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Short communication

Does drought affect inbreeding depression in the autogamous species *Convolvulus chilensis* (Convolvulaceae)?

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Abstract Inbreeding depression, the reduction in fitness of selfed progeny relative to outcrossed progeny, may be affected by the environment. The autogamous species *Convolvulus chilensis* (Convolvulaceae) occurs in habitats subject to drought, where seedling mortality is important in population dynamics. We compared in a greenhouse the performance of *C. chilensis* seedlings originated from different pollen sources (own or exogenous) and grown under different water availability treatments (regular or restricted watering). We measured the following plant traits: number of leaves, leaf area, internode length, stem length and diameter, shoot and root biomass, and root:shoot ratio. Water availability affected leaf area, shoot biomass, and stem diameter. There was no effect of pollen source on seedling traits in either soil moisture treatment, and no statistical interaction between pollen source and water availability was detected. We found no evidence of inbreeding depression at the seedling stage in *C. chilensis* under regular or restricted watering.

Keywords *Convolvulus chilensis*; Convolvulaceae; breeding systems; inbreeding depression; autogamy; drought stress

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INTRODUCTION

Autonomous selfing in plants may provide reproductive assurance when pollinators are scarce or absent (Stebbins 1950). However, actual reproductive assurance may be curtailed if selfed individuals are less viable or less fecund. Inbreeding depression, which is defined as the reduction in fitness of selfed progeny relative to outcrossed progeny, is generally considered the main force opposing the evolution of self-fertilisation (Lloyd 1979; Lande & Schemske 1985; Charlesworth & Charlesworth 1987). The condition for evolution of increased selfing rate in the population is an inbreeding depression value lower than 0.50. In other words, there is selection for increased selfing if the inbreeding depression is less than 50%, and there is selection for increased outcrossing if the inbreeding depression is greater than 50% (Lande & Schemske 1985; Charlesworth & Charlesworth 1987).

Theoretical studies have suggested a possible joint evolution of mating systems and inbreeding depression. Thus, inbred mating may contribute to purging deleterious mutations because recessive alleles involved in the expression of inbreeding depression are then exposed to selection (Lande & Schemske 1985). Consequently, strong and weak inbreeding depression should be associated with outbreeding and autogamy, respectively. Some experimental studies support the theory, showing that in mainly outbreeding species the differences in performance between selfed and outbred individuals is often higher than in mainly autogamous species (Husband & Schemske 1996). On the other hand, differences in performance between selfed and outbred individuals may be affected by environmental conditions as a consequence of differential expression of characters. Selfed individuals may be less tolerant of environmental stress, as has been reported for interspecific competition in *Crepis sancta* (Asteraceae) (Cheptou et al. 2000a) and habitat quality in *Sabiata angularis* (Gentianaceae) (Dudash 1990). In contrast, no variation in inbreeding depression with plant density has been found in *Impatiens capensis* (Balsaminaceae)

(Mitchell-Olds & Waller 1985) and two species of *Linanthus* (Polemoniaceae) (Goodwillie 2000).

Convolvulus chilensis Pers. (Convolvulaceae) is a perennial herb endemic to coastal and arid zones of Central-Northern Chile, often occurring in small populations. In a typical population of *C. chilensis*, we have shown that it is an autogamous species, with a high flower-to-fruit conversion after both experimental and autonomous selfing (93.5% and 75.73%, respectively; Suárez et al. 2004). Individuals in this population are prone to drought stress because in dry years annual precipitation may be less than 50 mm. Drought is a critical mortality factor during seedling establishment in this population of *C. chilensis* (Suárez et al. unpubl. data). We have shown that seedlings of *C. chilensis* do not show inbreeding depression under greenhouse conditions (regular watering; see Suárez et al. 2004). However, it is not known whether water availability can affect seedling performance in such a way that early inbreeding depression is expressed. Considering that *C. chilensis* is an autogamous species and that it inhabits arid and semi-arid ecosystems with frequent drought (Jaksic 2001), it could be expected that inbreeding depression should be low even under water shortage. The main goal of this study was to compare the performance of selfed and outcrossed seedlings of *C. chilensis* under both regular and restricted water availability.

MATERIALS AND METHODS

Plant material

Convolvulus chilensis is endemic to coastal and arid zones of Central-Northern Chile (29°–35°S), from sea level to 1800 m (O'Donell 1957; Hoffmann 1998). This species is a perennial trailing or climbing herb, lacking rhizomes. Stems are slender, long (up to 3 m), and seldom branched. Leaves are extremely variable in size (1–10 cm long) and shape. Plants produce hermaphroditic pink flowers, arranged individually or rarely in 2-flowered cymose inflorescences. Length of corolla reaches 15–30 mm, stamens 10–14 mm, anthers 2–3.5 mm, and style 6–10 mm (O'Donell 1957; Suárez et al. 2004). Flower longevity is extremely short. The funnel-like corolla is open for about 5.25 h and pollen remains exposed for only 1.95 h. Pollination may involve insects or wind. *C. chilensis* is an autogamous species which converts a high percentage of fruits after both manual self-pollination (93.5% flower to fruit conversion) and outcrossing (98.6%), and shows herkogamy ranging from -1.67 to 7.21 mm. (Suárez et al. 2004). *C. chilensis* exhibits phenotypic

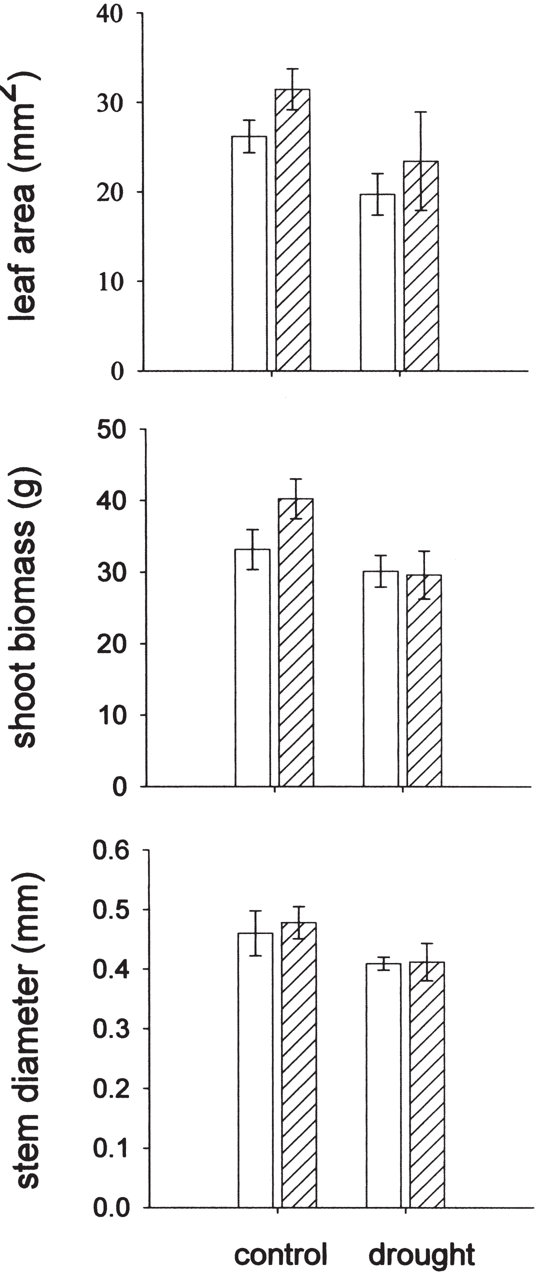

Fig. 1 Water availability effects on leaf area, shoot biomass, and stem diameter (mean ± SE) in *C. chilensis* seedlings. Open bars, selfed progeny; hatched bars, outcrossed progeny.

plasticity in response to shading (González & Gianoli 2004) and drought (Gianoli & González-Teuber in press).

Seeds of *Convolvulus chilensis* were collected in November 2003 from a population located in Aucó (31°29′S, 71°08′W, 600–700 m a.s.l.), Central-Northern Chile. The study population is very sparse (11.48 individuals ha^{-1}; 5.18 blooming individuals ha^{-1}). In October 2003, an experiment of controlled pollination was carried out in the field including manual cross-pollination, in which experimental flowers in focal individuals received pollen of other individuals, and manual self-pollination, in which experimental flowers in focal individuals received pollen of the same flower or another flower in the same focal plant (see Suárez et al. 2004).

Experimental design

Seeds obtained from five maternal plants including both pollination treatments were subjected to acid scarification before germination. Seeds were immersed in concentrated sulphuric acid for 30 min and then washed in running tap water for 5 min. Seeds were germinated individually on wet filter paper in Petri dishes at 22 ± 2°C in darkness. Twenty days after scarification, or when cotyledons were exposed and the radicle was >50 mm long, seedlings were planted in plastic pots (5 litre) filled with potting soil.

Experimental plants were grown in greenhouse conditions (Tmax = 24°C, Tmin = 10°C; Photoperiod: 14:10 L:D; 700 μ mol m^{-2} s^{-1} PAR average between 1200 and 1600 h) during austral summer (December 2003–March 2004). Plants were randomly assigned to each of two treatments differing in water availability: control (regular watering: two-three times a week) and drought (restricted watering: once every 15 days). Half of the plants within each treatment were selfed progeny and half outcrossed (n = 15 plants per group; total sample size = 60). The drought treatment started on Day 50 after scarification. This procedure followed the field situation (Suárez & Gianoli unpubl. data), where early seedlings experience mortality approximately two months after germination, which occurs when winter rainfall has already stopped. At the end of the experimental treatment (Day 146 after scarification) we examined differences in the performance of selfed and outcrossed progeny. We measured number of leaves, leaf area, internode length, stem length and diameter, shoot and root biomass, and root:shoot ratio. Measurements of internode length and stem diameter were made with a digital calliper (Mitutoyo Corporation, Kawasaki, Japan; resolution 0.01 mm). Leaf area (mm^2) was estimated from digital images using SigmaScan® software. Aboveground and belowground biomass were dried at 70°C for four days.

Statistical analysis

Two-way ANOVA was used to evaluate differences between plants from two pollen sources and two water availability treatments. Inbreeding depression would be proven if outcrossed seedlings showed better performance than selfed ones (significant effect of pollen source). Significant pollen source by water treatment interaction would indicate that inbreeding depression is affected by environmental conditions. It is acknowledged that the small sample sizes used in this study limit the statistical power to detect differences between groups, therefore caution is required when drawing conclusions.

Table 1 Effect of pollen source (PS), water availability (WA), and interaction between PS and WA (PS × WA) on seedling traits of *Convolvulus chilensis*. F-ratios (1, 48) after a two-way ANOVA are shown. *, $P < 0.05$; **, $P < 0.01$.

Trait	Pollen source (PS)	Water availability (WA)	PS × WA
Stem length	0.59	1.36	0.88
Internode length	0.14	0.34	0.46
Stem diameter	0.13	4.29*	0.07
Number of leaves	0.44	0.37	0.19
Leaf area	2.44	6.41**	0.07
Shoot biomass	1.39	6.06**	1.88
Root biomass	0.24	0.05	1.58
Root:shoot ratio	1.12	1.94	0.03

RESULTS AND DISCUSSION

As expected, experimental drought limited plant growth in three of the measured traits: leaf area, shoot biomass, and stem diameter (Fig. 1; Table 1). There was no difference in plant traits according to pollen source, nor a significant interaction between pollen source and water availability factors; inbred and outcrossed progeny showed a similar performance in both watering treatments (Table 1). Thus, we found no evidence of inbreeding depression at the seedling stage in *C. chilensis*, and drought did not affect this result. The observed tendency for greater inbreeding depression in control plants (Fig. 1), albeit non-significant, makes it unlikely that a greater sample size or a more prolonged/severe drought treatment would allow the detection of a significant pattern of inbreeding depression. There was no effect of pollen source on seedling survival. Eight seedlings died during the experiment, all from the drought treatment: four selfed and four outcrossed.

To our knowledge, only two studies have previously addressed the relationship between drought stress and inbreeding depression in plants. Hauser & Loeschcke (1996) reported that drought stress exacerbated inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae) in terms of survival, whereas no effect on fecundity was found. *L. flos-cuculi* is a mainly outcrossing perennial species that typically grows in moist, grazed meadows. The history of drought stress due to drainage in the *L. flos-cuculi* population studied was not reported (Hauser & Loeschcke 1996). Cheptou et al. (2000b) reported that drought intensified inbreeding depression in growth and fecundity variables, but not in terms of survival, in the outcrossing species *Crepis sancta* (Asteraceae), a typical herb of old fields. Therefore, the available evidence, including the present study, is insufficient to conclude that inbreeding depression is greater in plants under drought stress.

It has been hypothesised that inbred mating should contribute to purging of deleterious mutations because in that scenario selection may act upon recessive alleles responsible for inbreeding depression (Lande & Schemske 1985). Consequently, mainly autogamous species often exhibit lower levels of inbreeding depression than preferentially outcrossing species (Husband & Schemske 1996). For instance, Cheptou et al. (2000b) showed that, among four populations of *C. sancta*, the most self-fertile population had the lowest level of inbreeding depression. Given that selection pressures are often stronger under stressful environments, it could be speculated that drought might increase the purging rate of deleterious alleles associated with inbreeding depression above the rate expected under normal conditions (Lande & Schemske 1985). In the case of the studied population of *C. chilensis*, the common events of drought in the habitat (Jaksic 2001), together with the current high reproductive output from autonomous selfing (Suárez et al. 2004) and the observed critical survival during seedling establishment (Suárez et al. unpubl. data), might suggest that this population could have experienced purging of deleterious alleles in the past, via seedling mortality. This might explain why inbreeding depression is presently very low, at least in the seedling stage, in this perennial species. However, it is important to keep in mind that the lack of differences in this early stage does not necessarily imply that in the adult stage they cannot occur. Some authors have suggested that in highly selfing plant species, lethal genes that are expressed early in development have been purged (Latta & Ritland 1994; Husband & Schemske 1996), but inbreeding depression may be detected at later stages (Dole & Ritland 1993).

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