Is *Echeveria gibbiflora* (Crassulaceae) fecundity limited by pollen availability? An experimental study

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Summary

1. An experimental approach, manually pollinating all the flowers of individual plants, was used to measure the effect of pollen limitation on female fecundity of the hummingbird-pollinated perennial shrub *Echeveria gibbiflora* in the ecological preserve of Pedregal de San Angel around México City, México. Eleven randomly selected plants were manually over-pollinated in all their flowers and another 11 were left to be freely visited by natural pollinators.

2. Manually pollinated plants produced significantly more fruit and seeds than control plants (1·38 and 1·74 times, respectively). There was no change in average mass of fruits.

3. Considering individual fruit production per plant sampled three times in one season, decreases in fruit mass and average seeds per fruit were observed within the same reproductive season for both treatments. For the manually pollinated plants, from the start to the end of the reproductive season, seed set decreased 55·9%; while in control plants seed set decreased 33·4% in the same period. For both treatments, average fruit mass decreased 26%.

4. Vegetative growth was not significantly different between control and experimental plants but hand-pollinated plants showed a smaller reproduction probability for the following year.

5. It is concluded that female fecundity in *E. gibbiflora* is limited by pollen early in the reproductive season and by resources in the middle and the end of the season.

Key-words: Female fecundity, hummingbird pollination, México, pollen limitation, resource limitation

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Introduction

Two main evolutionary hypotheses are usually invoked when attempting to explain the low production of fruits and seeds per flower in many species of angiosperms: pollen limitation or resource limitation (Stephenson 1981; Sutherland 1986; Charlesworth 1989). Both hypotheses have traditionally been considered mutually exclusive (Stephenson 1981; Udovic & Aker 1981) but theoretical arguments and experimental evidence have demonstrated that pollen and resource limitation can interact to produce low seed and fruit sets (Aker 1982; Bertin 1985; Haig & Westoby 1988; Casper & Niesenbaum 1993). Pollen limitation may be the result, among other factors, of either low pollinator densities or poor quality available pollen (e.g. incompatibility with the recipient plant) (Aker 1982; Schemske & Pautler 1984; Waser & Price 1989; Vaughton 1991).

Experimental designs to differentiate the two types of limitation include the pollination of all the flowers (or a sample) of individual plants (Bierzychudek 1981; Bawa & Webb 1984). If hand-pollinated plants produce more fruits, the population is considered to be pollen limited, whereas if they are resource limited they will produce the same or fewer fruits and seeds in the same season. However, it is possible that the resources of other parts of the plant, such as roots or leaves, are allocated to fruits (Janzen *et al*. 1980) and cause low growth rates, as well as a decrease of resources for future reproduction events and/or higher mortality. Using hand-pollination experiments Ackerman & Montalvo (1990) and Primack & Hall (1990) have demonstrated these effects (but see Calvo & Horvitz 1990). Therefore, it is important to measure the differences in fecundity not only at a given moment (e.g. peak flowering) but also during the entire reproductive season, so that the long-term effects on growth, reproduction and survival of hand-pollinated plants and freely pollinated plants can be compared.

In this paper, we used an experimental approach to demonstrate pollen limitation in the hummingbird-pollinated *Echeveria gibbiflora* DC, manually pollinating all the flowers of individual plants and then studying the effects of this hand pollination on their future vegetative growth and reproduction. This Crassulaceae species has been described as pollen limited despite its high abundance (Parra 1988), and it is visited by only one species, the Hummingbird *Cynanthus latirostris* (Parra, Vargas & Eguiarte 1993).

Materials and methods

STUDY AREA

The study was carried out during the winter seasons of 1991–92 and 1992–93 in the Pedregal de San Angel Preserve, south-west México City (19°20′33″ N and 99°08′26″ W; 2290–2310 m a.s.l.). The Pedregal de San Angel presents a xerophytic shrub community with neotropical affinities. Plants grow on basalt rock substrate, which originated from the lava of a nearby volcano erupting *c.* 2500 years ago (Rojo 1994).

STUDIED SPECIES

Different reproductive, population, ecological and evolutionary aspects of *E. gibbiflora* (Crassulaceae) have been studied (Parra *et al.* 1993; Eguiarte, Parra & Vargas 1994; Larson, Eguiarte & Cordero 1994). *Echeveria gibbiflora* is a perennial succulent rosette of 15 leaves around a prostrated thick stem (±5 cm diameter). Paniculate inflorescences rise up to 1 m during the dry season (October to January). They bear an average of 160 floral buds and the mean number of inflorescences per flowering plant is 1·8 ± 1·7. Flowers are perfect, red, tubular and *c.* 2·5 cm long with 10 stamens and five styles. The flower is open for about 7–8 days when it can be pollinated by *C. latirostris*. Self-compatibility exists but hummingbird visits are needed to produce seeds. The fruits are dry, dehiscent and produce around 200 elongated 1 mm-long seeds per fruit. The population appears to have no detectable inbreeding depression (Parra *et al.* 1993).

HAND-POLLINATION AND POLLEN-LIMITATION EXPERIMENTS

Twenty-two plants of similar sizes were selected at the same site under similar soil and daylight conditions at the beginning of the reproductive season (Table 1). Eleven plants were randomly chosen and all their open flowers were manually pollinated, every 4–5 days while still open (Parra *et al.* 1993), with the pollen of three randomly chosen plants from the same site (1700 flowers were hand pollinated). The other 11 plants were regarded as controls that could be freely pollinated by natural visitors. Rosette diameter, stem length and the number of floral buds were recorded early in the reproductive season (Parra 1988). We were able to count the total number of floral buds because in *E. gibbiflora* it is possible to distinguish early in the reproductive season all the floral buds that the plant will produce during that season (Parra 1988).

The effect of hand pollination on female fecundity was evaluated at two levels: (a) the total number of fruits per individual plant in the entire reproductive season and (b) the number of mature seeds per ovule per mature fruit (seed set).

The total number of fruits per plant was counted as all the non-aborted and dehiscent fruits at the end of reproductive season. Fruit set of an individual plant was counted as the total number of fruits matured divided by the total number of floral buds. The mean mass of a fruit per plant was determined through the collection of five mature fruits from experimental and control plants on three occasions in a season (January, February and March). Fruits were weighed and for each fruit, mature and aborted seeds were counted using a dissecting microscope. Total ovule production was counted as the number of aborted and mature seeds; seed set was represented by the number of mature seeds per fruit divided by the total number of ovules per fruit. Seed set and average fruit mass differences between control and pollinated plants were tested with a repeated measures ANOVA (Zar 1984; StatView 1996) and differences in total fruit set were tested using a Student's *t*-test (Zar 1984).

EFFECTS ON GROWTH AND REPRODUCTION

Pollination treatment effects on vegetative growth and reproduction were determined by assessing both rosette-diameter and stem-length changes of individual plants at the beginning of the next reproductive season (November 1992). Rosette growth rates (GRR) were estimated as GRR (rosette) = [(FDR–IDR) ÷ IDR], where IDR was the initial rosette diameter in November 1991 and FDR was the final rosette diameter in November 1992. The same growth rates were estimated for the stem length.

Hand-pollination experiments can provoke an increased reproductive effort that may represent a cost by reducing future reproduction probability. Thus, in

Table 1. Mean and standard deviation for rosette diameter, stem length and number of floral buds in plants of *Echeveria gibbiflora* in the Pedregal de San Angel, México City, before a hand-pollination experiment: *n* = 11 for each treatment; NS, not significant

	Control		Hand-pollinated		
Variable	Mean	(SD)	Mean	(SD)	*t*-test
Rosette diameter (cm)	45·00	(7·14)	43·40	(8·52)	1·62 NS
Stem length (cm)	32·16	(13·30)	37·10	(10·30)	1·31 NS
Number of floral buds	171·80	(53·60)	161·10	(49·70)	0·64 NS

order to measure the probability of reproduction, we noted whether or not each plant produced an inflorescence during November and December 1992. The probability of reproduction of control and hand-pollination treatments was compared using the proportion of active reproductive plants in a chi-square test.

Results

Hand-pollinated fruit sets were 1·38 times higher than freely pollinated ones (75·2% ± 14·9 and 54·4% ± 17·6, respectively; $t = 3·88$, $P = 0·009$). Repeated measures ANOVA showed that hand-pollinated plants had significantly (1·74) higher seed set than control plants (54·3% ± 12·6 and 31·1% ± 15·6, respectively; Table 2). However, significant effects of the sampling month and the interaction were observed for seed set (Table 2). Thus, the seed set per mature fruit decreased through the study period for control and hand-pollinated plants (Fig. 1a). For the hand-pollinated plants, from January to March, seed set decreased 55·9%; while in control plants seed set decreased 33·4% in the same period (Fig. 1a). The mean number of seeds produced per fruit was also higher for pollinated plants than control (119·14± 22·1 and 93·6 ± 17·6, respectively). Thus, hand-pollinated plants produced 1·27 times more seeds in each mature fruit ($t = 2·85$, $P = 0·005$) than plants only pollinated by the natural visitor *C. latirostris*.

No significant effect between hand-pollinated and control plants was observed with respect to fruit mass but decreasing dry mass through the study period was noticed again for hand-pollinated and control plants (Table 2 and Fig. 1b). On average for both treatments, the fruit mass decreased 57 mg (26·1%) between January and March.

The probability of reproduction in the next year was 1·5 times smaller for hand-pollinated plants (54·6% *vs* 81·8%; $\chi^2 = 3·14$, $0·1 > P > 0·05$) than for control plants. However, both hand-pollinated and control plants had the same growth in the diameter of the rosette (0·25 cm year^{-1} ± 0·3 and 0·3 cm year^{-1} ± 0·41, respectively; $t = 0·41$, $P > 0·5$) and the same growth in the length of the stems (0·11 cm year^{-1} ± 0·08 and 0·12 cm year^{-1} ± 0·04, respectively; $t = 1·5$, $P > 0·1$).

Discussion

The addition of pollen to *E. gibbiflora* flowers produced a significant positive effect on female fecundity, increasing the probability of flowers becoming mature fruits and ovules becoming mature seeds within a single reproductive season. Yet, it is worth noticing that the different increases on their fecundity appeared to be caused by a larger pollen demand at the beginning of the reproductive season (January). Later additions in the reproductive season (February–March) had a lesser fecundity increase and, moreover, a smaller number of mature seeds was produced by each fruit of experimental and control plants (Fig. 1a). These results suggest a more intense resource limitation in the middle and at the end of the breeding season.

This late decrease of the number of seeds per fruit could not be explained by pollination reduction as the Hummingbird *C. latirostris* (Parra *et al.* 1993) is a resident species in the study site (Arizmendi *et al.* 1994) and was observed throughout the reproductive season (V. Parra-Tabla & C. F. Vargas, unpublished data).

The seed-set decrease per fruit and the average fruit mass decrease could also be explained as a consequence of the gradual reduction of resources towards the end of the reproductive season. Yet, smaller sizes (masses) and sets of late-formed fruits may be caused by physiological, genetical or morphological processes other than resource limitation (Lee 1988; Dudash 1993). Similar patterns have been described for several species in which an earlier flower opening results in a better opportunity to mature than a later one (Bawa & Webb 1984; Eguiarte & Búrquez 1987; Lee 1988).

Temporal variations in pollen and resource availability that affect plant breeding success can be a common factor for hermaphroditic species which depend on pollinators and on grounds with limited resources (Johnston 1991; Lawrence 1993). These variations

Table 2. Repeated measures ANOVA for (a) seed set (%) and (b) average fruit mass (mg) in *Echeveria gibbiflora*. The treatment refers to hand-pollinated plants and naturally pollinated plants. Samples were obtained in three months (January, February and March) during one reproductive season

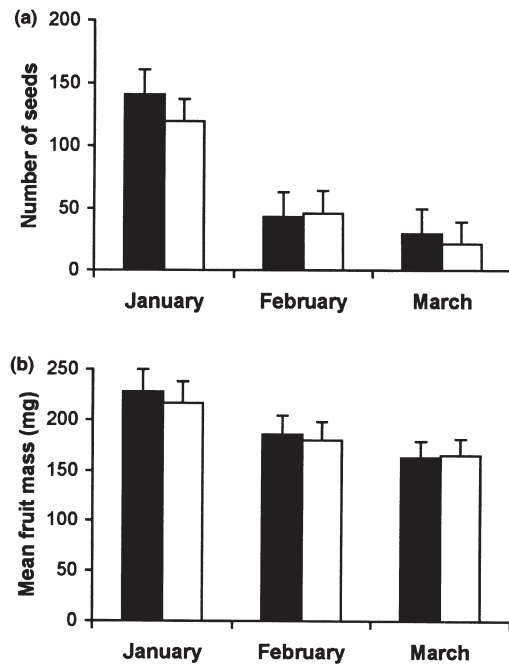| Variable	Source of variation	df	SS	MS	F	P
(a) Seed set (%)	Treatment	1	0·529	0·529	5·85	0·0252
	Month	2	1·925	0·963	48·2	<0·0001
	Treatment × month	2	0·132	0·066	3·29	0·0473
	Subject	20	1·809	0·09		
	Month × subject	40	0·799	0·02		
(b) Fruit mass (mg)	Treatment	1	1336·5	1336·5	0·10	0·753
	Month	2	135743	67871·5	21·3	<0·0001
	Treatment × month	2	1647·9	823·83	0·25	0·77
	Subject	20	263384	13169·2		
	Month × subject	40	127193	3179·85		

Fig. 1. Results of fruit samples of *Echeveria gibbiflora* on three successive occasions (January, February and March) in one season: (a) seed set (%); (b) average of mass per fruit (mg). Empty bars are control plants and solid bars are hand-pollinated plants. Standard error is shown in each bar.

become natural 'constraints' both during different reproductive seasons and within a reproductive season (Casper & Niesenbaum 1993). Pollen variation might be an important limiting factor within a single reproductive season, but it may also affect the pollen-flow dynamic (i.e. gene flow), which could partly explain reproductive success variance of a population (Waser & Price 1989) and have relevant consequences on the evolution of plant breeding systems (Lloyd 1979; Holsinger 1991).

In our study, the resource-limitation hypothesis led to the prediction that the growth rate and reproductive probabilities of rosettes would be negatively affected by pollen additions. Hence, diminished reproductive probability of *E. gibbiflora* suggests that the extra reproductive effort by pollen addition would lead to a trade-off between present and future reproduction and resource availability. As rosette growth rate did not decrease significantly with pollen addition, we considered that an effect at this level could only be detected within larger time intervals as in *Cypripedium acaule* (Primak & Hall 1990) and *Epidendrum ciliare* (Ackerman & Montalvo 1990).

In conclusion, results of experimental pollen additions indicate that resource limitation plays an important role for the reproductive success of the species. It is necessary to carry out specific experiments, such as additions or limitation of nutrients, water and light, to identify the most important limiting resources which constrain female fecundity (Campbell & Halama 1993), and their combined effects with manual-pollination experiments using different qualities and quantities of pollen at different times of the reproductive season (Calvo & Horvitz 1990; Casper & Niesenbaum 1993).

There are few demonstrations of fecundity limitation using hand pollination in all the flowers of a single plant because manual-pollination procedures are unsuited for many plant species (Janzen *et al*. 1980). This situation imposed small sample size but more pollination experiments using all the flowers of a single individual are needed rather than the pollination of some flowers in different individuals (Bawa & Webb 1984).

Pollen- and resource-limitation hypotheses have led to models about plants maximizing their fecundity and such models and empirical data have emphasized that seed production can be limited by either pollen or resources (Stephenson 1981; Charlesworth 1989). However, both factors must be jointly evaluated considering the role of spatial and temporal pollen and resource changes as limitations that condition the evolution of strategies that maximize reproductive success of hermaphroditic plants.

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