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The breeding system of *Lycium cestroides*: a Solanaceae with ovarian self-incompatibility

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Abstract To determine the breeding system of *Lycium cestroides*, several treatments were performed: self-, cross-, and geitonogamous pollinations, autonomous self-pollination, and a control (flowers exposed to natural pollinators). Production and quality of fruits and seeds as well as pollen tube growth were evaluated for each treatment. Experimental pollinations indicate that *L. cestroides* is self-incompatible since fruits were obtained only under cross-, and open-pollination treatments. However, in self- and geitonogamous hand pollinations, as well as in autonomous self-pollination, pollen tubes developed successfully and reached the ovules. The speed of pollen tube growth did not differ significantly among the different hand-pollinated flowers (cross-, self-, and geitonogamous). These facts indicate the presence of an ovarian self-incompatibility system. Significant differences were observed in fruit set, fruit size, and seed number per fruit between cross-pollinated flowers and open pollination (control). These results could be explained in terms of quantity and quality of pollination in each case.

Keywords Solanaceae · *Lycium cestroides* · Breeding system · Pollen tube growth · Ovarian self-incompatibility · Fruit quality · Seed set

Introduction

The term “ovarian self-incompatibility” (OSI) is used to refer to the situation where self-pollen tubes grow successfully in the style to the ovules, but flowers fail to produce fruits and seeds (e.g., Seavey and Bawa 1986; Sage et al. 1994; de Nettancourt 2001). Attempts to clarify the nature of OSI have had limited success and it seems that there may be several mechanisms involved

(cf. Seavey and Bawa 1986; Gibbs and Bianchi 1993, 1999; Sage et al. 1999). In the literature, it is also mentioned as late-acting self-incompatibility (e.g., Seavey and Bawa 1986). However, we prefer the term OSI which is topological and makes no implications about the causes of such rejections (Sage et al. 1994; de Nettancourt 2001).

The importance and prevalence of OSI in flowering plants has been underestimated (de Nettancourt 2001) and considered to be rare in angiosperms. However, studies in the last two decades indicate that it is more widespread than previously thought, operating in many woody and herbaceous species (e.g., Bawa et al. 1985; Seavey and Bawa 1986; Gibbs 1988; Oliveira et al. 1992; Gibbs and Bianchi 1999; de Nettancourt 2001). Seavey and Bawa (1986) reviewed about 20 species with this phenomenon, and since then some 64 species have been added (cf. Gibbs and Bianchi 1999). According to Gibbs and Bianchi (1999), species with OSI seem to cluster in certain families or family alliances rather than being randomly distributed in angiosperm taxa, although there are isolated cases as well.

No species of the family Solanaceae has been reported with certainty to have OSI, nor has there been one in the order Solanales in the ordinal classification proposed by APG (1998). Studies on the reproductive biology of *Lycium cestroides*, a common shrub of the Argentine Chaco, indicate that it has OSI. There have been several studies on this species related to its reproductive biology (Bernardello 1987; Bernardello and Luján 1997; Galetto and Bernardello 1995; Galetto et al. 1998). However, there are no data on its breeding system and on fruit/seed set and their quality under different pollination treatments. In this article, we characterized the floral biology of *L. cestroides* and determined its breeding system. We compared some fruit and seed traits and measured pollen tube growth under different pollination treatments. The data obtained are significant in the context of the reproductive biology of the species.

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Materials and methods

The species

Lycium cestroides Schtdl. (Lyctaeae, Solanoideae) is a shrub growing mainly in the Chaco region from the South of Bolivia to Uruguay (Bernardello 1986). Flowers have dark violet tubular corollas around 16 mm long. The androecium consists of five included stamens with filaments of different length adnated to the corolla tube, and the gynoecium has a pistil with an included style.

Methods

Experiments were carried out in October-December 1997 at two sites in Córdoba Province, Argentina. All measurements were taken at each site on a total of 18 individuals. Flower lifetime was characterized and subdivided into four stages of about 24 h each (Fig. 1). Stigmatic receptivity ($n=40$ flowers of each stage) was detected with benzidine and hydrogen peroxide (Galen et al. 1985). Pollen grains and ovules were counted in mature buds ($n=40$) and the pollen/ovule ratio (P/O) was calculated. Pollen viability ($n=48$ flowers per stage) was tested with fluorescein diacetate (Heslop-Harrison and Heslop-Harrison 1970).

Mature buds were bagged with bridal net and flowers (see Table 1 for sample sizes) were submitted to the following treatments to determine the breeding system: (a) self-pollination: hand-pollinated flowers with their own pollen; (b) geitonogamous polli-

nation: emasculated flowers hand-pollinated with pollen from flowers of the same ramet; (c) cross-pollination: emasculated flowers hand-pollinated with pollen from flowers from different genets located more than 100 m distant; (d) autonomous self-pollination: paper-bagged buds without any treatment; (e) control (open pollination): tagged buds exposed to natural pollinators. In hand-pollinated flowers (a, b, and c), the pollen load placed on each stigma was taken from three or four whole anthers from stage 2 flowers. Fruit set, size and mass, as well as seed germinability, seed number and mass per fruit were measured for each treatment.

Additional flower sets were subjected to identical hand-pollination treatments (a-c) to monitor pollen tube growth. Flowers were collected and fixed in FAA (formaldehyde, 5% glacial acetic acid, 70% ethanol) at 8, 24, 36, and 48 h after hand-pollination treatments ($n=50$ per fixation time). Pistils were softened and gently squashed on a slide in a droplet of leuco-aniline blue (Martin 1959). Pollen tubes were observed with fluorescence microscopy. We also observed pollen tube growth in 50 flowers subjected to autonomous self-pollination and 50 open pollinated flowers. In both cases, flowers were collected and fixed at stage 4.

Statistical Analyses

One- and two-way analyses of variance (ANOVA) were combined with Tukey tests for multiple comparisons at $P < 0.05$. Data were subjected to mathematical transformation to meet normality whenever needed. Mann-Whitney and t tests were used for comparing fruit and seed traits from artificial and open pollination. The statistical program package SPSS (1992) was used for the analyses.

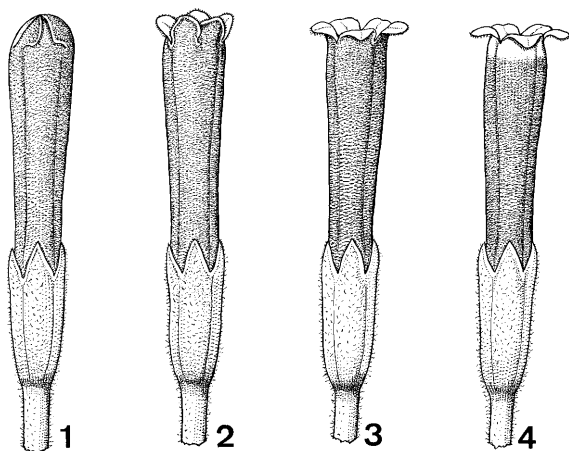


Fig. 1 Flowering stages of *Lycium cestroides*. 1 Mature bud with a dark violet corolla (0 h). 2 Open flower with corolla lobes parallel to the main axis and anthers dehiscent (24 h). 3 Open flower with corolla lobes perpendicular to the main axis; by the end of this stage the corolla turns light violet (48 h). 4 Old light-brown colored flower with the corolla ready to fall (over 72 h). All $\times 3$

Results

Flowers are homogamous and last 72 h (Fig. 1). Flower lifetime is unaffected by pollination. Stigmata were receptive throughout the flower lifetime and anthers dehisced immediately after the buds opened. Each flower had an average of 33.9 ± 3.3 ovules and $402,266 \pm 76,690$ pollen grains; consequently, the P/O ratio was $11,961 \pm 2,651$.

Percentage of pollen viability decreased as flowers aged (Fig. 2). In fact, statistical analyses showed significant differences among the stages ($F=145.1$; $P=0.001$).

Statistical analyses indicate that there were no significant differences in the growth rate among hand-pollination treatments ($F=1.23$; $P=0.51$, Fig. 3). The mean pollen tube growth rate for all treatments was 0.33 ± 0.02 mm h^{-1} .

Experimental pollinations indicate that *L. cestroides* is self-incompatible because no fruits were obtained in

Table 1 Fruit set, seed germinability, and fruit/seed traits of *Lycium cestroides* following hand cross-, and control (open pollination). Values are the means \pm SD

Variables	Pollination Treatments		Statistical results
	Cross-pollination	Open pollination	
Number of flowers treated	90	90	
Number of fruits obtained	78	71	
Fruit set	0.867	0.789	$U=7.54$; $P=0.02$
Seed germinability	0.027	0.025	$U=5.52$; $P=0.855$
Fruit length (mm)	5.54 ± 1.05	3.68 ± 0.71	$t=9.41$; $P=0.001$
Fruit width (mm)	4.92 ± 1.08	3.02 ± 0.95	$t=8.78$; $P=0.002$
Fruit mass (g)	0.05 ± 0.03	0.04 ± 0.02	$t=5.65$; $P=0.375$
Seed mass/fruit (g)	0.03 ± 0.01	0.02 ± 0.01	$t=4.33$; $P=0.233$
Seed number/fruit	13.2 ± 3.16	7.12 ± 2.58	$t=4.28$; $P=0.001$

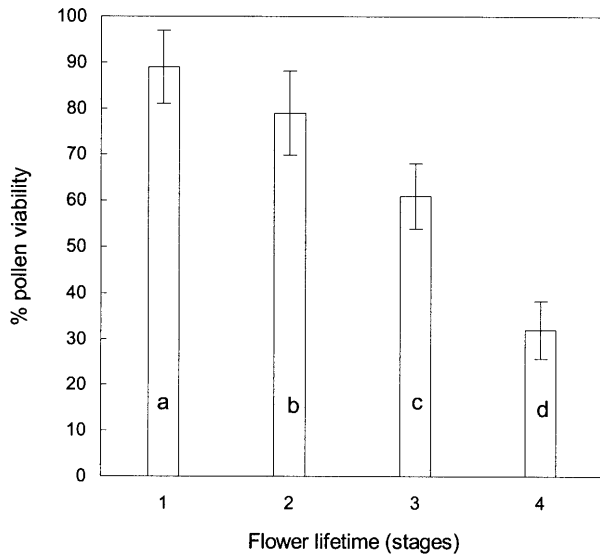


Fig. 2 Pollen viability throughout flower lifetime (stages 1–4) in *Lycium cestroides*. Values are the means±SD. Lower case letters in the bars indicate test results significant at $P < 0.05$

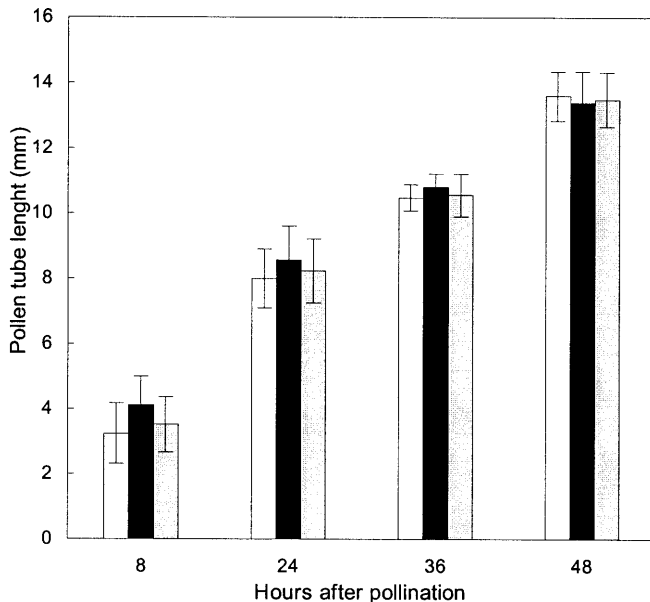


Fig. 3 Pollen tube growth (mm) in *Lycium cestroides* following self- (□), cross- (■), and geitonogamous (▒) hand-pollinations. Values are the means±SD. No significant differences at $P < 0.05$ were found in pollen tube growth among hand-pollinations

self- and geitonogamous hand treatments nor in autonomous self-pollination. Nevertheless, self-pollen tubes developed successfully through the style, ovary, and placenta, reaching the ovules (Fig. 4). In hand-pollination treatments (a–c in Methods), pollen placed on the stigma germinated and produced abundant pollen tubes that entered the transmitting tissue of the style, reaching the ovules through the micropyle around 48 h after pollination (Fig. 4). Pollen tubes were also seen penetrating the ovules in autonomous self-pollination and control

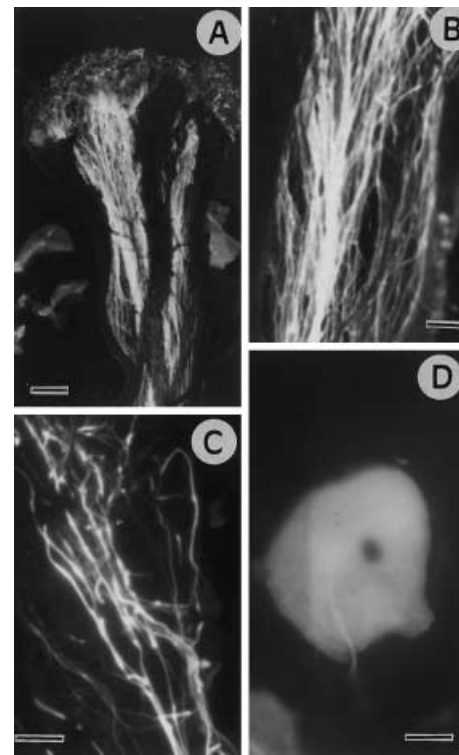


Fig. 4A–D Fluorescence micrographs of pistils of *L. cestroides* 48 h after hand self-pollination. **A** Abundant self-pollen tubes germinate and grow down the upper portion of the pistil. Bar 250 μ m. **B** (Bar 150 μ m) Self-pollen tubes are seen growing along the style, within the ovary (**C**, Bar 100 μ m) and penetrating one ovule (**D**, Bar 100 μ m)

flowers by the end of their lifetime (over 72 h). Although self-pollen tubes were seen penetrating the ovules (Fig. 4), we were unable to determine whether they discharged their nuclei and effected fertilization. In any case, rejection of self-pollen in *L. cestroides* occurs very late within the ovules.

The quality of berries and seeds obtained were compared. Fruit-set, fruit size (width and length), and seed number per fruit were consistently higher in cross-pollinated flowers (Table 1). On the other hand, fruit mass and seed mass per fruit were similar in both treatment and control. Similarly, seed germinability was always low and was not significantly different between treatment and control (Table 1).

The results of two-way ANOVA indicate that there was no interaction between populations and pollination treatments. No significant differences were found between the two populations studied for any of the variables measured.

Discussion

Lycium cestroides was previously thought to be self-compatible because of the presence of homogamy and the relative position of stigma and anthers, with the stigma below the anthers (Bernardello 1983). However,

fruit-set data obtained in the present study demonstrates the xenogamous condition of *L. cestroides*, and this correlates with the high P/O ratio as well (Cruden 1977). In addition, pollen tube observations indicate that it presents an OSI type of control of selfing.

Within *Lycium*, most species are hermaphroditic (Bernardello 1986; 1987) with a few functionally dioecious (Minne et al. 1994). Regarding its compatibility, there are previous reports of self-incompatibility (SI) in two *Lycium* species (East 1940; Richman 2000). Homomorphic gametophytic SI is frequent in the family, e.g. in *Nicotiana*, *Petunia*, *Phyalis*, *Solanum* (cf. de Nettancourt 2001; Clarke and Newbigin 1993). Thus, further studies are needed to detect whether OSI is widespread in Solanaceae and in *Lycium*. The OSI detected in *L. cestroides* could be another autapomorphy since this species is considered derived and is cladistically quite isolated among the species of the American continent (Bernardello and Chiang-Cabrera 1998).

Variability in pollination intensity (spatial and temporal) may have important effects on female reproductive performance affecting the likelihood of fruit production (e.g., Lee and Hartgerink 1986; Stephenson et al. 1986) and the number of seeds per fruit (e.g., McDade 1983; Shore and Barrett 1984). The differences found in seed number between artificial xenogamous treatment and open pollination could be explained in terms of different pollination intensities, as reported by several authors in other species (e.g., Silander and Primack 1978; Snow 1982; Bertin 1990). Although pollen loads in open pollination were not quantified, they were visually lower than loads applied in hand-pollinated flowers (from three or four whole anthers). In addition, we simultaneously applied this high load to each manipulated flower, a condition that pollinators would hardly achieve. Therefore, the positive correlation between the increase in number of pollen grains on the stigma and in seed production per fruit would explain these differences as well as those found in fruit size (i.e. higher number of seeds per fruit resulted in larger fruit sizes). Thus, pollinators seem to be not very effective in pollen deposition and the upper limit of seed production would be set by a pollen/pollinator limitation.

In addition, seed set in SI plants can be reduced significantly if self-pollen is deposited on stigmata before or even at the same time cross-pollen arrives (e.g., Bertin 1988; Sage et al. 1999). Some factors that may contribute to this reduction affecting the success of cross-pollen are: (1) the presence of self-pollen in mixed pollen loads of naturally pollinated flowers can reduce the effective number of cross-pollen grains by interfering physically in the stigma (see Shore and Barrett 1984; Bertin 1986, 1988); (2) self-tubes can also compete for stylar substances required for pollen tube growth (e.g., Labarca and Loewus 1973); and (3) loss of female fertility due to self-pollen tubes rendering some ovules non functional (Barrett et al. 1996). While present data do not allow a clear separation of these possibilities, partial evidence suggests that at least some interference occurs in the

micropyle: self-pollen tubes not only grow at the same rate as cross-pollen tubes but are capable of reaching and penetrating the ovules. Consequently, one or more of these factors have probably also induced the lower fruit- and seed-set found in open pollinated flowers.

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