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GENETIC SELF-INCOMPATIBILITY IN THE ENDEMIC PATAGONIAN GENUS *BENTHAMIELLA* (SOLANACEAE)

AUTOINCOMPATIBILIDAD GENETICA EN EL GENERO PATAGONICO ENDEMICO BENTHAMIELLA *(SOLANACEAE)*

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ABSTRACT

Results of controlled hand self- and cross-pollinations and spontaneous selfing trials to detect genetic self-incompatibility in the endemic Patagonian cushion plant, *Benthamiella nordenskjoldii* Dusén ex N.E. Br. (Solanaceae) are described for plants located in the high alpine on Cerro Santa Lucía, Sierra de los Baguales, latitude 50° S, Chilean Patagonia, together with natural fruiting levels. *Benthamiella nordenskjoldii* is totally self-incompatible. Hand-cross pollination increased fruit set over open pollination suggesting pollinator limitation.

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RESUMEN

Se describe los resultados de pruebas experimentales efectuadas bajo condiciones controladas, consistentes en la autopolinización manual, polinización cruzada manual y de pruebas de autopolinización automática, para la detección de la autoincompatibilidad genética en la planta en cojín, *Benthamiella nordenskjoldii* Dusén ex N.E. Br. (Solanaceae), especie endémica a la Patagonia, efectuadas en la zona alpina de Cerro Santa Lucía, Sierra de los Baguales, latitud 50°S en la Patagonia chilena. Asimismo se da los niveles de fructificación resultantes de las condiciones naturales de polinización de la comunidad. *B. nordenskjoldii* es totalmente autoincompatible. La formación de frutos fue más alta mediante la polinización cruzada manual en comparación con la polinización natural. El último sugiere que la formación de frutos en *B. nordenskjoldii* está limitada por sus polinizadores naturales.

KEYWORDS: Benthamiella, self-incompatability, Patagonia.

INTRODUCTION

In spite of the presence of many endemic genera, and a sizable number of endemic families, little is known about the reproductive biology of plant species in temperate South America. This contrasts strongly with the situation in other temperate regions, such as North America, Europe and Australasia, where, as of the 1960s, biosystematists made strenuous efforts to include information on breeding systems and pollination mechanisms in their treatments. Until recently (Arroyo *et al.*, 1982, 1983; Arroyo and Squeo, 1987) most of the published information on the breeding systems and pollination of temperate South America had been collected by non-resident foreign botanists (Moore, 1983).

Over the austral summers of 1984-85, 85-86 and 86-87, we undertook community-wide surveys of the breeding systems and pollination mechanisms of high elevation species in the Sierra de los Baguales, latitude 50°S, Patagonia, Chile. The overall thrust of this research has been to study the relationship between breeding systems, habitat harshness, and pollinator availability. We have argued that unfavorable abiotic conditions, as they affect seedling survival and adult maintenance in high elevation ecosystems, might place a high premium on outcrossing systems, in spite of the obvious reproductive disadvantage of such breeding systems in pollinator-depauperate environments.

The Patagonian alpine is not only interesting in that it provides a useful model for the evolution of plant breeding systems on harsh habitats, but for the presence there of a number of genera endemic to the Patagonia. One of these endemic genera, is *Benthamiella* Speg. (Solanaceae: Nicotianeae). *Benthamiella*, a genus of 12 cushion species, occurs between latitudes 37°S and 54°S, and is heavily concentrated in the southern Argentinian provinces of Santa Cruz and Chubut, and adjacent territories in the Province of Ultima Esperanza, Chile (Arroyo, 1980; Arroyo *et al.*, 1985, 1989; Boelcke *et al.*, 1985). During our survey of breeding sustems and pollination mechanisms in the Patagonian alpine, we had cause to study *Benthamiella nordenskjoldii* Dusén ex N.E. Br. Here we provide experimental evidence for self-incompatibility in *B. nordenskjoldii* and relate our findings to pollination.

MATERIALS AND METHODS

Work was conducted in field populations on Cerro Santa Lucía, 50° 46'S, 22° 21'W at the head of the Río Baguales, close to the border of Chile and Argentina. Here *Benthamiella nordenskjoldii* occurs in the lower and upper alpine (Arroyo *et al.*, 1989) from 1000-1400m, being most abundant at around 1300m. The upper vegetation limit on Cerro Santa Lucía is around 1600m. It prefers open, slightly moist habitats such as snow depressions. Common accompanying species are *Xerodraba pectinata* (Speg.) Skottsb., *Valeriana magellanica* Hombr. et Jacq., *Bolax gummifera* (Lam.) Spreng. and *Poa alopecurus* (Gaud) Kunth ssp. *alopecurus*. Flowering in *B. nordenskjoldii* occurs precociously during the last and first days of December and January, respectively. The flat cushions become covered with the strongly fragrant, white flowers, 9-11mm long, with included stamens and styles, containing a conspicuous drop of nectar secreted from an orange-red annular nectary located at the base of the style.

The experimental crosses reported here were made on 9 genets located between 1100-1200m elevation. Just before anthesis, the entire cushions were covered with cotton, conical shaped pollination enclosures. As the flowers opened, the plants were revisited to effect self-pollinations on one series of flowers using a mixture of pollen from the same flower and from neighboring flowers on the same individual. A second series of flowers, serving as a control for the self-pollinated flowers, was cross-pollinated using a mixture of pollen drawn from other individuals in the same population. Finally a third group of flowers was left unpollinated and unmanipulated to test for automatic selfing ability. The flowers were marked, and rebagged until the mature fruiting stage. Fruits were harvested and seed set per fruit determined. In order to determine whether the breeding system of *Benthamiella nordenskjoldii* limits fruit and seed production, fruit and seed set per fruit were also determined for field plants exposed to

natural conditions of pollination. The flowers controlled for natural pollination derived from 15 plants from the same location as the experimental plants.

RESULTS AND DISCUSSION

The results of the controlled hand crosses on *Benthamiella nordenskjoldii* are shown in Table 1. No fruits were obtained from any of the 162 self-pollinated flowers drawn from 6 different genets. The same result is evident for the 114 unpollinated flowers drawn from five genets. In contrast 39% of the hand cross-pollinated flowers yielded mature fruits. These results prove that *Benthamiella nordenskjoldii* is highly self-incompatible.

TABLE 1. Results of controlled hand self and cross-pollination and spontaneous selfing trials in field plants of *Benthamiella nordenskjoldii* (Solanaceae) on Cerro Santa Lucía, Chilean Patagonia. Flowers for all tests were bagged before anthesis and rebagged following manipulation.

Test	Plants	Flowers	Fruits	% Fruits	Seeds/flower/crossed		
					Mean	S.E.	Range
Hand Self-pollinated	6	162	0	0	0	—	—
Hand cross-pollinated	4	61	24	39.34	0.49 (1)	0.09	0-2
Non hand-pollinated	5	114	0	0	0	—	—

(1) Total seeds on fruits formed was 30.

Notwithstanding this result, an appreciable proportion of the crossed flowers failed to set seed (Table 1). This tendency is often found in field experiments due to the difficulty of determining the optimal time for stigma receptivity. However, fruit and seed abortion may occur in alpine situations, because of excessively cold temperatures. Thus the low % fruits following cross-pollination may reflect post-fertilization events. Self-incompatible species in general also exhibit high intrinsic levels of fruit and ovule abortion (Bawa & Webb, 1984), another factor that could be affecting this result.

TABLE 2. Natural fruit set and seed set per fruit as compared with fruit set and seed set per fruit following controlled hand cross-pollination in *Benthamiella nordenskjoldii* on Cerro Santa Lucía, Chilean Patagonia.

	Plants	Flowers	Fruits		Seed/fruit			
			n	%	n	Mean	S.E.	Rg.
Natural pollination	15	1043	159	15.24	45	1.97	0.001	1-3
Hand cross-pollination	4	61	24	39.34	24	1.25	0.001	1-2

Fruit production in naturally-pollinated plants was very low (15.24%; Table 2). Hand cross-pollination (39.34%) significantly increased fruit production ($t = 11.697$; $p<0.001$). The mean number of seeds per fruit, nevertheless was significantly higher for the naturally pollinated flowers ($t = 5.178$; $p<0.001$). However, the higher seed set per fruit, did not compensate for the lower fruit set (0.30 seeds/flower controlled for natural pollination versus 0.49 per flower crossed for hand cross-pollination). These results are consistent with strong pollinator-limited fruit set in *Benthamiella nordenskjoldii*.

In that it is highly self-incompatible, *Benthamiella nordenskjoldii*, is totally dependent on external agents for pollination and sexual reproduction. Morphologically, the long-tubular white fragrant flowers with abundant nectar, correspond to Faegri & van der Pijl's (1979) lepidopteran-pollination syndrome. During the daylight hours the high elevation butterfly *Hypsochila argyrodice* was the only insect seen on flowers of *B. nordenskjoldii*, however visits were sporadic and low in frequency. Observations made after dusk at the height of the flowering period revealed that *B. nordenskjoldii* is heavily visited by three species of Noctuideae (Squeo & Arroyo, unpublished observations). Such visits were restricted to warmer nights without wind and occurred between 9.00-10.00pm, before temperatures descended. The moths were observed to probe deeply into the flowers seeking nectar. Their movement between flowers on the cushions and between cushions was notably sluggish. That *B. nordenskjoldii* is predominantly nocturnally-pollinated, might be another factor influencing the fruits sets in our hand cross-pollination experiments. The experiments were conducted during the daylight hours, mostly in the late morning to mid-afternoon. Optimal stigma receptivity activity in *B. nordenskjoldii* probably occurs in the late-afternoon and early evening hours. Interestingly, seed set per fruit was higher in the naturally pollinated flowers (Table 2), indicating higher levels of fertilization per flower by the natural pollinators. If our results have been influenced by the time of pollination, we have probably underestimated the degree to which fruit set is pollinator-limited in *B. nordenskjoldii*.

Three additional species of *Benthamiella* occur on Cerro Santa Lucía (Arroyo *et al.*, 1989). The floral syndromes of *B. patagonica* and *B. lanata* are similar to that of *B. nordenskjoldii*. *Benthamiella azorella*, however, is notable for its highly reduced greenish-yellow corolla, and long-exserted stamens which give the flowers a yellow appearence. In contrast to *B. nordenskjoldii*, *B. patagonica* and *B. lanata*, the stigma is shortly-exserted from the corolla. This last species is visited by dipterans (Squeo and Arroyo, unpublished data). Given the diversity of style and stamen lengths in relation to corolla size in *Benthamiella* (Arroyo, 1980), further work on the pollination biology and breeding systems of this interesting Patagonian genus should be pursued.

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REFERENCES

ARROYO, S. 1980. The genus *Benthamiella* (Solanaceae). Bot. Not. 133: 67-76.

ARROYO, M. T. K., J. ARMESTO and R. PRIMACK. 1983. Tendencias altitudinales y latitudinales en mecanismos de polinización en la zona andina de Los Andes templados de Sudamérica. Revista Chilena Hist. Nat. 56:159-180.

ARROYO, M. T. K, C. MARTICORENA, A. LANDERO, O. MATTHEI, P. MIRANDA & F. SQUEO. 1989. Contribution to the high elevation flora of the Chilean Patagonia: A checklist of species on mountains on an east-west transect at latitude 50°S. Gayana, Bot. 46: (1-2): 121-151.

ARROYO, M.T.K, C. MARTICORENA & O. DOLLENZ. 1985. New records for the flora of Chile based on an expedition to the Sierra de los Baguales, Ultima Esperanza, Chile. Gayana, Bot. 42:41-45.

ARROYO, M. T. K., R. PRIMACK & J. ARMESTO. 1982. Community studies in pollination ecology in the high temperate Andes of Central Chile. I. Pollination mechanisms and altitudinal variation. Amer. J. Bot. 69:82-97.

Arroyo, M. T. K. & F. Squeo. 1987. Experimental detection of anemophily in *Pernettya mucronata* (Ericaceae) in western Patagonia. Bot. Jahrb. Syst. 108: 537-546.

Bawa, K. S. & C. J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. Amer. J. Bot. 71: 736-751.

Boelcke, O., D. M. Moore & F. A. Roig. (editores). 1985. Transecta Botánica de la Patagonia Austral, CONICET, Buenos Aires. XXVIII, 733 pp., 8 láms.

Faegri, K. and L. van der Pijl. 1979. The Principles of Pollination Ecology. Pergamon Press, New York. XII, 291 pp.

Moore, David M. 1983. Flora of Tierra del Fuego. Anthony Nelson, Ostwestry, St. Louis. IX, 396 pp., 8 láms.