

Effects of grazing disturbance on the reproduction of a perennial herb, *Cypella herbertii* (Lindl.) Herb. (Iridaceae)

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Abstract. A recent study in a natural grassland in Argentina revealed that grazing strongly reduced the available pollinators for a population of *Cypella herbertii*. Comparing grazed and fenced areas (exclosures) we tested the hypothesis that self-incompatibility would be selected against in a pollinator-poor environment leading to changes in breeding system. No differences in breeding system were found between populations from inside and outside exclosures. However, we detected: (i) *C. herbertii* clearly exhibits a mixed-mating system but has a remarkably low spontaneous fruit formation; (ii) this species though highly self-compatible expresses a significant decay at seed set stage when self-pollinated. We conclude that grazing impact on pollinators together with *C. herbertii*'s high dependence on pollinators should be considered when assessing the risk of extinction of this species.

Key words: *Cypella herbertii*, Iridaceae, grazing, disturbance, breeding system.

The importance of disturbances in shaping the structure and function of grasslands has been repeatedly emphasized (McNaughton 1983, Oesterheld et al. 1999 and references therein). Ecological factors, such as herbivory, may be important determinants of inbreeding depression, selfing rates, and

mating system evolution (Lloyd 1992, Herrera and Pellmyr 2002, Richards 1997 and references therein). Even though some effort has been devoted to studying the effect of disturbances on breeding systems (Cruden 1977, Feinsinger et al. 1987, Arroyo and Squeo 1990) there is still a lack of concrete data regarding the impact of cattle grazing on pollinator communities and its detrimental effect on plant reproduction. This issue, which is starting to be addressed world-wide, is of major importance to ecosystems function and conservation (Brazilian Ministry of the Environment 1999).

In the Flooding Pampa grasslands (Buenos Aires Province, Argentina) the disturbance produced by grazing has been found to strongly reduce the quantity and quality of pollinators available for a population of *Cypella herbertii*. Roitman (1998) found a highly significant 2.4 fold increase in the amount of available pollinators inside cattle exclosures compared to heavily grazed surroundings ($p < 0.0001$, $n = 48$). Remarkably, after three years of field observations *C. herbertii*'s specialist pollinator (an oil-collecting apid bee from the genus *Chalepogenus*) was only recorded visiting flowers inside

cattle-free enclosures and was never seen outside. The scarcity or even total absence of pollinators outside enclosures was attributed to the lower availability of nesting sites (due to soil compaction and vegetation changes) caused by cattle grazing (Gess and Gess 1993). This effect is likely to be more significant for ground nesting species such as *Chalepogenus* spp. Preliminary data suggested that this pollination shortage could account for an increase in self-compatibility in *C. herbertii* populations due to a negative selective pressure on self-incompatibility alleles (Roitman 1998).

The underlying hypothesis that was tested in the present study is that plants of *C. herbertii* from populations where pollinators are abundant are less autocompatible than plants from populations where pollinators are scarce or absent. The present work will attempt to describe the relationship between breeding system, inbreeding depression and reproductive success in *C. herbertii* populations under different disturbance regimes.

Materials and methods

The study was carried out in a floodplain rangeland located at the center of the Salado River basin (36°30'S, 58°30'W), which constitutes the main portion of the Flooding Pampa, that extends over 58,000 km² in Buenos Aires Province, Argentina. In this region, a temperate climate with a mean annual rainfall of 900 mm and halomorphic, poorly drained soils and summer droughts determine natural grasslands to be the dominant vegetation type (Cabrera 1976, León 1975, León et al. 1979). Palaeontological evidence suggests that the extinction of big mammals in late Pleistocene led native species to evolve under a low herbivore pressure (Owen-Smith 1987, Sala et al. 1986, Webb 1978). However, for the past century, these grasslands have been uninterruptedly grazed by cattle and the actual flora includes many adventitious species associated with grazing conditions (Sala et al. 1986).

Field work and material collection was carried out in a floodplain rangeland located at the "Las Chilcas" ranch where two permanent enclosures precluding cattle grazing were established 4 (Exc 4) and 19 (Exc 19) years prior to the beginning of the

essays. These two enclosures are ca. 2 ha each and are ca. 4 km apart. From each a sample of 15 plants of *C. herbertii* was collected. A third sample was obtained from the grazed area (GA) a hundred metres away from Exc 4. This area was year-long grazed by a cattle stocking rate of 0.5 animal/ha. Native tall grasses dominated the community inside the enclosures while the grazed site was dominated by forbs, mainly exotic (Chaneton et al. 1988). In May 1997 all plants were collected, transplanted to plastic boxes (80 × 40 × 25 cm) and placed in a glasshouse at the "Lucien Hauman" Botanical Garden (Faculty of Agronomy, University of Buenos Aires) (34°35'27"S 58°28'49"W) where all subsequent experiments were carried out starting in November 1997. Plants were watered twice a day during glasshouse culture, to avoid water stress effects.

Focal species. *C. herbertii* is one of several Iridaceae known to produce lipidic oils by special floral glands, called elaiophores, as a floral reward to their highly specific bee pollinators (Buchmann 1987, Vogel 1988). *C. herbertii* is a perennial bulbiferous herb that ranges from southern Brasil to north-eastern Argentina. The floral scape grows up to 30–100 cm. Its actinomorphic flowers have six orange-yellow tepals; the outer three being much bigger (3–4.5 cm long) than the inner ones (0.9–1.4 cm long). *C. herbertii* produces multiseeded capsules.

During the flowering season (November to January) the number of open flowers each day in single individuals is usually one, rarely 2 or 3 in exceptionally vigorous individuals. Flowers start opening early in the morning (ca. 7–8 AM) and remain open until 6–7 PM (M. Devoto, unpublished data).

Breeding system, reproductive success and inbreeding depression. For three seasons (1997, 1998, 1999) and starting in November, data were gathered on the efficacy of self and outcross hand pollinations (as described in Dafni 1992) in several fitness components (fruit set, seed set, germination rate, total germination and seedling survival). These were performed on all previously transplanted plants from sites Exc 4, Exc 19 and GA resulting in a six treatment factorial experiment (3 sites × 2 pollination modes). Maternal plant effect was not controlled in our experiment (Lynch 1988). Instead, pollinated flowers were randomly selected from all flowers available each day. This

resulted in several self- and cross-pollinations performed on each individual plant through the three years of experiments. Also, randomization of flower choice within plants avoided biases caused by differential resource allocation based on flowering time or the proximity to photosynthate producing leaves. In late bud stage (the afternoon before opening) flowers were tagged, emasculated (except for spontaneous and forced selfing treatments) and covered with translucent paper bags. Pollination was carried out the next morning using a fine paint-brush. Pollen from one to three dehiscent anthers was evenly applied across stigmas. Flowers were rebagged after pollination, and mature capsules were collected prior to dehiscence. Also, 15 flowers from several GA individuals were left unpollinated to test for spontaneous self-pollination/apomixis.

For seed germination tests (1998 and 1999) a sample of eight fruits from each treatment was randomly selected from different individuals. Seeds were stored in a dark dry place until early June when a sub-sample of twelve randomly selected seeds per individual was placed over moistened filter-paper in germination boxes to constant 20 °C. This resulted in 3 (sites) × 2 (pollination modes) × 8 (fruits) × 12 (seeds per fruit) = 576 tested seeds per year.

Germination was monitored every seven days for five weeks and seedlings with a radicle longer than 3 mm were transplanted to a 5 mm depth in plastic boxes (65 × 40 × 10 cm) filled with dark finely grained sterilized soil. After remaining six weeks under greenhouse conditions and regular watering, survival of transplanted seedlings was recorded.

Breeding system and reproductive success were estimated by calculating several comparative measures: fruit set (no. capsules / no. flowers pollinated), filled seed number per capsule, percentage seed germination (total no. of seeds germinated / 12*100), germination rate (calculated as the inverse of the mean germination time [MGT] measured in weeks obtained from the Kaplan-Meier analysis; see Statistical Analysis for details), and percentage of seedling survival.

Also, Inbreeding Depression (δ) (Kephart et al. 1999) was estimated for each site. For δ calculation fitnesses of selfed and outcrossed progeny were compared at five different stages: (i) fruit set after matings described above, (ii) seeds per capsule; (iii) progeny germination rate, (iv) percentage seed

germination and (v) seedling survival. Since all stages were assessed in the glasshouse where growing conditions were optimal, inbreeding depression estimates are probably conservative since higher values could occur under less favorable field conditions. Progeny transplants into natural environments were precluded by logistic impediments. At each stage and for each site, relative performance ratios of selfed (w_s) and outcrossed progeny (w_c) were calculated. A cumulative multiplicative ratio was then calculated as the product of the five stage ratios. This cumulative ratio was finally subtracted from 1 to obtain overall δ (Kephart et al. 1999).

Additionally, pollen-ovule ratio and outcrossing index (Cruden 1977) were calculated as an estimation of the expected breeding system. Pollen and ovule counts were performed on 10 flowers belonging to different individuals per site and the outcrossing index was estimated from 15 flowers at each site.

Statistical Analysis. Prior to analysis, data sets were tested for normality and homoscedasticity. The square-root transformation for seed set and the arcsine square-root transformation for percentage of fruit set, seed germination and survival were used for analysis (Sokal and Rohlf 1994). Mean Germination Time (MGT) was measured in weeks and calculated through a Kaplan-Meier (Kaplan and Meier 1958) survival test that was performed expressing available data as the non-germinated fraction for each date. MGT was also used to estimate germination rate as 1/MGT. All data were analysed using ANOVA tools of Statistica (Stat-Soft, Inc. 1999). Whenever possible, inter-annual variation was statistically tested. For all analyses, when the F test for treatments was significant ($p < 0.05$), Tukey's honest significant difference (HSD) multiple pairwise comparisons between means were performed. Deviations are standard errors (SE) unless otherwise noted.

Results

Effect of site and pollination treatment on fitness components. There was no significant effect of neither site nor pollination treatment on fruit set and seedling survival (Table 1). Plants from all three sites exhibited no differences between self and cross-pollination in these two fitness

Table 1. Effect of year, origin and pollination treatment on fitness components of *Cypella herbertii*. * $p < 0.05$; **** $p < 0.001$ n.s. not significant

Effect	Fruit Set	Seed Set	Germination rate	Total germination	Seedling survival
Origin	n.s.	n.s.	*	*	n.s.
Pollination mode	n.s.	****	n.s.	n.s.	n.s.
Origin \times Pollination mode	n.s.	n.s.	n.s.	n.s.	n.s.

components (Table 2). Among treatments fruit set ranged from 17.7 to 36.2%, germination percentage from 85.4 to 95.8% and seedling survival from 92.2 to 100% (Table 2).

Pollination treatment had a highly significant effect on seed set ($p < 0.001$) (Table 1). In all three sites, capsules obtained from self-pollination had fewer seeds than those from cross pollination ($p < 0.05$, Tukey's HSD) (Table 2). Overall, self-pollinations had produced an average fruit set of $24.3 \pm 4.5\%$, while cross pollinations had produced $35.1 \pm 7.5\%$ (Table 2). Site did not have any effect on seed set (Table 1).

Origin had a significant effect on germination rate and total germination (Table 1). Pairwise comparisons revealed Exc 19 seeds achieved a higher total germination than Exc 4 and GA seeds ($p < 0.05$) which resulted not different from each other (data not shown). Kaplan-Meier survival analysis revealed that

origin had a significant effect on germination rate (Table 1): seeds from the grazed site germinated slower than seeds from the old enclosure (Table 2).

Inbreeding depression (δ). Selfed progeny in both enclosures had lower fruit set than crossed progeny. The reverse was true for GA plants (Table 3). Selfed progeny in both enclosures had lower seed set than crossed progeny, while for GA plants there was little difference between selfed and crossed seed set (Table 3). Thus, δ measurements (Fig. 1) were positive for fruit set in Exc 19 and Exc 4 plants and negative (i.e. there was outbreeding depression) for fruit set in GA plants. Likewise, δ was higher for seed set in Exc 19 and Exc 4 than in GA plants. No remarkable differences between selfed and crossed progeny were observed in total germination, germination rate or seedling survival, which is also reflected in δ estimations close to 0 (Table 3 and Fig. 1). Averaging

Table 2. Results of selfings and crossings, and performance of selfed and outcrossed progeny of *Cypella herbertii* from sites that experienced different disturbance histories (pooled data from 3 years). Means followed by the same letter in a column are not significantly different ($p > 0.05$, Tukey's HSD). Fruit set and seed calculations are based on $N = 15$ individuals. Total germination, Mean germination time (MGT) and survival calculations are based on $N = 8$ and $N = 5$ samples of twelve seed(ling)s from each treatment, respectively. Seed set and MGT are expressed in seed number per fruit and weeks, respectively

Treatment	Fruit set (%) (N flowers)	Seed set (N fruits)	MGT (\pm SD)	Total Germination (%)	Seedling survival (%)
Exc 19 self-pollinated	17.7 ± 1.5 (40)	74.8 ± 8.58 (28) a	3.19 ± 1.38	94.7 ± 3.1	95.7 ± 2.3
Exc 19 cross-pollinated	23.9 ± 8.2 (64)	112.9 ± 10.72 (43) b	3.20 ± 1.43	95.8 ± 2.2	100 ± 0
Exc 4 self-pollinated	18.8 ± 10.2 (62)	83.6 ± 7.41 (40) a	3.14 ± 1.38	85.4 ± 3.7	92.2 ± 7.8
Exc 4 cross-pollinated	24.5 ± 12.5 (83)	119.5 ± 8.52 (52) b	3.17 ± 1.37	85.4 ± 8.4	100 ± 0
GA self-pollinated	36.2 ± 5.6 (74)	101.9 ± 10.31 (40) a	3.32 ± 1.37	89.5 ± 4.3	100 ± 0
GA cross-pollinated	26.3 ± 7.3 (91)	108.9 ± 8.54 (47) b	3.25 ± 1.38	88.5 ± 5.4	98.4 ± 1.6

Table 3. Relative performance ratios of selfed and outcrossed progeny of *C. herbertii* at five stages: fruit set (ISI), seed set, germination rate, total germination and seedling survival. Values of cumulative multiplicative ratio (CumFit), and overall δ are also given for each site

Origin	Fruit set	× Seed set	× Germination rate	× Total germination	× Seedling survival	= CumFit	$\delta = 1 - \text{CumFit}$
Exc 19	0.74	0.66	1.01	0.99	0.96	0.46	0.54
Exc 4	0.77	0.70	1.00	1.00	0.92	0.49	0.51
GA	1.38	0.94	0.98	1.01	1.02	1.32	-0.32
Average	0.96	0.77	1.00	1.00	0.97	0.76	0.24
Stage δ	0.04	0.33	0.00	0.00	0.03		

through sites the only stage where inbreeding depression was evident is in seed set (33%) while in fruit set, germination percentage and seedling survival δ values were extremely low (values close to 0; Table 3).

Self-compatibility and breeding system. The ratio no. fruits from self-pollination / no. fruits from cross-pollination (Index of Self-Incompatibility [ISI] *sensu* Ruiz and Arroyo 1978; modified by Dafni 1992) ranged from partial self-incompatibility (Exc 4 and Exc 19) to self-compatibility (GA) (first column Table 3). When averaging across sites overall ISI was 0.96 ± 0.2 which reflects partial self-incompatibility (almost complete self-compatibility) (first column Table 3).

Fruit set following hand self-pollination ranged from 17.7 to 36.2% across sites (Table 2). Following spontaneous self-pollination *C. herbertii* yielded a fruit set of

$3.7 \pm 2\%$ which resulted significantly lower than values of all other treatments ($p < 0.05$).

For *C. herbertii*, outcrossing index (with a value of 3) predicts self-compatibility and some demand for pollinators. Remarkably, P:O ratio (6250 ± 98) predicts obligate xenogamy.

Discussion

Grazing history and breeding system. This study failed to show any effect of severe decrease in pollinator abundance and diversity on the breeding system of a population of *Cypella herbertii*. The proposed hypothesis is not supported by our data: individuals from three sites with different grazing background exhibited the same responses to mating experiments.

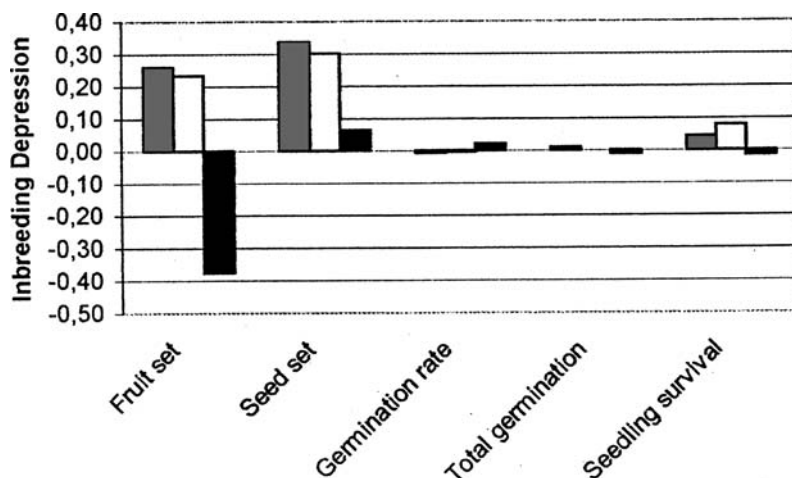


Fig. 1. Levels of inbreeding depression (δ) of *C. herbertii* at five life cycle stages based on the relative performance ratios of selfed (W_s) and outcrossed progeny (W_c) for three sites (see Table 3). δ was calculated as $1 - W_s/W_c$. Shaded bars, Exc 19; open bars, Exc 4; solid bars, GA

Even though previous studies revealed a severe decrease in pollinator abundance and diversity outside the exclosures used in this work (Roitman 1998), two main causes could be overriding this “exclosure effect” on the *C. herbertii* population. In the first place, the whole study area has a long grazing history that dates back at least to the end of the 16th century (León et al. 1984, Sala et al. 1986, Soriano 1991), while exclosures were established less than twenty years before data collection. The introduction of horses and cattle in the 16th century and the rapid increase of wild cattle in the whole pampas region at the beginning of 17th century (Soriano 1991) could have fostered in a relatively short term the loss of genetic features (e.g. self-incompatibility alleles) that are strongly selected against under grazing conditions (Bazzaz 1979, Loreti et al. 1994). Thus, the three sites compared might have a strong common background consisting of a generalized loss of self-incompatibility alleles which precludes any differences in breeding system between sites.

Secondly, the small size (ca. 2 ha) of the exclosures relative to the grazed area could also account for the lack of difference between sites. Gene flow across the entire population (including exclosures) could be more important than suggested by local pollinator abundances inside and outside exclosures. This effect could render homogeneous the pool of self-incompatibility alleles throughout the entire population (Broyles et al. 1991). Patterns of abundance of pollinators are not good predictors to estimate pollen flow across- and between populations (Willson 1984).

Breeding system. *C. herbertii* clearly exhibits a mixed-mating system. ISI estimations for each site, as well as overall ISI reflect partial (almost complete) self-compatibility. While fruit set following self-pollination ($24.3\% \pm 4.5$) ranks *C. herbertii* as slightly self-compatible, the low fruit set (3.7%) of unmanipulated flowers strongly suggests *C. herbertii* requires insect pollination. Natural fruit set from another large population of *C. herbertii* ca. 100 km north of Las Chilcas

population still in the Salado River basin ($35^{\circ}32'S$, $58^{\circ}00'W$) was found to be $12.5 \pm 2.6\%$ (M. Devoto, unpublished data). This fruit set was achieved in spite of the complete absence of pollinators even though this second population of *C. herbertii* was in full bloom. The three inner tepals while closing can occasionally remove pollen from the stamens rubbing it against stigma lobes (M. Devoto, unpublished obs.) and thus account for this low fruit set. This delayed selfing (sensu Lloyd and Schoen [1992]) mechanism apparently constitutes a reproductive assurance mechanism (Klips and Snow 1997 and references therein; Traveset 1999) and should receive further attention.

On the other side, the extended flowering season with most plants opening only one flower each day allow pollinators to carry pollen between plants, fostering xenogamy and rendering geitonogamy less likely (Traveset 1999). *C. herbertii*'s P:O ratio suggests facultative xenogamy and, likewise, there is a clear increase of fitness in xenogamous matings compared against selfings (Tables 2 and 3). Even though P:O ratio as a predictor of a plant breeding system has received criticism (Charnov 1979, 1982; Cruden et al. 1996) it remains widely employed in studies referred to hermaphrodite species (Cruden 2000, Wyatt et al. 2000, Pias and Guitián 2001) such as *C. herbertii*.

Inbreeding depression (δ). In *C. herbertii*, inbreeding depression effects are limited to seed set stage. Similar results, where inbreeding depression is manifested only in some stages throughout life-cycle, were found in other species (e.g. Charlesworth and Charlesworth 1987, Karron 1989, Kephart et al. 1999, Schaal 1984, Waller 1984). The detection of differences between self- and cross-pollination at fruit set and seed set stage but not at the germination and survival stage may be attributed to pre-zygotic partial autocompatibility, late acting incompatibility or post-zygotic inbreeding depression mechanisms (Traveset 1999). However, it is not possible to discern between these effects given the nature of our

experiment. (Hiscock and McInnis 2003, Richards 1997). From our results, field behavior of progeny is difficult to predict because micro-environment both inside and outside enclosures can be very different from glasshouse conditions which can influence the magnitude of inbreeding depression (Hauser and Loeschke 1994).

Conservation implications and conclusions. The risk of a plant species extinction can be assessed by considering (i) the probability of pollinator failure, (ii) reproductive dependence on the mutualism and (iii) demographic dependence on seeds (Bond 1994). Previous studies in the Flooding Pampa grasslands revealed that livestock grazing had a significant detrimental effect on pollinators (Roitman 1998). We report here the remarkably low efficiency of spontaneous seed formation and the negative effect of selfing on key fitness components of *Cypella herbertii*, a species that engages in a highly specific mutualism with an oil-collecting bee. Our results suggest that perpetuation of local *C. herbertii* populations is highly dependent on insect pollination to ensure a high quality progeny. However, the importance of seeds in the demography of *C. herbertii* has not been assessed and other mechanisms such as side-sprouting from bulbs might compensate for pollinator reduction and low reproductive success (Traveset 1999). A similar case is found in *Ixianthes retzioides* (Scrophulariaceae), a rare South African shrub with large oil-secreting flowers (Steiner 1993). Its only specialist pollinator is locally extinct in several populations causing a 37-fold decrease in reproductive success compared to the populations where the pollinator is present. However, the plant species can propagate vegetatively and is thus not immediately threatened with extinction (Steiner 1993, Steiner and Whitehead 1996). On the other side, in *Ixianthes*, as also happens in *Cypella*, long-term consequences of loss or severe reduction of a specialist pollinator are far more complex to evaluate (Bond 1994).

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