occur (Plitmann 1995). Moreover, as the morphological differences between CH and CL flowers become more obvious at maturity, in some cleistogamous species the two flower types can be distinguished only late in the development (Lord 1982, 1984; Mayers and Lord 1984). In other words, substantial morphological variation occurs among CL flowers of several species (Lord 1980, 1982). On the other hand, regarding pollinators and fertilization, different reproductive success in CL and CH flowers has been hypothesized, but little evidence has been found until recently (Berg and Redbo-Torstensson 1999, 2000). All these facts support the idea of cleistogamy as a complex and variable floral dimorphism (Lord 1980), but the mere occurrence of different floral traits is not enough to distinguish cleistogamy from other ways of selfing.

Cryptantha is one of the largest genera of Boraginaceae and is mainly distributed along a narrow strip in Western America (Johnston 1927, Kelley and Wilken 1993). Cleistogamy seems to have a South American origin within this genus, since all North American *Cryptantha* species bear CH flowers only (Grau 1983). Cleistogamous *Cryptantha* species display both CL and CH flowers and belong to two different sections, *Geocarya* and *Cryptantha*,

which are exclusively distributed in South America. Section *Geocarya* presents more specialized CL flowers and amphicarp (i.e. the production of two types of fruits which differ in morphology and ecological properties), whereas section *Cryptantha* shows less differentiated flower types (Johnston 1927, Pérez-Moreau 1976). Although the identification of CL flowers in the genus *Cryptantha* is not new, little is known about the reproductive biology of the species. Moreover, no previous work has assessed the occurrence of obligate autogamy in structurally modified flowers of this genus.

Cryptantha capituliflora (Clos) Reiche (section *Cryptantha*) is a perennial herb restricted to small areas in high Andean regions of Chile and Argentina (Pérez-Moreau 1976). Qualitative morphological differences between CL and CH flowers have been reported for all species of this section (Johnston 1927, Pérez-Moreau 1976). The position within the plant appears to be the most distinctive feature between flower types in this species, since CH flowers are located at terminal inflorescences and CL flowers are axially located (Pérez-Moreau 1976).

The aims of this work were (1) to determine if there are significant quantitative morphological differences between apical CH flowers and presumed CL flowers located in the axial leaf, (2) to evaluate self-pollen tube growth to assess the occurrence of selfing and (3) to quantify the fruit and seed production in both flower types.

Materials and methods

Cryptantha capituliflora flowers are white with yellow coronas. The corona is formed by five papillate-intruded appendages (fornices) at the apex of the corolla tube (Pérez-Moreau 1976). The flowers are small (approximately 0.5 cm long), with introrse anthers attached near the top of the corolla. The anthers are located at the same height as the stigma (Pérez-Moreau 1976). The ovary is biovulate. Apical CH flowers are arranged in simple or double densely germinate inflorescences

(Fig. 1), typical of several boraginaceous species. CL flowers are axial, solitary or arranged in few-flowered inflorescences, and generally located in the lower half of the flowering stem (Fig. 1) (Pérez-Moreau 1976).

C. capituliflora has a reduced distribution range and is confined to high altitudes. It mainly grows between 1500 and 3400 m above sea level. Considering collections from Chile and Argentina, *C. capituliflora* populations appear between 30°50' and 33°34'S and with narrow longitudinal distribution (from 70°49' to 70°03'W), always within the same altitudinal range.

Flower morphology. Plants for this study were collected in February 2000 in a population located near Cristo Redentor, Mendoza province, Argentina, at 3350 m above sea level. Because of the scarcity of these plants all this work was carried out with the flowers and fruits from six plants of this population. Six flowering stems were collected per plant, and fixed in ethanol 70%. A voucher of this population is deposited at CORD (Galetto and Calviño 1007). In order

to assess the degree of floral dimorphism between CH and CL flowers, newly open CH flowers and CH buds from terminal inflorescences were compared with CL ones. One CH bud and one open CH flower were measured per inflorescence (N=36 for each developmental stage). Axial flowers with closed corollas and from inflorescences located in the lower half of the stem were classified as CL. The translucent closed corolla allowed us to observe the dehiscent anthers and helped us to identify these flowers. Because there are few flowers per plant, only 22 CL flowers could be measured. Flowers were dissected and measured under a light microscope at 35×. The corolla width and length, anther number and length, gynoecium length, and the presence of nectaries were recorded in each flower type. To detect and count the stomata of the nectary, 10 gynoecia of each flower type were cleared with NaOH (10% aqueous solution), washed with ethyl alcohol:acetic acid (3:1), and stained with IKI solution. Stomata were counted under light microscope at 400×.



Fig. 1. Semi-schematic representation of a flowering individual of *Cryptantha capituliflora* showing the location of the different flower types. Squares = indicate geminate chasmogamous inflorescences. Circles = mixed inflorescences with chasmogamous and cleistogamous flowers. Black dots = location of solitary cleistogamous flowers in the leaf axis. Bar = 1 cm

Scanning electron micrographs were obtained to examine the features of the stigma surface (e.g. the size and shape of the papillae). The flower samples were dehydrated in an acetone series, critical-point dried with liquid CO₂, mounted on aluminium stubs, coated with gold – palladium, and observed with a microscope Zeiss DSM 940A at 5 kV. Pollen and papillae size were estimated using the scanning images. Measurements were made on 20 papillae from two stigmas for each flower type. Pollen volume was calculated as $\pi PE^2/6$ (Harder 1998) where P and E are the polar and equatorial diameter respectively.

Pollen tube growth. To assess the occurrence of autogamy in both flower types, 51 gynoecia (12 CL and 39 CH, obtained from unopened buds) were softened in sodium hydroxide 8N and stained with aniline blue 0.1% in potassium acetate for the detection of pollen tubes (Martin 1959). Stigmas were observed under a Zeiss Axiophot fluorescence microscope at UVH 365 nm.

Fruit and seed production. As the corolla remains attached to the fruit, those fruits with small closed corollas could be assigned to CL flowers. Fruits from natural pollinated flowers (74 CH and 36 CL) were dissected and the number of seeds with viable embryos was counted under a stereoscopic magnifying glass. Empty and shrivelled seeds were considered aborted, whereas those with well developed embryos were considered viable.

Statistical analysis. A MANOVA was carried out to test the overall differences between flower types. One-way ANOVAs were performed to evaluate the differences between flower types for each of the different traits considered. The gynoecium and anther length were log-transformed to achieve normality assumptions. Mann-Whitney U-tests were used to compare papillae and pollen grain size between CH and CL flowers.

Results

Leaf-axis inflorescences with CL flowers usually did not bear more than 4 flowers. In some axillary inflorescences, one or two of the flowers were CH. Thus, some leaf-axis inflorescences bear both flower types. On the contrary, apical inflorescences only displayed CH flowers. As a plant may produce up to ten apical inflorescences, with a mean of c. 30 flowers

(29.5 ± 4.35 flowers) per inflorescence, the number of CL flowers per plant is considerably lower than CH ones.

Flower morphology. The general flower morphology showed distinctive features for CH and CL flowers (Fig. 2). Significant differences were observed when comparing flower types (MANOVA Hotelling's T value = 4.10, $F = 16.40$, $P = 0.0001$; Table 1). Most of the traits considered were significantly different between flower types (Table 1). In general, CL flowers were smaller than CH ones (Fig. 2), and CH flower buds showed a size intermediate between CH and CL flowers, allowing to distinguish CH buds from CL flowers (Table 1).

CL flowers presented a reduced gynoecium and usually a lower anther number. However, some CL flowers developed five anthers as in the CH ones. In general, CL flower characters had a wider range of variation than CH buds and CH open flowers (Table 1). The largest size variation was observed in the CL gynoecium, which ranged from 0.20 to 1.58 mm length (Table 1). Style length varied

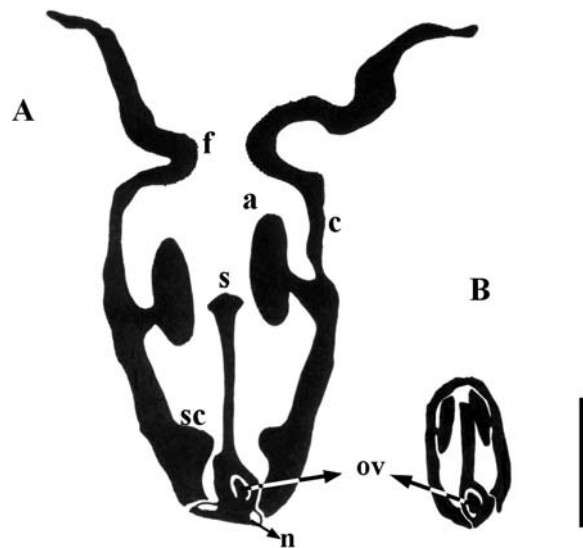


Fig. 2. Longitudinal flower schemes of *Cryptantha capituliflora*. (A) chasmogamous and (B) cleistogamous flowers. Abbreviations: (a) anther, (c) corolla, (f) fornices, (n) nectary, (ov) ovule, (s) stigma, (sc) scale. Bar = 1 mm

Table 1. Morphological characters and dimensions (mean \pm SD) of chasmogamous and cleistogamous flowers of *Cryptantha capituliflora*. The coefficient of variation (%) is given within parentheses. Values in the same row followed by the same letter are not significantly different at 0.05 level (Tukey test). n/d: no data

Floral characters	CL flower	CH flower	CH flower bud	Statistic
Corolla width (mm)	1.52 \pm 0.64 ^a (42)	3.93 \pm 0.66 ^b (17)	3.47 \pm 0.41 ^b (12)	$F_{[2,45]} = 64.00$ $P < 0.001$
Corolla length (mm)	1.47 \pm 0.75 ^a (51)	3.25 \pm 0.42 ^b (13)	2.69 \pm 0.46 ^c (17)	$F_{[2,45]} = 47.02$ $P < 0.001$
Gynoecium length (mm)	0.72 \pm 0.47 ^a (65)	1.46 \pm 0.20 ^b (14)	1.31 \pm 0.24 ^c (18)	$F_{[2,44]} = 26.66$ $P < 0.001$
Style length (mm)	0.56 \pm 0.25 ^a (44)	1.00 \pm 0.17 ^b (17)	0.78 \pm 0.23 ^c (29)	$F_{[2,45]} = 28.25$ $P < 0.001$
Anther length (mm)	0.47 \pm 0.20 ^a (42)	0.70 \pm 0.09 ^b (13)	0.81 \pm 0.05 ^c (1)	$F_{[2,45]} = 19.33$ $P < 0.001$
Anther number	3–5	5 (invariable)	5 (invariable)	
Nectary	Inconspicuous	Well developed	Well distinguishable	
Corolla appendages (fornices)	Generally absent	Conspicuous	Well distinguishable	
Location within the stem	Axial	Apical	Apical	
Pollen size (μm^3)	91.78 \pm 43.43	57.45 \pm 7.90	n/d	$U = 3.26$ $P = 0.001$
Papillae length (μm)	3.93 \pm 0.17	4.58 \pm 0.55	n/d	$U = -1.65$ $P = 0.09$
Papillar cap width (μm)	4.99 \pm 1.26	6.90 \pm 1.26	n/d	$U = -1.76$ $P = 0.07$
Number of lobes	Absent	3–6 per papillae	n/d	

considerably less in both CH buds and CH open flowers. The style of a few CH flowers was taller than the anthers.

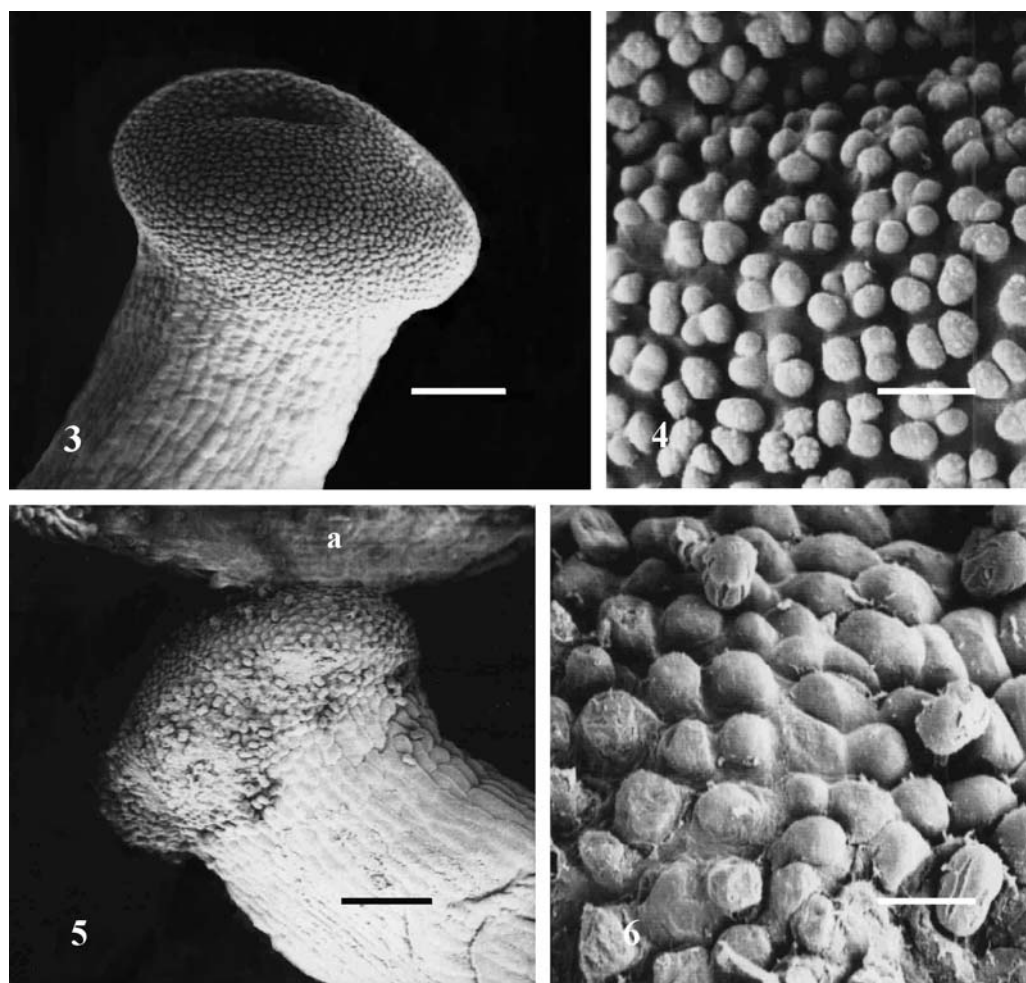
Pollen grains from CH flowers were significantly smaller than those from CL flowers (Table 1). The number of pollen grains in CH flowers was above 30000. CL flowers showed completely dehiscent anthers, so no exact estimations of the number of pollen grains per flower could be made. The fact that the anthers of CH flowers were dehiscent at the time of collection (even those of newly open flowers), may be responsible of their smaller size compared with the anthers of flower buds (Table 1).

CH and CL flowers of *C. capituliflora* have capitate stigmas with a flattened head and the papillae uniformly distributed (Figs. 3–6). The shape and size of the papillae differed between

flower types. A CH stigma (from both buds and open flowers) is covered by langeniform papillae, which are formed by a short distinct neck and a cap with a variable lobe number (Fig. 4, Table 1). Papillae from CL flowers are capless and shorter (Fig. 6), but differences in size between CL and CH papillae were not significant (Table 1).

The nectary of CH flowers is an inconspicuous disc located at the base of the ovary. The nectariferous tissue did not differ from the surrounding tissues, so its presence could primarily be assessed staining the stomata. The number of stomata per nectariferous disc varied from 18 to 25 in both flower types.

Pollen tube growth. Pollen tubes were observed growing in the styles of some CH apical buds (only in 3 styles out of 39), but the tubes did not reach the ovules (Fig. 8). On the



Figs. 3–6. Stigma shape and stigmatic papillae of *Cryptantha capituliflora* flowers. **3.** General view of the stigma of a chasmogamous flower. **4.** Stigmatic papillae of the chasmogamous flower. **5.** General view of the stigma of a cleistogamous flower. **6.** Stigmatic papillae of the cleistogamous flower. Abbreviations: (a) anther. **3, 5** Bars = 50 µm. **4, 6** Bars = 10 µm

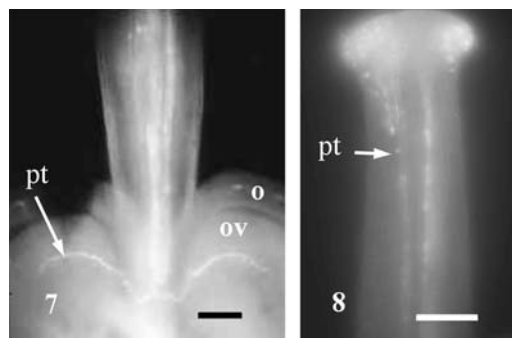
contrary, pollen tubes reached the ovules in all the CL flowers analyzed (Fig. 7).

Fruit and seed production. 97% of the CL flowers set fruit compared to 82% of the CH flowers. Mean seed number did not differ between fruits from CH and CL flowers (1.43 ± 0.77 and 1.38 ± 0.58 for CH and CL flowers, respectively; $U = -0.834$, $P = 0.403$). Consequently, the mean seed/ovule ratio between flower types was very similar (0.69 and 0.71 for fruits from CL and CH flowers respectively). In all the aborted seeds of fruits from both flower types it was possible to observe an embryo at an early developmental

stage, rather than an unfertilized ovule. Nevertheless, further histological studies are necessary to confirm this finding.

Discussion

Cleistogamous species with a clear distinction between floral types is the rule (Plitmann 1995), but slight morphological differences between CL and CH flower types have also been described for some species (Lord 1981, Bernhardt 1983). Although floral forms intermediate between CL and CH do occur in *C. capituliflora*, many floral traits allow CL



Figs. 7–8. Pollen tubes growing in the styles of *Cryptantha capituliflora*. **7.** Partial view of a cleistogamous flower bud showing pollen tubes reaching the ovules. Bar = 50 μ m. **8.** Partial view of the style of a chasmogamous flower bud. Bar = 100 μ m. Abbreviations: (o) ovary, (ov) ovule, (pt) pollen tube

flower identification. Among these traits, the reduced anther number was the most distinctive character.

Morphological differences between CL and CH flowers regarding the stigmatic papillae are of special interest when functional implications in the post-pollination process are considered (Lord 1984, Lord and Eckard 1986). A specific morphological structure of the papillae has been interpreted as an adaptation to facilitate pollen deposition and hydration (Heslop-Harrison 1981, Bigazzi and Selvi 2000). Then, differences between CL and CH flowers in the morphology of the papillae in *C. capituliflora* may reflect different mechanical implications related to pollen-stigma association and/or solar protection of the pollen grains. Regarding pollen protection, the grains on the stigmas of CH flowers will be exposed to solar radiation and consequently would run a higher risk of desiccation and UV damage (Torabinejad et al. 1998, Feng et al. 2000). Capless stigmatic papillae of CL flowers are incapable of protecting pollen grains, whereas CH stigmatic papillae may act as ‘parasols’, as has been suggested for other boraginaceous species (Heslop-Harrison 1981, Bigazzi and Selvi 2000). However, pollen grains deposited on stigmas of CL flowers are primarily protected by the closed corolla and secondarily by the axial

leaf, which covers the entire inflorescence in most cases.

Regarding the structure of the nectariferous disc, according to the classification proposed by Fahn (1979) nectaries from *C. capituliflora* belong to the nonstructural type. A tissue without differentiated cells characterizes this type of nectary. The shape of the nectary in CH flowers resembles those observed in many other boraginaceous species (Fahn 1979, Smets 1986, Galetto 1993). Because CL flowers are independent of pollinator activity for pollen delivery and deposition, a reduction of the nectary is expected. A conspicuous nectary could not be identified in CL flowers, but the presence of stomata on the ovary base suggests the possibility of a non-structured nectary. Nevertheless, its functional activity remains uncertain. In summary, *C. capituliflora* show several traits that characterize autogamous species, such as the generally small size of the flowers, the inconspicuous nectaries, and the short anther-stigma separation (Ornduff 1969, Porrás and Álvarez 1999).

The growth of self-pollen tubes in CH flowers buds indicates the occurrence of prior selfing in *C. capituliflora*. Although observed in some few CH buds, this fact jointly with floral characters makes selfing the most likely way of reproduction for CH flowers in this species. Prior selfing has been observed in other chasmogamous flowers with a short anther-stigma separation (Porrás and Álvarez 1999), and also in other *Cryptantha* species from Argentina with CH flowers only (A. Calviño, pers. obs.). In terms of the reproductive assurance hypothesis, self-pollination of CH flowers is expected as a strategy for increasing seed set when the chances of insect-mediated pollination are reduced (Lord 1984, Schoen and Brown 1991, Jarne and Charlesworth 1993, Porrás and Álvarez 1999, Culley 2000, Herlihy and Eckert 2002). Thus, the occurrence of self-fertilization appears related to poor and unpredictable pollination environments such as alpine and subalpine regions (Schoen and Brown 1991, Escaravage et al.

1997, Ruiz de Clavijo 1997, Culley 2000). Andean communities where *C. capituliflora* grows are covered by snow from 3 to 5 months during winter and support dry conditions during the summer (Arroyo et al. 1981). A strong decrease in mean flowers visitation rates above 3100 m has been documented for high Andean communities (Arroyo et al. 1985). With this background, a scarcity of pollinators and/or reduced visitation rates can be expected for *C. capituliflora* populations and consequently a reduction of the chances of insect-mediated pollination in CH flowers. When cross pollination is very unlikely, a circumstantial increase in self-pollination may occur (Lloyd 1992), more even when floral traits favour the occurrence of self-pollination. As was suggested for other species, it is highly probable that CH flowers of *C. capituliflora* that do not receive outcross pollen would self-pollinate with a resulting additional increment in fruit set (i.e. Schoen and Brown 1991, Lloyd 1992). Thus, fruit set in CH *C. capituliflora* flowers may largely result from this way of autonomous selfing together with prior selfing, and only a few flowers (i.e. those with slightly longer styles than anthers) would rely on pollination services (i.e. outcrossing) for fruit production. If this is the case, the relatively high fruit set of CH flowers may be due mainly to self-pollination mechanisms (Lloyd 1987). A high CH fruit set has also been observed in other cleistogamous species, where the production of CL flowers was negatively affected by a high CH fruit production (Redbo-Torstensson and Berg 1995). This fact agrees with a recent theoretical model, that explains the occurrence of CL flowers relative to CH reproductive success and predicts lower CL flower production when fertility rates of CH flowers are high (Masuda et al. 2001). The present study only focusses on the variability of the flower dimorphism at the flower level, but the low production of CL flowers in *C. capituliflora* may be due in part to the high fruit-set of CH flowers. To evaluate this apparent trend in a more adjusted way, seasonal as well as spatial variation in the

CL/CH flower production should be measured.

Fruits from CL and CH flowers showed similar seed abortion rates. Because low levels of cross-pollination are expected for CH flowers of *C. capituliflora*, the similar abortion rates may be due mainly to high homozygosity in the embryos. It has been suggested that autogamy and even cleistogamy are favoured when selfing become established as a successful way of reproduction (Richards 1997). In this context, autogamy may be the first step for the acquisition of cleistogamy. Nevertheless, it is less obvious to interpret how environmental factors could have enhanced the within-flower autogamy in structurally modified flowers. The production of CL flowers appears associated with a lower energetic cost due to the small size of these flowers, and to constraints related to resource shortage. These mechanisms were proposed to explain the presence and location of CL flowers within the plant (Ellstrand et al. 1984, Waller 1988). Differences in the position of CL and CH flowers within the same plant are expected when different parts of the plant experience different levels of pollination, predation, etc. (Schoen and Lloyd 1984, Waller 1988). Under some circumstances, this fact may determine that those flowers without any opportunities for outcrossing become CL, a common pattern in cleistogamous species with amphicarpny (Schoen and Lloyd 1984, Lloyd and Schoen 1992). Since CL flowers in *C. capituliflora* could only be observed in axial inflorescences, which are completely covered by the leaf, flowers from these inflorescences do not have any chances of insect-mediated pollination. Then, if the opportunities for outcrossing are not equal within the same plant, different reproductive strategies (such CL and CH flowers) may reflect the effect of such dissimilar opportunities. Other species of section *Cryptantha* also showed the same trend, with CL flowers located in the lower half of the stems (Grau 1983). Amphicarpic *Cryptantha* species of section *Geocarya* with CL flowers at the soil level also reinforces the idea that the production of CL flowers in the

genus tends to be concentrated to the base of the stems.

In summary, CL flowers of *C. capituliflora* can be identified primarily by their lower anther number and by their location within the plant. Selfing can be achieved through CL and CH flowers in different ways, but this species also let some chances of outcrossing displaying CH flowers. The presence of cleistogamy in *C. capituliflora* can be viewed as an economic way to ensure fruit and seed production. Considering that cross-pollination is very unlikely to occur in natural populations, this species seems to respond by maintaining different strategies that ensure fruit production.

The authors thank C. Carrizo, A. Sársic and C. Torres for their technical assistance, C. Marticorena for the information submitted about collections of this species in Chile, and two anonymous reviewers for useful suggestions on early version of this manuscript. Ana Calviño thanks the fellowship to the Agencia Córdoba Ciencia S.E. (ACC). Our research was supported by grants from Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), ACC, Agencia Nacional de Promoción Científica y Técnica y Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba. L. Galetto is supported by CONICET.

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