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The breeding system of *Linaria ricardoi* (*Scrophulariaceae*), a threatened Portuguese endemism

Abstract

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Linaria ricardoi Cout. (*Scrophulariaceae*) is a Portuguese endemism considered as a prioritary species by the European Community Habitats and Species Directive (Directive 92/43/CEE), that grows in dry arable fields in a restricted area from SE Portugal. Its actual distribution is limited to few known areas where anthropogenic action, namely agriculture, is less intensive. A study on pollen viability, stigma receptivity and breeding system of *Linaria ricardoi* was begun to improve the knowledge on this species and to contribute to its conservation. Results shows that L. ricardoi can reproduce by both cross- and self-pollination, although xenogamy looks to be more efficient.

Introduction

Linaria ricardoi Cout. is a narrowly distributed endemic species from Baixo Alentejo, Portugal. It is an annual species associated with arable dry fields. In the last two years the occurrence of only few populations was confirmed. It is considered as a prioritary species by the European Community Habitats and Species Directive (92/43/CEE) and is formally listed in Annexes II and IV. According to the IUCN categories (IUCN, 1997), this species was classified as Vulnerable (V) although it should be classified as Critically Endangered (CR) (IUCN, 2000), because of its fragmented distribution and continuous declining in population size due to the use of intensive farming techniques. This species occurs in the future area of irrigation of the Alqueva dam, in the Guadiana basin. So its flooding will cause more intense land use as well as climatic changes, that may lead to disturbance of the habitat and the structure of the species populations.

In this paper, data on the pollen viability, stigma receptivity, floral phases and breeding system of this species are presented. Knowledge of the species breeding system is essential in order to optimise *ex situ* plant propagation, should conservation actions as re-introductions or population re-inforcements be necessary.

Material and methods

In May 2001 fruits were collected from Cuba population (UTM: 29SNC9922, Baixo

Alentejo, Portugal). Seeds were stored in "Banco de Sementes Belo Correia do Museu, Laboratório e Jardim Botânico da Universidade de Lisboa" (Belo Correia Seed Bank from Museum, Laboratory and Botanical Garden of Lisbon University). In the plant nursery of this institution, 3000 seeds were sown in February and 1152 in April 2001. 31 of the germinated plants were monitored along the flowering and fruiting season.

FLORAL PHASES

Mean flower duration from May (n=70), June (n=24) and July (n=113) was compared.

Flowers on 1, 3, 4, 6 and 8 of anthesis were monitored in concern with the following features: open/closed long and short stamens and open/closed stigma. Stigmatic receptivity was tested in the same flowers using hydrogen peroxide (3%) (Galen & Kevan 1980). This is a qualitative technique and the bubble formation is related with the stigma receptivity. Value 0 means a non-receptive stigma, 1 a receptive stigma and 2 a highly receptive stigma.

Viability of pollen from the two types of stamens and from flowers being open for 1, 3 and 6 days, was tested. Pollen viability was measured using *in vitro* germination rate in 60% sucrose solution in a mixture of 50% of 2 X 10^{-3} M H_3BO_3 and 50% of 6 X 10^{-3} M $Ca(NO_3)_2$ (Dafni 1992). Pollen viability was estimated as the total percentage of pollen germination in three optical fields for each slide and the mean of 3 replicates for each pollen sample was calculated.

BREEDING SYSTEM

Fifteen plants were subjected to the following pollination treatments:

open pollination (control): flowers were not manipulated and were left to pollinators (n = 19);

spontaneous self-pollination: bagged flowers were left without treatment (n = 29);

induced self-pollination: flowers were pollinated with own pollen (n = 21);

geitonogamy: flowers were pollinated with pollen from another flower of the same plant (n = 22);

hand cross-pollination: pollinations were performed by hand with pollen from different individuals (n = 19).

In treatments (B), (C), (D) and (E), inflorescences with unopened buds were covered with cellophane bags. In (C), (D), and (E), flowers were pollinated and rebagged until before fruit dehiscence. In treatments (D) and (E), flowers were not emasculated to avoid possible manipulation damages due to the reduce size of flowers. Fruits and seeds were collected as they matured. The number of mature seeds and aborted seeds were counted. All manipulations and controls were done at least once on the same plant to minimize the effects of intraspecific genetic variability (Dafni 1992).

Data from stigma receptivity (see results) and previous tests showed that flowers on day 4-6 of anthesis should be pollinated with pollen of day 3 of anthesis for optimal results. Self-incompatibility index (ISI) was calculated by dividing fruit set (Fruit/Flower ratio) from self-pollination by fruit set from cross-pollination (Becerra & Lloyd 1992; Lloyd & Schoen 1992). For each treatment, pollen viability was tested to find out if it was limiting fruit production, using the method described above.

Results and discussion

FLORAL PHASES

Mean flower duration for the three months was 6,07± 3,29 days. Flowering was significantly longer at the beginning of the flowering season and shorter at the end of that period (Kruskal-Wallis $H = 95{,}288$, $p < 0{,}001$) (Table 1). Temperature could be one explanation for the observed decrease of flower longevity across the months of May, June and July, as also concluded by Ortega-Olivencia & Devesa (1998) for *Scrophularia fontqueri* Ortega-Olivencia & Devesa.

Anthers of both long and short stamens were nearly open since the 1st day of flower; anthesis and pollen was completely released by the 6th day. Hydrogen peroxide test showed that stigmas were receptive from the beginning of anthesis through the last days although from the 4th day onwards they were even more receptive (Fig. 1). By the 8th day stigmas were completely dusted with pollen. *In vitro* pollen germination was higher on the 1st day of anthesis and significantly decreased with flower age (Kruskal-Wallis $H = 38{,}800$, $p < 0{,}001$; Fig. 1), although previous tests showed higher pollen viability from 3-day flowers. In fact, Stone & al. (1995) refers that pollen viability is known to decline, sometimes rapidly, with age and exposure to environmental stresses. There were no differences between

Table 1. Values of flower longevity from May to July (mean ± st. dev) and mean air temperatures (www[1]); n = sample size; minimum and maximum values between brackets.

Month	Flower longevity (days)	Mean air temperature (°C)
May (n = 70)	9,06 ± 2,58 (4,0 - 17,0)	17,8
June (n = 24)	6,04 ± 2,92 (1,0 – 11,0)	21,5
July (n = 113)	4,21 ± 2,24 (1,0 – 11,0)	22,0

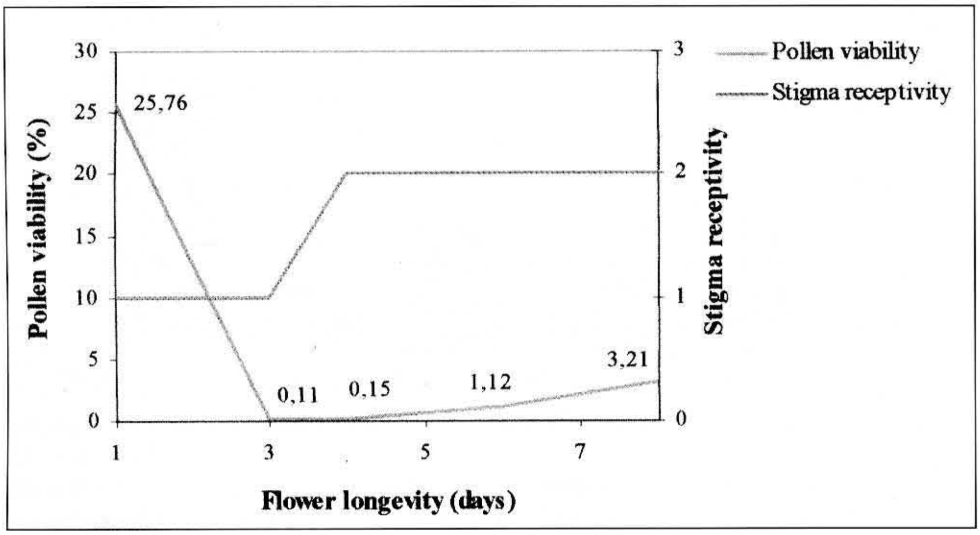

Fig. 1. Pollen viability and stigma receptivity during the lifetime of flowers.

pollen germination from short and long stamens (short: 11,42 ± 18,04%; long: 13,61 ± 23,77%; n = 33; Mann-Whitney $U = 542,00$, $p > 0,05$).

BREEDING SYSTEM

Fruit set from experimental pollinations in *L. ricardoi* was higher after hand cross-pollination (Fig. 2). Although there were no significant differences among treatments (Kruskal-Wallis $H = 12,325$, $p > 0,01$), this may be due to the low number of fruits formed. Results shows that self-pollination can occur but fruit set is quite inferior. This is supported by the ISI index obtained (0.59), which considers *L. ricardoi* as a partially self-incompatible species (Becerra & Lloyd 1992; Lloyd & Schoen 1992). Many Linaria species are self-incompatible (Dilleman, 1949; Valdés, 1970; Arnold, 1982) and Valdés (1970) also refers the gametophytic self-incompatible system as the most common in this genus. Gametophytic self-incompatibility may be broken by excess of pollen produced by flowers and plant age, so this could explain why there was some seed production by autogamy (Valdés 1970). Seed production by autogamy has been reported for many *Linaria* species (Darwin 1883; Champagnat 1955, 1961; Valdés 1970, 1996).

Control flowers produced a low number of fruits when compared to cross-pollinated flowers (Fig. 2). This suggests that there may be pollen limitation possibly due to a reduced

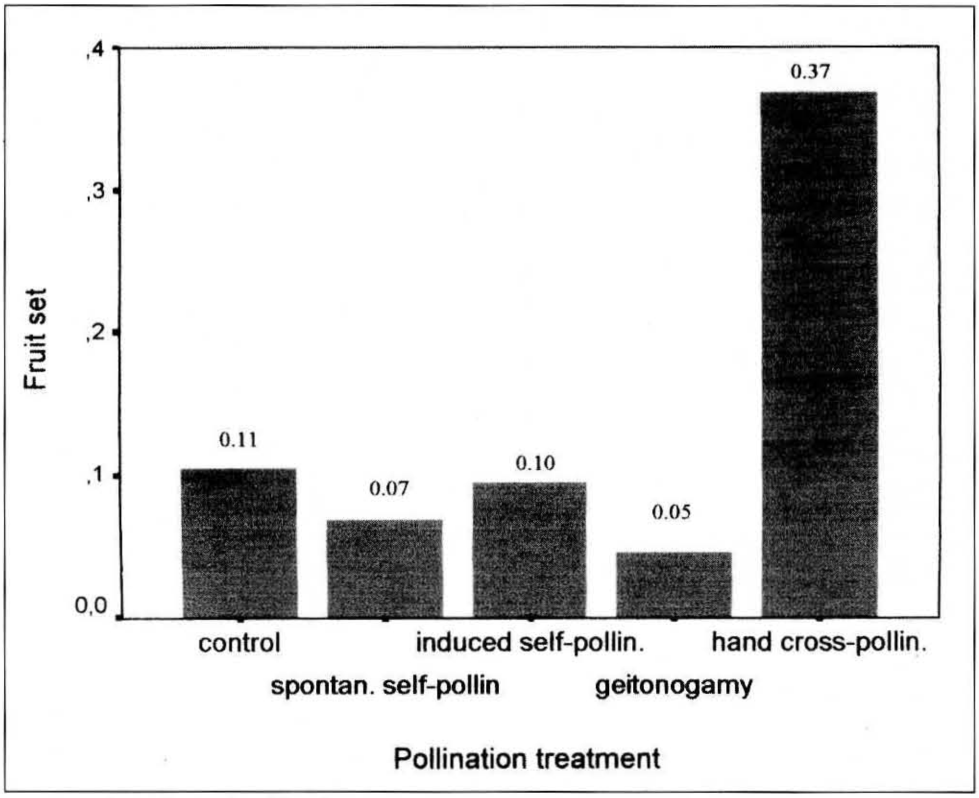

Fig. 2. Fruit set for each pollination treatment.

activity of pollinators (Mateu & Figueres 1998) or maybe the bagging system affects their activity.

Low values of fruit set may be caused by stigma damage or changes in intrafloral conditions due to manipulation. It could also be attributed to low pollen viability: there were no significant differences in pollen germination among treatments (*one way* ANOVA $F = 1{,}405$, $p > 0{,}05$; data *arsin* transformed; mean 23,24 ± 15,65%), so pollen viability could be limiting fruit production but in the same way for all the treatments.

Although low values of fruit formation did not allow statistical analysis, values of mature and aborted seeds counted in fruits also revealed that the species is mainly xenogamous and, secondary, fruit and seed formation by self-pollination is also possible (mean number of mature and aborted seeds from self-pollination: 3,0 ± 6,2 and 3,8 ± 4,3, n = 5; and from hand cross-pollination: 23,3 ± 9,8 and 1,4 ± 1,8, n = 7).

Results from manual pollinations were consistent with those of pollen viability and stigma receptivity. Two floral phases can be established. First, in the period between the 1st and 3rd days of anthesis, pollen viability is higher and the stigma is receptive. This seems to be favourable for autogamy. Second, between the 3rd and 8th days, although stigma is highly receptive, pollen viability is very low and this may favour xenogamy.

We conclude that *L. ricardoi* can reproduce by both, cross- and self-pollination, although xenogamy seems to be more efficient, and furthermore it provides higher genetic variability. Autogamy may be a good chance to reproduce, especially in critical situations.Population size and alteration of the plant-pollinator system due to habitat destruction may seriously limit the species reproduction.

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References

Arnold, R. M. 1982: Pollination, predation and seed set in *Linaria vulgaris* (*Scrophulariaceae*). — American Midland Naturalist, **107**: 360-369.

Becerra, J. X. & Lloyd, D. G. 1992: Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (*Agavaceae*): a second action of self-incompatibility at the whole-flower level? — Evolution, **46**: 458-469.

Champagnat, M. 1955: Pélorie et autostérilité dans une souche de *Linaria reflexa* Desf. — Monde Pl., **11**: 303-314.

— 1961: Recherches de morphologie descriptive et expérimentale sur le genre *Linaria*. — Ann. Sci. Nat. Bot. (Paris), 12ª ser. **2**: 1-170.

Dafni, A. 1992: Pollination Ecology, a Practical Approach. — Oxford.

Darwin, C. 1883: The effect of cross and self-fertilization in the vegetable kingdom, ed. 2, — London

Dilleman, G. 1949: L'absence de fécondation spontanée chez *Linaria heterophylla* DESF. Dans la region parisienne. — Monde Pl., **256**: 15.

Directiva 92/43/CEE do Conselho, relativa à preservação dos habitats naturais e da fauna e da flora selvagens. — Jornal Oficial das Comunidades Europeias Nº L 206/7.

Franco, J. A. 1984: Nova Flora de Portugal (Continente e Açores), **II**. — Lisboa.

Galen, C. & Kevan, P. G. 1980: Scent and color, floral polymorphisms and pollination biology in *Polemonium viscosum* Nutt. — American Midland Naturalist. **104**: 281-298.

IUCN 1997: IUCN Red List of Threatened Plants. Walter, K. S. Gillet, H. J. (eds.). Compiled by the World Conservation Monitoring Centre. IUCN. — The World Conservation Union, Gland, Switzerland and Cambridge, UK.

— 2000: IUCN Red List Categories: Version 3.1. Prepared by the IUCN Species Survival Commission. — IUCN, Switzerland and Cambridge, UK.

Lloyd, D. G. & Schoen, D. J. 1992: Self- and cross-fertilization in plants. I. Functionals dimensions. — International Journal of Plant Sciences, **153**: 370-380.

Mateu, I. & Figueres, S. 1998: Breeding system of three taxa of *Chaenorhinum* (D. C.) Rehb. (*Scrophulariaceae*) of the Iberian Peninsula: *C. origanifolium* (L.) Fourr. subsp. *cadevallii* (Bolos & Vigo) Lainz, *C. origanifolium* (L.) Fourr. subsp. *crassifolium* (Cav.) Rivas Goday & Borja and *C. tenellum* (Cav.) Lange. — Acta Bot. Gallica, **145(1)**: 69-79.

Ortega-Olivencia, A. & Devesa, J. A. 1998: Taxonomy and breeding system in a new species of *Scrophularia* L. (*Scrophulariaceae*) from Morocco. — Botanical Journal of the Linnean Society, **128**: 185-202.

Stone, J. L., Thomson, J. D. & Dent-Acosta, S. J. 1995: Assessment of pollen viability in hand-pollination experiments: a review. — American Journal of Botany, **82(9)**: 1186-1197.

Valdés, B. 1970: Taxonomía experimental del género *Linaria*. IV. Reproducción sexual: autogamia y polinización intraespecífica. — Bol. R. Española Hist. Nat. (Biol.), **68**: 79-89.

— & Lifante, Z. D. 1996: Habitual autogamy in *Linaria tursica* Valdés et Cabezudo (*Scrophulariaceae*). — Flora, **191**: 329-333.

www[1] - Informação climática [online] <http://www.meteo.pt/ Informaçãoclimática/ Meses/ Meses.htm> [consulta: 23-08-2001].

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