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The breeding system of *Hippeastrum advenum* (Ker-Gawl.)
Herb. (Amaryllidaceae), an endemic bulbous geophyte of
the mediterranean-type climate region in central Chile

By

Francisca Saavedra, Mary T. Kalin Arroyo and Alicia J. Hoffmann

With 3 tables in the text

Abstract

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Experimental self- and cross-pollinations and observations on flower structure and pollinators were conducted on *Hippeastrum advenum* (Ker-Gawl.) Herb. (Amaryllidaceae), an endemic bulbous geophyte of the mediterranean-type climate of central Chile. No fruit formed by spontaneous self-pollination. Hand cross-pollination resulted in significantly higher fruit set and a higher number of seed per flower and seed per fruit than did hand self-pollination. Natural pollination resulted in a significantly higher number of fruit and a slightly higher number of seed per flower than hand cross-pollination, but both treatments resulted in a similar number of seed per fruit. The flowers, with exerted styles, are visited by hymenoptera and diptera. A self-incompatibility index of 0.25 was established, indicating that the species may be considered to be marginally self-compatible, pollinators are required for seed set. The breeding systems of bulbous geophytes were then related to growth-form and longevity, according to available data.

Over 90% of species are self-incompatible, confirming a strong tendency for xenogamy in this group. Life-span of bulbs is of up to seven years, but due to vegetative reproduction the genome may be long-lived.

Introduction

Geophytes, a life form that is particularly well adapted to seasonal, unpredictable climates (DAFNI et al. 1981a, DAFNI et al. 1981b), are important components of mediterranean-type and semi-desert climate vegetations (RAUNKIAER 1934, DAFNI et al. 1981a, DAFNI et al. 1981b, DAFNI 1986, REES 1989, DANIN & ORSHAN 1990) but are generally rare in extreme desert climates (SHMIDA

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& BURGESS 1988). The northern coastal deserts of Chile and central Chile, with a mediterranean-type climate, are characterized by a rich representation of geophytes, especially Amaryllidaceae, Liliaceae, Iridaceae and Orchidaceae. Higher cover of geophytes in the coastal desert and coastal sclerophyllous matorral of central Chile than in the Californian chaparral has been reported by MOONEY (1977).

For a total Chilean native flora of around 5100 taxa (MARTICORENA 1990), HOFFMANN (1989) provides a list of not fewer than 180 geophyte monocotyledonous species. For Chile many geophytes are endemic (MARTICORENA 1990). Several species, moreover, have limited geographical distributions and are placed in the rare and vulnerable category by conservationists (HOFFMANN 1989). However, little is known about their reproductive biology.

Differences in breeding systems tend to be correlated with successional stages and life form. Thus plants of early successional stages, including some forest trees, are often self-compatible (CRUDEN 1977, PARRISH & BAZZAZ 1979, BAWA et al. 1985). As to life form, at one extreme, annual herbs tend to be self-compatible, while at the other, trees tend to be self-incompatible or dioecious. The ultimate variable correlated with breeding system is probably longevity (ARROYO & SQUEO 1990b). Geophytes are specially interesting in this context. Many are likely to be long-lived, and high levels of self-incompatibility have been found among several Chilean geophytes (ARROYO & USLAR 1993).

In this paper we first report the results of experimental crosses to detect compatibility level in the endemic species *Hippeastrum advenum* (*Rhodophiala advena*) (Ker-Gawl.) Herb. (Amaryllidaceae). *Hippeastrum* is the second largest of 10 genera of Amaryllidaceae, after *Alstroemeria*, in the native Chilean flora. Presently it is considered to contain 24 species, 22 of which are endemic to Chile (MARTICORENA 1990). These are distributed between 28° 26' S and 40° 09' S. Knowledge on reproductive systems of *Hippeastrum* is apparently restricted to *H. bicolor* (ARROYO & USLAR 1993) and *H. aulicum* (DE NETTANCOURT 1977), both of which are reported as self-incompatible. Second we relate the breeding systems of bulbous geophytes to growth-form and longevity, according to the available data on breeding systems of this group.

Methods

The plants studied grow at Peñalolén, on the lower slopes of Cerro San Ramón, (33° 27' S, 70° 42' W; 800 m) on the outskirts of Santiago, Chile. The climate is typically mediterranean. For Santiago, mean annual precipitation is 356,2 mm and mean annual temperature 13.9° C (DI CASTRI & HAJEK 1976).

Hippeastrum advenum is distributed between 29° 26' S and 33° 02' S (ARROYO unpublished data). It occurs in small patches at open sites between the dominant matorral trees and shrubs. It is a large-bulbed geophyte, with an hysteranthus life cycle pattern as defined by DAFNI et al. (1981b). Leaves appear in winter

(June–July) and flowers in early summer (December), following leaf die-back. The erect inflorescence, averaging 21 cm in height, bears 2 to 8 pale-rose flowers with briefly exerted stigmas. Maximum flowering occurs in late January and ends in early March, at which time seeds are shed (HOFFMANN & SAAVEDRA unpublished data).

Work was conducted during summer and early fall of 1990. Stamens and styles were measured to detect morphological impediments to automatic self-pollination. To detect compatibility levels, inflorescences of plants from 6 patches in an area of approximately 10 000 m² were isolated before anthesis with cotton pollination-bags to exclude flower visitors. Flowers were subjected to the following treatments:

- a) Obligate agamospermy: flowers were emasculated prior to anthesis.
- b) Hand self-pollination: flowers were pollinated manually with pollen of the same flower or other flowers from the same individual.
- c) Spontaneous self-pollination: flowers were isolated without further manipulation.
- d) Hand cross-pollination: previously emasculated flowers were hand-pollinated with a mixture of pollen taken from other individuals of the same population.

Additionally, flowers were checked for:

- e) Natural pollination: unbagged flowers were marked without any experimental manipulation.

All flowers for treatments a-d were rebagged until signs of fruit formation or until the flowers withered. Given that the seeds of *H. advenum* are apparently wind-dispersed (HOFFMANN & SAAVEDRA unpublished data), the young emerging fruits were covered individually with small bags made of fine cotton fabric so as to prevent seed dispersal. Mature fruits were harvested around 6 weeks after pollination, and seed set per fruit was determined. All apparently aborted seeds were examined for the presence of an embryo. Around 1000 of the filled seeds were also checked for embryo development in the same manner.

Self-compatibility and selfing potential were determined as per RUIZ & ARROYO (1978). A self-incompatibility index (ISI) was calculated as seed number per hand self-pollinated flowers divided by seed number per hand cross-pollinated flower. Selfing potential was calculated as seed per flower treated for spontaneous selfing divided by seed number per hand self-pollinated flower. Fruit sets per treatment were compared using the G-test (SOKAL & ROHLF 1981). Seed number per fruit was compared with the WILCOXON 2 sample Test. A Voucher specimen of *H. advenum* has been deposited in the herbarium of the Museo de Historia Natural Santiago, Chile.

Results and discussion

Among the morphological features of the flowers of *Hippeastrum advenum*, the long styles are noteworthy. They are exerted beyond the stamens, thus suggesting that intraflower-selfing is unlikely.

Table 1. Results of the treatments performed to detect compatibility level in a population of *Hippeastrum advenum* growing at the lower slopes of Cerro San Ramón, central Chile.

Treatment	Plants	Flowers	Fruits	%Fruits	No.Seed/Flower Mean (SD)	No.Seed/Fruit Mean (SD)
Obligate agamospermy	12	29	0	0	0	0
Spontaneous self-pollination	12	42	0	0	0	0
Hand self-pollination	17	43	16	37.21	5.77 (11.84)	17.31 (14.01)
Hand cross-pollination	19	43	26	60.47	27.42 (20.17)	37.35 (15.19)
Natural pollination	18	67	59	88.06	33.20 (22.15)	37.07 (20.25)

The treatments conducted to examine compatibility levels had different consequences on fruit and seed set (Table 1). The emasculated flowers failed to form fruit, indicating that *H. advenum* is not an obligate apomictic. Spontaneous self-pollination resulted in no fruit production.

Over one third of hand self-pollinated flowers formed fruits, although the seed set per flower and per fruit was low compared to that obtained by hand cross-pollination or natural pollination.

Close to two thirds of the hand cross-pollinated flowers formed fruits: hand cross-pollination resulted in 60.5 % fruit set, a significantly higher value ($G = 4.62$; $p < 0.05$) than by hand self-pollination. The number of seed per flower and seed per fruit also was higher ($t = 3.717$; $p < 0.001$) than by hand self-pollination.

Natural pollination resulted in 88.1 % fruit set, significantly higher ($G = 10.94$; $p < 0.005$) than in hand cross-pollination, and in a slightly higher number of seed per flower than by hand cross-pollination, but both treatments resulted in a similar number of seed per fruit ($t = 0.863$; $p > 0.05$). Differences of this nature could be due to the difficulty of determining the exact timing of optimal stigma receptivity in field experiments (ARROYO & SQUEO 1990a). The number of fruits produced by hermaphrodite species is frequently lower than the number of fruits that potentially may be matured, fruiting being limited by resources when pollination levels are high (STEPHENSON 1981, AKER 1982, WIENS 1984). In geophytes, moreover, reproductive biomass is strongly correlated with vegetative development, including below-ground storage (BOEKEN 1990).

The self-incompatibility index (ISI) for *H. advenum* is 0.25, i.e., very close to 0.2, the limit proposed by RUIZ & ARROYO (1978) for self-incompatible species. Therefore, *H. advenum* may be considered to be a marginally self-compatible species. The index of automatic selfing is 0. The lack of spontaneous self-pollination in *H. advenum* is consistent with the flower morphology of this species. These results suggest that although *H. advenum* has some self-compati-

Table 2. Breeding systems in geophytes. Number of species in which self-incompatibility (SI); self-compatibility (SC); or self-incompatibility with some self-compatibility (SI-SC), was established in the indicated source.

Source	SI No.	SC No.	SI-SC No.
FRYXELL (1957)	46	0	2
FRANKEL & GALUM (1977)	2	1	0
DE NETTANCOURT (1977)	3	0	0
ARROYO & USLAR (1993)	3	1	0
ARROYO & SQUEO (1990b)	2	1	0
Total species	56	3	2

bility, pollinators are required for seed set. During the study, flowers were seen to be visited by hymenoptera and diptera. However, more work is needed to ascertain the effective pollinators of this species.

A survey of the available data on breeding systems of bulbous geophytes (Table 2) shows that among a total of 61 species, 91.8 % are self-incompatible. In contrast, among 104 nongeophyte perennial herbs, drawn from many genera, only 27 % are self-incompatible (ARROYO & SQUEO 1990b). This comparison confirms the strong tendency for xenogamy existing among the bulbous geophytes. Also there seems to be as much self-incompatibility in geophytes as in tropical trees (BAWA et al. 1985).

The breeding system of geophytes seems to be correlated with longevity. Several authors have reported a tendency for self-incompatibility in long-lived species, mainly trees (RAVEN 1979, ARROYO 1981, ARROYO & SQUEO 1990b). Although the life-span of bulbs and corms varies from one to seven years (DAFNI et al. 1981a), due to vegetative reproduction the genome may be many years older in plants with this growth form.

Table 3. Breeding systems of bulbous geophytes. — SI = self-incompatible; SC = self-compatible; SI-SC = self-incompatible, some self-compatible.

Family	Taxa	Breeding systems	Reference
Amaryllidaceae	<i>Allium multibulbosum</i>	SI	FRYXELL, P. A.
	<i>Allium nutans</i>	SI-SC	FRYXELL, P. A.
	<i>Allium schoenoprasum</i>	SI	FRYXELL, P. A.
	<i>Hymenocallis occidentalis</i>	SI	FRYXELL, P. A.
	<i>Narcissus triandrus</i>	SI	FRYXELL, P. A.
	<i>Zephyranthes atamasco</i>	SI	FRYXELL, P. A.
	<i>Zephyranthes caudata</i>	SI	FRYXELL, P. A.
	<i>Zephyranthes grandiflora</i> (<i>carinata</i>)	SI	FRYXELL, P. A.
	Liliaceae	<i>Hyacinthus orientalis</i>	SI
<i>Lilium aurantum</i>		SI	FRYXELL, P. A.

	<i>Lilium bulbiferum</i>	SI	FRYXELL, P. A.
	<i>Lilium bulbiferum</i> <i>var. croceum</i>	SI	FRYXELL, P. A.
	<i>Lilium canadense</i>	SI	FRYXELL, P. A.
	<i>Lilium chalcedonicum</i>	SI	FRYXELL, P. A.
	<i>Lilium dauricum</i>	SI	FRYXELL, P. A.
	<i>Lilium formosanum</i>	SI	FRYXELL, P. A.
	<i>Lilium hansonii</i>	SI	FRYXELL, P. A.
	<i>Lilium henryi</i>	SI	FRYXELL, P. A.
	<i>Lilium humboldtii</i>	SI	FRYXELL, P. A.
	<i>Lilium japonicum</i>	SI	FRYXELL, P. A.
	<i>Lilium kelloggii</i>	SI	FRYXELL, P. A.
	<i>Lilium leichtlinii</i>	SI	FRYXELL, P. A.
	<i>Lilium longiflorum</i>	SI-SC	FRYXELL, P. A.
	<i>Lilium maculatum</i> (<i>elegans</i>)	SI	FRYXELL, P. A.
	<i>Lilium martagon</i>	SI	FRYXELL, P. A.
	<i>Lilium myriophyllum</i> (<i>sulphureum</i>)	SI	FRYXELL, P. A.
	<i>Lilium pardalinum</i>	SI	FRYXELL, P. A.
	<i>Lilium parryi</i>	SI	FRYXELL, P. A.
	<i>Lilium parvum</i>	SI	FRYXELL, P. A.
	<i>Lilium philadelphicum</i>	SI	FRYXELL, P. A.
	<i>Lilium pumilum</i> (<i>tenuifolium</i>)	SI	FRYXELL, P. A.
	<i>Lilium regale</i>	SI	FRYXELL, P. A.
	<i>Lilium sargentiae</i>	SI	FRYXELL, P. A.
	<i>Lilium speciosum</i>	SI	FRYXELL, P. A.
	<i>Lilium superbum</i>	SI	FRYXELL, P. A.
	<i>Lilium tigrinum</i>	SI	FRYXELL, P. A.
	<i>Lilium willmottiae</i> (<i>warleyense</i>)	SI	FRYXELL, P. A.
	<i>Tulipa armena</i>	SI	FRYXELL, P. A.
	<i>Tulipa celsiana (persica)</i>	SI	FRYXELL, P. A.
	<i>Tulipa chrysantha</i>	SI	FRYXELL, P. A.
	<i>Tulipa fosteriana</i>	SI	FRYXELL, P. A.
	<i>Tulipa gesneriana</i>	SI	FRYXELL, P. A.
	<i>Tulipa hageri</i>	SI	FRYXELL, P. A.
	<i>Tulipa kolpakowskiana</i>	SI	FRYXELL, P. A.
	<i>Tulipa micheliana</i>	SI	FRYXELL, P. A.
	<i>Tulipa oculis solis</i>	SI	FRYXELL, P. A.
	<i>Tulipa violacea</i>	SI	FRYXELL, P. A.
	<i>Urginea (Scilla) maritima</i>	SI	FRYXELL, P. A.
Amaryllidaceae	<i>Allium cepa</i>	SC	FRANKEL, R. & GALUM, E.
Lilaceae	<i>Narcissus tazetta</i>	SI	FRANKEL, R. & GALUM, E.
	<i>Lilium longiflorum</i>	SI	FRANKEL, R. & GALUM, E.
Amaryllidaceae	<i>Amaryllis belladonna</i>	SI	DE NETTANCOURT, D.
	<i>Hippeastrum aulicum</i>	SI	DE NETTANCOURT, D.
	<i>Zephyranthes carinata</i>	SI	DE NETTANCOURT, D.
Amaryllidaceae	<i>Alstroemeria patagonica</i>	SC	ARROYO, M. T. K. & SQUEO, F.
Iridaceae	<i>Phaiophleps</i> <i>biflora var. biflora</i>	SI	ARROYO, M. T. K. & SQUEO, F.

	<i>Phaiophleps biflora</i> var. <i>lyckholmii</i>	SI	ARROYO, M. T. K. & SQUEO, F.
Amaryllidaceae	<i>Astroemeria ligtu</i>	SI	ARROYO, M. T. K. & USLAR, P.
	<i>Astroemeria pallida</i>	SC	ARROYO, M. T. K. & USLAR, P.
	<i>Hippeastrum bicolor</i>	SI	ARROYO, M. T. K. & USLAR, P.
Liliaceae	<i>Leucocoryne ixioides</i>	SI	ARROYO, M. T. K. & USLAR, P.

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Addresses of the authors:

- F. SAAVEDRA, Department of Geography, University of Florida, Gainesville, Florida 32611-2036, USA.
- M. T. K. ARROYO, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.
- A. J. HOFFMANN, Departamento de Ecología, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile.