



Breeding system of *Bulnesia retama* (Gillies ex Hook & Arn.) Gris. (Zygophyllaceae) in the Central Monte Desert (Mendoza, Argentina)

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Reproductive and phenological patterns of *Bulnesia retama* were analysed in the Ñacuñán Biosphere Reserve. Flowering spanned approximately 4 weeks, while mature fruits dispersed in 75 days. Six flower stages were determined, protogyny occurred in early stages, receptive stigmas and nectar production were detected during stages 1–4. Pollinator and flower predator behaviors are described. Mating treatments namely autogamy, geitonogamy, cross-pollination, and an open pollinated control showed statistical differences in seed and fruit production. Seed viability was high in all four treatments. Although *B. retama* has been considered self-compatible, few seeds were produced by selfing compared with cross-pollination, suggesting inbreeding depression.

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Introduction

The genus *Bulnesia* Gay (Zygophyllaceae) comprises nine species that grow exclusively in South America. Porter (1974) has proposed a tropical origin for this genus. Palacios & Hunziker (1984) considered two subgenera, and suggested an early divergent evolution among them based on evidences from flower, fruit, and seed characters. The subgenus *Bulnesia* has the southernmost geographic distribution and two species, *Bulnesia chilensis* Gay and *B. retama* (Gillies ex Hook & Arn) Gris., highly adapted to dry environments and considered evolutionarily the most advanced (Crisci *et al.*, 1979; Hunziker, 1980; Palacios & Hunziker, 1984). *Bulnesia retama* (called

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'retamo' in Argentina) has a discontinuous geographic distribution, ranging from Ica to Nazca in Perú, and from Catamarca to La Pampa in Argentina (Palacios & Hunziker, 1984). This species constitutes a characteristic woody perennial of the Monte Desert in Argentina (Morello, 1958) occupying approximately 6,400,000 ha (Tinto & Pardo, 1956) in the driest areas, typically with less than 150 mm of annual rainfall (Hunziker, 1980; Palacios & Hunziker, 1984). Continuous wood extraction for vineyard posts as well as intensive pruning for wax production has greatly diminished natural populations (Dalmasso & Llera, 1996). Although this species is an important resource from both ecological and economic points of view, there are no detailed data about its phenological and reproductive patterns.

In a comparative analysis of the breeding system of dominant perennial plants from the Sonoran and Monte deserts, *B. retama* was considered as a self-compatible species (Simpson, 1972, 1977; Neff *et al.*, 1977). In an experiment carried out in the Monte Desert (Andalgalá, Argentina), Simpson (1972) found that *B. retama* can produce seeds in the absence of pollinators, but her conclusions were based on only four bagged flowers that produced fruits and at least one seed per fruit.

Although this species has been always considered highly self-compatible (Simpson, 1972, 1977), more detailed studies are necessary to understand its reproductive behavior. It is important to point out that the mating system of a plant species is not a static property of an individual, population or species, but a dynamic one subject to modification by many forces operating on both ecological and evolutionary time-scales (Barret & Eckert, 1990).

This work is part of a broad study focused on the reproduction of the woody perennial species of the Zygophyllaceae family in the Monte Desert. The main objective was to study the phenological patterns, flower development, and mating system of a *B. retama* population in the Ñacuñán Biosphere Reserve. Other goals were to compare the behavior of flower visitors and pre-dispersal predators, and to discuss its possible role in *B. retama* reproductive output.

Methods

Species studied

Bulnesia retama is a perennial shrub 1.5–3 m high, although in some cases plants can reach 6 m. The slender stems are covered by wax and the leaves are opposite, even-pinnately compound with reduced and short-lived leaflets. Photosynthesis is carried out mainly by young twigs. Flowers are solitary or arranged in small groups of 2–4, pentamerous, slightly zygomorphic and dish-shaped with a bright yellow corolla. Ten stamens are arranged in two cycles, each stamen has a basal scale fused to the filament that constitutes, as a whole, a nectar receptacle. The gynoecium is pentacarpelar, with 10–12 ovules per carpel. The ovary is fusiform with a terminal style and a single stigma. Schizocarpic fruits separate at maturity in five winged mericarps, with only one seed each. Simpson (1972) and Palacios & Hunziker (1984) have mentioned the production of parthenocarpic fruits in this species.

Study site

Fieldwork was carried out in the Ñacuñán Biosphere Reserve, located in the mid-east of the Province of Mendoza, Argentina (34°02'S, 67°58'W). Regionally, this Reserve is situated in the center of the Monte Desert and has been excluded from grazing and logging during more than 30 years. The climate is arid, with a mean annual rainfall of 329.4 mm (1972–1992 periods), showing a high inter-annual variability. These

rainfall events are concentrated mainly in spring (24% in September–December) and summer seasons (48% in January–March; Estrella, 1995). Mean monthly temperature of the coldest month (July) is 6.9°C and 22.4°C in the warmest month (January).

The two main plant communities are the ‘algarrobal’, an open forest of *Prosopis flexuosa* D.C. (‘algarrobo’) with a dense shrub layer dominated by *Larrea divaricata* Cav. (‘jarilla’); and the ‘jarillal’, a dense shrubland community dominated by *L. cuneifolia* Cav. (‘jarilla’). *Bulnesia retama* populations (‘retamal’) grow in flat areas with superficial sand deposition within the *Prosopis* forest community (Roig, 1971, 1995).

Phenological patterns

The phenological events were registered for ten randomly chosen *B. retama* plants of similar growth during late October of 1996 and mid-February of 1997. We registered the presence and development of the following structures: (a) new twigs, (b) floral buds, (c) flowers, (d) young fruits, and (e) fruit dispersion. Plants were considered at the beginning of each phenological event at the first appearance of the respective structure. The intensity of the phenological events was estimated considering the proportion of twigs, floral buds, flowers, and fruits observable in the canopy of each individual plant. For example, a *B. retama* shrub showing one-third of their crown covered by flowers was considered as having a flowering intensity of 30–40%. The registered percentage of each phenological event was assigned a numerical value according to the following scale: 1 (1–20%), 2 (21–40%), 3 (41–60%), 4 (61–80%), and 5 (81–100%). Mean and S.D. of each phenological event were calculated at each date.

The sequential flower development was followed in marked flowers and different floral stages were established. During each stage, the stigma was tested for receptivity using the peroxidase enzyme test, which consists in applying hydrogen peroxide to stigmas and considering the presence of bubbling as an indication of peroxidase activity (Kearns & Inouye, 1993). In addition, we measured the volume of nectar produced in each floral stage. For this purpose, we selected several branches in four plants with flowers in different degrees of development. These branches were excluded from insect visitors with a mesh gauze during 24 h. After this time, at least five flowers of each floral stage were selected to measure their nectar volume using 1 µl microcapillaries.

Assessment of the mating system

While flower buds were growing, four branches with similar positions and orientations were selected in each of eight individuals of *B. retama*. One of these branches was kept unaltered in order to assess the pollination in the presence of natural agents. The other three branches were bagged and subjected to the following treatments:

Autogamy: Unopened flowers were counted before bagging with a mesh gauze, and were kept unmanipulated in order to determine the seed set due to spontaneous self-pollination.

Geitonogamy: A set of flowers was repeatedly hand-pollinated with pollen from flowers of the same plant, and bagged again until fruit dispersion.

Cross-pollination: A set of flowers was repeatedly hand-pollinated with a mixture of pollen from flowers of at least ten surrounding plants, and also bagged until fruit dispersion.

The pollen used in hand-pollination treatments was tested for viability with Muntzig stain (glycerin 1:acetic carmine 1), considering as viable those pollen grains that were well stained. On average, we found a viability of 99.5% for all the plants we used.

In order to assess the degree of compatibility of *B. retama*, we calculate the self-compatibility index (Lloyd & Schoen, 1992), the average of fruits or seeds produced in geitonogamy divided by the seed set of cross-pollination treatments. We also estimate the auto-fertility index (Lloyd & Schoen, 1992), the seed set of autogamy treatment divided by the seed set of artificial cross-pollination treatment. Inbreeding depression was calculated as the maternal fecundity component of inbreeding depression ($IBD = 1 - [\text{seed set in geitonogamy} / \text{seed set in outcrossed flowers}]$, Holsinger, 1988). In addition, the relationship between cross-pollination and open-pollination gave us a picture of the degree of pollen limitation in this plant species (Burd, 1994).

The seeds obtained were subjected to the Tetrazolium test (Grabe, 1970) to determine if the different treatments had some effect on their viability. In addition, we registered the behavior and visit frequency of the principal flower visitors, although their low frequencies did not allow us to make a detailed statistical analysis.

Statistical analysis

Our main interest was to analyse the number of flowers yielding fruits and seeds in each treatment. As data of this kind come as proportions (i.e. number of fruits or seeds in relation to the number of flowers pollinated), we analyse them by means of logistic models (Crawley, 1993). When the residual errors in these models showed overdispersion (i.e. the variance of the residuals was significantly higher than that predicted by the binomial distribution), the data were rescaled to correct for biases in the statistical test of hypotheses (Crawley, 1993). In the case of continuous variables such as the nectar production, we used ANOVA in order to assess differences between different floral stages. In all cases, the analyses were made with the generalized linear interactive modeling (GLIM) statistical package, version 3.77 (NAG, 1986).

Results

Phenology

Phenological events were registered during a 4-month period. The observations began on October 25 of 1996, when the first flowers opened, and lasted until February 15 of 1997 when the majority of the fruits had been dispersed. Vegetative growth, evaluated as the production of new twigs and leaves, was observed in all individuals during the entire period. However, the vegetative development intensity was different among shrubs, ranging from 10% to 30%.

Flowering spanned a short period of 4–5 weeks, and its intensity was always below 50% (Fig. 1). In contrast, fruit growth and seed maturation were more prolonged, and the fruit dispersal event reached a peak 10 weeks after the appearance of the first fruits (Fig. 1).

The sequential development of *B. retama* flowers has been characterized as follows:

Stage 0: The flower bud is still unopened, sepals begin to separate but the internal whorls are not visible (Figs 2(a)–(c)).

Stage 1: Sepals continue their opening and the style–stigma exert more than 2 mm above the apex of the flower (protogyny, Figs 2(d)–(e)). The stigma is already receptive and shows a positive reaction to the peroxidase test.

Stage 2: Sepals open, ten large, yellow, and indehiscent anthers are revealed, and stand near the base of the style (Fig. 2(f)). The stigma remains receptive.

Stage 3: Sepals display a gradual opening until the rest of the cycles become evident. Petals are smaller than sepals and therefore not apparent at the beginning of this stage. The filaments of the outer whorl stamens elongate before those of the inner whorl and their anthers dehisce first near the receptive stigma (Fig. 2(g)).

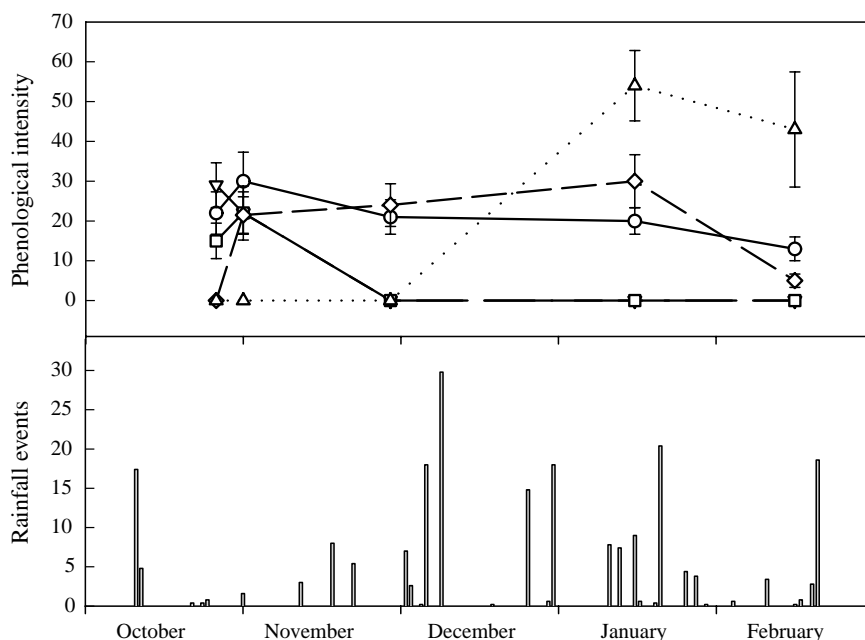


Figure 1. Development of phenological events during reproductive cycle of 1996–97 in the Nacuñán Biosphere Reserve. Bottom graph shows rainfall events occurred during the same period: New twigs (—○—), buds (—▽—), flowers (—□—), fruits (—◇—), fruit dispersal (··Δ··).

Stage 4: All the ten stamens become erect with their anthers dehiscent and making contact with the still-receptive stigma. The yellowish petals enlarge and extend completely (Fig. 2(h)).

Stage 5: Sepals and petals lose turgor and become darker. The filaments of the stamens lie attached to the grooves of the ovary, which rapidly begins to grow. The stigma is no longer receptive.

Mating system

We found highly significant differences between treatments in fruit ($\chi^2 = 43.6$; df.=3; $p < 0.00001$) and seed production ($\chi^2 = 151.9$; df. = 3; $p < 0.00001$). When the flowers were bagged without further manipulation (autogamy treatment), they yielded the lowest number of fruits and seeds. Persistent hand-pollination with pollen of the same plant (geitonogamy treatment) showed a small increment over the autogamy treatment in fruit number that was marginally significant ($p < 0.05$; Table 1). However, no significant increase was observed in seed production. When we manually cross-pollinated the flowers, we observed a remarkable increase in fertilization. This increment in fruits and seeds in the cross-pollination treatment was highly significant when compared to the rest of the treatments ($p < 0.0001$ in all cases, Table 1). This improvement in fecundity due to cross-pollination yielded a low value for the self-compatibility index = 0.086, lower than the threshold value proposed by many authors (e.g. Ruiz-Zapata & Kalin-Arroyo, 1978; Lloyd & Schoen, 1992) for a self-incompatible mating system. In addition, the auto-fertility index was also very low (0.007). The estimated maternal fecundity component of inbreeding depression was 0.91, indicating a seed set limitation due to failure of self-fertilization.

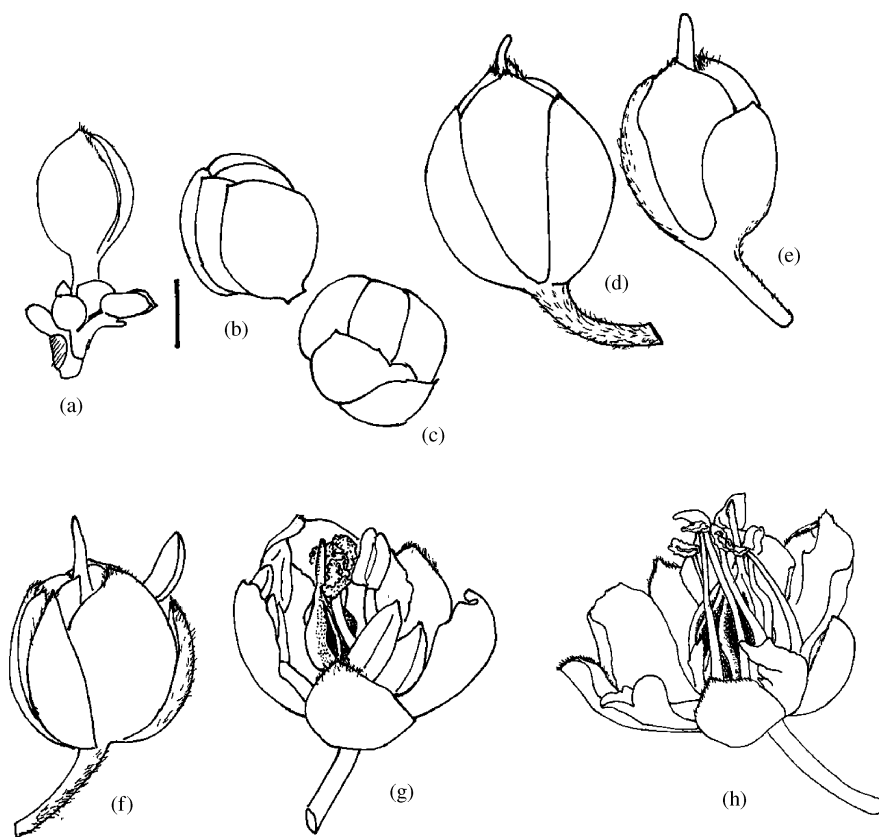


Figure 2. Phenological stages of *B. retama* flowers. Bars = 2.5 mm.

Flowers exposed to natural pollination produced fruits and seeds at intermediate levels between the self- and cross-pollination treatments, showing differences only with the latter ($p < 0.001$ for both mericarps and seeds; Table 1). This low fruit and seed output in naturally pollinated flowers is a consequence of a depressed fauna of flower visitors. We registered only four species of Apoidea visiting *B. retama* flowers during the 1996–97 period: *Caupolicana* sp. (mainly early males looking for mates and nectar) and *Colletes* sp. (Colletidae), *Dialictus* sp. (Halictidae), and *Apis mellifera* (Apidae). We also recorded an undetermined long-tongued bee fly (Bombyliidae) visiting the flowers.

Table 1. *Bulnesia retama*, controlled and open pollination treatments

	No. of flowers	No. of fruits	No. of seeds	Mericarps/ flower*	Seeds/ flower*
Open pollination	302	29	19	0.0838 (± 0.0176)a	0.0107 (± 0.00033)a
Autogamy	370	17	5	0.0498 (± 0.0036)a, b	0.0023 (± 0.00001)a
Geitonogamy	203	43	33	0.2176 (± 0.0407)a, c	0.0442 (± 0.00515)a
Cross-pollination	199	113	373	0.5825 (± 0.0414)d	0.3991 (± 0.04839)b

Different letters show significant differences between treatments (t -tests, $p < 0.001$).

*Proportion calculated between the total number of flower mericarps (no. of flowers \times 5) and the number of mericarps or seeds produced.

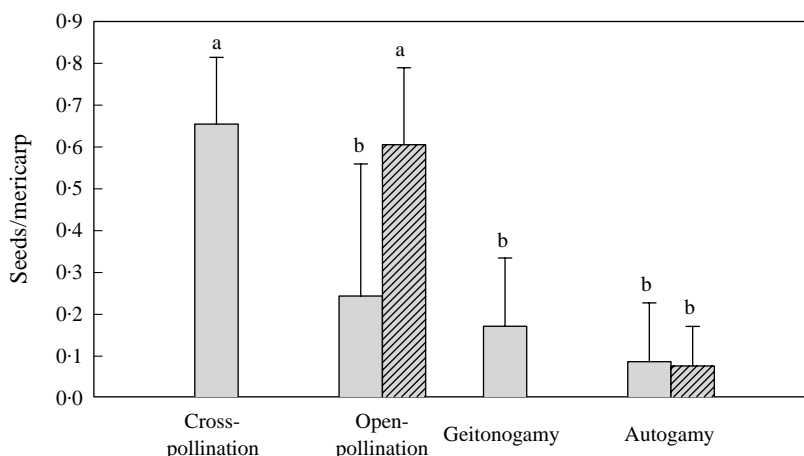


Figure 3. Proportion of seeds per fruit produced by *B. retama* under different pollination treatments. (□) correspond to 1996–97 data and (▨) to 1995–96 (mean \pm S.D.). Different letters (a, b) indicate significant differences ($p < 0.05$).

When we compared the seeds yielded by mericarps, we also found highly significant differences between treatments ($\chi^2 = 72.86$; df. = 5; $p < 0.00001$). We also included in the analysis data obtained in a previous survey in the same study area during the 1995–96 flower period for the open pollination and autogamy treatments. Seed set per fruit yielded by naturally pollinated flowers during the 1995–96 season was significantly higher than the same treatment in 1996–97 ($p < 0.01$), and similar to the cross-pollination results of 1996–97 ($p = 0.53$; Fig. 3). Most probably, the lack of pollinators observed in 1996–97 may explain the low seed set produced per fruit during that season. In contrast, the autogamy treatments did not show differences in the number of seeds per fruit between both flowering periods, indicating that the degree of self-compatibility did not vary (Fig. 3).

Although we found important differences in seed production, the viabilities of the seeds produced by all treatments were not statistically different (G test, $p = 0.31$). The average viability was 88%, ranging from 63% to 100%.

Nectar volumes showed an increase from stage 1 to stage 4 (Fig. 4), although nectar production among different floral stages was not statistically significant ($F = 2.20$; df. = 4.26; $p = 0.05$). The decrease of nectar at stage 5 might be attributed to a cessation in nectar production, and it is possible that the registered volume is a remnant of the nectar produced at stage 4.

Discussion

In the Ñacuñán Biosphere Reserve, all the individuals of *B. retama* produced new twigs and leaves during the entire observation period. A similar phenological pattern was also observed in three closely related species of *Larrea* in Nacuñán and in the piedmont near Mendoza City (Rossi *et al.*, 1999).

Regarding flowering patterns, Solbrig & Yang (1977) categorized *B. retama* as a 'mixed' bloomer, a species that is capable of blooming heavily with the onset of summer rains but also occasionally during the dry season if there are off-season rains. In our study site, flowering of *B. retama* began in late October, one month after the first spring rains (late September). The reproductive growth in *B. retama* could be a response to rainfall events, a common pattern observed in *Larrea* species

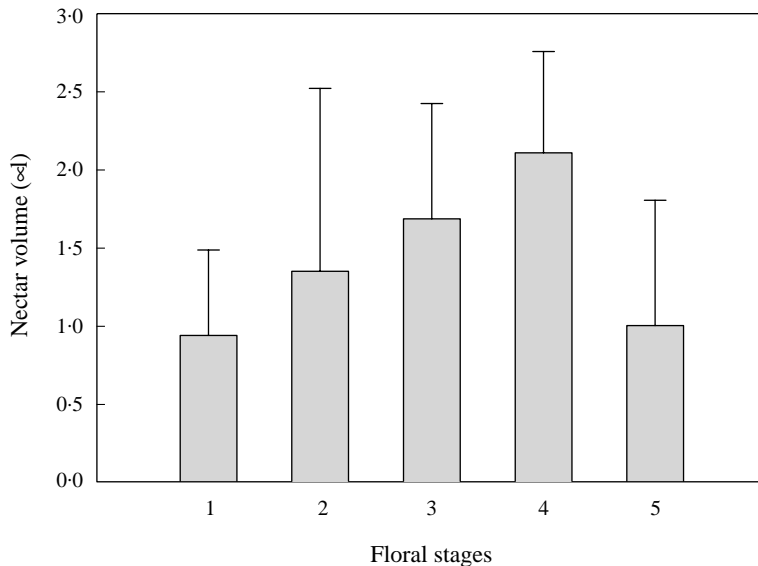


Figure 4. Nectar production during the development of *B. retama* flowers. The volume did not show significant differences between flower stages (mean \pm S.D.).

(Barbour *et al.*, 1977; Solbrig & Yang, 1977; Ackerman *et al.*, 1980; Kemp, 1983; Bowers & Dimmit, 1984; Rossi *et al.*, 1999). *Larrea cuneifolia*, which co-exists with *B. retama* in the Nacuñán Biosphere Reserve, showed a faster response to rainfall events, flowering 15–20 days earlier than *B. retama* (Rossi *et al.*, 1999). During the study season, there were several summer rainfall events in Nacuñán (i.e. December, January, and February; Fig. 1) that could have been favorable for further flowering. Nevertheless, we did not observe any subsequent bloom in *B. retama* nor in *L. cuneifolia*, a well-known opportunistic bloomer (Rossi *et al.*, 1999).

The basic pattern and development of *Bulnesia* flowers is very similar to that in *L. divaricata* and *L. nitida* (Rossi *et al.*, 1999). The main difference was observed at stage 3, where petals do not rotate 90° and, therefore, stamens of the inner cycle are not protected by them. The flower-opening sequence of *B. retama* favors cross-pollination during stages 1 and 2, when the receptive stigma appears at the top of the flower bud, and there is nectar production. In these two stages, autonomous selfing is not possible because the anthers are still indehiscent. The stigma remains receptive during the two subsequent stages and, based on morphological observations, during these stages the stigma surface could receive pollen from its own dehiscent anthers or from other pollen sources. A temporal separation between female and male phases was observed. During the first two stages, the receptive stigmas have the opportunity to receive pollen from other flowers. At stages 3 and 4, when the anthers release pollen, facilitated selfing as well as cross-pollination may occur due to pollinator activity, although autonomous self-pollination could also be possible (Lloyd & Schoen, 1992). Regarding this particular dichogamy mechanism, *Bulnesia* and *Larrea* species can be considered as incompletely protogynous (Lloyd & Schoen, 1992; Rossi *et al.*, 1999). It is important to point out that similar morphological and phenological features of flowers in the related species of *Bulnesia* and *Larrea* might indicate phylogenetic constraints in the development and function of flower structures.

Autonomous selfing is rare in *B. retama*. The low fruit and seed set obtained in the autogamy treatment suggests either that pollen transfer within the same flower is very unlikely, as the auto-fertility index showed, or that pollen grains landing on the

stigmas of the same flower do not prosper. It is also possible to attribute these results to an experimental error due to accidental pollen transfer between flowers of the same bag. However, we found similar results for the autogamy treatment during two consecutive seasons. Geitonogamy is the most distinct of the chasmogamous modes of selfing, having both the ecological properties of cross-fertilization and the genetic properties of self-fertilization (Lloyd & Schoen, 1992). In Ñacuñán, *B. retama* set few fruits and seeds after artificial self-pollination with flowers of the same plant, compared with the intra-population cross-pollination treatment. According to the low value of the self-compatible index, this species can be considered as self-incompatible (Ruiz-Zapata & Kalin-Arroyo, 1978; Lloyd & Schoen, 1992). Genetic self-incompatibility mechanism operates before fertilization (Richards, 1997), and has not been reported in *Bulnesia*. On the other hand, self-compatible plants usually set seeds especially when they are artificially self-pollinated between flowers of the same plant (Richards, 1997). Based on our results, we can conclude that the population of *B. retama* studied has a weakly self-compatible mating system or facultative inbreeding (Simpson *et al.*, 1977). When we compared our data with the number of fruit and seed produced by autogamy in the *B. retama* population from Andalgalá (Simpson, 1972, 1977), we found differences in the level of compatibility. It has been recognized that self-fertilization, a factor causing non-random mating in populations, can vary with the degree of self-compatibility, the limitation and behavior of pollen vectors, the environmental conditions and flower morphology and phenology (Wilson, 1984).

It is interesting to point out the high value of inbreeding depression, indicating that other post-fertilization mechanisms may play a role in the low seed set produced by selfing (Richards, 1997). Inbreeding depression was high but comparable with the values of another self-compatible woody species (Rathcke & Real, 1993). Although seeds obtained by self-fertilization were viable, we did not further evaluate vigor or other reproductive behavior of the progeny as a measure of inbreeding depression. Cytological studies are needed in *B. retama* to elucidate pre- and post-zygotic mechanism acting after self-fertilization.

We can conclude that the mating system found in a *B. retama* population of Ñacuñán favors cross-pollination among individuals, indicated by flower morphology and phenology, and by the low self-compatibility index that showed similar values to those found in woody perennial species of the Chilean matorral (Arroyo & Uslar, 1993). Mating patterns in plants affect the genetic structure of populations, and the potential for local differentiation of populations in response to selection pressures and random drift (Wilson, 1984). In the studied *B. retama* population, allogamy is favored and we would expect a higher genetic diversity as reported in *Larrea* (Schuster *et al.*, 1994), and also an increase of the out-crossing rate as has been described for other woody perennial species (Barret & Eckert, 1990).

Flowers used to evaluate open-pollination produced numbers of fruits and seeds more similar to the geitonogamy treatment than to the cross-pollination one. The relationship between open-pollination and hand-cross-pollination treatments gives us a picture of pollen limitation in a plant (Sih & Baltus, 1987; Corbet, 1991; Burd, 1994). In our case, the highly significant difference between these treatments indicates clear pollen limitation due to the lack of pollen vectors during the 1996–97 flowering period, a phenomenon not observed during the previous year. Two main processes occurring in the Ñacuñán Biosphere Reserve during 1996 may account for this response: an unusual low frequency of flower visitors, and, at the same time, a high rate of flower predation. Visit frequency rates by pollinators were atypically low, taking into account the high level of rewards offered by *B. retama* flowers. Neff *et al.* (1977) and Simpson (1977) registered a rich insect fauna visiting the flowers of *B. retama* near Andalgalá, Catamarca, and our observations in previous years can also confirm a high diversity of potential pollinators in the Ñacuñán Biosphere Reserve. This low diversity of flower visitors might be a consequence of a prolonged dry period that occurred

during the previous year. This drought affected the normal flowering development of many perennial plants, including *Larrea* species, which are the dominant entomophilous flowering shrubs in the Reserve. During that dry period, we observed in *Larrea* species that most newly opened flowers had brownish anthers, which probably did not produce pollen at all. In addition, the most frequent flower visitor to *B. retama* was *A. mellifera*, a recent introduction in the study site (personal observation). Honeybees are known for their short displacements within the plant when there is a rich nectar patch, favoring the transfer of pollen from one flower to another in the same individual (Schaffer *et al.*, 1983; Corbet, 1991; Toro *et al.*, 1993). In contrast, the behavior registered for the native bees visiting *B. retama* and *Larrea* species flowers (Debandi *et al.*, unpublished data) was different. These native bees tend to visit a few flowers in each plant and then move to another plant, favoring the transfer of pollen coming from neighbors.

Pre-dispersal predation is the other process that can explain the low seed production of naturally pollinated flowers. Flower buds, flowers at all stages, and developing fruits were predated by *Pseudomeloe* sp. (Coleoptera: Meloidae). Of 30 marked flower buds, only 20% of them reached the stage of mature fruits, while the remaining percentage was consumed in different stages by this beetle.

The recent introduction of honeybees in the boundaries of the Reserve can be dangerous for both native plants and native bees. For the local population of *B. retama*, the pollen transfer among flowers of the same plant by *A. mellifera* may severely reduce seed production. This could be more accentuated if honeybees outcompete the native bees in gathering pollen and/or nectar from *B. retama* flowers. Reproductive output could also be diminished by a continuous rate of pre-dispersal predation by *Pseudomeloe* sp.

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